FEMALE DALL'S SHEEP SUMMER NUTRITION IN THE CHUGACH MOUNTAINS, ALASKA

By

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Abstract

As part of recent research efforts by the Alaska Department of Fish and Game (ADF&G) to identify issues related to current population declines in Alaskan Dall's sheep (*Ovis dalli dalli*), we conducted a summer nutrition study on a subpopulation of female Dall's sheep (ewes) in the Chugach Mountains of Southcentral Alaska. Over the summers of 2016, 2017, and 2018, we assessed ewe diet compositions and identified forage types through video observation of foraging ewes. During this time, we collected forage and fecal samples to evaluate ewe forage quality [crude protein (%), gross energy (kcal/g), and apparent digestibility (D_a)] and identify forage types (deciduous shrubs, evergreen shrubs, forbs, graminoids, lichen and mushrooms). Lastly, we conducted compound specific stable isotope ($\delta^{13}C$) analysis of amino acids (CSIA-AA) on a subset of our forage samples (n = 35) to evaluate it as a tool in ewe diet sourcing. We also addressed if our alpine samples differ from previous plant samples from other terrestrial habitats. We found that ewe diet composition varied from one summer to the next as well as from early to late summer. Forage crude protein also varied intra-annually and inter-annually between all years, while gross energy varied within seasons. Our CSIA-AA fingerprints showed that our samples did not group together as conventional forage types; in addition, our forage samples differed from previously mapped end-members (e.g., some alpine terrestrial C_3 plants mapped as fungi). Nutrition studies like ours will help establish carrying capacities and identify critical habitat of the Dall's sheep within Alaska as current dynamic changes continue to occur in these alpine ecosystems.

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Chapter 1: General Introduction

1.1 Background

Dall's sheep (*Ovis dalli dalli*) are one of the most iconic species in the Arctic and are well adapted to the cold, dry climates of the arctic and subarctic mountain ranges (Nichols & Bunnel, 1999). As alpine specialists, Dall's sheep act as bellwethers for the health of alpine ecosystems as a whole, and their current status is one of rising concern (Van de Kerk et al., 2018). A large portion of the world's Dall's sheep population resides in Alaska. In recent decades, many Alaskan Dall's sheep subpopulations have declined by as much as 50-70% (ADF&G, 2022). These declines raise the question: What factors are driving the population trends and trajectories?

The Alaska Department of Fish and Game (ADF&G) manages wildlife in Alaska, and most management decisions are made at the game management unit (GMU) level. GMUs separate large geographic areas into smaller units usually defined by terrain features that tend to incorporate similar weather patterns and habitat, and as such, animal populations, allowing biologists to adjust harvest and management strategies according to specific needs in that area. The Chugach Mountains span approximately 300 miles across Southcentral Alaska and incorporate four different Game Management Units and/or Subunits, with sheep distributed throughout the mountain range and GMUs. The westernmost portion of the Chugach Mountains is in GMU 14C and is home to one of the most visible Dall's sheep subpopulations, which is highly prized as a hunting and wildlife viewing resource due in part to its' proximity to the city of Anchorage.

Like many other Dall's sheep subpopulations across the state, the Dall's sheep in GMU 14C have seen population declines in recent decades. In 2000, aerial surveys estimated the Dall's sheep subpopulation in GMU 14C as 2097, and since then, it has declined by roughly half, with a low of 904 in 2007 and a current estimated population of 1132 (ADF&G, 2022). ADF&G initiated research targeted at investigating potential causes of the declines in the Northern Chugach Mountains in 2009 and expanded the project to include GMU 14C in 2012. Early research results suggested animals were nutritionally limited and had low and variable pregnancy rates, while predation and disease did not appear to have population level effects (Lohuis, 2021).

These results suggested that declines in Chugach Mountains' sheep subpopulations were likely related to nutritional and habitat issues.

ADF&G theorizes that these environmentally related nutritional issues could potentially be related to the effects of climate change in Dall's sheep habitat and forage. The topic of climate change and Alaskan Dall's sheep is well studied, with previous studies on the effects on habitat selection (Aycrigg et al., 2021), movement (Mahony et al., 2018), survival (Van de Kerk et al., 2020), and recruitment and abundance (Rattenbury et al., 2018). Dall's sheep are of particular interest in the study of climate change, because climate change may be exacerbated at the high altitudes and/or latitudes that characterize their habitat (Van de Kerk et al., 2018). In general, in arctic terrestrial ecosystems, winter weather is expected to become warmer and wetter, relative to historical trends, while summer growing conditions are expected to become longer, warmer, and drier (Cooper, 2014). These warmer, dry summer growing conditions have already been shown to decrease the nutritional content of the forage of caribou (*Rangifer tarandus granti*), a similar arctic herbivore (Lenart et al., 2002; Walsh et al., 1997; Zamin et al., 2017). Shifts in the summer growing season include earlier plant senescence (Cooper, 2014) which could mean the decreased nutritional value and/or accessibility of Dall's sheep forage during key times of the year (Aycrigg et al., 2021; Khapalova et al., 2018). In the Chugach Mountains, changing summer growing conditions are also thought to have resulted in shrub encroachment into Dall's sheep habitat (Dial et al., 2016), potentially decreasing their carrying capacity. Recent work by Smith (2020) generated range selection functions and a digital map of sheep habitat in GMU 14C that can be used to quantify potential habitat loss. But there is still a need for targeted research to assess the potential effects of climate change on summertime Dall's sheep diet with regards to habitat quality. This work can expand on the 'animal indicator concept' (Franzmann & Schwartz, 1997) that states that an animal is a product of its environment, and its body condition, and by extension its foraging behavior, represents the quality of that habitat.

1.2 Project Description and Goals

We investigated female Dall's sheep summer diet composition and forage quality, to understand if and how they could change within the summer season (early and late) and from one summer to the next. We also investigated multiple forage quality factors (crude protein, gross energy, and apparent digestibility) of different forage types, and then compared them with diet composition to understand the interactions between the two. These findings were then put into context of temperature and precipitation patterns during each summer. This information will create novel Dall's sheep diet and nutritional data in the Chugach Mountains and provide some insight as to why these populations are declining.

The specific goals of this research were:

I. For the designated time frames of total summer (June 1-August 31), early summer (June 1-July 16) and late summer (July 17-August 31), for 2016, 2017, and 2018:
   - **a.** Describe the diet of female Dall's sheep in the Chugach Mountains, Alaska, and identify diet composition by forage type.
   - **b.** Assess forage quality of each forage type and by specific diet for all designated time frames, measuring crude protein (%) and gross energy (kcal/g) content of female Dall's sheep summer diet in the Chugach Mountains.
     - **i.** Evaluate if there is potential variation from one summer to the next or from early to late summer in aspects of forage quality, followed with an assessment of correlation with local mean air temperatures (°C) and total precipitation (cm) during the sampling time frame.
   - **c.** Estimate the apparent digestibility of the forage quality aspects: crude protein (%) and gross energy (kcal/g) in the summer forage of female Dall's sheep.

II. Conduct compound-specific stable isotope analysis ($\delta^{13}C$) of amino acids (CSIA-AA) of 40 samples selected from known Dall's sheep forage items (identified in goal #I) across multiple forage types (i.e., deciduous shrubs, evergreen shrubs, forbs, graminoids, lichens, and mushrooms).
   - **a.** Evaluate if CSIA-AA can be used as an effective tool to trace nutrient sources of Dall's sheep diet.

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Chapter 2: Female Dall's Sheep Summer Diet Composition and Forage Quality in the Chugach Mountains[1]

2.1 Abstract

Dall's sheep (*Ovis dalli dalli*) are a bellwether alpine species across arctic and sub-arctic North America, whose populations have declined across their range. One subpopulation that has dropped 46% since 2000 resides in the western Chugach Mountains in southcentral Alaska. To better understand recent population declines in this population, and how they might be related to climate change, we evaluated total summer (June 1-August 31), early (June 1-July 16), and late (July 17-August 31) diet composition and forage quality from 2016-2018. We used direct video observations of feeding free-ranging Dall's sheep ewes to assess diet composition of our select time frames and sample forage samples for subsequent analyses. We collected forage (n = 343) and fecal (n = 160) samples to determine forage quality measured by crude protein (%) and gross energy (kcal/g), and the apparent digestibility (D_a) of both. Then to observe the interaction of our results and weather, we compared results with co-occurring local mean air temperature (˚C) and precipitation (cm) data. In this study, female Dall's sheep consumed 6 major forage types: deciduous shrubs, evergreen shrubs, forbs, graminoids, lichens, and mushrooms. Ewe total summer diet composition shifted inter-annually, but across all years graminoids and forbs were the dominant forage types, accounting for 46 %-60 % of ewe diet. Early to late summer season diet was variable across years, the only consistent shift was that all early season diets contained evergreen shrubs (predominantly *Dryas octopetala*) as a key forage type, while late season did not. Crude protein was statistically different across all years ($P = 0.008$), declining from 2016-2018, while gross energy was only statistically different among some years ($P < .001$). Highest crude protein in 2016 coincided with moderate summer temperatures (~20˚C) and moderate total precipitation (~12 cm). Associations of high-quality forage with moderate weather conditions suggest that the hotter, dryer summer conditions typically associated with climate change in high latitudes, alpine ecosystems could reduce Dall's sheep forage quality and in turn population health.

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2.2 Introduction

Wild sheep (*Ovis* spp.) have lived in North America for over 30,000 years (Valdez & Krausman, 1999). In the last century, they have garnered one of the most diverse and enthusiastic followings in wildlife conservation. Despite this interest, wild sheep are relatively understudied when compared to other species of terrestrial North American megafauna. With many wild sheep populations in decline, additional research and better understanding of North American wild sheep biology is critical. Dall's sheep (*O. dalli dalli*) and Stone sheep (*O. dalli stonei*) are the two subspecies of wild thinhorn sheep and are the northernmost, and most numerous of all North American wild sheep (Bowyer & Leslie, 1992; Valdez & Krausman, 1999). Because Dall's sheep depend on high elevation and high latitude habitat, they are of interest in the study of arctic and alpine environments (Van de Kerk et al., 2020), particularly in the face of climate change which may be amplified at higher altitudes and latitudes (Aycrigg et al., 2021; Khapalova et al., 2018). Alaska is home to the majority of the world's Dall's sheep population (Valdez & Krausman, 1999). Most Alaskan Dall's sheep subpopulations have declined 50-70% in recent decades (Lohuis et al., 2022).

Dall's sheep are native to the Chugach Mountains in southcentral Alaska and have been monitored and managed by the Alaska Department of Fish and Game (ADF&G) since statehood in 1959. ADF&G uses aerial surveys to obtain minimum counts of Dall's sheep populations. Although these surveys do not correct for sightability, Dall's sheep numbers in some parts of the Chugach Mountains have been in decline since the 1970s (Smith, 2020). The westernmost subpopulation of sheep in the Chugach Mountains is in ADF&G's Game Management Unit (GMU) 14C. Dall's sheep numbers in GMU 14C historically has been cyclical, but since the last population peak in 1996 (2430 sheep) numbers have declined, reaching as low as 904 sheep in 2007 (Battle & Stantorf, 2018).

In response to declining numbers of Dall's sheep numbers, ADF&G began a study in the Northern Chugach Mountains in 2009. This research investigated the health and population dynamics of the Dall's sheep in the Chugach Mountains by radio collaring and tracking ewes to measure body condition, pregnancy and disease status, and record cause-specific mortality rates. In 2012, this work was expanded to include sheep in GMU 14C. Researchers reported poor body condition scores (BCS) for adult ewes (BCS = 1.5-2, on a scale of 1-5) across the Chugach

Mountains, with ewes showing low amounts of subcutaneous (SQ) fat, and evidence of muscle catabolism and depleted bone marrow fat, both indicative of extreme nutritional limitation (Lohuis, 2021). The body condition of wild animals is primarily dependent on environmental conditions acting alone or in conjunction with forage quality (Cook et al., 2001). In a study on caribou (*Rangifer tarandus granti*), decreases in SQ rump fat correlated with decreases in forage quality (Parker et al., 2005).

Despite poor body condition, the ADF&G Chugach Mountains study found similar adult survival rates to previous thinhorn wild sheep studies. Researchers reported a mean adult ewe survival rate of 87 % in GMUs 13D and 14C (Lohuis, 2021). In other projects, researchers found similar adult ewe survival rates, in the Alaska Range (1999-2003) 86 % (Arthur, 2003) and in the Brooks Range (2009-2011) 83 % (Arthur, 2013). In a Stone sheep study in Northern Canada from 1994 to 2004, Wood et al. (2010) found similarly high adult ewe survival rates (89 %). While adult survival rates were similar across mountain ranges and years, cause-specific mortality varied. In the Chugach Mountains only 24 % of adult mortality was caused by predation, while in areas like the Alaska range (1999-2003) 100 % of adult mortality was attributed to predation (Arthur, 2003).

