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Isotopic analysis of arctic ground squirrel tissues and potential food sources

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ISOTOPIC ANALYSIS OF ARCTIC GROUND SQUIRREL TISSUES AND POTENTIAL FOOD SOURCES

BY

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THESIS

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ABSTRACT

ISOTOPIC ANALYSIS OF ARCTIC GROUND SQUIRREL TISSUES AND POTENTIAL FOOD SOURCES

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Given limited knowledge of the food sources of Arctic ground squirrels, an important arctic prey species, it is difficult to predict the implications of changes in food source availability that could result from climate change. I hypothesized that Arctic ground squirrels at two colonies, Atigun and Toolik, would have similar feeding habits and mushrooms would contribute to their diet The Arctic ground squirrels at Toolik had significantly higher del15N (3.7 per mill difference) and del13C values (1.3 per mill difference) than those at Atigun. Mixing models indicated that the signatures observed in the Atigun hair could result from a combination of several foods. The relatively high isotope values of the Toolik hair indicated that squirrels there are feeding on a food source with higher isotope values than most of the sampled vegetation. Mushrooms could provide a proportional contribution between 0.09 and 0.60 to the diet of Toolik squirrels.

CHAPTER 1

BACKGROUND

Arctic Ground Squirrels

Arctic ground squirrels, *Spermophilus parryii*, are the northernmost mammalian hibernators and can weigh more than 1000g (Buck & Barnes 1999a). This mass can be difficult to attain over a short active season during which the growing season of vegetation lasts only 6 to 10 weeks (Chapin & Shaver 1985). Arctic ground squirrels spend seven to nine months hibernating during which their body temperatures can drop as low as -2.9˚C (Barnes 1989). They must stay actively thermogenic for more than five months of hibernation due to burrow temperatures of less than -3˚C (Buck & Barnes 1999b). The costs of hibernation may vary between males and females and also juveniles and adults due to differences in burrow temperatures. Females were found to inhabit burrows that were warmer than those of males and adults were found to inhabit burrows that were warmer than juveniles (Buck & Barnes 1999b).

Several studies have offered data on Arctic ground squirrel food choices (Mayer 1953, Batzli and Sobaski 1980, McLean 1985, McLean and Towns 1981). Mayer (1953) reported field observations of Arctic ground squirrels consuming *Dryas integrifolia*, *Pedicularis sp.*, *Carex sp.*, *Eriophorum sp.*, leaves

of *Poa sp.* and seeds of *Polygonum viviparum.* Arctic ground squirrels in captivity were found to consume almost any greens offered. The author also listed foods as berries of *Arctostaphylos alpina* & *Vaccinium vitus-idaea*, seeds of *Polygonum* & *Astragalus*, mushrooms, *Polygonum* roots and one animal with stomach content of 60% insect material.

McLean (1985) found legumes to be the most common item in fecal samples (77.4%) in April and May by Arctic ground squirrels, *S. parryii plesius* in southwestern Yukon. Other common fecal contents were grasses (9.0%) and *Antennaria* (7.6%). While *Artemisia* only had a 0.3% frequency in feces in April and May, it jumped to a 74% frequency in fecal samples of females in August. A similar jump occurred for males (74% frequency in feces) but not until September.

In another study, stomach content analysis revealed forbs to be prevalent in all months (May-August) with the highest percent of forbs (72.7±6.2%) occurring in July (Batzli and Sobaski 1980). Presence of seeds in stomach contents increased from 14.6±5.3% in May/June to 34.2±7.4% in August. Dicotyledon roots decreased from 12.4±7.8% of stomach contents in May/June to only 0.3±0.3% in August. Shoots on monocotyledons (12.1±4.3%) and leaves of deciduous shrubs (11.8±7.0%) were both at their highest in May/June. Batzli and Sobaski (1980) found the five most palatable plants in the Atkasook area of Alaska to be *Lupinus arcticus* (94.61% consumed), *Astragulus alpinus* (92.25% consumed), *Pedicularis capitata* (83.87% consumed), *Salix alaxensis* (75.36% consumed) *and Equiseteum arvense* (74.74 % consumed).

Additional studies have recorded foods carried in cheek pouches for caching and contents of retrieved caches (Krog 1954, Gilles et al. 2005, Zazula et al. 2006). Analysis of cheek pouch contents of Arctic ground squirrels at a southwestern Yukon site found *Polygonum viviparum* seeds or rhizomes in over 90% of squirrels examined. Seeds of sedges and rushes were also common in cheek pouches (Gillis et al. 2005). Excavated caches from a site in central Yukon revealed fruits and seeds of *Geocaulon lividum* and *Rosa acicularis* to be the most common items (Zazula et al. 2006). The dominant vegetation items in both of these studies were relatively rare in the local plant communities. One ground squirrel cache excavated in northern Alaska contained *Salix sp.* leaves and seeds of grasses and rushes (Krog 1954).

Isotope modeling of diets

Isotopic modeling of food items offers an alternative to the more traditional dietary analysis techniques of stomach content analysis, fecal analysis and observation of foods consumed. These traditional methods provide information only on foods consumed at a specific time and are likely to miss some smaller or more difficult to identify food items. Isotope analysis can provide accurate, quantitative data that is averaged over different time periods according to the tissue analyzed (Koch 2007, Crawford et al. 2008). Assimilation of dietary nutrients is calculated in contrast to consumption of food items. This provides

information on nutritionally important food items and disregards items that may be consumed unintentionally that are assimilated poorly. Isotope analysis can also be less invasive than these other techniques if tissues such as hair or feathers are utilized.

Isotopes, Diet and Physiology

This study used isotope ratio measurements of carbon and nitrogen to determine potential dietary contributions of available food sources. However, there are factors other than diet that can influence tissue isotope ratios. Physiological factors that may influence tissue isotope ratios are discussed in this section.

Several studies have focused on the variation of ^{15}N discrimination values between diet and tissue (Vanderklift 2003, Robbins et al. 2005). Hobson et al. (1993) found that bird tissues (liver, muscle, blood and bone collagen) showed significant increases in $\delta^{15}N$ during fasting or limited food intake in both wild and captive birds. The fasting Ross' geese analyzed in this study showed decreases in total body mass from 1739.2±42.5 g at arrival on their nesting sites to 954± 57.3 g post-incubation. Values of $\delta^{13}C$ did not change significantly after fasting. This early research has led to studies analyzing the separate influences of diet protein quantity and protein quality on $\delta^{15}N$ values of tissues.

Difference in consumer and diet $\delta^{15}N$ is caused by fractionation due to transamination and deamination reactions within the body. During deamination

^{14}N amine groups are preferentially removed and excreted in urine (urea, uric acid or ammonia). This results in amino acids that are enriched in ^{15}N relative to diet (Gannes et al. 1998). Starving animals are forced to catabolize their own tissues resulting in deamination of body proteins to be used as energy and for constructing amino acids (Gannes et al. 1998).

More recent data have shown that animals that are in nutritional equilibrium with their diet can also have variable discrimination factors due to diet. The quantity hypothesis states that if an animal consumes a diet with high protein content, a relatively large portion of the nitrogen in the diet is lost in urine. This lost nitrogen has a disproportionately large amount of ^{14}N compared to ^{15}N. This results in a large discrimination value between diet and body tissues. However, in instances of lower dietary protein intake most of the nitrogen consumed is used up in building body tissues and very little nitrogen is excreted in urine. This results in much smaller discrimination values between diet and body tissues (Koch 2007).

The quantity hypothesis has recently been disputed by researchers showing that protein quality is more important than quantity (Robbins et al. 2005). Mixed diets containing foods with complementary amino acid profiles are assumed to be higher quality than those with non-complementary values. Robbins et al. (2010) found that rats had lower diet to blood discrimination values when fed a diet of complementary amino acids than when fed a diet of non-complementary amino acids. This supports the theory that diets of higher quality lead to lower discrimination values between diet and tissue.

Although ^{15}N generally comes from dietary protein, ^{13}C sources can be more difficult to distinguish. Bulk diet may not reflect tissue $\delta^{13}C$ values due to nutrient routing. This effect may be particularly evident in animals such as herbivores and omnivores with low-protein diets which synthesize many of their non-essential amino acids. Researchers have recently focused on isotope analysis of specific amino acids to distinguish the source of the observed $\delta^{13}C$ signature (O'Brien et al. 2002, Fogel & Tuross 2003).

CHAPTER 2

AN ISOTOPIC ANALYSIS OF ARCTIC GROUND SQUIRREL DIET

Introduction

Food choices made by hibernating mammals in the arctic can have significant consequences on their survival as a result of the short growing season and harsh hibernation conditions. Arctic ground squirrels (*Spermophilus parryii*) are selective omnivores that make food choices based on a combination of their energy requirements and the seasonality and local availability of food items. Changes taking place in the Arctic as a result of climate change may affect vegetation availability and phenology resulting in changes to Arctic ground squirrel population sizes and distributions. As a major prey species for many Arctic and migratory predators these changes could cascade up the food chain.

It is expected that the Arctic will be more rapidly affected by climate change than other areas, and temperature changes and related phenomena have already been documented over the past couple of decades (Shaver et al. 2001, Hinzman et al. 2005, Wahren et al. 2005). Nitrogen and phosphorus availability are expected to increase with elevated temperatures (Chapin et al. 1995). One study has shown increased temperatures and nutrient availability to result in an overall increase of ectomycorrhizal fungi in the Arctic (Clemmensen

et al. 2006). In the warming climate, shrubs have already been expanding their territory on the tundra (Tape et al. 2006), while forbs are expected to decrease (Chapin et al. 1995). Experiments have revealed a 30-50% decrease in vegetation species diversity with a disproportionate decline of forbs (Chapin et al. 1995).

Toolik Field Station is one of the best-studied Arctic sites where climate change has been analyzed (Knapp et al. 2008, Walker et al. 2006, Wahren et al. 2006, Shaver et al. 2001, Mack et al. 2004). Two well-studied Arctic ground squirrel colonies exist near the Toolik Field Station at Toolik Lake and Atigun River (Buck and Barnes 1999a, Buck and Barnes 1999b). Previous research on these colonies has focused mainly on hibernation physiology of the animals and little is known about the feeding habits of the Arctic ground squirrels in these colonies.

Arctic Ground Squirrels

Given their short active season (3 to 5 months) and extreme hibernation conditions (average burrow temperatures of -8.9 ˚C), food availability and timing is particularly important for Arctic ground squirrels (Buck and Barnes 1999a). In two studies the major food source for Arctic ground squirrels was legumes (McLean 1985; Batzli and Sobaski 1980). They are also known to consume roots, seeds, berries, grasses, fungi and occasionally insects (Mayer 1953; McLean 1985; Batzli and Sobaski 1980). Changing vegetation conditions may

affect how Arctic ground squirrels choose food items for both immediate consumption requirements and caching for post-hibernation nutritional needs. Decreases in the abundance of forbs, an important food source, could be particularly detrimental to Arctic ground squirrels. As a major prey source for many predators in the Arctic, changes in Arctic ground squirrel behaviors and populations could affect the entire system. Arctic ground squirrel predators include ravens, ermine, long-tailed jaegers, snowy owls, short-eared owls, golden eagles, northern harriers, gyrfalcons, wolverines, red foxes, wolves and grizzly bears (Buck and Barnes 1999a).

Females lose between 11 and 45% of their body mass during hibernation, while males emerge with body masses similar to those recorded at immergence in the fall (Buck and Barnes 1999a). This indicates that male Arctic ground squirrels may be consuming food cached in their hibernacula during the previous fall. Gillis et al. (2005) found that males (4.4%) were more likely to be carrying food in their cheek pouches when trapped than females (0.6%). These food items were likely to have been deposited in burrows rather than consumed due to the short amount of time spent in the burrow while depositing the items (Gilles 2005).

The different energy requirements between sexes and age groups indicated by caching behavior (Gilles 2005, McLean and Towns 1981, Buck and Barnes 1999b), differences in hibernation timing (Buck &Barnes 1999a), reproduction requirements (McLean & Towns 1981), and burrow temperatures (Buck &Barnes 1999b) indicates that these groups may make different food

choices. However, McLean (1985) found sex differences in feeding behavior only during August. This was primarily a result of males switching to consuming *Artemisia* sp. a month later than females at that study site and could be related to differences in hibernation timing. Females enter hibernation before males and adults enter before juveniles. Reproductive males emerged from hibernation before females and adults emerged before yearlings (Buck and Barnes 1999a).

Arctic ground squirrels may be able to adapt to a variety of habitats making food choices based on the foods available near their burrows. In order to contain a burrow system, depth of thaw to permafrost needs to be deep enough for the animals to build hibernacula that are sufficiently insulated for harsh winter conditions (Buck and Barnes 1999b). This limits the sites available for Arctic ground squirrel colonies. Foraging close to burrow locations would likely reduce the chances of encountering predators. Therefore, plants chosen by members of a colony may be influenced by the best available food source within a certain area (Zazula et al. 2006). This indicates that Arctic ground squirrels residing in different colonies could make different food choices. Large differences in food choices made by separate colonies could also indicate adaptability to a variety of conditions and resilience in the face of climate change.