Further, in the Chugach study, from 2012-2020, Lohuis et al. (2021) reported pregnancy rates ranging from 19-85 % that was highly variable (n = 9, SD = 26). While pregnancy rates for Dall's sheep in the Alaska (Arthur, 2003) and Brooks Range (Arthur, 2013), and in Stone sheep in British Columbia (Wood et al., 2010) were consistently higher with less variability (89-95 %) (Arthur 2003, 2013).

Initial research suggested that the declines were most likely caused by limited nutrition as Dall's sheep were in poor body condition and exhibited low and variable pregnancy rates when compared to other ungulate populations (Lohuis, 2021). When assessing the habitat selection of Dall's sheep, high quality forage availability has proven to be one of the most influential factors (Aycrigg et al., 2021; Smith, 2020). Recent work has also demonstrated that forage quality of arctic herbivores is influenced by temperature and precipitation patterns (Lenart et al., 2002; Walsh et al., 1997; Zamin et al., 2017). In fact, the interactions between population dynamics of wild ungulates and their habitat and nutrition are well documented (Parker et al., 2009). The combination of comparably lower and more variable pregnancy rates with observed poor ewe body condition during the ADF&G study in GMU 14C, strongly suggested some combination of habitat and forage as the underlying problem behind the poor Dall's sheep population numbers (Lohuis, 2021). Interrelationships between habitat, forage, adult female body condition, and population dynamics drive population trajectories in Dall's sheep and other North American herbivores. For example, in GMU 20A near Fairbanks, AK, low nutritional status (based on available browse to forage), was correlated with overall poor population health and low recruitment rates in Alaska moose (*Alces alces gigas)* (e.g., low twinning rates) (Boertje et al., 2007). In the Sierra Nevada Mountain Range in California, body condition of female mule deer (*Odocoileus hemionus)* has been used as a metric for forage quality and for predicting survival and recruitment rates for the entire population (Stephenson et al., 2002). For Dall's sheep and a majority of wild ungulates, poor forage quality can lead to conservative allocation of resources in pregnant females, which can have a life-long effect on the body size of offspring, and, in turn, the health of the cohort as a whole (Monteith et al., 2018). The combination of comparably lower and variable pregnancy rates with poor ewe body condition strongly suggested some combination of habitat and forage as the underlying problem behind declining Dall's sheep populations (Lohuis, 2021).

Forage is typically assessed on a scale of abundance and quality. In recent years, there have been two major investigative approaches to wildlife nutrition; nutritional ecology, which focuses on the relationship of an animal with its environment through forage based nutritional interactions (Barboza et al., 2009), and nutritional physiology, which focuses on how the animal is ingesting and incorporating food components into their body (Birnie-Gauvin et al., 2017). Our research strove to incorporate both nutritional ecology in the form of diet composition and forage quality and nutritional physiology by estimating the apparent digestibility of ingested forage.

The annual life cycles of female arctic ungulates depend on summer forage to restore sufficient body stores of energy and nitrogen for the individual to survive the winter, nurse their young and be successful the during subsequent reproductive cycle (Barboza et al., 2009). It follows that female body mass in fall and parturition rates the following spring are correlated (Reimers, 1983; Skogland, 1985). Dall's sheep ewes give birth from late May to early June (Nichols & Bunnel, 1999). Parturition alleviates the energetic burden of pregnancy but gives way to the even more energetic demanding phase of lactation (Oftedal, 1985). Dall's sheep ewes nurse their young from the day of birth typically through October (Geist, 1971). During lactation, wild female herbivore's daily metabolic demand is at its highest (Parker et al., 2009), and daily energy requirements increase by 65-215 % compared to non-maternal females (Oftedal, 1985; Robbins, 1993). Supporting offspring, while at the same time trying to rebuild fat reserves to survive the following winter is a balancing act for many northern ungulates (Parker et al., 2009). This suggests that nutrition for arctic ungulates during the summer forage season is of critical importance to both the survival of an individual female and her reproductive success. Arctic ungulates use body stores of energy and protein to support both pregnancy over winter and lactation in spring. Females must also recover sufficient body stores over the summer to survive the next winter and establish the reserves for another reproductive cycle.

Dall's sheep summer diet composition has been assessed in several other mountain ranges, but never in the Chugach Mountains. Previous analysis suggests that any specific subpopulation of Dall's sheep can consume between 50-120 different species of forage (Egorov, 1967; Hoefs & Cowan, 1979; Jones et al., 1963; Valdez & Krausman, 1999). A study in the Kenai Mountains found that graminoids (i.e., sedges and grasses) comprised the majority (66 %) of the mean total summer Dall's sheep diet, with the remainder being an even split between shrubs and forbs (17 %), and lichens and mosses (17 %). Mountain avens (*Dryas octopetula*), an evergreen shrub, is prevalent in all the Dall's sheep diet of all the major mountain ranges in Alaska (Nichols, 1971). While Dall's sheep in the Kenai Mountains avoid Mountain avens (Nichols, 1971), in the Alaska and Brooks Mountain ranges, Dall's sheep consume them readily (Whitten, 1975; Winters, 1980; Ayres, 1986). When studying Dall's sheep on Sheep Mountain in the Yukon Territory, Hoefs & Cohen (1979) found that their total summer diet consisted of 46 % graminoids and 53 % shrubs and forbs, with less than 1 % lichens and mosses. These studies indicate variability in Dall's sheep diet composition based on location, suggesting that an assessment of the Chugach Mountains' Dall's sheep summer diet composition would be novel and necessary to understanding the effects of climate change on this subpopulation.

Climate change and associated effects on forage have the potential to influence large arctic herbivore populations. For example, caribou population dynamics can be influenced by altered food supply and nutritional quality with warmer, drier summer growing conditions decreasing forage nutritional content (Lenart et al., 2002). Warming climates in the arctic and subarctic biomes have also been shown to cause recent increases in shrub canopy, in turn leading to a decrease in plant species richness, which could impact nutrition and quality (Pajunen et al., 2012; Tape et al., 2006; Thompson & Barboza, 2015; Tremblay et al., 2012). In some areas of

Dall's sheep habitat in the Chugach and Kenai mountains, tree-and shrub lines have been advancing by as much as 1 meter per year, reducing the area of alpine habitat (Dial et al., 2016) and potentially carrying capacity. Female Dall's sheep may be particularly vulnerable to changes in forage quantity or quality, because they must acquire enough nutrient stores to successfully reproduce and survive harsh winters, in an extremely brief growing season that can vary from one year to the next (Bowyer et al., 2000; Rachlow & Bowyer, 1994).

In ruminants, forage quality is typically quantified by crude protein, neutral detergent fiber, gross energy, mineral, and fat content. Energy and protein are often considered the most limiting of these factors (Van Soest, 1994). Ewe survival and pregnancy success are partially determined by elements of Dall's sheep nutrition like aspects of proximate composition, e.g., macro-, and micro- molecules, determination of crude protein (%), and gross energy (kcal/g) of dominant forage types, and changes in these metrics that can result in metabolic deficits are critically important.

Obtaining and maintaining energy and protein directly affects a wild herbivore's body composition and overall fitness (Allaye-Chan, 1991; Parker et al., 2005). Nitrogenous compounds in crude protein are the basis for the bodily structure and function of animals (White, 1993). Digestible crude protein is especially critical in herbivore diets, because compared to other trophic guilds, their diet has a lower crude protein content (Richard et al., 2017). Digestion balances based around digestible nutrient intake indicate how much nutrients are being absorbed by the animal and are ultimately link resources and animal performance (Parker et al., 1996). Apparent digestibility (D_a) is the simplest way to evaluate this metric by comparing basic nutrient gain relative to fecal output (Van Soest, 1994). An evaluation of forage quality measured by crude protein (%) and gross energy (kcal/g), combined with a calculation of the D_a of these two factors provides a fairly comprehensive evaluation of ewe forage quality.

In this study, we assess the summer diet composition of female Dall's sheep in the Chugach Mountains using direct video observations. We collected forage and fecal samples based on these observations to then assess forage quality in the form of gross energy (kcal/g) and crude protein content. We evaluated the apparent digestibility (D_a) of both of forage quality factors to understand how well Dall's sheep ewes absorb their summer forage, and how these factors varied from one complete summer season (June 1-August 31) to the next, and within a summer season (early: June 1-July 16 to late: July 17-August 31). Finally, to attempt to understand if forage quality was related to climate conditions, we compared summer (June 1-August 31) precipitation and mean temperature data obtained from a local weather station in the Chugach Mountains.

2.3 Methods

2.3.1 Study Area and Time Frame

We performed our study in the western Chugach Mountains in southcentral Alaska, within ADF&G GMU 14C (Fig. 2.1). The study area was located northeast of the city of Anchorage, Alaska, mainly within the Chugach State Park. GMU 14C extends east into the Chugach Mountains, borders Anchorage and Eagle River to the west, the waterway of Turnagain Arm to the south, and the Knik River to the north (Downs et al., 2018; Smith, 2020). Over the course of this study, the estimated minimum counts for the Dall's sheep population in GMU 14C were 968 sheep in 2016 and 1069 sheep in 2017, respectively, there were no survey data for 2018 (Battle & Stantorf, 2018). The study area encompassed an area roughly located in the center of GMU 14C (Fig. 2.1) and includes locations that are both open and closed to Dall's sheep hunting. Data collection, i.e., video footage of Dall's sheep foraging, forage sample collection, and gathering of sheep fecal pellets took place across this area during the summer forage season from 2016 to 2018.

We conducted all our research in alpine terrain, ≥500 meters (m) in elevation, on ridges, cliffs, and talus slopes occupied by Dall's sheep. Previous research shows Dall's sheep in the Chugach Mountains primarily selected for areas on steep slopes devoid of trees, at mid-elevations (900-1800 m) (Smith, 2020), but our observations recorded sheep feeding as low as 500 m in elevation. The Chugach Mountains support multiple ecosystem types, but the alpine terrain (≥ 600 m) is primarily tundra interspersed with meadows of low and dwarf shrub (typically *Dryas*) dominated plant communities, bedrock, and snowfields (Dial et al., 2016). Dall's sheep preferentially select steep, exposed areas, because the extreme terrain serves as a means of escape and has an extensive line of sight. These conditions give them advantages over their terrestrial predators, primarily coyotes (*Canis latrans*), wolves (*C. lupus*), and wolverines (*Gulo gulo*) (Geist 1971).

Data collection was completed during summers of 2016, 2017, and 2018 (Fig. 2.2). Summer growing season was defined as the arctic summer growing season, which begins at the start of June and lasts for 2.5-3.5 months (Inauen et al., 2012). We further separated the summer growing season into two time periods, early (June 1-July 16) and late (July16-August 31). All of our observations were conducted during or within a week of this time frame. We split the summer into early and late time periods, because we observed shifts in plant phenology and weather during these times. We defined ‘total summer’ as the entire summer growing season, which included all samples collected during that calendar year.

2.3.2 Video Observation and Diet Composition

This study focuses on the summer diet composition and quality of the adult female Dall’s sheep population in the Chugach Mountains. We accessed the study area on foot or by helicopter during the summer forage season (June 1-August 31) in 2016, 2017, and 2018. During these trips, we gathered opportunistic video footage of actively foraging ewes. Bands of ewes and lambs were located and cautiously approached on foot, as to not disturb the behavior of the animals. For video footage to be clear enough to identify different diet items without disturbing the sheep, we determined that a distance to the animals of 75 m or less as measured by laser rangefinder (Leica Camera Inc, Rangemaster 1200) was sufficient. Upon reaching the desired distance, we selected an individual ewe that was actively feeding. We used a Nikon Coolpix P900 video camera mounted on a tripod, with an 83x optical zoom and a 24-2000 mm lens to record feeding, as long as there was continued line of sight to the foraging ewe. The mean recording session during observations was 15:27 (Minutes: Seconds), and the mean number of sheep observed in a session across the study was 7 ewes. Individual video recordings were of varying length ranging from 00:20-10:35 with a mean duration of 2:26 over the course of the study. Total number of female Dall’s sheep observed foraging over the course of the study was 140 animals.

Forage types (i.e., deciduous shrubs, evergreen shrubs, forbs, graminoids, lichens, and mushrooms) were identified and grouped based on known information from previous research of Dall’s sheep forage (Seip & Bunnell, 2011), with the exception of mushrooms, which were added as a category after direct observations during this study. No previous studies have recorded sheep feeding on mushrooms. We identified and classified diet items consumed by sheep (both in video footage and collected in the field) using field guides: for terrestrial plants: deciduous shrubs, evergreen shrubs, forbs, and graminoids (Pratt, 2005, Johnson et al., 1995), and for lichens and mushrooms (Laursen & Seppelt, 2010).