Isotopes

Though carbon and nitrogen isotopes have been used in animal ecology studies since the late 1970s (DeNiro and Epstein 1978a, DeNiro and Epstein 1978b, DeNiro and Epstein 1980), stable isotope analysis has increasingly been

used to analyze aspects of mammalian feeding ecology over the past two decades (Wolf et al. 2009, Gannes et al. 1998, Post 2002, Kelly 2000). Major uses have been to determine diet (Hilderbrand et al. 1996), determine trophic position (Post 2002), and analyze migration (Hobson 1999).

Stable Isotope mixing models can provide insight into food sources from which nutrients are assimilated into body tissues. In order to obtain this information, the food sources available must be known. Simple stable isotope mixing models can be used when the number of food sources is equal to the number of isotope ratios sampled plus one. However, more complex models are needed to calculate food proportions when greater numbers of food sources are utilized.

In omnivores it can be difficult to know the exact food sources because there are many from which to select. In these cases food items may be grouped to provide a workable number of sources (Phillips et al. 2005). Grouped items must have similar isotope values and C:N ratios. Foods with similar C:N ratios should contribute similar amounts of carbon and nitrogen to the diet. Similar isotope ratios are important because these will be averaged to provide one value as input to the model. Further, food sources within one group should be related in some way so information can be extracted from the model output.

It is important to know the turnover time of the tissues sampled. Isotope values of tissues will vary according to diet at the time that tissue was synthesized. Some tissues such as bone are relatively inert after they are

produced and can provide an isotope signature over the course of the bone growth (Koch 2007). Other tissues such as blood plasma can turn over in a matter of days (Hilderbrand et al. 1996, Pearson et al. 2003). Hair is inert once it is grown but isotope values will change with hair growth and during molt, which can take place over several days or even weeks.

Finally, the discrimination values from food source to body tissue must be known or accurately estimated. Discrimination values result from physiological processes such as transamination and deamination of amino acids. These processes result in a loss of the lighter nitrogen (^{14}N) which is excreted from the body as urine. This results in a standard ^{15}N enrichment of 2 - 4‰ with each increasing trophic level (Crawford et al. 2008, Kelly 2000). Discrimination values for $\delta^{13}C$ can vary from negligible to significant depending on whether nutrients are preferentially routed from specific foods to the sampled tissue (Gannes et al. 1998, Podlesak & McWilliams 2006).

In the past, standard discrimination values derived from a variety of captive animal studies have been applied to studies on wild populations (Koch 2007). This can lead to spurious results due to variations in the physiology of different animals and differences in metabolic routing to different tissues. Important differences may include urine excretion method, environment, diet, taxonomic class and tissue type (Vanderklift & Ponsard 2003 and Caut et al. 2009). Ideally, discrimination values are experimentally determined using the exact animal, tissue and diet that values will be applied to the in wild. However, this is not always possible.

The Isosource mixing model (Phillips & Gregg 2003) calculates a distribution of feasible solutions for food source contributions. This model allows the use of up to 10 sources and five isotopes. The model was developed based on mass balance equations used in linear mixing models (Phillips 2001) for one isotope ratio and two sources:

$$\delta_M = f_A\delta_A + f_B\delta_B$$

$$1 = f_A + f_B$$

In the model, δ_M is the mixture isotope (consumer tissue), f_A is the proportion of the food source A and δ_A is the isotope of food source A. For more than n+1 sources these equations can then be expanded to include each possible combination of food source proportions by some assigned increment totaling 100%. The isotope signature of the mixture is then used to determine if that combination of food sources is possible within some predetermined tolerance.

The objectives of this study were to improve the understanding of Arctic ground squirrel food choices by quantifying food items available to Arctic ground squirrels, measuring the variability in food consumption patterns within and among Arctic ground squirrel colonies and estimating percent utilization of available foods. Isotope values should reflect differences in food choices made by segments of the populations indicating which, if any, groups may be most adversely affected by climate change. Differences in percent utilization of foods will allow us to predict how expected changes in food sources could influence Arctic ground squirrel populations.

I hypothesized that males and females would have similar isotope values due to previous studies finding similar feeding patterns (McLean1985); juveniles would have higher $\delta^{15}N$ values than adults as a result of feeding on milk during their development and similar $\delta^{13}C$ values; and all colonies would have similar values based on close proximity and similarity of available foods. As omnivores, I expected Arctic ground squirrels at both sites to consume a variety of food sources to meet their nutritional needs. I also hypothesized that fungi would make some contribution to diet of the Arctic ground squirrels based on evidence of fungivory in other rodents (Maser et al. 2008) and one record of fungal consumption by Arctic ground squirrels (Mayer 1953). These food contributions to Arctic ground squirrel tissues were expected to vary according to differences in food sources available at the two study sites.

In this study I surveyed vegetation in the Atigun Arctic ground squirrel colony and used available vegetation data around the Toolik colony to determine available food sources. Potential food sources of vegetation and fungi along with hair samples from Arctic ground squirrels were collected. These samples were analyzed for isotope ratios of ^{15}N and ^{13}C and variation within and among colonies was quantitatively compared. Simple isotopic mass balance models were used to estimate proportions of dietary components.

Methods

Study Sites

Hair samples were collected from Arctic ground squirrels at two sites in the northern foothills of the Brooks Range in Alaska. The Toolik site is located near the Toolik Field Station along the Dalton Highway 254 kilometers north of the Arctic Circle (68˚37'40"N, 149˚35'41"W) at an elevation of 720 meters. The Atigun site is located 12 kilometers south of Toolik Field Station along the Dalton Highway in the Atigun river drainage (68˚26'58"N, 149˚21'43"W) at an elevation of 804 meters. Both sites (Toolik and Atigun) are underlain by continuous permafrost with a depth of thaw that is generally <1m deep.

Vegetation maps are available for the Toolik site (Walker et al. 2008) which were used in subsequent analyses to infer potential habitat use near the Toolik colony. The burrows at the Toolik site are located along an approximately two kilometer strip on the east side of Toolik lake with some burrows located in the field station area. A majority of the burrows are located in prostrate-shrub tundra with some burrows in erect-shrub tundra and barrens around the Toolik station. Dominant species in the prostrate-shrub tundra include *Dryas octopetala*, *Arctostaphylos alpina*, *Salix phlebophylla*, *Cassiope tetragona*, *Salix rotundifolia, Dryas integrifolia, Betula nana, Salix pulchra, Ledum palustre ssp. decumbens, Empetrum nigrum, Vaccinium uliginosum, Vaccinium vitus-ideae*, graminoids and mosses. Erect-shrub tundra is dominated by *Betula nana, Salix pulchra, Rubus chamaemorus*, graminoids and mosses. Barrens are areas that

are currently or previously affected by anthropogenic disturbance. Vegetation at the Atigun River site had not previously been analyzed.

Atigun Vegetation Analysis

Vegetation cover data were collected during August of 2008 at the Atigun study site. All Arctic ground squirrel burrows within the Atigun study site on the east side of the Dalton Highway were previously marked with GPS (Geographic Positioning System) points. The points were downloaded to Arcmap software and the perimeter of this site was then mapped within a 50-meter radius from all burrows nearest the edge of the colony. GIS was then used to overlay a 50-meter grid on the study site map (Figure 2.1). Vegetation present at each of the points on the 50-meter grid was recorded for both overstory and understory.

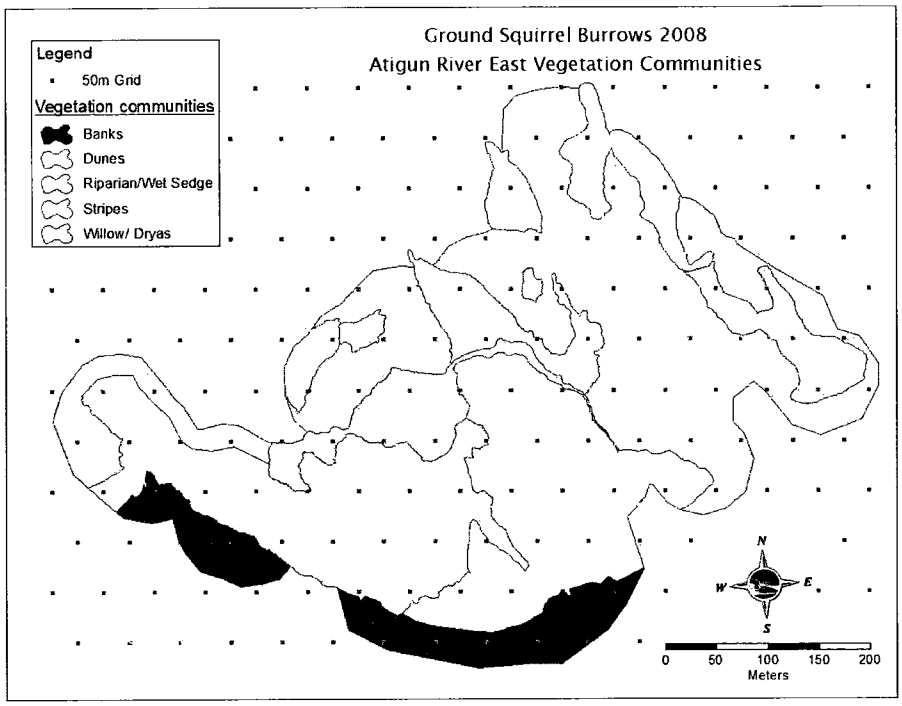

Figure 2.1 Atigun study site with vegetation communities delineated and 50-meter grid showing points where vegetation species was identified. Both overstory and understory vegetation was recorded at each point.

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Hair Sample Collection

Arctic ground squirrels were trapped using Tomahawk live traps baited with carrots during the spring, summer and fall of 2007 and 2008 at the Toolik and Atigun study sites. The squirrels were brought back to Toolik Field Station and anesthetized using isoflurane mixed with oxygen. The animals were then weighed, measured and hair samples were collected. The animals were held overnight and returned to the burrows where they were captured. All live animal procedures were approved by the Institute of Animal Care and Use (UAF IACUC #06-25 and #08-59, UNH IACUC #080904A).

Hair samples were placed in small manila envelopes and transported back to the Stable Isotope Lab at the University of New Hampshire where they were clipped between base and tip. Hair samples were separated in order to determine if there was a difference in recently grown and older hair (Jones et al. 2006). Tips and bases were separately weighed out to approximately 1 mg and placed in tin cups giving one sample of the hair base and one sample of the hair tip. ^{15}N and ^{13}C values were determined using a Costech 4010 Elemental Analyzer and a Delta XP Mass Spectrometer with an accuracy of 0.1‰.

Isotope data is reported using delta notation indicating the difference between the standard and the sample in parts per thousand (‰):

$$\delta^{H}X = [(R_{sample}/R_{standard})-1] \times 1000$$

where H is the heavy isotope, X is the element and R is the ratio of the heavy to light isotope (^{13}C:^{12}C or ^{15}N:^{14}N). Samples are compared to internationally recognized standards of atmospheric nitrogen and Peedee Belemnite Limestone carbon. Samples with more of the heavy isotope in relation to the standard are referred to as "enriched" and those with less of the heavy isotope are referred to as "depleted" (Crawford et al. 2008).

Lab standards of mushroom and tuna with known isotope values along with NIST1515 and NIST1575a standards were included with each run. A minimum of 15 standards were included with each run of 28 samples. A minimum of two sample duplicates were also included in each run. All data were normalized and corrected for drift and linearity.

Statistics

The hair isotope values were plotted on a scatterplot to look for any trends that may appear within the data points. Hair tip and base samples were then compared using a matched pairs analysis to determine if there was a consistent difference between the two hair sections, which would indicate a seasonal variation in feeding choices. The two samples from each squirrel were averaged to produce a value for each squirrel. Student's two sample t-tests were then used to compare site, sex, age and seasonal differences. Normal Quantile Plots were used to check normalcy of data and Wilconson's Rank Sum (2 sample) tests were used when the range of variation differed substantially between data sets. All analyses were conducted separately for the Toolik and Atigun sites. All statistical analyses were completed using JMP 8.0 (2008 SAS Institute Inc.) with an α=0.05 unless otherwise stated.

Each sample was assigned a season based on the date the hair was shaved. Only a few samples were collected during the summer months of June and July so these samples were excluded from seasonality analysis. Because Arctic ground squirrels molt twice a year, in the spring and fall, the samples were separated into those collected in April and early May defined as "spring samples" and those collected in August and September defined as "fall samples." Although molt stages were recorded at the time of hair collection it was not

possible to separate the hair based on exact molt stage. Molt took place over a period of a few weeks and animals were often mid-molt during hair collection.