Bite counts per forage type were recorded for each individual video observation. Bites were then totaled seasonally (early and late) and for total summer (2016-2018) for each forage type across all observations during that time frame (Seip & Bunnell, 2011; Risenhoover, 1989). To describe diet compositions, we used 'frequency of occurrence' of each forage type, which we defined as the total number of bites allocated to that forage type in relation to the total number of bites, expressed as a percentage, over the course of the season (early and late) and total summer (2016-2018). All known methods of diet composition estimates are inherently biased, but for larger herbivores, the use of 'frequency of occurrence' of diet items in direct observations has proven comparable or more effective than other options (Parker & Bernard, 2006).

2.3.3 Forage Quality - Crude Protein and Gross Energy

We collected a total of 343 forage samples during early and late season 2016-2018. Samples were comprised of 87 different species across 6 different forage types: 12 deciduous shrubs, 5 evergreen shrubs, 45 forbs, 10 graminoids, 11 lichens, and 4 mushrooms during early and late season 2016-2018 (Table 2.1). Forage samples were collected and processed as a whole, not separating stems, leaves, or litter. We did accept that this could be underrepresenting the forage quality with regards to selection of specific parts of forage, we actually showed this by comparing flower, stem and leaves of a main forage item *Dryas octopetala* (Table 2.2). This study focused on crude protein and gross energy in select Dall's sheep forage types and diets, as identified during video observation (Section 2.3.2).

Forage samples were collected in paper coin envelopes (5.7 x 8.9 cm) and allowed to air dry in the field (0-22 ˚C). Within 24 hours of returning from the field, samples were dried in a Blue M Stabil-Therm gravity convection oven at 60 ˚C for 24 hours (Barboza et al., 2018). The dried forage samples were then ground in a Thomas Scientific Wiley mill (Risenhoover, 1989) and placed in reinforced 1.8 ml micro-vials containing BB-sized steel beads. Samples were then inserted into a BioSpec Mini-Beadbeater for about 4 minutes until completely homogenized.

We collected fresh Dall's sheep fecal samples throughout the summers 2016, 2017, and 2018 from individual ewes following an observed defecation. Unfortunately, the 2018 samples were lost in a freezer failure. We therefore analyzed 82 fecal samples from 2016 and 78 from 2017.

Due to logistical challenges in the field, our forage samples were not directly weighed at time of collection and had to be analyzed based on dry matter (DM) content. However, to best describe in-situ conditions, we used published water content for each forage type to report our results as wet weight (ww) (Reiling, 2011). Mean water content values used for each forage group were obtained from the literature and are as follows: evergreen shrubs, deciduous shrubs, graminoids, and shrubs were assumed to be 59.0 % water (Lee, 2018), while lichens were 22.0 % (Klamerus-Iwan et al., 2020), and mushrooms were 90.0 % water (Kurtzman, 1997). These values were then applied to our data to account for water content within our calculations.

Fecal samples were collected in the field in plastic Whirl-Pak sample bags, then transferred to Falcon 50 mL conical centrifuge tubes in the lab. Samples were freeze-dried for no less than 24 hours using a Labconco Freezone 2.5 L freeze dry system. Water content was determined by weighing all fecal samples before and after freeze-drying, then calculating the mean water content across all of our fecal samples. We applied that value in our equations to account for fecal water content.

For nitrogen content analysis, homogenized forage samples were analyzed using an Isoprime isotope ratio mass spectrometer (Isoprime Ltd, Cheadle Hulme, UK) coupled to a CN elemental analyzer (Eurovector, Milan, Italy) using continuous flow and helium as a carrier gas (Blok et al., 2015). We used percent nitrogen (%N) obtained from bulk stable nitrogen ($\delta^{15}N$) isotope analysis (data not included in this study) after validation (see below), to estimate crude protein (%) content of the forage samples. We compared %N values from bulk stable isotope analysis to those obtained from a subset (n = 15) of forage samples with adequate mass using a LECO FP-528 nitrogen/protein analyzer (LECO Corporation, Michigan, USA) at the University of Alaska Fairbanks. Both methods resulted in comparable %N values (<5 % variability). Therefore, due to the limited amount of sample mass available, %N obtained from the bulk stable isotope measurements was used for all subsequent data analyses. All Dall's sheep fecal samples from 2016 and 2017 were analyzed for fecal nitrogen using a LECO TruSpec Carbon Nitrogen Analyzer at the Wildlife Habitat Nutrition Laboratory, Washington State University, Pullman, Washington, USA.

We determined the mean inorganic matter content of forage and fecal samples by ashing no less than 0.5 mg of 60 forage and fecal samples using a Lindberg/Blue LGO Element Box Furnace at 600 °C for 2 hours. Due to limited forage sample mass, we were only able to ash 10 samples from each forage type. Our fecal sample ash data sets were divided evenly among the two years, (n = 30) from 2016 and (n = 30) from 2017. We used the Kjeldahl method to estimate crude protein based on nitrogen content of forage and feces (Barboza et al., 2009). Crude protein contains roughly 16 % nitrogen, thus a conversion factor of 6.25 is applied to the ash-free dry mass of %N values to calculate crude protein (Van Alfen, 2014).

We used bomb calorimetry to determine the gross energy (kcal/g) of forage (n = 172) and fecal samples (n = 40), using a Parr 6300 oxygen bomb calorimeter (Parr Instrument Company, Illinois, USA). Triplicates of a benzene standard were run for at least two calibration runs per day to determine instrument variability (SD = 0.03 kcal/g).

2.3.4 Apparent Digestibility

We determined the D_a of crude protein (%) and gross energy (kcal/g) in GMU 14C female Dall's sheep summer forage. We calculated D_a as daily intake (DI) of crude protein or gross energy minus daily fecal excretion (FE) of crude protein or gross energy, divided by the daily intake (DI) of crude protein or gross energy (Equation 2.1, Van Soest, 1994).

Equation 2.1

$$D_a = \frac{[DI(a) - FE(b)]}{DI(a)}$$

We determined the variable *a* by combining the seasonal diet composition (early and late) or total summer diet composition (2016, 2017) and the crude protein or gross energy of each assessed diet (see Section 2.4.1). The mean crude protein and gross energy values were calculated for each forage type for each time frame (seasonal and total summer), adjusted for the known water content of that forage type, and were then multiplied by frequency of occurrence of each forage type in the Dall's sheep diet (see Section 2.4.1). The variable *b* was calculated by running each sample individually and then calculating the mean value of either crude protein or gross energy of fecal samples collected during the relevant time frame.
We calculated DI using:

Equation 2.2

$$DI = 774\ (BC * BS)$$

Bite count (BC) was based on the mean bites per minute across all individual Dall's sheep video observations during seasonal or total summer time frames. Bite size (BS) was determined using an allometric equation based on body mass most representative for mammalian herbivores (Shipley et al., 1994).

Equation 2.3

$$BS = BM^{0.71}$$

We used 55 kg as the mean body mass (BM) of female Dall's sheep (Lohuis, 2021). Because there have been no previous summer Dall's sheep studies in the Chugach Mountains, we used values from a similar Dall's sheep nutrition study in the Brooks Range mountains, Alaska (Hansen, 1996) to supplement some of our unknown variables. The product of (BS * BC) estimates intake rate per minute, which was then multiplied by the 774 ± 36 minutes a day as mean length of time that Dall's sheep are actively feeding during the summer season (Hansen, 1996) (*Equation 2.2*). We determined the combined crude protein and gross energy content of specific diets (a) based on the proportion of each forage type in that diet, multiplied by the mean crude protein or gross energy that we measured for each forage type. We compared our DI values with DI (dry matter) estimates of Dall's sheep ewes in Alaska's Brooks Range (1902 g/day) reported previously (Hansen, 1996) to ensure our methodological approach was consistent with other work.

To estimate daily fecal loss (FE), we applied the allometric equation from (Lavigne, 1982), describing daily fecal production (g/day) based on whole animal body weight:

Equation 2.4

$$FE = 0.85 * BM^{0.63}$$

where FE is daily dry matter (DM) fecal production in grams and (BM) is the mean summer body mass (g) of Dall's sheep ewes in late summer. When converted to wet weight (ww), this value was comparable to the 612 g (ww) per day (DM) of Dall's sheep ewes in the Brooks Range calculated by Hansen (1996).

2.3.5 Mean Temperature and Total Precipitation

We obtained mean daily air temperature (˚C) and total precipitation (cm) values from a local weather station near the Chugach Mountains for the period of June 1-August 31 of 2016, 2017, and 2018. The weather station, site number 1070, is a SNOTEL site operated by the United States Department of Agriculture (USDA) National Water and Climate Center and located on the hillside region of Anchorage at 61.007° N, 149.040° W at an elevation of 634 m. The weather station has been in operation since October 10, 2004.

2.3.6 Analysis

Forage quality was assessed by measuring crude protein and gross energy values, and calculating the D_a of both, for each major forage type and bulk diets. Mean crude protein and gross energy values for each forage type were estimated for each forage and fecal sample collected throughout the project. We then calculated mean crude protein and gross energy for each forage type for total summer, early summer, and late summer. Calculated mean crude protein and gross energy of each forage type were then multiplied by the diet composition. We visually evaluated these data using bar charts and determined that they were not normally distributed. With our non-normally distributed data set, we used SPSS® software version 26 to perform a Kruskal-Wallis test ($\alpha = 0.05$) to determine if there were statistical differences in crude protein and gross energy values across forage types and diets. All graphs and tables were created using Microsoft Excel (Microsoft Corporation, 2021).

2.4 Results

2.4.1 Diet Composition

During the summer (June-August) 2016-2018, we conducted direct observation of actively foraging Dall's sheep ewes to describe diet composition through frequency of occurrence of forage types (i.e., deciduous shrubs, evergreen shrubs, forbs, graminoids, lichens, and mushrooms). We collected a total of 4 hours, 53 minutes, and 40 seconds of video footage of individual ewes ($n = 140$) feeding over the three years of our study (Table 2.2). We identified individual ewes based on geographic location and morphological differences. There was a chance for repeated observations of some individuals from year to year because some ewes were observed as yearlings and would look physically different following their second year.

Diet composition was recorded for the entire summer forage season for each year (2016-2018) and for the early (June 1-July 16) and late (July 17-August 31) season of each summer. In 2016, we recorded 2 hours 2 minutes and 35 seconds of video of 48 individual adult ewes. Total summer diet composition for 2016 showed ewes primarily consuming deciduous shrubs (33 %) and graminoids (30 %), the remainder of their diet was composed of forbs (16 %), evergreen shrubs (11 %), and mushrooms (10 %) (Fig. 2.3). Sheep forage in early season 2016 was dominated by evergreen shrubs (68 %) with forbs (19 %), graminoids (10 %), and deciduous shrubs (3 %) comprising the rest. Diet in the 2016 late foraging season primarily consisted of deciduous shrubs (39 %) and graminoids (34 %), with forbs (15 %) and mushrooms (12 %) representing the remainder.

In 2017, we recorded 1 hour 11 minutes and 16 seconds of video of 50 individual adult ewes. Total summer diet composition for 2017 was mainly comprised of three forage types, forbs (33 %), graminoids (27 %), and evergreen shrubs (26 %), with small contributions of deciduous shrubs (7 %) and mushrooms (10 %) (Fig. 2.3). The 2017 early season showed higher contributions of evergreen shrubs (35 %); the remainder was made up of graminoids (32 %) and forbs (30 %) and deciduous shrubs (3 %), which contributed little to the early season diet. Diet composition in the 2017 late season was dominated by forbs (41 %) followed by mushrooms (23 %), deciduous shrubs (17 %), graminoids (14 %), deciduous shrubs (17 %), and evergreen shrubs (5 %).

In 2018, we recorded 1 hour 39 minutes 49 seconds video of 42 individual adult ewes. Similar to 2016 and 2017, total summer diet was dominated by two forage types, graminoids (38 %) and evergreen shrubs (32 %). The remainder was composed of mostly forbs (22 %) with small contributions of deciduous shrubs (4 %) and mushrooms (4 %) (Fig. 2.3). Graminoids (41 %) and evergreen shrubs (40 %) were represented in similar proportions in the 2018 early season followed by forbs (17 %) and deciduous shrubs (2 %). Graminoids (35 %) continued to comprise the majority of sheep diet in the 2018 late season followed by forbs (28 %), evergreen shrubs (22 %), mushrooms (8 %), and deciduous shrubs (7 %). Although lichens are a known Dall's sheep forage item in other regions, like the Yukon Territory (Hoefs & Cowan, 1979) and the Brooks

Range (Ayers, 1986), we did not observe them representing more than 1 % of the total diet in any of our seasonal or total summer observations in the Chugach Mountains.