Although species and tissue sampled were identical for all samples, data could be affected by metabolism or body condition. In order to examine these factors body mass was recorded for each individual over the course of the trapping season. Body mass values were separated by sex and month before comparing the two study sites. Arctic ground squirrel body masses fluctuate widely throughout the active season (Buck and Barnes 1999a), making it important to compare the two sites using the same time period. The month of August was the only month with sufficient data to separate the samples by sex before comparing sites. Weights were compared using Student's 2-sample T-test.

Isotope Modeling

Food sources were determined using a literature review to determine preferred foods and available foods at each study site. Food sources were sorted into groups based on growth characteristics, similarity of isotope ratios and C:N ratios in order to limit the number of sources to ten. Isotope ratios were measured individually for each item and then combined and averaged with like items.

Based on these methods, food items included in the original model included graminoid leaves and seed heads, N-fixing plant leaves, *Artemisia sp.* leaves, *Salix spp.* leaves, other shrub leaves, *Shepherdia canadensis* berries,

other berries, mushrooms and roots (Figure 2.2). The category "other shrubs" included *Arctostaphylos sp.* leaves, *Dryas sp.* leaves, *Vaccinium uliginosum* leaves and *Betula nana* leaves. The category "roots" included *Oxytropis sp.* roots, *Hedysarum sp.* roots, *Dryas sp.* roots, *Epilobium latifolium* roots and *Dryas sp.* roots. The category "other berries" included *Vaccinium uliginosum* berries, *Empetrum nigrum* berries and *Arctostaphylos sp.* berries. *Shepherdia canadensis* berries were grouped separately due to their widely disparate isotope ratio. The category "mushrooms" included mushrooms from the families Boletaceae and Russulaceae. Mushrooms from these two families are known to be consumed by other rodents (D'Alva et al. 2007 and Maser et al. 2008).

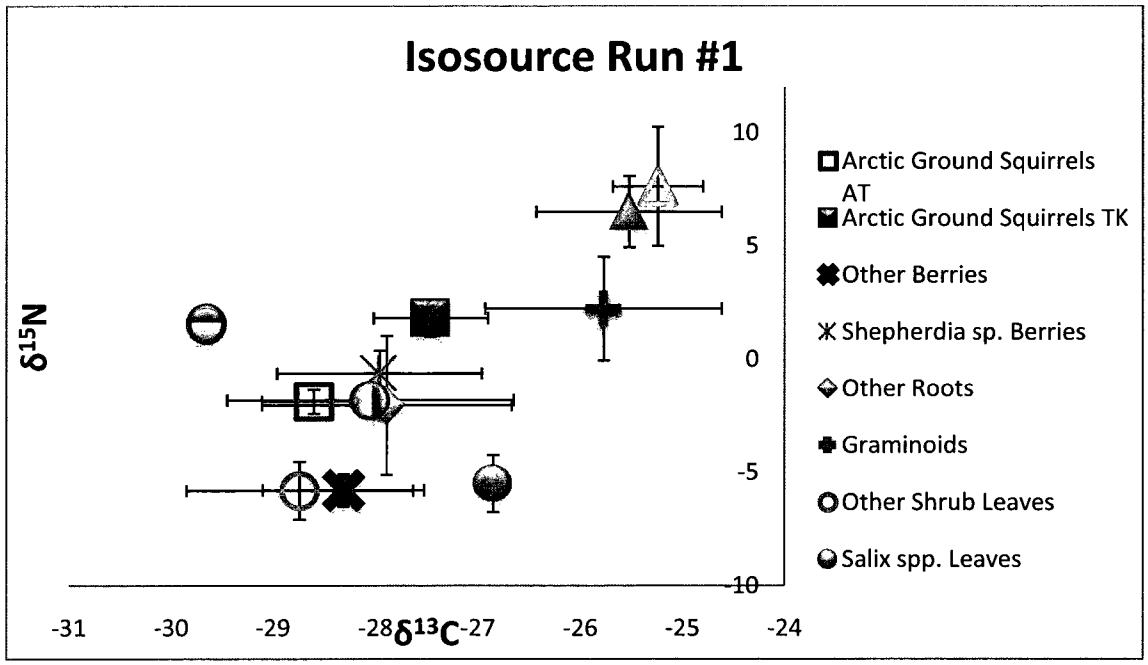

Figure 2.2. $\delta^{15}N$ and $\delta^{13}C$ isotope values for food sources used to calculate Isosource values in the first run for both Toolik and Atigun.

Groups calculated as very low dietary contributors in the original output were dropped and other groups were expanded to increase the amount of

information gained from the analysis. *Artemisia sp.* was dropped from the second analysis because it is very uncommon in the study sites. The second analysis grouped potential food sources into graminoid leaves and seed heads, *Shepherdia canadensis* berries, other berries, mushrooms, *Oxytropis sp.* and *Hedysarum sp.* roots, *Dryas sp.* and *Arctostaphylos sp.* roots, *Epilobium latifolium* roots, *Salix spp.* leaves, N-fixer leaves and shrub leaves (Figure 2.3).

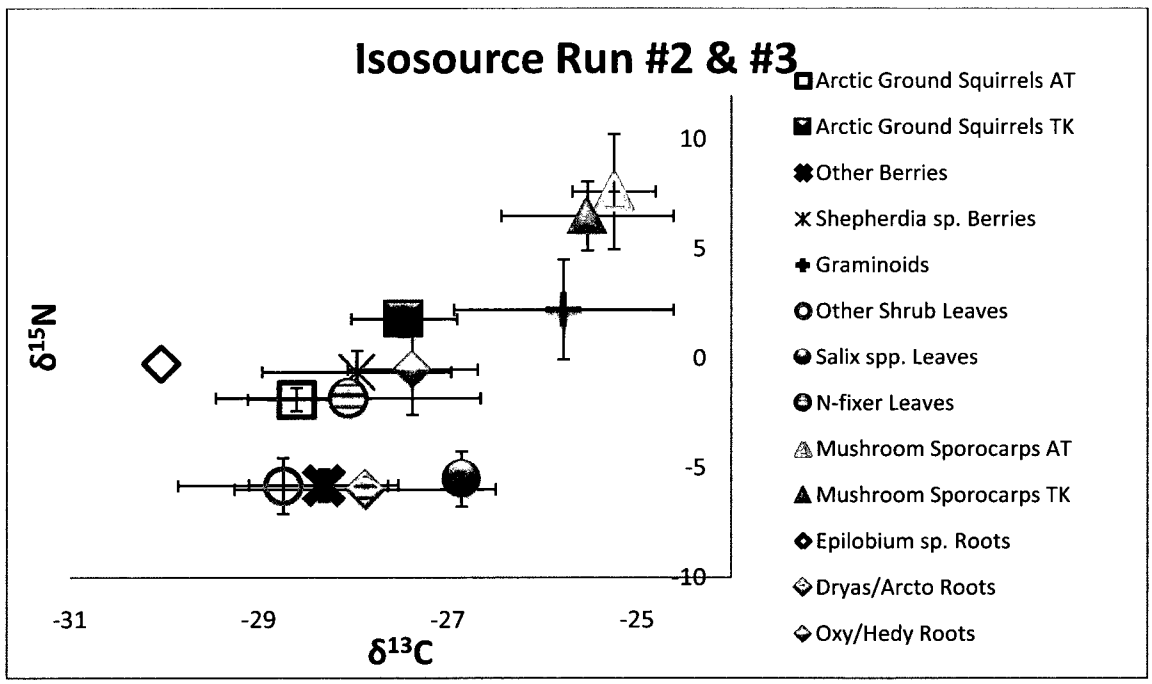

Figure 2.3. $\delta^{15}N$ and $\delta^{13}C$ isotope values for food sources used to calculate Isosource values in the second and third run for both Toolik and Atigun.

Food items were collected from the Atigun and Toolik study sites in the spring, summer and fall of 2007 and 2008. Vegetation was dried in a drying oven at 60°C and mushrooms were dried in a food dehydrator at Toolik Field Station. All items were returned to the University of New Hampshire isotope lab for analysis. Vegetation and mushrooms were ground in a Wig-L-Bug grinder and

weighed into tin cups for analysis. Vegetation samples collected from four to five different plants were ground and analyzed as a single sample to obtain an average value for the species.

"Diet Dependent Discrimination Factors" (DDDFs) were calculated using values and equations for mammal hair from Caut et al. (2009). The value for $\Delta^{13}C$ was calculated using the following equation:

$$\Delta 13C = -0.474\delta^{13}C - 9.064$$

The value for $\delta^{13}C$ is calculated from the average $\delta^{13}C$ of the diet. The equation was developed based on a regression analysis of discrimination values compared to dietary isotope ratios in a meta-analysis of 290 $\Delta^{13}C$ values in the literature. In the first analysis a $\Delta^{13}C$ discrimination value of 4.02‰ was used for the Atigun squirrels and a $\Delta^{13}C$ value of 4.04‰ was used for the Toolik squirrels. Values for Δ13C were recalculated for the second analysis in order to account for the dropped food source groups. Values of $\Delta^{13}C$ for the second analysis were calculated as 3.97‰ for Atigun squirrels and 3.98‰ for Toolik squirrels.

Caut et al. (2009) did not find nitrogen discrimination values in mammalian hair to vary with diet isotopic ratios. Therefore a $\Delta^{15}N$ discrimination value of 2.6‰ was used which corresponds with the value Caut et al. averaged for studies using mammalian hair. In another meta-analysis of consumer-diet $\delta^{15}N$ enrichment, a similar value of 2.56‰ was obtained for studies of animals consuming mixed diets (Vanderklift and Ponsard 2003). These Isosource values were calculated at 5% intervals with a tolerance of 0.01‰.

Values of $\delta^{13}C$ calculated using the DDDF analysis were quite high compared to most literature values for $\delta^{13}C$ discrimination, possibly due to spurious values obtained in some of the studies used to calculate the regression line (Caut et al. 2009). Many of the studies used high-protein artificial feed as the food source used to calculate discrimination values. Hair (C:N ratio = ~3) is nearly all protein and the anabolic processes used to produce non-essential amino acids likely draw carbon from a number of sources when animals consume a diet lower in protein. As a precaution, an additional discrimination value was used to run the model a third time. This value was calculated from rabbit (*Oryctolagus cuniculus*) hair fed a diet of alfalfa (Sponheimer et al. 2003a), which is the most similar species with a hair discrimination value and fed a natural diet available in the literature. A $\Delta^{13}C$ value of 3.4‰ (Sponheimer et al. 2003a) and a Δ15N value of 2.4‰ (Sponheimer et al. 2003b) were used in this analysis. These Isosource values were calculated at 3% intervals with a tolerance of 0.1‰.

Results

Atigun Vegetation

A total of 97 grid points were surveyed with 163 total vegetation items recorded including understory plants when they were present. The ten most common species within the 50-meter grid included *Salix glauca*, mosses, *Dryas octopetala*, grasses and sedges, *Betula nana*, *Salix reticulata*, *Vaccinium*

uliginosum, *Shepherdia canadensis*, *Arctostaphylus rubra* and lichens (Figure 2.4). Sand without vegetation was recorded at 22 percent of the grid points surveyed.

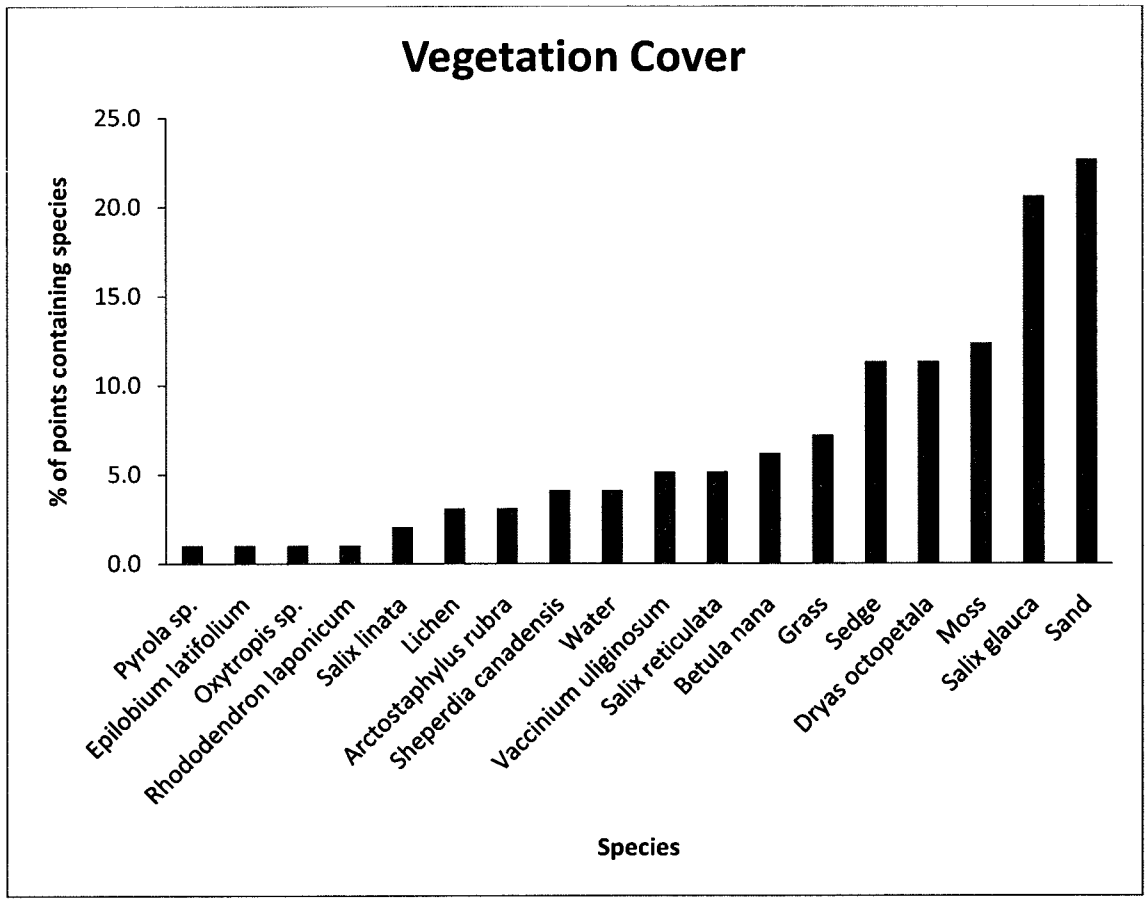

Figure 2.4. Species present at each of the points surveyed on the 50-meter grid of the East Atigun study site. Items are displayed as the percent of the total points surveyed that contain each species.