2.4.2 Forage Quality

During this study, for the purpose of forage quality analysis, we collected a total of 341 forage samples during the summer forage seasons of 2016 (n = 115), 2017 (n = 130), and 2018 (n = 96). We selected the forage samples based on species that female Dall's sheep were observed feeding on during video observations. We collected and analyzed a total of 160 fecal samples over the course of 2016 (n = 82) and 2017 (n = 78), the fecal samples we collected in 2018 were lost in a freezer failure.

2.4.2.1 Crude Protein

We assessed crude protein (%) based on wet weight (ww) of individual forage types and of seasonal and total summer diets based on diet composition calculated during those time frames. Of the different forage types assessed across 2016-2018, forbs had the highest mean crude protein (7.1 ± 2.6 %) and mushrooms had the lowest (2.9 ± 1.0 %) when averaged across all three summers. The forage type that was most variable in its amount of crude protein from one sample to another were lichen (0.9 – 16.7 %). Female Dall's sheep diet mean total summer crude protein intake was significantly different among years ($P = 0.008$). Those differences in total summer crude protein of Dall's sheep diet were from 2016 (6.1 %) to 2017 (5.5 %) to 2018 (4.8 %). Graminoids and forbs were the two forage types with mean crude protein values that most closely followed total summer diet crude protein values (Fig. 2.4 (A)). Crude protein in early and late season diets also differed significantly ($P < 0.0001$). 2016 had highest crude protein in the late season diet (5.7 %), while 2017 and 2018 had higher crude protein in early season diet (6.0 % and 5.6 % respectively). The forage types with the highest mean total summer diet crude protein across the project were forbs (6.6 ± 1.3 %) and graminoids (5.6 ± 1.6 %). Forbs and graminoids also showed a decrease in total summer crude protein from 2016-2018. The forage types with the lowest mean total summer crude protein were mushrooms (3.0 ± 1.0 %) and evergreen shrubs (3.9 ± 0.8 %).

2.4.2.2 Gross Energy

Due to inadequate mass of most forage and fecal samples, we analyzed wet weight (ww) gross energy (kcal/g) of a subset of forage items collected in 2016 (n = 34), 2017 (n = 93), and 2018 (n = 63), and fecal samples obtained in 2016 (n = 17) and 2017 (n = 17). The mean total summer gross energy (kcal/g) of female Dall's sheep diet differed significantly among some years ($P < 0.0001$). Gross energy (kcal/g) content of sheep diet was lowest in 2017 (1.84 kcal/g %) and similar in 2016 (2.04 kcal/g) and 2018 (2.05 kcal/g). The forage type that most closely followed total summer total diet trends was graminoids (Fig. 2.4 (B)). Gross energy of Dall's sheep diets consistently increased from early to late season (2016-2018). The forage types with the highest mean total summer gross energy were mushrooms (3.24 ± 0.75 kcal/g) and lichen (2.83 ± 0.02 kcal/g), while the forage types with the lowest mean total summer gross energy were forbs (1.75 ± 0.12 kcal/g) and graminoids (1.79 ± 0.12 kcal/g).

2.4.3 Apparent Digestibility

We found bite size (BS) based on our allometric equation to be 0.17 grams per bite and from video observations the bite count (BC) to range from 41-48 bites per minute. Our ww DI across all seasons and years ranged from 5,139-6,016 grams of forage intake per day (g/day) with a mean of 5,703 ± 361 g/day. Our calculated ww FE was 824 grams per day. From 2016-2018 ewes consumed on mean of 312 ± 54 grams of ww crude protein and 11,235 ± 708 kcals of ww gross energy per day. The D_a across both crude protein (%) and gross energy (kcal/g) was relatively high for all observed years and seasons. Neither the D_a of crude protein nor gross energy were lower than 78 % either during seasonal summer periods or total summer. Seasonal D_a ranged from 78.53 % - 86.20 % with a mean of 82.69 %, while total summer D_a ranged from 80.98 % - 85.36 % with a mean of 82.64 %. Seasonal D_a was more variable, but the mean of both seasonal and total summer D_a differed by less than 0.05 %.

2.4.3.1 Crude Protein

In our two-year comparison, the total summer D_a of crude protein in the Dall's sheep diet was very similar despite the mean amount of crude protein being different. While mean amount of total summer crude protein in Dall's sheep diet dropped from 5.5 % in 2016 to 5.0 % in 2017, the D_a of crude protein was not significantly different from 2016 (81.0 %) to 2017 (81.0 %). In

2016, the mean amount of seasonal crude protein in the seasonal diet increased from early to late (4.4 % to 5.4 %), and D_a of crude protein also increased from 78.9 % in the early season to 83.9 % in the late season. In 2017, trends were opposite, with the early season diet having a higher mean amount of crude protein (5.7 %) and higher D_a of crude protein (82.9 %) and the late season having lower crude protein (5.0%) and lower D_a (78.5 %).

2.4.3.2 Gross Energy

The mean amount of gross energy (kcal/g) and the D_a of gross energy showed similar trends to that of crude protein with seasonal and total summer shifts in D_a and gross energy mirroring each other. The mean amount and D_a of total summer gross energy of the Dall's sheep forage in 2016 was slightly higher (GE = 2.04 kcal/g and D_a = 85.3 %), than that in 2017 (GE = 1.84 kcal/g and D_a = 83.2 %). Seasonal gross energy in the 2016 early season was (2.73 kcal/g) and D_a (84.6 %), while gross energy (3.13 kcal/g) and D_a (86.2 %) were higher in the late season. Gross energy in the early and late season in 2017 (2.64 kcal/g and 2.77 kcal/g, respectively) showed less of an increase across the summer, while the seasonal D_a increased more than in 2016 from 87.5 % to 90.0 %.

2.4.4 Mean Temperature and Total Precipitation

Mean total summer temperature (˚C) and total precipitation during the 2016, 2017, and 2018 summer growing seasons (June 1-August 31) varied from year-to-year, as well as from early (June 1-July 16) to late (July 17-August 31) season. In 2016, total summer mean temperature was highest at 12.3 ± 2.1 ˚C, while total summer mean temperature in 2017 was 10.9 ± 2.4 ˚C, and during 2018 was 11.0 ± 2.5 ˚C. In 2016, early season and late season mean temperatures were highest (11.8 ± 2.3 ˚C and 12.8 ± 1.8 ˚C, respectively). In 2017, early season mean temperature was lowest (10.4 ± 2.3 ˚C), while 2018 had the lowest late season mean temperature (11.4 ± 2.1 ˚C).

Total precipitation across the summer season (June 1-August 31) was highest in 2018 (24.6 cm), followed by 2016 (20.8 cm), and then 2017 (15.5 cm). The majority of precipitation in 2016 (14.2 cm, 68.3 %), 2017 (9.4 cm, 60.7 %), and 2018 (17.0 cm, 69.1 %) fell in the late season (July 17-August 31). The early season (June 1-July 16) was drier and accounted for less than 40 % of the total precipitation across the entire summer in all three years.

Samples from 2016 overall had the best forage quality, with the highest levels of crude protein and second highest levels of gross energy (kcal/g) in total summer diet. Climate in 2016 showed the highest average daily temperatures and the second highest precipitation levels. Regarding our study and sampling timeframe, it would appear that high temperatures along with moderate precipitation rates resulted in highest summer Dall's sheep forage quality.

2.5 Discussion

2.5.1 Diet Composition

In this study, female Dall's sheep diet composition varied both seasonally and across the entire summer from year-to-year. Throughout all three years of the study, forbs and graminoids combined made up 46 % to 60 % of the total observed summer Dall's sheep diet, with graminoids alone consistently accounting for more than a quarter of the total summer diet (Fig. 2.3). Our results are supported by previous literature on thinhorn sheep which states that thinhorn sheep are mixed feeders but are primarily grazers relying on forage types other than shrubs (Nichols & Bunnell, 1999). Hoefs & Cowan's (1979) study in the Yukon Territory, Canada reported an annual total summer Dall's sheep diet consisting of 46.3 % graminoids and 36.8 % forbs, even higher than the portion of diet composition we observed in our study. Slightly lower diet proportions of forbs and graminoids in our study, could be due to variation in the location of these studies, or it could be due to the loss of forb and graminoid dominant habitats in the Chugach Mountains due to shrub encroachment. With recent warming temperatures, the Chugach Mountains have seen an encroachment of tall shrubs into montane environments and Dall's sheep habitat (Dial et al., 2016).

We observed female Dall's sheep increasing their total summer consumption of evergreen shrubs from 2016-2018. Simultaneously, we saw an increase in the crude protein content of those evergreen shrubs from 2016-2018. Caribou, a large arctic herbivore species that inhabits and forages in similar habitats to Dall's sheep, select forage based on highly digestible nitrogen, a component of crude protein (Johnson et al., 2021). Dall's sheep could be employing a similar selective feeding strategy, choosing a diet that is high in crude protein. It has been shown in other populations that the movement of thinhorn sheep during the summer is dictated by the selection of habitat that has the most nutritious forage (Nichols & Bunnel, 1999).

Seasonal (early and late summer) diet compositions of female Dall's sheep in the Chugach Mountains varied intra-annually and inter-annually, but there were some consistent patterns. Across all years, ewes selected more evergreen shrubs in the early summer and more deciduous shrubs in the late summer.

A noteworthy observation that we made while in the field that our established collection and evaluation techniques regarding samples did not account for was Dall's sheep selecting for the reproductive parts of flowering and fruiting plants. For example, we noted that evergreen shrubs like *Dryas octopetala*, were the first plants to produce flowers in the early season, which the sheep actively selected for. Sheep also targeted bacciferous deciduous shrubs that appeared in late summer. High turnover rate plant organs like flowers and fruits have higher nitrogen content and in turn higher crude protein than more quiescent tissues (Mattson, 1980). Berries are a high-energy food source that arctic animals depend on to build energy reserves necessary for reproduction, physiological function, and winter survival (Welch et al., 1997). Our field collection strategies were already established and were based on a whole plant (stem, flower, and leaves combined) of each plant, and as such could be underrepresenting the higher crude protein or gross energy (kcal/g) resulting from this selection. The selection of fruiting bodies is a behavior that is potentially driven by the fact that Dall's sheep are animals that live nutritionally on the edge and are constantly seeking the highest quality forage. Future Dall's sheep nutrition studies should account for this observed selectivity and collect samples accordingly.

2.5.2 Forage Quality

Our forage quality sampling technique measured bulk forage items, meaning we ground all parts of the plants we sampled. From our later observations, Dall's sheep appeared to be selective feeders, and as such our values could be underrepresenting the actual crude protein and gross energy intake.

We observed fluctuations in forage quality between seasons within and among years. Both crude protein and gross energy values changed across these time periods. Gross energy (ww) showed low variability across years and forage types, ranging from 2.36 - 3.86 kcal/g across all forage types and all years. Annual fluctuations in crude protein were statistically significant. We measured annual differences in crude protein of over 1 %, which when extrapolated to a mean of 5703 g/day DI of forage over the course of a summer growing season amounts to a potential difference of 5246 grams (5.25 kg) of crude protein per year. The loss of 5.25 kg per year is equivalent to 9.5 % of the average total body mass of a Dall's sheep ewe, certainly enough loss to potentially affect reproductive success. Forbs (6.56 ± 1.34 %) and graminoids (5.61 ± 1.60 %) consistently had the highest annual crude protein and were the two dominant forage types in ewe annual diet composition. Ewes are already at a nutritional disadvantage during this time due to lactation, where their daily bodily requirements can more than double compared to normal maintenance demands (Oftedal, 1985; Parker et al., 2009; Robbins, 1993). Crude protein values in forbs (7.99 % to 5.32 %) and graminoids (7.75 % to 4.47 %) decreased across years from 2016-2018, while the amount of crude protein in evergreen shrubs (3.36 % to 5.01 %) increased. Note that despite this increase, evergreen shrubs in their highest year (2018) still had comparable amounts of crude protein to forbs and graminoids. Consequently, we observed in ewe diet across years a continued high consumption rate of forbs and graminoids, along with a marked increased consumption of evergreen shrubs. This supports our earlier suggestion that total summer diet of female Dall's sheep in the Chugach Mountains is driven by strong selectivity for flowering and fruiting bodies and associated crude protein. We showed with one of our key diet items: *Dryas octopetala* that there is indeed variation in the ww percent crude protein among the parts of the forage item (Table 2.2)

Total seasonal crude protein in female Dall's sheep diet decreased from early to late summer season in 2017 and 2018, while seasonal crude protein increased from early to late season 2016. These annual differences could be explained by variability at the landscape level that could be affecting diet selection behavior. Similar behaviors, with large scale trends in diet choice driven by landscape forage quantity and quality have been reported in other ungulates. For example, caribou in early summer prioritize areas that are highest in forage quality, in particular digestible nitrogen, while later in the summer, caribou select forage quantity over quality (Johnson et al., 2021). Our observed decreases in crude protein from early to late season could be due to Dall's sheep foraging in a similar manner. The alternative is that Dall's sheep could be continuing to be highly selective throughout the summer, focusing on high quality forages that perhaps become more or less available throughout the course of the summer depending on the conditions that year or the population size at that time.

Gross energy content of sheep diets was less variable across years but had an interesting dynamic with diet selection. The forage types that constituted the two largest portions of ewe diet (forbs and graminoids) had the lowest gross energy, which could suggest crude protein is more of a driver than gross energy in ewe diet. Despite their comparatively high-water content (90.0 %, Kurtzman, 1997), mushrooms emerged as the highest mean gross energy (3.24 ± 0.75 kcal/g) forage type in our study. No previous Dall's sheep diet study has identified mushrooms as a forage type, although mushrooms have been found to be key forage for other arctic herbivores, such as caribou (Thompson & Barbosa, 2014) and moose (LeResche & Davis, 1973)., Muskoxen (*Ovibos moschatus*) allocate a majority of their time during the arctic summer months maximizing energy intake (Beumer et al., 2020). Mushrooms consistently emerged in the late season (July 16-August 31) and consequently, we observed Dall's sheep showing a preference for them when available. Although we didn't measure it across our forage types, it is worth noting that mushrooms are also very high in other key nutrients like B vitamins, copper and selenium (Barboza et al., 2009). Potentially, this indicates an evolved adaptation in Dall's sheep, taking advantage of a nutrient rich diet source as it appears on the landscape.

2.5.3 Apparent Digestibility

Our apparent digestibility (D_a) estimates and calculated D_a values were similar to those computed in studies on other arctic herbivores and with Hansen's (1996) findings on Dall's sheep in the Brooks Range. The DI rates of our study were ~5,500-6,000 g/day, allometrically comparable when considering that moose can consume up to 32,000 g/day (Burton, 1998). Within years seasonal D_a of both crude protein and gross energy increased from early to late season in 2016 and decreased in 2017. These changes are reflective on the intake of the forage quality shifts in two main forage types, evergreen shrubs and graminoids corresponding to those years. Across 2016 and 2017, the total summer and seasonal crude protein and gross energy of the diets showed a positive relationship to their corresponding D_a. This suggests that ewes had higher consumption rates of forage types as their forage quality and consequently the D_a of the diet as a whole was higher. Similarly, Hansen (1996) found that D_a increased during the spring, peaked alongside highest forage quality in June, and began to decline along with forage quality heading into July.

Our assessment of D_a of forage begins to evaluate the question of forage quantity versus forage quality. More forage does not always mean better nutrition. An increase in herbivore forage abundance can mean more cellulose content. An assessment of mule deer (*Odocoileus hemionus*) forage found that the available energy in forage was inversely related to cellulose content (Short, 1966). The Normalized Difference Vegetation Index (NDVI) provides information on the productivity of vegetation in ecological studies and has become a metric for evaluating habitat quality (Hamel et al., 2009). However, this popular technique to assess ungulate forage quality based on abundance, especially in the arctic, may not be as useful as previously thought (Johnson et al., 2018). In the arctic, over the last few decades, summer season that has shown the greatest increase in NDVI (Hobbie et al., 2017). These increases could be beneficial, a previous study on Dall's sheep cross multiple mountain ranges found that maximum summer NDVI was the most important environmental covariate related to lamb survival (Aycrigg et al., 2021). But the increased summer NDVI in the arctic alpine could also have negative effects on Dall's sheep through their forage, leading to accelerated plant growth, faster lignification, and reduced concentrations of digestible protein (Lenart et al., 2002), as well as earlier plant senescence, which produces lower forage digestibility and quality (Cooper, 2014). These increases in NDVI are also often associated with increased abundance of less desirable shrubs as forage for Dall's sheep. In controlled studies with ungulates an increase in dry matter intake actually led to a decrease in digestibility (Ammann et al., 1973). Therefore, changes in apparent digestibility could be due to changes in forage quality, as demonstrated in this study, or could be the result of changes in the quantity of forage on the landscape; either could be a consequence of changes in climate to the region.

2.5.4 Mean Temperature and Total Precipitation

When comparing forage quality with mean temperature (˚C) and total precipitation (cm), our observations suggested that moderate weather potentially coincided with better forage. Across the two forage quality measures in this study, 2016 had the highest crude protein and the second highest gross energy, making it the year with the overall highest forage quality. The mean daily temperatures across the total summer in 2016 (12.3 ± 2.1 ˚C) were the highest over the course of our three-year project, but when compared to similar Dall's sheep habitats, these temperatures were relatively moderate. For example, in the Northern Richardson Mountains, an area with recorded temperatures dating back to 1926, mean July temperatures in Dall's sheep habitat were 13.6 °C (Koizumi et al., 2011). The effect of temperature on Dall's sheep and their behavior occurs both directly and indirectly and can occur in a variety of time steps. In the Lake Clark Park and Preserve, Alaska, Dall's sheep habitat selection throughout the year was influenced by multiple ecological requirements, some directly or indirectly influenced by temperature (Aycrigg et al., 2021). Temperature directly affected habitat selection during the spring with sheep selecting for areas with warmer temperatures and less snow, subsequently then warmer summer temperatures indirectly affected Dall's sheep habitat selection through the expansion of taller shrubs (Dial et al., 2016) into certain areas causing sheep to avoid those areas (Aycrigg et al., 2021).

In our study, the year with the second highest total summer rainfall (2016, 20.8 cm), had the highest quality forage. It is hard to determine if rainfall during a summer season would directly influence the quality of Dall's sheep forage. There is not much evidence as to how summer precipitation affects Dall's sheep specifically, but precipitation in other seasons has demonstrated to affect Dall's sheep populations in a number of ways. Summer precipitation influenced similar arctic herbivores through effects on alpine plant forage. In the winter, precipitation directly influences Dall's sheep movement and accessibility to forage through snow cover and density (Sivy et al., 2018). Spring precipitation, combined with erratic temperatures or rain on snow events, can cover already deep snow with a thin layer of ice that makes forage inaccessible and hinders movement and predator avoidance (Pan et al., 2018). A study on the effects of climate change on caribou forage found that increased summer precipitation and the shady cool conditions associated with delayed or reduced growth of grasses and forbs in an alpine habitat (Lenart et al., 2002). A longer timescale study on how the effect of summer precipitation levels affect Dall's sheep and their forage becomes more and more necessary as the related effects of climate change continue to grow.

2.6 Conclusion

As conditions in arctic and subarctic environments continue to change, it is necessary to assess how these changes are affecting habitat conditions for Dall's sheep. Alpine habitats in these areas may be some of the last regions in which the ancestral ecological processes are still intact and where further knowledge of these processes and how climate may affect them, could contribute to management strategies to improve resilience and adaptation (Chapin et al., 2006). Dall's sheep are alpine specialists that rely on alpine terrain at high elevations, and as such have poor adaptability and dispersal abilities (Geist, 1971). Warmer summers brought on by climate change are increasing tall shrub encroachment (e.g., *Betula nana*, *Betula glandulosa* etc.) into high alpine meadows, previously dominated by other forage species that Dall's sheep depend on (Dial et al., 2016). This could have the direct effect of reducing Dall's sheep summer forage availability and the potential indirect effect of the retention of deeper winter snows that take longer to recede, leading to shifts in the timing and ecological balances of summer Dall's sheep foraging grounds (Dial et al., 2016). Because Dall's sheep depend on a fine balance of seasonal and phenological timing, the loss of high-quality forage and seasonal shifts brought on by climate change are effects that have the potential to negatively impact the fitness of these animals (Aycrigg et al., 2021). We suspect that there are ongoing changes in Dall's sheep habitat. Our research reported here shows the potential for altered forage quality as temperature and precipitation patterns change, presumably as a result of climate change. To better understand these interactions and their potential future effects on Dall's sheep, it is important to begin to fill the current data gap with baseline data and evaluations of current conditions.

In this study, we identified the forage Dall's sheep are consuming, then examined how that forage use and quality changes over time. We identified the six key forage types consumed by Dall's sheep in the Chugach Mountains and then evaluated the quality and digestibility (D_a) of those forages across multiple summer seasons, a critical time of year for Dall's sheep, especially adult females. We described variability in summer diet patterns as well as forage quality and D_a, within and across years. This variability revealed that Dall's sheep habitat and associated nutrition are changing as a result of any number of factors, including factors that could be exacerbated by climate change. Snow phenology (Aycrigg et al., 2021; Van de Kerk et al., 2018), late spring ice on snow events (Pan et al., 2018), and the uphill encroachment of shrubline (Dial et al., 2016) all play a potential role in current Dall's sheep population dynamics and trajectory. So, there is a strong potential that climate change could be negatively affecting Dall's sheep through forage related issues.

Our research could be advanced with continued long-term monitoring of Dall's sheep diet and potentially more fine scale evaluations of specific areas. Movement patterns and fine scale evaluations of habitat and forage selection relative to availability and forage quality could give a better idea of current carrying capacity of habitat. Dynamic and integrative management at a landscape scale could be the possible response to assist Dall's sheep to adjust to the recent changes in distribution and quality of their habitat (Aycrigg et al., 2021). Range enhancements, including controlled burning, could be a viable option, and it has been used to aid Stone sheep populations in British Columbia (Nichols & Bunnel, 1999). Researchers on Round Mountain on the Kenai Peninsula, Alaska also found that Dall's sheep utilized burned areas from a 1974 wildfire more than twice as much as adjacent unburned hillsides and showed population increases in following years (Elliot, 1978). But realistically, because Dall's sheep are fairly dispersed in their habitat, the scale with which controlled burning would have to be implemented to have population level effects would be considerable.

It is evident that due to the rapidly changing climate in arctic regions, carrying capacity is not static and evolves as habitat changes. Management strategies might involve lessening anthropogenic impacts through the closure of recreational areas surrounding high-quality habitats in regions with population declines, allowing for better connectivity among habitats for those threatened subpopulations (Aycrigg et al., 2021) or implementing harvest objectives in line with carrying capacity in a given area (Van de Kerk et al., 2020). Regardless of what management strategies are implemented, it is clear that a response is necessary to the current condition of Dall's sheep. Dall's sheep are a sentinel species in arctic and subarctic alpine ecosystems with a wide variety of invested stakeholders. Projects like ours and other current Dall's sheep research, are puzzle pieces that are furthering our understanding of the bigger picture of the impact of climate change on Dall's sheep and other environmentally vulnerable species.

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2.8 Tables

Table 2.1: *Forage samples collected during an evaluation of summer female Dall's sheep nutrition in the Chugach Mountains in Alaska from June1-August 31 during 2016, 2017, and 2018. The samples are organized by forage type and listed by species.*

Forage Type	Scientific Name	Common Name
Deciduous shrub	*Arctostaphylos alpina*	Alpine Bearberry
	Betula nana	Dwarf Birch
	Loiseleuria procumbens	Alpine Azalea
	Luetkea pectinata	Alpine Spiraea
	Pontentilla fruticosa	Shrubby Cinquefoil
	Salix alaxensis	Felt-leaf Willow
	Salix arctica	Arctic Willow
	Salix barclayi	Barclay's Willow
	Salix glauca	Grayleaf Willow
	Salix monticola	Mountain Willow
	Salix reticulata	Net-leaved Willow
	Vaccinium uliginosum	Alpine Blueberry
Evergreen shrub	*Cassiope tetragona*	Bell Heather
	Diapensia lapponicum	Lapland Diapensia
	Dryas octopetula	White Mountain Avens
	Empetrum nigrum	Crowberry
	Harrimanella stelleriana	Alaskan Mountain-Heather
Forb	*Aconitum delphiniifolium*	Monkshood
	Anemone narcissiflora	Narcissus Anemone
	Anemone richardsonii	Yellow Anemone
	Angelica lucida	Wild Celery
	Antennaria monocephala	Cats Paw
	Aster sibiricus	Siberian Aster
	Astragalus alpinus	Hairy Alpine Milk Vetch
	Campanula lasiocarpa	Mountain Harebell
	Castilleja unalaschcensis	Yellow Paintbrush
	Cerastium arvense	Mouse-ear Chickweed
	Chamerion latifolium	Dwarf Fireweed