Variation within and among colonies

A scatterplot of all the hair tip and hair base averaged values for 99 squirrels (Fig 2.5) revealed an obvious difference in both $\delta^{15}N$ and $\delta^{13}C$ between squirrels trapped at the Atigun and Toolik study sites. A matched pairs analysis showed no significant variation between base and tip of hair samples for $\delta^{15}N$ ($p=0.2569$). Matched pairs analysis showed slightly higher values for $\delta^{13}C$ in the tips of the hair when compared to the base with a mean difference of 0.1‰ ($SE=0.025$, $p<0.0001$, $n=99$, $t=-4.66$).

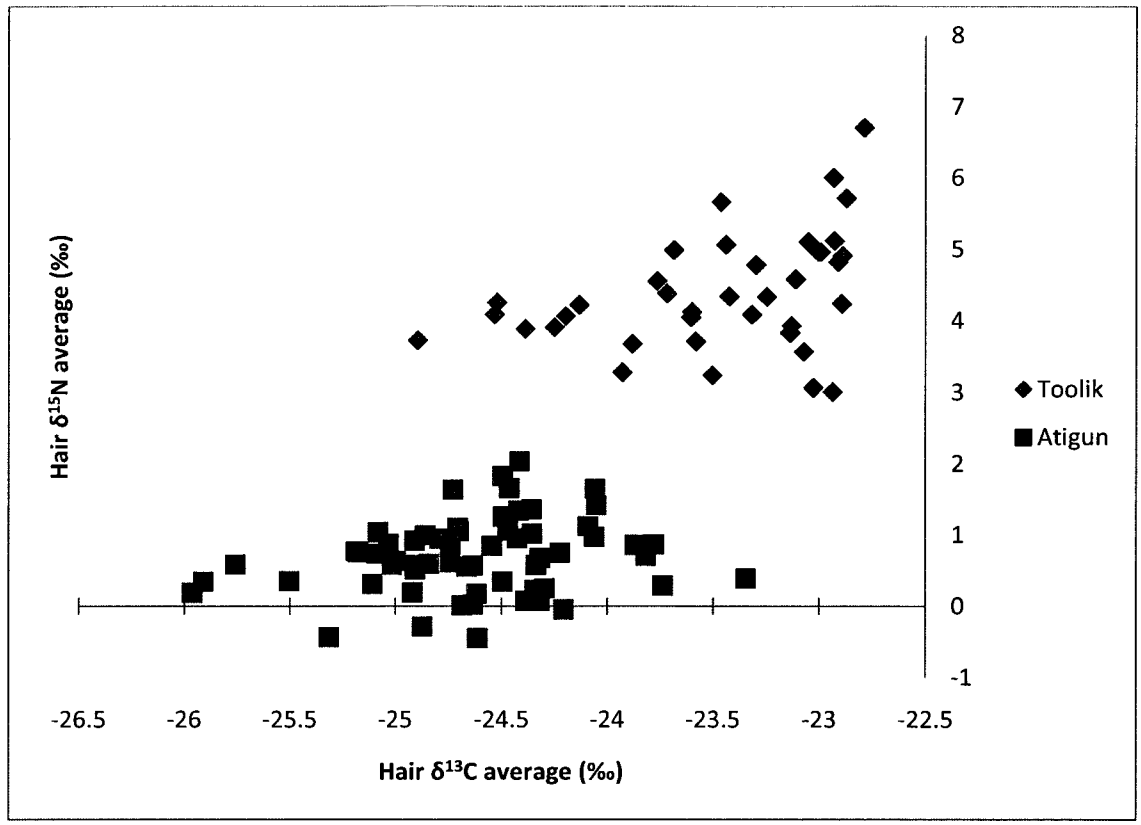

Figure 2.5. Scatterplot Matrix showing all of the Arctic Ground Squirrel hair isotope samples analyzed (n=99). The Toolik animals are shown as blue diamonds and the Atigun animals are shown as red squares.

A Student's T-test of $\delta^{15}N$ means between sites showed a significant difference of 3.7‰ between the Toolik and Atigun study sites ($p<0.0001$, SE=0.18). The mean $\delta^{15}N$ value for the Toolik site was 4.4‰ (SD=0.77, n=24) and for the Atigun site was 0.7‰ (SD=0.56, n=42).

A Wilcoxson's rank sum test for the $\delta^{13}C$ mean between sites also showed a significant difference between the Toolik site and the Atigun site ($p<0.0001$). The mean $\delta^{13}C$ value for the Toolik site was -23.3‰ (SD=0.34, n=25), while the mean $\delta^{13}C$ value for the Atigun site was -24.6‰ (SD=0.37, n=43). Given the

significant difference between study sites, all remaining comparisons are presented separately for the Atigun and Toolik sites.

No differences in body mass were detected between study sites for either males (p= 0.24, SE= 46.99, Mean Diff.= -56.31g) or females (p= 0.95 SE= 56.59, Mean Diff.= 3.42 g) in August. The mean August weight for female squirrels at the Atigun River site was 615.88 g (SD=143.27, n=8) and at the Toolik site the mean was 619.30 g (SD= 79.77, n=10). The mean August weight for male squirrels at the Atigun River site was 740.42 g (SD=145.14, n=19) and at the Toolik site the mean was 684.11 g (SD=99.46, n=9).

There was no significant difference between males and females for $\delta^{15}N$ or $\delta^{13}C$ at either site (Table 2.1). There was no significant difference in hair $\delta^{15}N$ between adults and juveniles at either site (Table 2.1). Adults had higher hair $\delta^{13}C$ than juveniles at the Atigun site (p=0.0469, Table 2.1), but there was no significant difference at the Toolik site.

Site	Isotope	Male	Female	Adult	Juvenile
Toolik	15N Mean (‰)	4.5	4.3	4.4	4.3
	13C Mean (‰)	-23.5	-23.5	-23.2	-23.6
Atigun	15N Mean (‰)	0.8	0.5	0.9	0.5
	13C Mean (‰)	-24.7	-24.4	*-24.4	*-24.7

Table 2.1. Mean ^{15}N and ^{13}C values for sex and age categories at both Toolik and Atigun sites. All data points are presented with an α=0.05 and data with significantly different values are marked with an *.

Seasonally sorted data showed a decrease of 0.55‰ from spring to fall in female Arctic ground squirrel $\delta^{15}N$ values at the Atigun site (p=0.0405, SE=0.23). No seasonal significant difference was detected in male $\delta^{15}N$ at Atigun or either sex Toolik Arctic ground squirrels. Male $\delta^{13}C$ values showed a decrease of 0.38‰ from spring to fall at the Toolik site (p=0.0139, SE=0.14). None of the other $\delta^{13}C$ values differed significantly by season.

Isosource Modeling

The first Atigun analysis showed *Artemisia sp.* to have the highest mean value followed by shrub leaves. *Artemisia sp.* leaves were also estimated to contribute a minimum of 0.30 or 30% to Atigun Arctic ground squirrel diet. Roots, berries, N-fixer leaves, *Shepherdia canadensis* berries and *Salix spp.* leaves made potentially moderate contributions to Arctic ground squirrel diets. Graminoids and mushrooms made small potential dietary contributions (Table 2.2).

Food Source	Range	Mean	SD	99th %ile
Shrub leaves	0.00-0.40	0.195	0.131	0.4
Salix leaves	0.00-0.15	0.034	0.047	0.15
N-fixer leaves	0.00-0.30	0.082	0.087	0.3
Artemisia leaves	0.30-0.45	0.35	0.044	0.45
Other berries	0.00-0.40	0.129	0.117	0.4
Shepherdia berries	0.00-0.25	0.042	0.069	0.25
Other roots	0.00-0.35	0.145	0.119	0.35
Graminoids	0.00-0.05	0.013	0.023	0.05
Mushrooms	0.00-0.10	0.011	0.027	0.1

Table 2.2. Potential food source contributions as proportion of diet for Atigun River Arctic ground squirrels Isosource model run number one using discrimination values calculated from Caut et al. 2009.

The second Atigun analysis, in which *Artemisia sp.* leaves were removed from the analysis due to low abundance at the site, estimated that *Epilobium latifolium* roots were the largest contributor with a mean value of 0.387 followed by N-fixer leaves with a mean value of 0.12. The analysis estimated *Epilobium latifolium* roots to contribute a range minimum of 0.20 or 20% of Atigun Arctic ground squirrel diet. *Shepherdia canadensis* berries, *Oxytropis sp.* and *Hedysarum sp.* roots, other shrub leaves, other berries and *Dryas sp. and Arctostaphylos sp.* roots were all calculated to make moderate potential contributions. Graminoids, *Salix spp.* leaves and mushrooms also made some potential contribution (Table 2.3).

Food Source	Range	Mean	SD	99th %ile
Shrub leaves	0.00-0.50	0.098	0.089	0.35
N-fixer leaves	0.00-0.65	0.102	0.108	0.45
Salix leaves	0.00-0.35	0.051	0.061	0.25
Other berries	0.00-0.45	0.083	0.082	0.3
Shepherdia berries	0.00-0.55	0.094	0.099	0.4
Dryas/Arctostaphylos roots	0.00-0.40	0.068	0.072	0.3
Oxytropis/Hedysarum roots	0.00-0.35	0.066	0.073	0.3
Epilobium roots	0.20-0.60	0.383	0.058	0.5
Graminoids	0.00-0.20	0.032	0.041	0.15
Mushrooms	0.00-0.15	0.021	0.03	0.1

Table 2.3. Potential dietary contributions as proportion of diet for Atigun River Arctic ground squirrels Isosource model run number two using discrimination values from Caut et al. 2009.

The third Atigun analysis, in which discrimination factors were adjusted, estimated mean values similar to the second analysis with higher range maximums for all sources with the exception of *Epilobium latifolium* roots which

had a lower range maximum. *Epilobium latifolium* roots were again the highest mean dietary contributor followed by *Shepherdia canadensis* berries and N-fixer leaves. However, range minimums were all 0.00 in this analysis. *Oxytropis sp.* and *Hedysarum sp.* roots and shrub leaves also made moderate potential contributions. Other berries, graminoids, *Salix spp.* leaves and mushrooms were potential contributors as well (Table 2.4).

Food Source	Range	Mean	SD	99th %ile
Shrub leaves	0.00-0.63	0.1	0.089	0.36
N-fixer leaves	0.00-0.99	0.13	0.127	0.54
Salix leaves	0.00-0.45	0.061	0.064	0.27
Other berries	0.00-0.57	0.088	0.082	0.33
Shepherdia berries	0.00-0.78	0.136	0.128	0.54
Dryas/Arctostaphylos roots	0.00-0.51	0.076	0.074	0.3
Oxytropis/Hedysarum roots	0.00-0.63	0.103	0.1	0.42
Epilobium roots	0.00-0.45	0.183	0.066	0.33
Graminoids	0.00-0.33	0.062	0.059	0.24
Mushrooms	0.00-0.24	0.051	0.045	0.18

Table 2.4. Potential dietary contributions as proportion of diet for Atigun River Arctic ground squirrels Isosource model run number three using discrimination values from Sponheimer et al. 2003.

The first Toolik analysis showed *Artemisia sp.* to have the highest dietary contribution followed by mushrooms. *Artemisia sp.* leaves and mushrooms were estimated to contribute range minimums of 0.20 or 20% and 0.10 or 10% of diet respectively. Graminoids, *Shepherdia canadensis* berries, N-fixer leaves and roots were all calculated as potentially moderate dietary contributors. Other berries, other shrubs and *Salix spp.* leaves also made some potential contribution (Table 2.5).