Table 2.1 Continued

Forb	*Claytonia sarmentosa*	Spring Beauty
	Cornus canadensis	Dwarf Dogwood
	Delphinium glaucum	Larkspur
	Draba incerta	Whitlow Grass
	Epilobium angustifolium	Common Fireweed
	Epilobium latifolium	Dwarf Fireweed
	Equisetum arvense	Horsetail
	Erigeron peregrinus	Coastal Fleabane
	Geranium erianthum	Woolly Geranium
	Geum rossii	Ross Avens
	Heracleum lanatum	Cow Parsnip
	Kumlienia cooleyae	Cooley's False Buttercup
	Lloydia serotina	Common Alplily
	Lloydia serotina	Alp Lilly
	Lupinus arcticus	Arctic Lupine
	Minuartia arctica	Arctic Sandwort
	Myosotis alpestris	Alpine Forget-Me-Not
	Oxytropis nigrescens	Purple Oxytrope
	Parrya nudicaulis	Parry's Wallflower
	Polemonium pulcherrimum	Jacob's Ladder
	Polygonum viviparum	Alpine Meadow Bistort
	Potentilla nivea	Snow Potentilla
	Pyrola minor	Alpine Pyrola
	Rhodiola integrifolia	King's Crown
	Sanguisorba stipulata	Sitka Burnet
	Saxifraga bronchialis	Yellow Spotted Saxifrage
	Saxifraga flagellaris	Spider Saxifrage
	Saxifraga lyallii	Red Stemmed Saxifrage
	Saxifraga punctata	Dotted Saxifrage
	Saxifraga punctata	Brook Saxifrage
	Saxifraga tricuspidata	Prickly Saxifrage
	Silene acaulis	Moss Campion
	Solidago multiradiata	Northern Goldenrod
	Taraxacum platycarpum	Dandelion

Table 2.1 Continued

Graminoid	*Arctagrostis latifolia*	Polargrass
	Bromus pumpellianus	Arctic Brome
	Calamagrostis canadensis	Bluejoint Reedgrass
	Carex aenea	Bronze Sedge
	Carex bigelowii	Bigelow's sedge
	Carex microchaeta	Short Stalk Sedge
	Festuca ovina	Rocky Mountain Fescue
	Poa alpina	Alpine Bluegrass
	Podagrostis humilis	Alpine Bent
	Vahlodea atropurpurea	Mountain Hairgrass
Lichen	*Cladonia atbuscula*	Little Tree Reindeer Lichen
	Cladonia rangiferina	Grey Reindeer Lichen
	Cladonia stellaris	Alpine Reindeer Lichen
	Cladonia stygia	Black footed Reindeer Lichen
	Flavocetraria cucullata	Curled Snow Lichen
	Nephroma arctica	Arctic Kidney Lichen
	Peltigera britannica	Flaky Freckle Pelt
	Sphaerophorus globosus	Coral Lichen
	Thamnolia spp.	Tundra Spahetti
	Umbilicaria spp.	Rock Tripe
	Vulpicida juniperinus	Yellow Lichen
Mushroom	*Boletus edulis*	King Bolete
	Boletus subtomentosus	Suede Bolete
	Laccaria laccata	Lackluster Laccaria
	Russula xerampelina	Shrimp Russula

Table 2.2: *A comparison of mean wet weight crude protein (%) for different parts of a key forage species Dryas octopetala, from samples collected as part of our Dall's sheep nutrition study in the Chugach Mountains in Southcentral Alaska. The forage parts are parts different body parts of the plant, these are to show the potential variation missed in measured crude protein (%) when sampling in bulk as we did in our study.*

Scientific Name	**Common Name**	**Forage Part**	**Crude Protein (%)**	**Standard Deviation**
Dryas octopetala	Eightpetal Mountain-Avens	Flower	7.44	2.30
		Leaf	8.36	0.96
		Stem	5.46	0.73

2.9 Figures

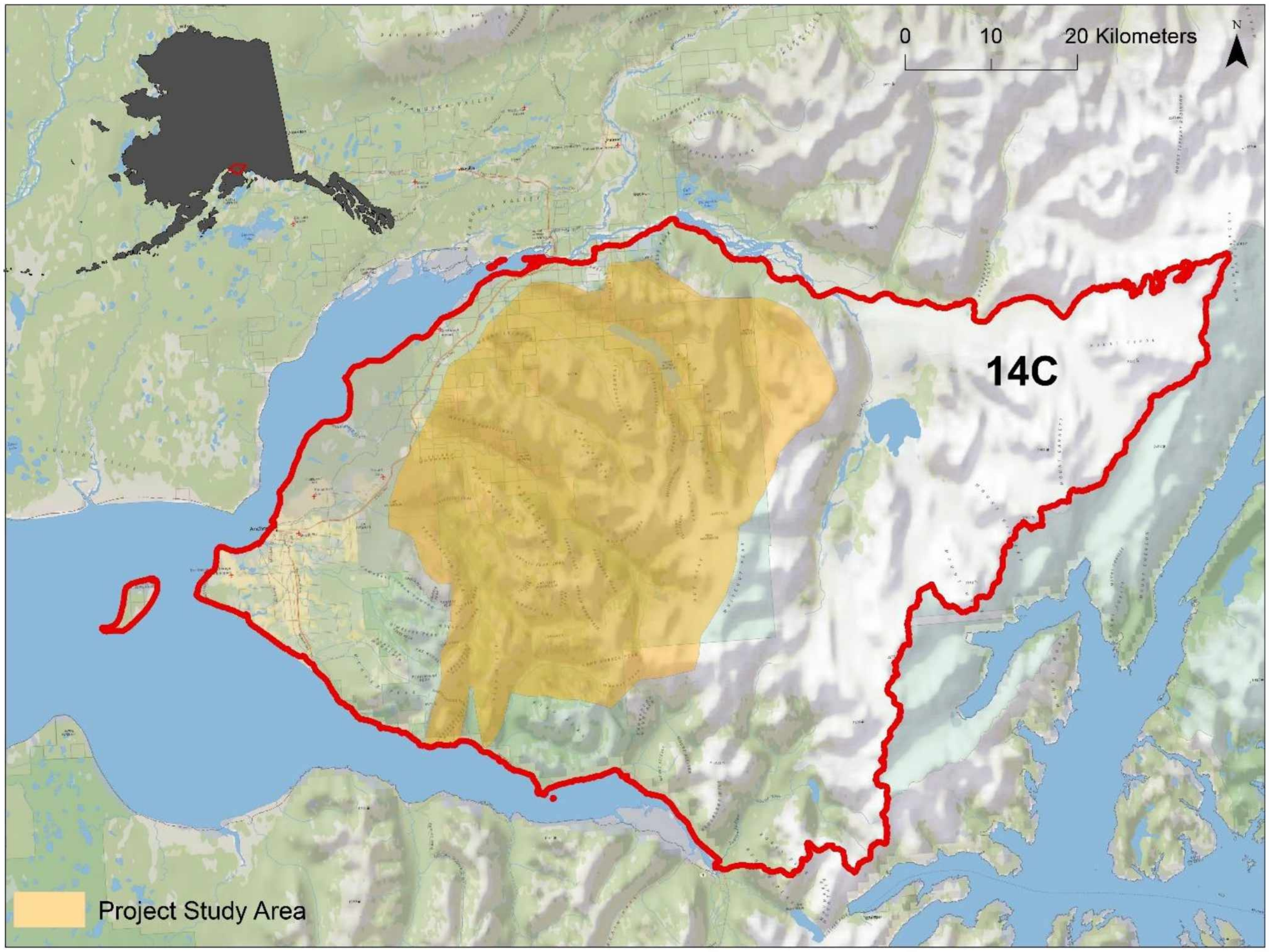

Figure 2.1: *Map of the study area in the Chugach Mountains, where video observation and forage sample collection of female Dall's sheep summer diet and nutrition were conducted. The red border is the entirety of the Alaska Department of Fish and Game's Game Management Unit 14C with the project study area highlighted in yellow. The gray outline in the upper left corner is a reference of where the study area is located in relation to the entire State of Alaska.*

Figure 2.2: *(a) Mountain avens (Dryas octopetala) on triangle peak in the Chugach Mountains, Alaska, an evergreen shrub that is a key diet item in summer for female Dall's sheep in this area. (b) Collecting fecal samples for forage quality assessment in Ewe Valley, one of the main study sites for our study of female Dall's sheep summer diet and forage in the Chugach Mountains. (c) Recording video footage of a ewe feeding to determine sheep diet composition and subsequent forage sample collection.*

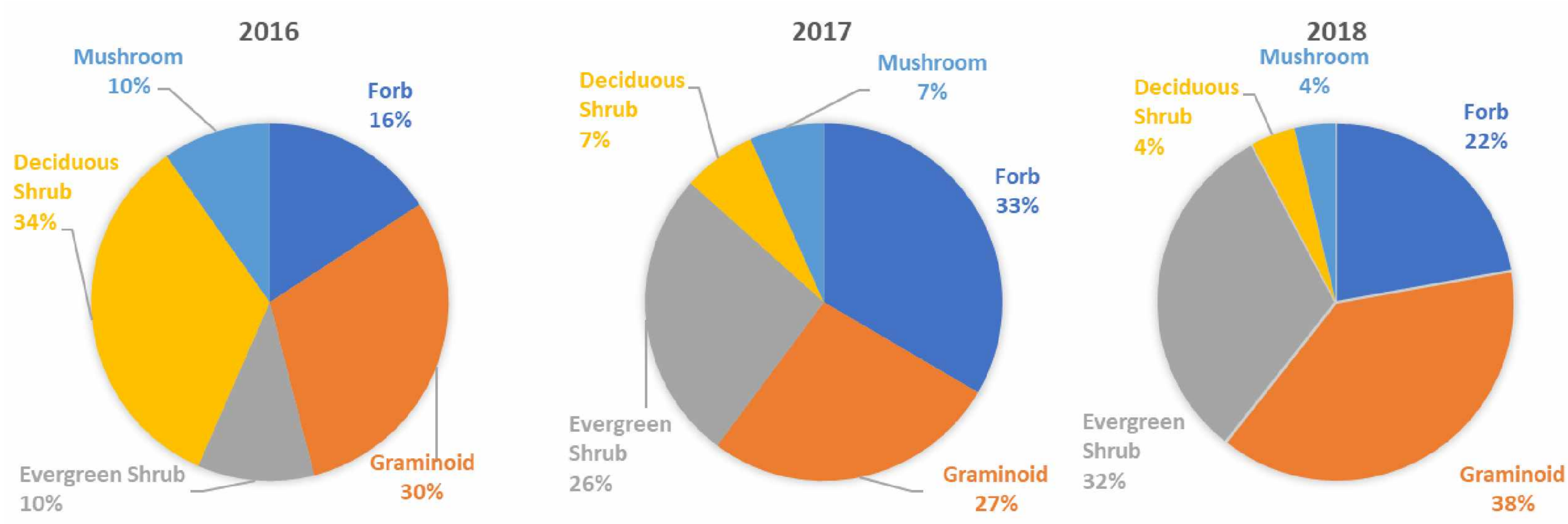

Figure 2.3: *Total summer diet composition of adult female (ewes) Dall's sheep in the Chugach Mountains during the arctic summer growing season (June 1-August 31) of 2016-2018. Proportions are calculated by totaling frequency of occurance of known and observed forage types (deciduous shrubs, evergreen shrubs, forbs, graminoids, lichens, and mushrooms) obtained during video observations of Dall's sheep ewes actively feeding.*