Food Source	Range	Mean	SD	99th %ile
Shrub leaves	0.00-0.10	0.027	0.037	0.1
Salix leaves	0.00-0.10	0.02	0.037	0.1
N-fixer leaves	0.00-0.30	0.057	0.08	0.3
Artemisia leaves	0.20-0.40	0.287	0.064	0.4
Other berries	0.00-0.15	0.027	0.046	0.15
Shepherdia berries	0.00-0.40	0.1	0.115	0.4
Other roots	0.00-0.20	0.06	0.06	0.2
Graminoids	0.00-0.40	0.16	0.142	0.4
Mushrooms	0.10-0.40	0.263	0.097	0.4

Table 2.5. Potential dietary contributions as proportion of diet for Toolik Lake Arctic ground squirrels Isosource model run number one using discrimination values from Caut et al. 2009.

The second Toolik analysis, in which *Artemisia sp.* was dropped due to low abundance at the site, calculated mushrooms to have the highest mean contribution followed by *Epilobium latifolium* roots. Mushrooms and *Epilobium latifolium* roots both had estimated range minimums of 0.20 or 20%. *Shepherdia canadensis* berries, graminoids, *Oxytropis sp./Hedysarum sp.* roots and N-fixer leaves all had moderate potential contributions. *Salix spp.* leaves, other shrub leaves, other berries, and *Dryas sp./Arctostaphylos sp.* roots also made some potential contribution (Table 2.6).

Food Source	Range	Mean	SD	99th %ile
Shrub leaves	0.00-0.05	0.02	0.026	0.05
N-fixer leaves	0.00-0.20	0.05	0.071	0.2
Salix leaves	0.00-0.10	0.02	0.035	0.1
Other berries	0.00-0.15	0.02	0.048	0.15
Shepherdia berries	0.00-0.25	0.115	0.078	0.25
Dryas/Arctostaphylos roots	0.00-0.05	0.01	0.021	0.05
Oxytropis/Hedysarum roots	0.00-0.15	0.055	0.064	0.15
Epilobium roots	0.20-0.35	0.275	0.042	0.35
Graminoids	0.00-0.30	0.085	0.094	0.3
Mushrooms	0.20-0.45	0.35	0.071	0.45

Table 2.6. Potential dietary contributions as proportion of diet for Toolik Lake Arctic ground squirrels Isosource model run number two using discrimination values from Caut et al. 2009.

Mushrooms had the highest mean value in the third analysis, in which discrimination values were adjusted, with a higher mean and range maximum but a lower minimum than calculated in analysis number two. *Epilobium latifolium* roots had a lower mean value and range maximum in the third analysis while graminoids and N-fixer leaves had similar means but higher range maximums. Range minimums for mushrooms and *Epilobium latifolium* roots were estimated as 0.9 or 9% and 0.00 respectively (Table 2.7).

Food Source	Range	Mean	SD	99th %ile
Shrub leaves	0.00-0.33	0.04	0.046	0.18
N-fixer leaves	0.00-0.54	0.073	0.076	0.33
Salix leaves	0.00-0.24	0.027	0.034	0.15
Other berries	0.00-0.30	0.036	0.043	0.18
Shepherdia berries	0.00-0.57	0.095	0.093	0.39
Dryas/Arctostaphylos roots	0.00-0.27	0.031	0.039	0.15
Oxytropis/Hedysarum roots	0.00-0.57	0.08	0.083	0.36
Epilobium roots	0.00-0.27	0.094	0.048	0.21
Graminoids	0.00-0.63	0.089	0.09	0.39
Mushrooms	0.09-0.60	0.424	0.068	0.54

Table 2.7. Potential dietary contributions as proportion of diet for Toolik Lake Arctic ground squirrels Isosource model run number three using discrimination values from Sponheimer et al. 2003.

Discussion

Variation within and among colonies

The large variation in $\delta^{15}N$ hair values found between the Toolik and Atigun study sites likely represents some variability in the feeding habits of the two colonies. Arctic ground squirrels are selective omnivores that choose from a

variety of available food sources. However, this nearly 4‰ difference between the two sites is quite large and equivalent to or greater than the generally accepted Δ value between trophic levels (Crawford et al. 2008). This indicates that Arctic ground squirrels at Toolik are either eating at a higher trophic level than those at Atigun or consuming some other food item with high $\delta^{15}N$ values. Mushrooms were the only food source items of those surveyed that exhibited $\delta^{15}N$ values sufficient to produce such high $\delta^{15}N$ values in Arctic ground squirrel hair.

It is highly unlikely that the higher $\delta^{15}N$ in the Toolik squirrels was due to starvation given similarities in body weights between the two sites. Ben-David et al. (1998) found that nutritional state did not correlate with $\delta^{15}N$ of female arctic ground squirrel blood. The $\delta^{15}N$ values varied by as much as 1.8‰ within one group with similar body masses and other indicators of nutrition. Further, the one animal that did exhibit nutritional stress did not have a $\delta^{15}N$ value significantly higher than the rest of the group. This relatively wide variation in $\delta^{15}N$ was attributed to variation in food choices and the authors dismissed the use of isotope analysis as a good indicator of nutritional stress in omnivorous animals.

The Toolik squirrels also showed $\delta^{13}C$ values that were significantly higher than the Atigun squirrels. Fogel and Tuross (2003) found that dietary protein essential amino acids strongly influenced bone collagen essential amino acid isotopic composition in humans. If hair $\delta^{13}C$ in omnivores is similarly reflective of dietary protein $\delta^{13}C$, we could expect that the difference in isotope values of Arctic ground squirrels at the two sites is largely the product of some variation in

the protein source available. Possible alternative protein sources could include mushrooms, insects or bird eggs.

The lack of an isotopic difference between males and females was expected given previous studies indicating similar feeding habits (McLean 1985). These results provide further evidence that differences in hibernation timing and reproductive requirements do not significantly affect dietary nutrient intake at the time of molt. Males typically emerge from hibernation weighing the same as they did at immergence in the fall (Buck and Barnes 1999a). In one study they gained 30% body mass in the few weeks prior to emergence (Buck and Barnes 1999a) from what was presumed to be consumption of cached food items (Gilles et al. 2005, Zazula et al. 2006). The males then lost 21% of their body mass during the subsequent mating season (Buck and Barnes 1999a). Females emerged from hibernation at their lowest body mass and reached peak body mass in late July (Buck and Barnes 1999a). Peak body masses were not significantly different for males and females, but females reached peak mass one month prior to males reflecting their earlier immergence into hibernation. Despite these differences, McLean (1985) found no significant difference in foods consumed by males and females except during August. The isotopic data from this study supports those findings

Variation in $\delta^{15}N$ between juveniles and adults was expected to result from early mass gain from milk. No difference in δ13C was predicted. Juveniles do not emerge until the last week of June. They then gain enough to nearly triple their body mass by immergence in mid-September (Buck and Barnes 1999a).

Both sexes of juvenile Arctic ground squirrels temporarily lose body mass during late July and early August which coincides with dispersal (Buck and Barnes 1999a). No difference in $\delta^{15}N$ values and only a small difference in the $\delta^{13}C$ values was detected between adults and juveniles at the Atigun site. This indicates that changes in body mass over the course of the season and early milk consumption does not significantly contribute to the nutrients assimilated in hair growth. Neither sex nor age group appears to alter food choice based on changes in mass gain, which may reflect only an increase or decrease in the amount of food consumed rather than choosing species with higher fat or protein content.

Body mass flux for sexes and ages may partially explain the seasonal difference in Arctic ground squirrel isotope values. Higher spring δ15N could be a result of the loss of lean muscle mass during hibernation (Buck and Barnes 1999b). Hair molt soon after emergence from hibernation could reflect δ15N increases similar to those observed during starvation (Hobson et al. 1993).

The phenology of food items contributes to seasonal variation in Arctic ground squirrel isotopes as well. Several known food items such as seeds, berries, roots and mushrooms exhibit seasonal availability. Additionally, McLean (1985) found that arctic ground squirrels switched from a diet that consisted mainly of legumes over most of the active season to *Artemisia sp.* in the fall. Caching behavior also takes place in the fall for males. These items are thought to be consumed by males in the spring when other food items are unavailable (Gilles et al. 2005). Some of these items, such as *Artemesia sp.* leaves (Figure

2.2), have relatively high $\delta^{15}N$ values, while others, such as mushrooms and graminoid seeds (Figure 2.3), have both relatively high $\delta^{15}N$ and $\delta^{13}C$ values.

Isosource Modeling

The Isosource mixing model presents a range of possible food sources Arctic ground squirrels could be consuming in order to produce the isotope signatures present in their tissues. However, mixing models are very sensitive to missing sources (Phillips et al. 2005). It is difficult to know all the sources omnivores could be consuming. A mixture of small portions of a variety of vegetative food sources appear to be sufficient to produce the isotope values present in the tissues of Atigun Arctic ground squirrels. However, given the high values of both $\delta^{15}N$ and $\delta^{13}C$ in the Toolik colony, it is unlikely they could be consuming strictly plant material.

Mushrooms are one example of a food source high in $\delta^{15}N$ that could be enriching tissue signatures. Other potential food sources could include bird eggs or insects. Fungivory is common in rodents (Maser et al. 2008), and the literature suggests that Arctic ground squirrels consume some portion of mushrooms (Mayer 1953). Tooth marks have also been observed in *Leccinum sp.* sporocarps in the Toolik area and other sporocarps have been observed piled near burrow entrances.

Although mushrooms were not quantitatively surveyed at the two sites, there may be differences in abundance due to vegetation characteristics. A

majority of the burrows in the Toolik colony were located in prostrate-shrub vegetation communities. These shrub communities contain *Dryas spp.*, *Arctostaphylos alpina*, *Betula nana* and *Salix spp.* (Walker et al. 2008), all of which are ectomycorrhizal hosts for either Boletes or Russulas (Gardes and Dahlberg 1996). These shrubs were also present in the Atigun site and more extensive vegetation surveys are needed to determine whether they are more abundant at one site than the other. The expected expansion of shrubs across the arctic (Tape et al. 2006), several of which are ectomycorrhizal (Gardes and Dahlberg 1996), could lead to increases in mushroom abundance.

Mushroom sporocarps have been found to have high nitrogen content, but nutrients may be difficult to access because they are in the form of indigestible cell walls (Cork & Kenagy 1989). However, ground squirrels (*Spermophilus saturates*) were able to digest cell-wall carbohydrates and chitin. Although mice (*Peromyscus maniculatus* & *P. alstoni*) lost body mass when consuming only mushrooms, they gained 20-30% body mass while consuming a combination of fungal sporocarps and other vegetation and preferred fungal sporocarps to other dietary items (D'Alva et al. 2007). McIlwee & Johnson (1998) were able to distinguish rates of fungal consumption between three fungivorous marsupials using stable isotope analysis combined with fecal analysis.

Enrichment due to epigeous mushroom consumption could be seasonally reflected but no such seasonal effect of higher $\delta^{15}N$ and $\delta^{13}C$ in the fall was recorded. Epigeous fungi are seasonally available in the arctic in late summer and fall. It is possible that Arctic ground squirrels also consume hypogeous

fungi, which are a common dietary component for northern flying squirrels (*Glaucomys sabrinus*), Californian red-backed voles (*Clethrionomys californicus)* and other rodents (Johnson 1996, Maser et al. 2008).

Insects have also been documented as a food source for Arctic ground squirrels (Batzli & Sobaski 1980) but only in one individual. Enrichment due to infanticide is also unlikely due to the uniform enrichment in males, females and juveniles.

Bird eggs are another potential protein source for Arctic ground squirrels at the Toolik site. The Toolik site is located on the shores of Toolik Lake where many shorebirds and ducks make their nests and egg depredation has been reported in the study area (B. Barnes personal communication). However, eggs could serve as a food source only during the few weeks of incubation in the spring. This seasonal signal should be present in the Arctic ground squirrel isotope signatures. However, no seasonal $\delta^{15}N$ signature change was observed at the Toolik site.

Conclusions

The large differences in isotope values between the two Arctic ground squirrel colonies analyzed in this study indicate that colonies choose food based on availability. This feeding strategy should allow Arctic ground squirrels to adapt to changing conditions despite the fact that forbs, one of their important food sources (McLean 1985, Batzli and Sobaski 1980), are expected to decrease

across the Arctic (Chapin et al. 1995). Expanding ectomycorrhizal shrub habitat in the arctic may lead to increased Arctic ground squirrel fungivory if mushroom sporocarps become more common. Accurate data on important Arctic ground squirrel food items can be used to monitor potential dietary changes with the changing food source availability.

Although Arctic ground squirrels show adaptability to regionally available food resources, they may be less adaptable to changes in plant phenology. Buck and Barnes (1999a) found very little change in dates of emergence from hibernation from year to year. The length of the short active season is likely to be quite important in order to allow time for breeding and building enough body mass to survive the following winter. Adaptability of Arctic ground squirrels to change will be important to population numbers and distributions which will affect the many predators that depend on them for food.

Further research is needed to determine whether there may be missing food sources that need to be included in isotopic mixing models. Better qualitative knowledge of food sources could be obtained through fecal analysis and/or observational studies. A combination of isotope modeling with more traditional dietary analyses such as fecal or stomach content analysis could provide more complete and accurate estimates of food source contributions to consumer tissues. In the case of omnivores such as Arctic ground squirrels, it is important to gather site-specific data to account for dietary variation with food availability. Future dietary analyses should focus on stomach content and/or fecal analyses of Arctic ground squirrels at the Toolik and Atigun sites. This data

could then be used to narrow the food source inputs to the Isosource model thus producing more accurate results. An analysis of Arctic ground squirrel feces could also be used to determine if fungal spores are more prevalent at the Toolik site than the Atigun site. This finding could support the data indicating more fungal consumption at the Toolik site.

LIST OF REFERENCES

Barnes, BM (1989) Freeze avoidance in a mammal: body temperatures below 0 C in an arctic hibernator, Science, 244(4912): 1593-1595.

Batzli, GO & ST Sobaski (1980) Distribution, abundance, and foraging patterns of ground squirrels near Atkasook, Alaska, Arctic and Alpine Research, 12(4); 501-510.

Ben-David M, CJ McColl, R Boonstra, TJ Karels (1999) ^{15}N signatures do not reflect body condition in Arctic ground squirrels, Canadian Journal of Zoology 77: 1373-1378.

Buck, CL & BM Barnes (1999a) Annual cycle of body composition and hibernation in free-living Arctic Ground Squirrels, Journal of Mammalogy, 80(2): 430-442.

Buck, CL & BM Barnes (1999b) Temperatures of hibernacula and changes in body composition of Arctic Ground Squirrels over winter, Journal of Mammalogy, 80(4): 1264-1276.

Caut, S, E Angulo, F Courchamp (2008) Discrimination factors (Δ15N and Δ13C) in an omnivorous consumer: effect of diet isotopic ratio, Functional Ecology 22: 255-263.

Caut, S, E Angula, F Courchamp (2009) Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction, Journal of Applied Ecology 46: 443-453.

Chapin, FS, GR Shaver, AE Giblin, KJ Nadelhoffer & JA Laundre (1995), Responses of Arctic Tundra to Experimental and Observed Changes in Climate, Ecology, 76(3): 694-711.

Claridge, AW, JM Trappe, SJ Cork, DL Claridge (1999) Mycophagy by small mammals in the coniferous forests of North America: nutritional value of sporocarps of *Rhizopogon vinicolor*, a common hypogeous fungus, Journal of Comparative Physiological Biology 169: 172-178.

Clemmensen, KE, A Michelsen, S Jonasson, GR Shaver (2006) Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems, New Phytologist, 171: 391-404.

Cork, SJ & GJ Kenagy (1989) Nutritional value of hypogeous fungus for a forest-dwelling ground squirrel, Ecology 70(3): 577-586.

Crawford K, McDonald RA, Bearhop S (2008) Applications of stable isotope techniques to the ecology of mammals, Mammal Rev, 38(1): 87-107.

D'Alva, T, C Lara, A Estrada-Torres, C Castillo-Guevara (2007) Digestive responses of two omnivorous rodents (*Peromyscus maniculatus* and *P. alstoni*) feeding on epigeous fungus (*Russula occidentalis*), Journal of Comparative Physiological Biology 177: 707-712.

DeNiro, MJ & Epstein S (1978) Carbon Isotopic Evidence for Different Feeding Patterns in Two Hyrax Species Occupying the Same Habitat, Science, 201: 906-907.

DeNiro, MJ & Epstein S (1978b) Influence of diet on the distribution of carbon isotopes in animals, Geochimica Acta, 42: 495-506.

DeNiro, MJ & S Epstein (1980) Influence of diet on the distribution of nitrogen isotopes in animals, Geochemica et Cosmochemica Acta, 45: 341-351.

Fogel ML & Tuross N (2003) Extending the limits of paleodietary studies of humans with compound specific carbon isotope analysis of amino acids, Journal of Archaeological Science 30: 535-545.

Gannes, LZ, C Martinez del Rio, P Koch (1998) Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology, Comparative Biochemical Physiology, 119A(3): 725-737.

Gardes, M & A Dahlberg (1996) Mycorrhizal diversity in arctic and alpine tundra: An open question, New Phytologist 133(1): 147-157.

Gillis, EA, SF Morrison, GD Zazula, DS Hik (2005) Evidence for selective caching by Arctic Ground Squirrels living in alpine meadows in the Yukon, Arctic, 58(4): 354-360.

Hilderbrand, GV, SD Farley, CT Robbins, TA Hanley, K Titus, C Servheen (1996) Use of stable isotopes to determine diets of living and extinct bears, Canadian Journal of Zoology 74: 2080-2088.

Hinzman, LD, ND Bettez, WR Bolton, FS Chapin, MB Dyurgerov, CL Fastie, B Griffith, RD Hollister, A Hope, HP Huntington, AM Jensen, GJ Jia, T Jorgenson, DL Kane, DR Klein, G Kofinas, AH Lynch, AH Lloyd, AD McGuire, FE Nelson, WC Oechel, TE Osterkamp, CH Racine, VE Romanovsky, RS Stone, DA Stow, M Sturm, CE Tweedie, GL Vourlitis, MD Walker, DA Walker, PJ Webber, JM Welker, KS Winker, K Yoshikawa (2005) Climatic Change 72: 251-298.

Hobson, KA, RT Alisauskas, RG Clark (1993) Stable-Nitrogen Isotope Enrichment in Avian Tissues Due to Fasting and Nutritional Stress: Implications for Isotopic Analyses of Diet. The Condor 95(2): 388-394.

Hobson, KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review, Oecologia ,120: 314-326.

Izzo, AD, M Meyer, JM Trappe, M North, TD Bruns (2005) Hypogeous ectomycorrhizal fungal species on roots and in small mammal diet in a mixed-conifer forest, Forest Science 51(3): 243-254.

Johnson, CN (1994) Nutritional ecology of a mycophagous marsupial in relation to production of hypogeous fungi, Ecology 75(7): 2015-2021.

Johnson, CN (1996) Interactions between mammals and ectomycorrhizal fungi, Trends in Ecology and Evolution 11(12): 503-507.

Jones ES, Heard DC & Gillingham MP (2006) Temporal Variation in Stable Carbon and Nitrogen Isotopes of Grizzly Bear Guardhair and Underfur, Wildlife Society Bulletin 34(5): 1320-1325.

Kelly, JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology, Canadian Journal of Zoology, 78: 1-27.

Knapp, AK, JM Briggs, SL Collins, SR Archers, MS Bret-Harte, BE Ewers, DP Peters, DR Young, GR Shaver, E Pendall, MB Cleary (2008) Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs, Global Change Biology 14: 615-623.

Koch, PL (2007) Isotopic study of the biology of modern and fossil vertebrates in Stable Isotopes in Ecology and Environmental Science 2nd Edition, Ed. Robert Michener & Kate Lajtha, Blackwell Publishing, 2007, Malden, MA

Krog, J (1954) Storing of food items in the winter nest of the Alaskan ground squirrel, *Citellus undulates,* Journal of Mammalogy 35(4): 586.

Leberfinger, K & I Bohman (2010) Grass, mosses, algae, or leaves? Food preference among shredders from open-canopy streams, Aquatic Ecology 44: 195-203.

MacAvoy, SE, LS Arneson, E Bassett (2006) Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals, Oecologia 150: 190-201.

Mack, MC, EAG Schuur, MS Bret-Harte, GR Shaver, FS Chaping III (2004) Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization, Nature 431: 440-443.

Maser, C, AW Claridge, JM Trappe (2008) Trees, truffles, and beasts: How forests function, Rutgers University Press, Piscataway, NJ

Mayer, WV (1953) A preliminary study of the Barrow Ground Squirrel, *Citellus parryi barrowensis,* Journal of Mammalogy, 34(3): 334-345.

McIlwee, AP & CN Johnson (1998) The contribution of fungus to the diets of three mycophagous marsupials in *Eucalyptus* forests, revealed by stable isotope analysis, Functional Ecology 12: 223-231.

McLean, IG & AJ Towns (1981) Differences in Weight Changes and the Annual Cycle of Male and Female Arctic Ground Squirrels, Arctic, 34(3): 249-254.

McLean, IG (1985) Seasonal patterns and sexual differences in the feeding ecology of arctic ground squirrels (*Spermophilis parryii plesius*), Canadian Journal of Zoology, 63: 1298-1301.

O'Brien DM, Fogel ML, Boggs CL (2002) Renewable and non-renewable resources: amino acid turnover and allocation to reproduction in Lepidoptera, Proceedings of the National Academy of Sciences of the United States of America 99: 4413-4418.

Pearson, SF, DJ Levey, CH Greenberg, C Martinez del Rio (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird, Oecologia 135: 516-523.

Phillips, DL (2001) Mixing models in analysis of diet using multiple stable isotopes: a critique, Oecologia 127: 166-170.

Phillips, DL, JW Gregg (2003) Source partitioning using stable isotopes: coping with too many sources, Oecologia 136: 261-269.

Phillips, DL, SD Newsome, JW Gregg (2005) Combining sources in stable isotope mixing models: alternative methods, Oecologia 144: 520-527.

Podlesak, DW & SR McWilliams (2006) Metabolic routing of dietary nutrients in birds: Effects of diet quality and macronutrient composition revealed using stable isotopes, Physiological and Biochemical Zoology 79(3): 534-549.

Post, DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions, Ecology, 83(3): 703-718.

Robbins CT, Felicetti LA, Florin ST (2010) The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation, Oecologia 162: 571-579.

Robbins CT, Felicetti LA, Sponheimer M (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds, Oecologia 144: 534-540.

Shaver, GR, MS Bret-Harte, MH Jones, J Johnstone, L Gough, J Laundre, FS Chapin III (2001) Species composition interacts with fertilizer to control long term change in tundra productivity, Ecology 82(11): 3163-3181.

Shaver, GR, F Chapin III, BL Gartner (1986) Factors limiting seasonal growth and peak biomass accumulation in *Eriophorum vaginatum* in Alaskan tussock tundra, Journal of Ecology 74: 257-278.

Shaver, GR, MS Bret-Harte, MH Jones, J Johnstone, L Gough, J Laundre, FS Chapin III (2001) Species composition interacts with fertilization to control long-term change in tundra productivity, Ecology 82(11): 3163-3181.

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

Sponheimer, M, T Robinson, L Ayliffe, B Roeder, J Hammer, B Passey, A West, T Cerling, D Dearing, J Ehleringer (2003b) Nitrogen isotopes in mammalian herbivores: Hair $\delta^{15}N$ values from a controlled feeding study, International Journal of Osteoarchaeology 13: 80-87.

Tape, K, M Sturm, C Racine (2006) The evidence for shrub expansion in northern Alaska and the pan-Arctic, Global Change Biology, 12(4): 686-702.

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet δ15N enrichment: a meta-analysis, Oecologia, 136: 169-182.

Wahren CHA, Walker MD, Bret-Harte MS (2005) Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment, Global Change Biology, 11: 537-552.

Walker, MD, CH Wahren, RD Hollister, GHR Henry, LE Ahlquist, JM Alatalo, MS Bret-Harte, MP Calef, TV Callaghan, AB Carroll, HE Epstein, IS Jonsdottir, JA Klein, B Magnusson, U Molau, SF Oberbauer, SP Rewa, CH Robinson, GR Shaver, KN Suding CC Thompson, A Tolvanen, O Totland, PL Turner, CE Tweedie, PJ Webber, PA Wookey (2006) Plant community responses to experimental warming across the tundra biome, Proceedings from the National Academy of Sciences 103: 1342-1346.

Walker, DA & HA Maier (2008) Geobotanical maps in the vicinity of the Toolik Lake Field Station, Alaska, Biological Papers of the University of Alaska 28.

Wolf, N, SA Carleton, C Martinez del Rio (2009) Ten years of experimental animal isotopic ecology, Functional Ecology, 23: 17-26.

Zazula, GR, DG Froese, SA Elias, S Kuzmina, RW Mathewes (2007) Arctic ground squirrels of the mammoth-steppe: paleoecology of Late Pleistocene middens (~24,000-29,450 14C yr BP), Yukon Territory, Canada, Quaternary Science Reviews, 26: 979-1003.

Zazula, GD, RW Mathewes, AS Harestad (2006) Cache selection by Arctic Ground Squirrels inhabiting Boreal-steppe meadows of southwest Yukon Territory, Canada, Arctic, Antarctic, and Alpine Research, 38(4): 631-638.