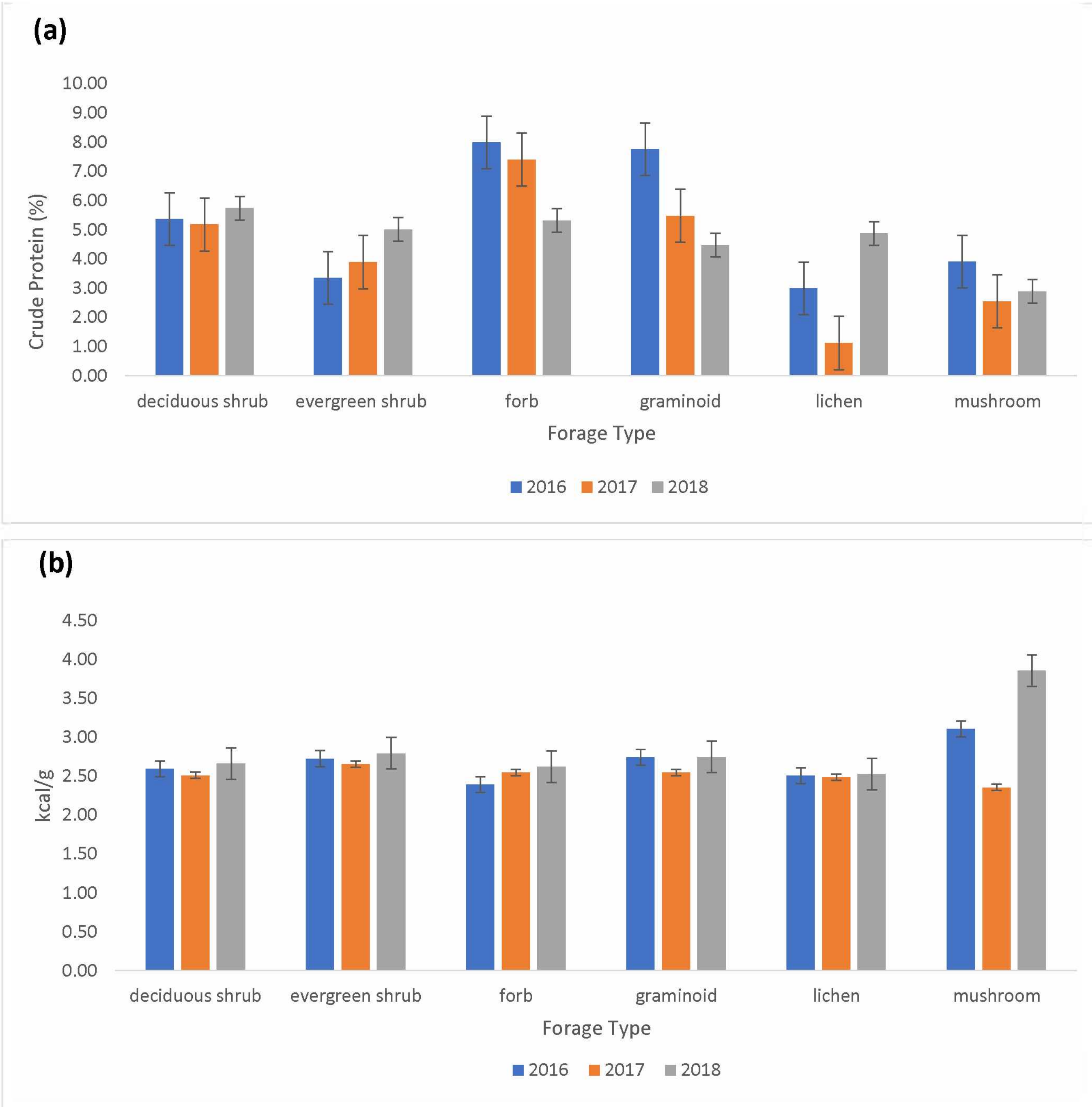

Figure 2.4: *Mean wet weight (A) crude protein (%) and (B) gross energy (kcal/g) for the total summer of different forage types (deciduous shrubs, evergreen shrubs, forbs, graminoids, lichens, and mushrooms) from adult female (ewes) Dall's sheep total summer diet in the Chugach Mountains, 2016-2018. 'Total summer' is defined here as the arctic summer growing season (June 1-August 31).* The whiskers on the graph show standard error.

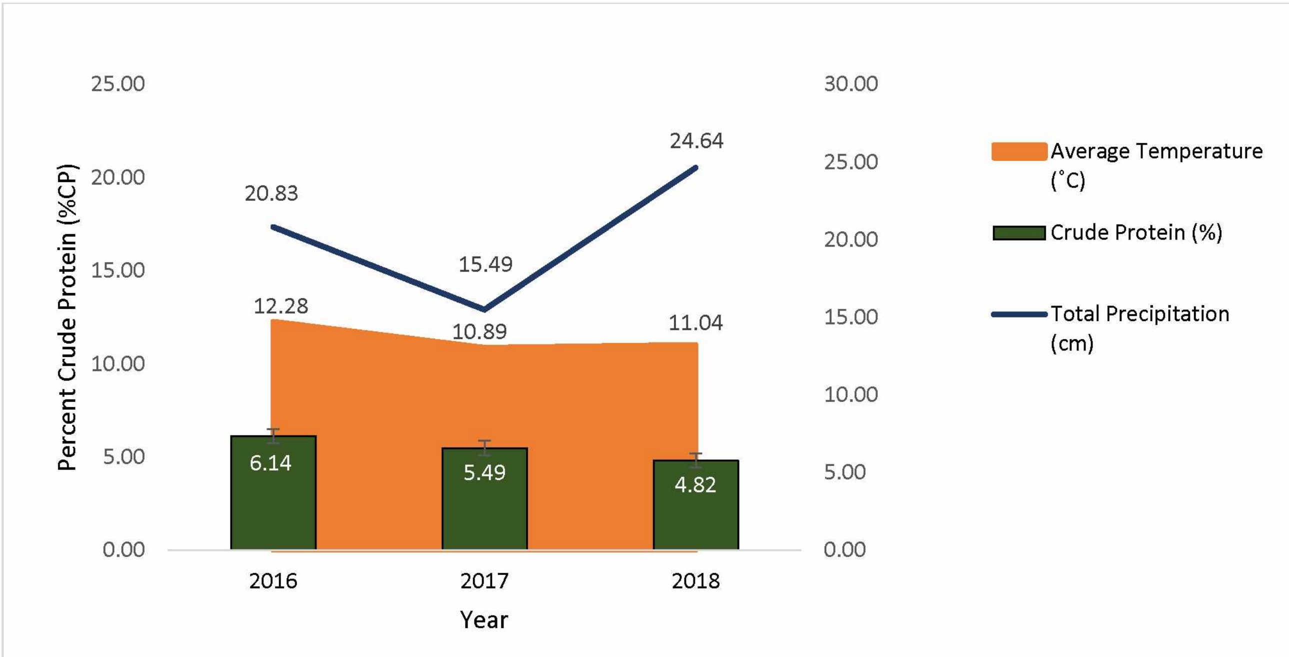

Figure 2.5: *Mean crude protein (%) of total summer (June 1-August 31) adult female (ewes) Dall's sheep diet in the Chugach Mountains versus the mean temperature (˚C) and total precipitation (cm) during the arctic summer growing season from 2016-2018. Calculated by multiplying the frequency of occurrence of known and observed Dall's sheep forage types (i.e., deciduous shrubs, evergreen shrubs, forbs, graminoids, lichens, and mushrooms) in total summer ewe diet composition and crude protein based on wet weight. Temperature and precipitation data were collected in a United States Department of Agriculture weather station located on the hillside region of Anchorage, Alaska*

Chapter 3: Compound-Specific Carbon Isotope ($\delta^{13}C$) Analysis of Amino Acids in Adult Female Dall's Sheep Forage in the Chugach Mountains[2]

3.1 Abstract

Recent population declines in Alaskan Dall's sheep (*Ovis dalli dalli*) are thought to be tied to the effects of climate change on their arctic alpine habitat and forage. The study of the patterns of carbon stable isotope ($\delta^{13}C$) signatures of amino acids (AAs) through compound-specific isotope analysis (CSIA) has proven a valuable ex-situ tool in foraging ecology. The demand for more ex-situ tools is high in Dall's sheep research due to logistical risks associated with it. In this study, we evaluated the use of CSIA-AA as a tool in Dall's sheep diet and assessed how our alpine primary producer forage types compared to stable isotope fingerprints of those previously mapped from different terrestrial environments. We explored if different forage types in Dall's sheep diet could be differentiated using a linear discriminant analysis (LDA) of CSIA-AA, in particular the alpine terrestrial C_3 plant forage types. With this technique we mapped the $\delta^{13}C$ values of essential AAs of a sample set ($n = 35$) of summer Dall's sheep forage samples alongside previously classified end-members (plants, bacteria, and fungi). We found that our alpine terrestrial C_3 plant forage types could not be differentiated in the LDA. However, when using the LDA to classify our samples compared to known end-member data, only half of alpine terrestrial C_3 plant Dall's sheep forage items classified as terrestrial C_3 plants ($P <$ 0.0001), the other half mapping instead as fungi. We theorized that this could be due to either AA exchange from mycorrhizal fungal partners or be a result of the influences of the free pool of AAs in arctic soils. Recent studies have found that climate related changes are altering alpine terrestrial C_3 plant community composition and activity. This has the potential to alter the presence and activity of mycorrhizal species within those environments, as well as the dependence of those mycorrhizal species on their symbiotic partners. If there is a mycorrhizal influence on our CSIA-AA results, further research could draw parallels to specific types of mycorrhizal plants present within Dall's sheep forage therefore creating an avenue to trace those

[2] Journal: Rapid Communications in Mass Spectrometry Authors: Luke Metherell, Benjamin Barst Ph.D., Thomas Lohuis Ph.D., and Lara Horstmann Ph.D.

diet sources. Either way, our results indicate a potential need for future research to better understand how terrestrial habitats may influence the isotopic fingerprints of primary producers.

3.2 Introduction

Dall's sheep (*Ovis dalli dalli*) live in alpine areas of the arctic and subarctic regions of North America.[1] As climate change continues to alter conditions across the globe, these arctic regions are some of the most rapidly changing.[2] These changes have the potential to affect Dall's sheep and aspects of their habitat including forage, but more research is needed to understand the direction and magnitude of these alterations. Dall's sheep habitat is dangerous and access to these hazardous, alpine environments makes *in situ* forage studies logistically and financially challenging. Dall's sheep primarily forage on and/or near steep cliffs and other extreme terrain to avoid terrestrial predators. [3] The severe weather conditions and long hours of darkness outside of spring and summer in these high-latitude, high-altitude environments also limit the amount of time during the year in which direct studies can take place. In addition, Dall's sheep are particularly vigilant,[4] thus making direct observation difficult.

Previous studies report that Dall's sheep consume a wide variety of forage types: evergreen shrubs, deciduous shrubs, forbs, and graminoids, as well as some lichens and mosses.[3,5-7] Much of Dall's sheep forage consists of many varied species of alpine terrestrial plants, all of which use the C_3 photosynthetic pathway.[1] For example, in the southwest Yukon Territory, Dall's sheep eat as many as 110 different plant species.[8] We witnessed Dall's sheep in the Chugach Mountains of southcentral Alaska consuming all of these forage types, except for mosses (Chapter 2). However, we also observed female Dall's sheep actively eating mushrooms, which has not been previously reported (Chapter 2). Female Dall's sheep summer diet in the Chugach Mountains can be classified into six broad forage types (evergreen shrubs, deciduous shrubs, forbs, graminoids, lichens, and mushrooms). Although this knowledge was gained through direct observation, monitoring the effects of climate change on Dall's sheep forage could also be approached through an indirect approach, such as chemical feeding ecology.

Bulk stable isotope analysis, typically $\delta^{13}C$ and $\delta^{15}N$, of various tissues is a cost-effective method of chemical feeding ecology widely used to study diet sources of marine and terrestrial animals of all phyla and taxa. [9-10] $\delta^{15}N$ is generally applied to study an organism's trophic level and food web linkages, while $\delta^{13}C$ is commonly used to trace carbon sources.[11] However, there are drawbacks to the use and interpretation of bulk stable isotopes in forage ecology, such as trophic fractionation and the specificity of diet sources.[12-13] The use of bulk stable isotope signatures in herbivores like Dall's sheep, whose diet consists in large part of terrestrial plants, can be particularly confounding, because of the isotopic overlap of co-occurring diet sources.[14] Bulk $\delta^{13}C$ values of C_3 and C_4 plants show distinct differences, because C_3 plants discriminate against the heavier ^{13}C isotope during photosynthesis, while C_4 plants do not.[15] Bulk $\delta^{13}C$ ‰ (per mil) of terrestrial C_3 plants becomes more enriched with plant water stress, and typically ranges between -30 to -20 ‰.[16] This variation makes differentiating specific isotopic signatures of terrestrial C_3 plant food sources difficult. [17]

One of the potential ways to circumvent the issue of diet source specificity in bulk stable isotope analysis is compound specific analysis of unique $\delta^{13}C$ patterns (fingerprints) of molecules like amino acids (AAs) within diet items (CSIA-AA) (Whiteman et al., 2019). There are twenty-one canonical AAs that act as functional units in natural proteins[18] that are ubiquitous across all biological systems and act in critical roles in structure and metabolic, enzymatic, and cellular processes.[19] Of these AAs, nine can only be synthesized by bacteria, fungi, and photoautotrophs, and therefore have to be consumed by animals, making these essential AA (EAAs).[20] The stable isotope signatures of these EAAs are typically unaltered when passed from producer to consumer, and therefore are well-suited for potentially identifying the forage sources from which they were derived. [21] Patterns of amino acid $\delta^{13}C$ have been previously shown to accurately trace the diet sources of primary consumers.[22] During synthesis, organisms that share similar fractionation against the heavier ^{13}C isotope group together, resulting in subtle differences among primary producer types and lineages in the $\delta^{13}C$ signature of individual AAs.[22-24] This creates large variation when tracing their $\delta^{13}C$ values in consumer tissues.[12] Thus, examination of the $\delta^{13}C$ patterns of EAAs could be a means to trace the source forage types within female Dall's sheep diet.