APPENDICES

APPENDIX A

ATIGUN VEGETATION COMMUNITIES

Methods

The study area was qualitatively analyzed to define different vegetation community types falling within the boundaries. The boundaries of these community types were defined using GPS and a cover map was created using Arcmap software(Figure A.1). Percent cover for the study area was analyzed using a one square meter point-frame quadrat. Eight to thirteen random sampling points were established within each community based on the approximate size of the community using Arcmap software. Each of these points was then analyzed for percent cover of overstory and understory vegetation falling within the quadrat.

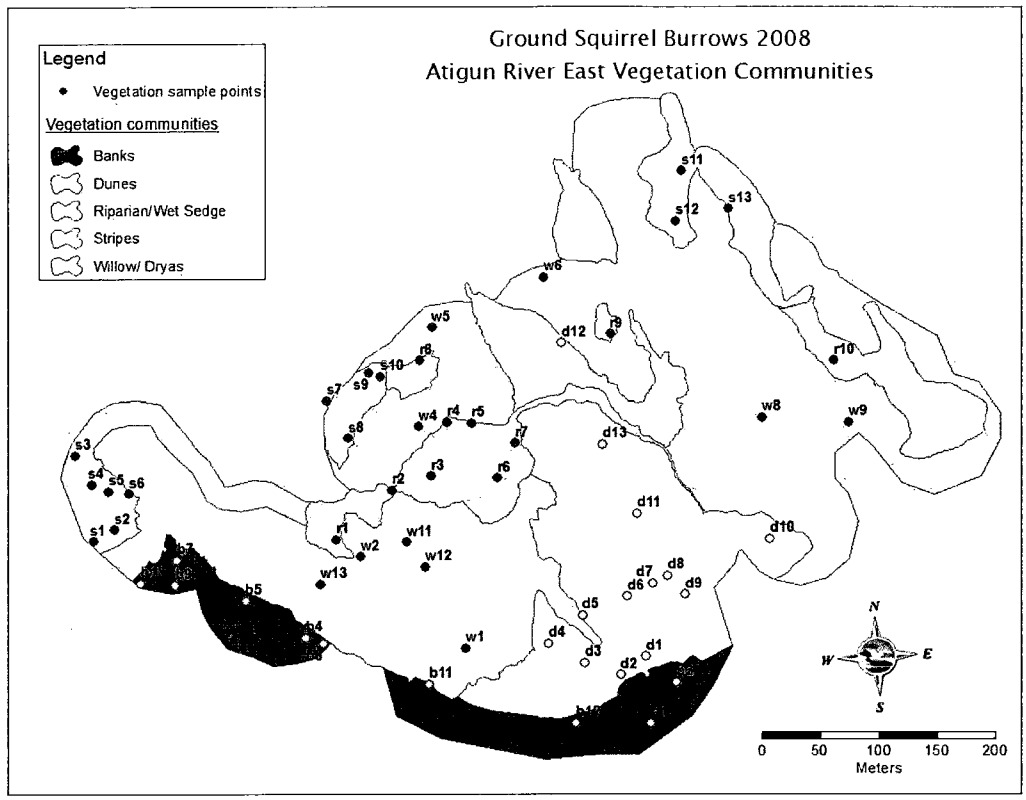

Figure A.1. Atigun study site with delineated vegetation communities and random sample quadrat points.

Results

The five communities delineated within the study area were given the names Bank, Dunes, Riparian, Stripes and Willow/Dryas. A total of 34 species were encountered in the percent cover analysis (Table A.1). This group includes grasses, sedges, rushes, mosses, lichens and fungi which were not identified to species, and would likely make this figure much higher. The Bank community contained seven species with the three most common species or substrates being sand (82.8%), *Salix glauca* (11.8%) and *Salix alexensis* (4.5%). The Dunes community contained 15 species with the three most common species or

substrates being sand (54.1%), *Salix glauca* (14.1%) and *Dryas octopetala* (7.5%). The Riparian community contained 14 species with the three most common species or substrates being mosses (65.3%), sedges (30.6%) and *Vaccinium uliginosum* (6.4%). The Stripes community contained 18 species with the three most common being mosses (52.6%), *Salix reticulata* (13.7%) and sedges (12.5%). The Willow/Dryas community contained 19 species with the three most common species or substrates being *Dryas octopetala* (20.1%), moss (16.6%) and litter (14.8%).

Species	Bank	Dunes	Riparian	Stripes	Willow/Dryas
Arctostaphylos rubra		0.8	0.5	9.3	3.8
Artemisia sp.	1.8	1.3			
Betula nana				0.9	
Cassiope tetragona					0.5
Dryas octopetala		7.5	1	6.6	20.1
Empetrum nigrans					0.6
Epilobium latifolium	1.1	0.9			
Equisetum sp.	0.1	1.3	2	0.7	0.5
Grass	1.5	2.5		0.9	2.8
Hedysarum sp.			0.5	2.8	0.2
Lichen				0.4	4.8
Litter	0.9	4.4	4.1	2	14.8
Moss		0.2	65.3	52.6	16.6
Oxytropis sp.	0.3	3.5		0.2	0.5
Pedicularis sp.			0.1	0.1	
Potentilla fruiticosa			0.3		0.6
Pyrola sp.		0.1	0.1	0.1	
Rhododendron laponicum				4.3	2.5
Rush		0.1		0.1	
Salix alexensis	4.5	1.1			
Salix glauca	11.8	14.1	1.1		8.6
Salix linata		0.4	4	4.2	
Salix reticulata			3.3	13.7	5.7
Salix sp. 1				0.3	
Salix sp. 2			0.4		0.5
Sand	82.8	54.1		0.9	7
Saxifraga tricuspidata					0.1
Sedge		0.2	30.6	12.5	3.7
Shepherdia canadensis		2.1			2.6
Vaccinium uliginosum			6.4	5.7	4.4
Water		0.8	0.9		

Table A.1 Proportion of total site vegetation represented by individual species for the five community types designated in the Atigun River burrow site. Proportions were determined using a 1 square-meter quadrat

APPENDIX B

FEEDING STUDY

Methods

Data Collection

Fourteen male Arctic ground squirrels were housed in animal quarters at the University of Alaska Fairbanks for two weeks and fed controlled diets in addition to being offered rodent chow. The animals were separated into three groups, each of which was offered a different combination of plants and mushrooms either known to be consumed by Arctic ground squirrels or present in areas of high burrow density. Group one contained four squirrels fed only vegetation of grass leaves, *Salix spp.* leaves and a combination of *Epilobium latifolium*, *Dryas sp.* and *Hedysarum sp.* roots. Groups two and three both contained five squirrels that were fed mushrooms. In addition to mushrooms, group two squirrels were fed *Artemisia sp.* leaves, grass leaves, *Salix spp.* leaves and *Oxytropis sp.* roots. The rodent chow that the Arctic ground squirrels are fed in the lab was replaced with the wild foods for two hours each morning for the duration of the experiment.

Wild vegetation was harvested at the Atigun study site in August 2008 and immediately frozen at Toolik Field Station for later feeding. The frozen vegetation was transported in a cooler from Toolik Field Station back to the University of Alaska Fairbanks. Vegetation genera included *Artemisia sp.* leaves, graminoid leaves, *Salix sp.* leaves, *Dryas sp.* roots, *Epilobium latifolium.* roots, *Hedysarum sp.* roots and *Oxytropis sp.* roots. Mushroom genera offered included *Leccinum* and *Russula* which have both been observed near Arctic ground squirrel burrows

with tooth marks. The mushrooms were dried at 60˚C at Toolik Field Station and returned to the University of Alaska Fairbanks. These vegetation and mushrooms will hereafter be referred to as "wild foods."

Dry weights of the wild foods were obtained before and after they were offered to the animals on a daily basis. For non-dry food sources offered (frozen and thawed vegetation), identical volumes of the food source offered were dried and weighed to obtain an approximate dry weight. Due to the high water content and variability in water/volume ratios of mushrooms, these were offered dried. All remaining wild food items were collected from cages at the end of the feeding period each day. The items were then separated and dried to obtain the dry weight of each item not consumed. This was subtracted from the dry weight of the food item offered.

The Arctic ground squirrels were also weighed twice a week to measure any weight changes that resulted from their diet and to ensure they maintained a healthy weight. Weights were measured while animals were anesthetized for blood extraction.

Blood samples were taken originally by cardiac puncture and subsequently by toenail clipping two times per week in order to monitor carbon and nitrogen isotope changes as a result of a varied diet. Animals were anesthetized with isoflurane prior to blood extraction. During toenail clipping the toenail of a rear toe was clipped far enough to produce blood and collected in heparinized capillary tubes to prevent clotting. Blood samples (0.5-1.0 ml) were

obtained by cardiac puncture with a 25 gauge 5/8" sterile needle. Both types of samples were centrifuged to separate plasma from whole blood. Samples were them kept frozen in a -80˚C freezer at the University of Alaska Fairbanks. The plasma samples were later extracted and weighed out into approximately 1 mg samples in tin cups and dried at 40˚C. These samples were then returned to the Stable Isotope Lab at the University of New Hampshire for analysis.

Hair samples were collected prior to the start of the study to compare to the rodent chow fed to Arctic ground squirrels in the University of Alaska Fairbanks animal lab. A small patch of hair was shaved from the abdomen of each animal. These samples were stored in small manila envelopes and returned to the University of New Hampshire Isotope Lab for analysis.

Statistics

Total item consumed and proportion consumed of the amount offered was calculated for each day. These values were then averaged over the course of the study period for each item offered to the animals in each group. Means and standard deviations were calculated for each item by group. However, because data did not meet assumptions of normality and did not vary in a consistent manner, neither parametric nor non-parametric analyses could be completed on the feeding results.

Blood plasma isotopes were measured through the course of the experiment and matched pairs analysis was used to determine if the values

changed from the beginning of the feeding period to the end. Only six of the 14 animals could be used in this analysis because blood could not be obtained at either the beginning or the end of the experiment from some animals. This was due either to difficulty extracting blood or animals entering hibernation at the end of the study.

Both hair samples and blood samples were used to determine fractionation from diet to tissue by comparing the isotope values at the start of the feeding study with those of the rodent chow diet. Discrimination between rodent Chow to hair and rodent chow to blood were determined separately and compared to determine differences in values between the two tissues. Due to the long turnover time of hair only one animal that was born in the lab could be used to compare hair to diet values. All other animals in the study were trapped in the field the previous spring or summer and we could not verify whether molt took place in the lab or previously in the field.

Discrimination values were calculated using the following equation:

$$\Delta = \delta^{15}N_{consumer} - \delta^{15}N_{diet}$$

The $\delta^{15}N_{consumer}$ represents the Arctic ground squirrel blood or hair and the $\delta^{15}N_{diet}$ represents the rodent chow fed to the lab animals. This equation is also used to determine $\Delta^{13}C$ by replacing $\delta^{15}N$ with $\delta^{13}C$ for both consumer and diet.

All statistical analyses were performed using JMP 8.0 (SAS inc.) with an α=0.05.

Results

Food Items Consumed

The Arctic ground squirrels in group one consumed a mean proportion of 0.59 (n=4, SD=0.31) of the roots they were offered (Fig B.1). The mean proportion of *Salix spp.* leaves consumed was 0.20 (n=4, SD=0.18) and grass leaves was 0.17 (n=4, SD=0.17).

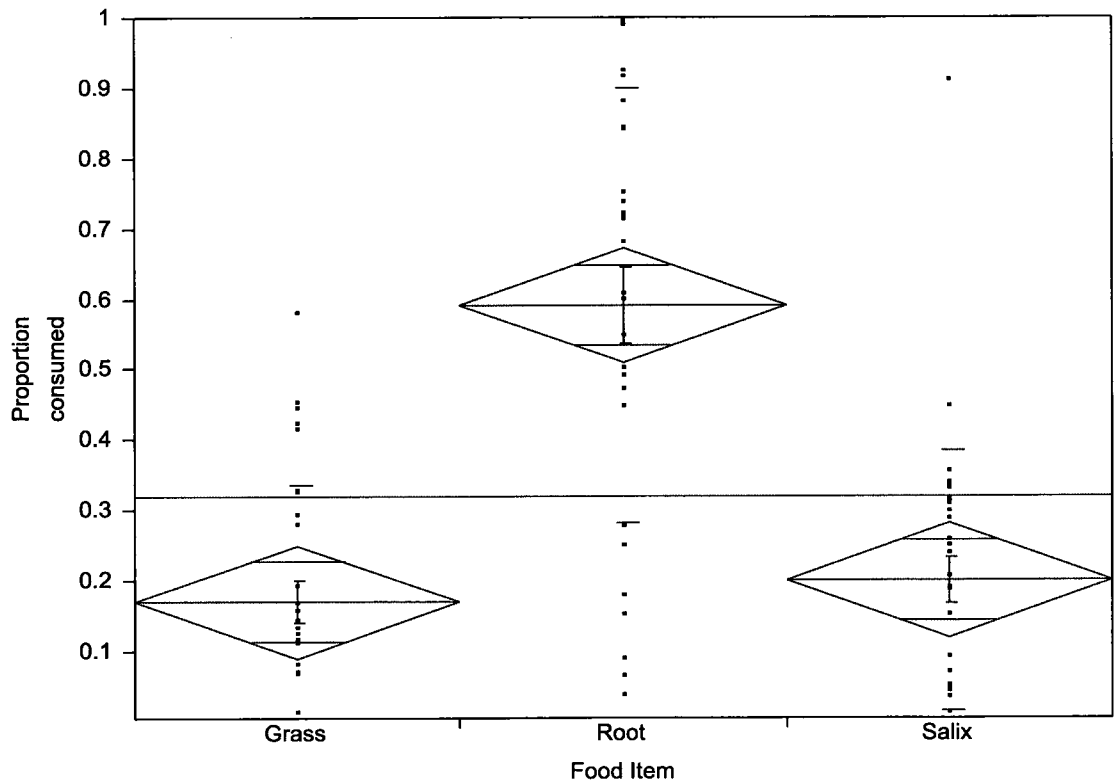

Figure B.1. Proportion of food items offered that were consumed by Arctic ground squirrels in feeding group one. Food Item groups are displayed with mean diamonds indicating the mean, 95% confidence interval and overlap marks. The overall mean is displayed by a horizontal line. The blue lines indicate error bars (inner) and one standard deviation (outer).

The Arctic ground squirrels in group two consumed *Artemisia sp.* leaves at a proportion of 0.76, (n=5, SD=0.23) *Oxytropis sp.* roots at a proportion of 0.74 (n=5, SD=0.28) and mushrooms at a proportion of 0.36 (n=5, SD=0.28, Figure B.2). Grass leaves were consumed at a proportion of 0.15 (n=5, SD=0.16) with *Salix spp.* leaves at 0.17 (n=5, SD=0.18).

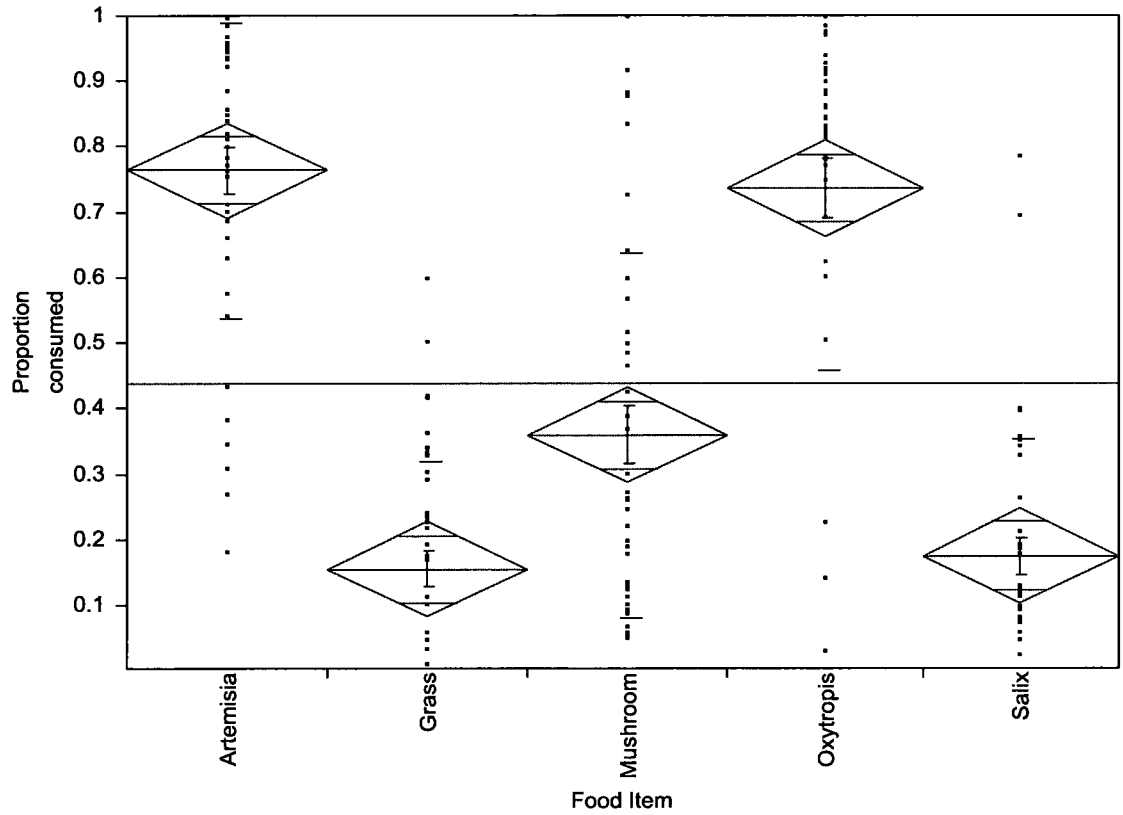

Figure B.2 Proportion of food items offered that were consumed by Arctic ground squirrels in feeding group two. Food Item groups are displayed with mean diamonds indicating the mean, 95% confidence interval and overlap marks. The overall mean is displayed by a horizontal line. The blue lines indicate error bars (inner) and one standard deviation (outer).

Arctic ground squirrels in group three consumed a mean proportion of 0.08 of the mushrooms they were offered (n=5, SD=0.17). This amount was significantly less than the proportion of 0.36 consumed by group two animals with a difference of 0.28 ($p<0.0001$, SE=0.05).

Discrimination Factors

Rodent chow had a mean $\delta^{15}N$ value of 2.5‰ (n=2, SD=0.4) and a mean $\delta^{13}C$ value of -20.2‰ (n=2, SD=0.0). Discrimination factors of $\Delta^{15}N$ for hair ranged from -1.9‰ to 2.2‰ with a mean of -0.8‰ (SD=1.2, n=14). Discrimination factors of $\Delta^{13}C$ for hair ranged from -5.1‰ to 1.0‰ with a mean of -3.4‰ (SD=1.9, n=14). Blood $\Delta^{15}N$ discrimination factors ranged from 3.2‰ to 4.3‰ with a mean of 3.9‰ (SD=0.4, n=8). Blood $\Delta^{13}C$ discrimination factors ranged from -2.2‰ to -0.9‰ with a mean of -1.6‰ (SD=0.4, n=8, Table 3.1).

AGS ID#	Tissue	$\Delta^{15}N$ (‰)	Δ^{13}(C‰)	$\delta^{15}N$ Pre-study (‰)	$\delta^{13}C$ Pre-study (‰)
836	Hair	2.2	1.0	4.7	-19.3
8126	Hair	1.2	-1.0	3.7	-21.2
8127	Hair	-0.6	-3.1	1.9	-23.4
8134	Hair	-1.9	-4.9	0.6	-25.1
8136	Hair	-1.7	-5.1	0.8	-25.3
8137	Hair	-1.1	-4.1	1.4	-24.3
8138	Hair	-1.7	-5.0	0.8	-25.2
8139	Hair	0.2	-1.5	2.7	-21.7
8144	Hair	-0.9	-2.6	1.6	-22.9
8147	Hair	-0.6	-2.3	1.9	-22.5
8148	Hair	-1.5	-4.7	1.0	-25.0
8149	Hair	-1.5	-4.7	1.0	-24.9
8151	Hair	-1.4	-4.8	1.1	-25.0
8154	Hair	-1.8	-5.1	0.7	-25.4
8127	Blood plasma	4.0	-1.5	6.5	-21.8
8134	Blood plasma	4.0	-1.8	6.6	-22.0
8137	Blood plasma	3.7	-1.5	6.2	-21.7
8144	Blood plasma	3.8	-1.5	6.3	-21.7
8148	Blood plasma	4.3	-1.7	6.8	-21.9
8149	Blood plasma	4.2	-0.9	6.8	-21.1
8151	Blood plasma	3.8	-1.6	6.3	-21.9
8138	Blood plasma	3.2	-2.2	5.7	-22.4

Table B.1. Discrimination factors for nitrogen and carbon from rodent chow diet to hair and blood plasma tissues of lab Arctic ground squirrels

Weights of most Arctic ground squirrels declined over the course of the feeding study from pre-study to day 9. All of the animals had some weight fluctuation which ranged from a loss of 66 grams to a gain of 30 grams (Table 3.2). On average, animals lost 28 grams (SD=34, n=13).

AGS ID#	Weight (g) pre-study	Day 3	Day 9	3 weeks post-study	weight Δ pre-study to Day 9
836	859	824	807	703	-52
8126	838	815	773	712	-65
8127	927	?	873	774	-54
8134	823	842	833	876	10
8136	835	821	803	803	-32
8137	908	922	938	872	30
8138	842	801	776	721	-66
8139	752	719	705	695	-47
8144	791	791	?	723	?
8147	987	992	997	915	10
8148	834	806	790	724	-44
8149	921	894	878	848	-43
8151	810	?	775	766	-35
8154	879	876	904	814	25

Table B.2. Weight change of Arctic ground squirrels in grams over the course of the study from pre-study to day 9 of the study. AGS ID# is the identification number assigned to the individual at birth or time of capture.

Matched pairs analysis revealed a significant change in blood plasma $\delta^{15}N$ values from pre-study to the end of the experiment with a mean increase of 0.67‰ ($p=0.0022$, $SE=0.12$, $n=6$). Values of $\delta^{13}C$ were also significantly different with a mean increase of 0.99‰ ($p=0.0007$, $SE=0.13$, $n=6$) over the course of the experiment.

It was not possible to determine a discrimination value for mushroom to Arctic ground squirrel hair due to the small amount of mushroom sporocarp consumed each day in relation to the amount of rodent chow consumed. Average rodent chow consumed in one day was 22.59 g ($SD=11.75$, $n=14$) and average mushroom sporocarp consumed in one day by group three animals was 0.42 g ($SD=0.81$, $n=35$).

APPENDIX C

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) APPROVAL

University *of* New Hampshire

Research Conduct and Compliance Services, Office of Sponsored Research
Service Building, 51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

01-Oct-2008

Hobbie, Erik A
Complex Systems Research Center, Morse Hall
Durham, NH 03824

IACUC #: 080904A
Project: A Feeding Study for the Calibration of Multidisciplinary Diet Analysis Techniques in Arctic Ground Squirrels (Spermophilus parryii)
Category: C
Approval Date: 22-Sep-2008

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the research potentially involves minor short-term pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics or other assessments.* The IACUC made the following comment(s) on this protocol:

All work being conducted at the University of Alaska-Fairbanks by graduate student, Julee Shamhart. No animal contact at UNH.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File
Shamhart, Julee

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UAF
FAIRBANKS

Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

April 28, 2008

To: Brian Barnes, PhD
Principal Investigator

From: Erich H. Follmann, PhD
IACUC Chair

Re: IACUC Modification Request

On behalf of the University of Alaska Fairbanks Institutional Animal Care and Use Committee (IACUC) I have reviewed and approved the following request for modification:

Protocol#:	06-25
Title:	*Hibernation genomics and proteomics/ arctic ground squirrels*
Modification:	The addition of Julee Shamhart.
Received:	April 5, 2008
Approved:	April 28, 2008

Thank you for keeping your Assurance of Animal Care form Current.

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FAIRBANKS

Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

September 9, 2008

To: Brian Barnes, PhD
Principal Investigator

From: Michael Castellini, PhD
Interim IACUC Chair

Re: IACUC Assurance Application

The University of Alaska Fairbanks Institutional Animal Care and Use Committee (IACUC) reviewed the following Assurance at their August 25, 2008, meeting. This Assurance was approved pending receipt of a revised assurance addressing the committee's questions. The assurance received on September 8, 2008 was determined to be satisfactory; therefore I am pleased to issue approval.

Protocol#: 08-59

Title: *A feeding study for the calibration of multidisciplinary diet analysis techniques in Arctic Ground Squirrels (Spermophilus parryii)*

Received: August 18, 2008 (orig)
September 8, 2008 (revision)

Approved: September 9, 2008

Review Due: September 9, 2009

The PI is responsible for acquiring and maintaining all required permits and permissions prior to beginning work on this assurance. Failure to obtain or maintain valid permits is considered a violation of an IACUC assurance, and could result in revocation of IACUC approval.

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FAIRBANKS

Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

October 2, 2008

To: Brian Barnes, PhD
Principal Investigator

From: Michael Castellini, PhD
Interim IACUC Chair

Re: IACUC Modification Request

On behalf of the University of Alaska Fairbanks Institutional Animal Care and Use Committee (IACUC) I have reviewed and approved the following request for modification:

Protocol#:	08-59
Title:	*A feeding study for calibration of multidisciplinary diet analysis techniques in Arctic Ground Squirrels (spermophilus parryii)*
Modification:	To analyze plasma samples, instead of plasma and whole blood and thereby shortening time frame to 8 days.
Received:	September 24, 2008
Approved:	October 2, 2008

Thank you for keeping your Assurance of Animal Care form Current.