Plants, fungi, and bacteria are the end-members that have been used to trace diet sources in forage ecology, because they each show characteristic AA $\delta^{13}C$ patterns due to the unique pathways utilized during AA synthesis.[22] However, there has not been extensive investigation into differentiating the $\delta^{13}C_{EAA}$ patterns of arctic alpine terrestrial plant groups, or plants from different terrestrial environments. Previous studies have characterized $\delta^{13}C_{EAA}$ patterns of C_3 plants as a whole but have not examined differentiation among different plant types (e.g., graminoids, forbs, deciduous shrubs etc.) within this group. Larsen et al.[21] and Rowe et al.[25]

found discernible differences in $\delta^{13}C$ patterns among $\delta^{13}C_{EAA}$ of C_3 plants sourced from aquatic versus terrestrial environments in and around lacustrine systems in the North Slope of Alaska and in the Chukchi Sea. Terrestrial plants from different habitat types (e.g., alpine vs. subalpine) could thus potentially show differences in EAA stable isotope signatures among plant species and/or plant functional groups.

Many plant species growing in nutrient-limited environments have symbiotic relationships with mycorrhizal fungi to provide nutrients, including nitrogen in the form of intact AAs, in exchange for recent photosynthate.[26] Essential AAs transferred intact to the plant host by fungal partners may be derived directly from the soil by mycorrhizal fungi and/or from mycorrhizal fungal biosynthesis. Free EAAs in soils are a mix of fungal and bacterial biosynthesis. Because AAs transferred to plants by mycorrhizal partners are likely derived from both soils and biosynthesis within the fungal partner, it is feasible that the unique signature of $\delta^{13}C_{EAA}$ patterns derived from fungal partners could translate to differences in $\delta^{13}C_{EAA}$ patterns between mycorrhizal vs non-mycorrhizal terrestrial C_3 plants. This differentiation in the stable isotope fingerprints of EAAs could emerge as a tool with finer resolution in diet sourcing and give insight into the foraging ecology of Dall's sheep and other terrestrial C_3 plant consumers. There have been other types of studies to improve techniques in the field of Dall's sheep diet analysis, such as fecal metabarcoding,[27] but these techniques are generally limited to identification of short-term diets at the time of fecal collection, e.g., late spring to early fall in the case of Dall's sheep. The key advantage of bulk and compound-specific stable isotopes is that they represent an integrated long-term view of diet.[28] In addition, if CSIA-AA prove to be an effective tool to identify Dall's sheep diet, it would open the door to use CSIA-AA on incrementally growing structures, such as horns and teeth, to map migratory, seasonal, and lifelong diet changes.[29-30]

The aim of this study was to investigate the potential use of CSIA-AA as a tool in diet sourcing studies of Dall's sheep. Because Dall's sheep forage types originate in arctic alpine environments, where resources are limited, there is the potential that $\delta^{13}C_{EAA}$ fingerprints of forage species might differ from previously mapped end-members. Traditionally all of the $\delta^{13}C_{EAA}$ fingerprints of C_3 terrestrial plants mapped by end-member type (i.e., plants, bacteria, and fungi) but not by groups within that end-member type (i.e., in plants; graminoids, forbs etc.). But if there is a connection between adaptation to element sourcing by plant type, there is a chance that our terrestrial C_3 plants sourced in our unique environment (arctic, alpine) may show distinct grouping, which, since our forage types are based on those groups would create forage type clusters. In that case CSIA-AA could work as a valid tool in mapping the diet sources of Dall's sheep. We selected a subset of Dall's sheep forage samples across all forage types (n=40), out of an original sample set (n = 341) collected during our observational study (Chapter 2). We analyzed the $\delta^{13}C_{EAA}$ fingerprints of those forage samples and mapped them using linear discriminant analysis (LDA) alongside known end-members: bacteria, terrestrial C_3 plants, and fungi previously identified by Larsen et al.[21-22] All $\delta^{13}C_{EAAs}$ data were normalized to their sample means before carrying out the LDA.[22,31] These results were then visually assessed to discern if and how forage samples differed from known end-members, as well as if forage samples clustered by plant functional type. We hypothesized that $\delta^{13}C_{EAAs}$ plant functional types of Dall's sheep forage will differ from other ecosystems, because of the extreme and unique conditions of the arctic alpine environment from which they were collected. We also hypothesized that the CSIA-AA results of our individual forage types (forb, graminoid, deciduous shrub, and evergreen shrub) would group distinctly based on forage type.

3.3 Methods

3.3.1 Sample Collection and Selection

We selected a subset of 40 Dall's sheep forage samples for CSIA-AA from 2016, 2017, and 2018 from an original collection (n = 341), only 35 of which we were able to run (n = 35). The forage samples were comprised of different tissue types (e.g., plant samples: leaves and stems, mushroom samples: cap, stem, and gills) all different species from six distinct forage types: deciduous shrubs (n = 8) *Arctostaphylos alpina, Betula nana, Diapensia lapponicum, Pontentilla fruticose, Salix alaxensis, Salix glauca, Salix monticola, Vaccinium uglinosum*, evergreen shrubs (n = 4) *Empetrum nigrum, Geranium erianthum, Harrimanella stelleriana, Loiseleuria procumbens*, forbs (n = 12) *Aconitum delphiniifolium, Anemone narcissiflora, Campanula lasiocarpa, Castilleja unalaschcensis, Delphinium glaucum, Epilobium angustifolium, Epilobium latifolium, Geranium erianthum, Geum rossi, Lupinus arcticus, Polemonium pulcherrimum, Saxifraga bronchialis*, graminoids (n = 8) *Bromus pumpellianus, Calamagrostis canadensis, Carex aenea, Carex bigelowii, Carex macrochaeta, Festuca ovina, Poa alpina, Vahlodea atropurpurea,* lichens (n = 1) *Nephroma Arctica*, and mushrooms (n = 2) *Boletus edulis, Russula xerampelina*. These samples were collected following video observations (Chapter 2) and are all confirmed diet items of female Dall's sheep during summer. They were collected at the same time and location where sheep were foraging.

3.3.2 Bulk and Compound-Specific Stable Carbon Isotope ($\delta^{13}C$) Analysis of Amino Acids

We analyzed Dall's sheep forage samples at the Alaska Stable Isotope Facility (ASIF) at the University of Alaska Fairbanks. All forage samples were collected in paper coin envelopes (5.7 x 8.9 cm) and allowed to air dry in the field (0-22 °C). Within 24 hours of returning from the field, samples were dried in a Blue M Stabil-Therm gravity convection oven at 60 °C for 24 hours.[32] The dried forage samples were then ground in a Thomas Scientific Wiley mill[33] and placed in reinforced 1.8 ml micro-vials containing BB-sized steel beads. Samples were then inserted into a BioSpec Mini-Beadbeater for about 4 minutes until completely homogenized. For bulk stable isotope analysis, our forage samples were analyzed by an Isoprime isotope ratio mass spectrometer (Isoprime Ltd, Cheadle Hulme, UK) coupled to a CN elemental analyzer (Eurovector, Milan, Italy) using continuous flow, with helium as carrier gas.[34]

The compound-specific stable isotope ($\delta^{13}C$) analysis of individual AA was completed following procedures outlined in Barst et al.[31] All standards and reagents were purchased from Sigma-Aldrich® Brand. Briefly, forage samples (2-6 mg) were transferred to 13×100 mm Pyrex VWR screw cap culture tubes with polytetrafluoroethylene-lined caps. The samples were combined with 1 ml of 6 N HCl, flushed with N_2 gas for 30 seconds, and then heated at 110 °C for 20 hours. Hydrophilic AAs were then separated from hydrophobic compounds using liquid-liquid extraction with 2 ml of hexane/dichloromethane (6:5 v/v). Next, 15 μl of norleucine (nLeu) (6.6 mg/ml) internal standard was added to each isolated acidic fraction. The acidic fractions were then evaporated under a gentle stream of N_2 gas, and the resulting residues were stored in capped dram vials at 4 °C until AA derivatization.

Residual AAs were methylated with a mixture of 0.65 ml acidified methanol (anhydrous MeOH/acetyl chloride, 6:1) in each vial for 1 hour at 75 °C. The methylation mixture was then evaporated to almost complete dryness with N_2 gas. The methylated AAs were then further derivatized by acetylation using a mixture of acetic anhydride (1.0 ml), trimethylamine (2.0 ml), and acetone (5.0 ml) for 10 minutes at 60 °C. The acetylation mixture was then evaporated until almost complete dryness with N_2 gas.

The derivatized AAs were purified by washing with a mixture of potassium phosphate buffer (p-buffer, pH 7.4) and chloroform (1:1). The organic fraction containing the AAs was then removed and evaporated under N_2 gas, and the residuals were reconstituted in ethyl acetate. Each derivatized AA sample was spiked with 3 µl of nonadecane (10 mg/ml methanol) and 6 µl caffeine (10 mg/ml methanol) as internal standards.

Derivatized AAs were analyzed at ASIF using gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS). A Thermo-Scientific TriPlus RSH smart autosampler injected samples into a borosilicate glass double gooseneck (4 × 6.5 × 78.5 mm) Restek liner for splitless injection held at 280 °C for 2 minutes. The derivatized AAs were separated on an Agilent VF-35ms column (30 m × 0.32 mm × 1µm) installed in a TRACE 1310 GC coupled via a GC Isolink II and a ConfloIV to Delta V Plus IRMS. The following GC temperature program was applied: 80 °C (hold 2 min), increase to 135 °C at a rate of 20 °C/min, increase to 160 °C at a rate of 5 °C/min (hold 3 min), and increase to 300 °C at a rate of 8 °C/min (hold 3 min). Samples were run in triplicates and derivatized in a single batch with two mixed AA standards with differing known AA $\delta^{13}C$ values for follow-up correction of added carbon. In this study, we isolated defined peaks from 12 AAs total, but only considered the EAAs threonine (Thr), valine (Val), lysine (Lys), isoleucine (Ile), leucine (Leu), methionine (Met), and phenylalanine (Phe) in all analyses.

3.3.4 Carbon Correction and Analysis

Mean $\delta^{13}C$ values ± 1 standard deviation were generated for each AA using the $\delta^{13}C$ values of triplicate injections.[21,31] We derivatized and analyzed each sample in batches along with two mixed AA standards, UAF AA1 and UAF AA2. The mixed AA standard comprised of equal concentrations of the AAs alanine (Ala), glycine (Gly), threonine (Thr), serine (Ser), valine (Val), leucine (Leu), isoleucine (Ile), nor-leucine (Nle), proline (Pro), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), lysine (Lys), tyrosine (Tyr), and histidine (His) with known $\delta^{13}C$ values. This allowed us to account for added carbon during derivatization and any variation in carbon isotopic fractionation occurring during derivatization and analysis. The $\delta^{13}C$ values of AAs in the standard UAF AA1 were used to correct the $\delta^{13}C$ values of AAs in UAF AA2, then the corrected values in UAF AA2 were compared to known AA $\delta^{13}C$ values. This allowed us to determine how accurate the carbon correction was prior to correcting the $\delta^{13}C$ values of AAs in the same way moving forward. [31,35-37] AA $\delta^{13}C$ values of the UAF AA2 standard carbon corrected using UAF AA1 standard were highly correlated with the known AA $\delta^{13}C$ values of UAF AA2 ($R^2 = 0.998$; slope = 1.003, Fig. 3.1). The measured $\delta^{13}C$ values of the internal standards were consistent across all batches of samples (n = 99) with nLeu (SD = 0.7), nonadecane (SD = 0.2), and caffeine (SD = 0.3), and standards (n = 6) with nLeu (SD = 0.6), nonadecane (SD = 0.4), and caffeine (SD = 0.1).

The measured corrected $\delta^{13}C$ values were then compared to the known $\delta^{13}C$ values of the AAs previously determined through bulk □^{13}C stable isotope analysis. Our derivatization methods added carbon to the AAs, such that the measured AA $\delta^{13}C$ values reflect a combination of the intrinsic AA carbon from the original molecule measured and reagent carbon added during this process. The carbon corrected $\delta^{13}C$ values were then mean centered.

These carbon-corrected and normalized $\delta^{13}C$ values were compared to the $\delta^{13}C$ values of end-member data from Larsen et al.[21-22] (Table 3.1) in a Linear Discriminant Analysis (LDA) to classify the different Dall's sheep forage types.[21-22,31,38] Forage samples were divided into the 6 forage types (deciduous shrubs, evergreen shrubs, forbs, graminoids, lichens, and mushrooms). Stable carbon isotope values of AA were expressed in delta ($\delta^{13}C_{EAAs}$) notation relative to the isotopic ratio of an internationally recognized standard Vienna PeeDee Belemnite (VPDB) in per mil (‰).

Delta values were defined by the equation:

Equation 3.1

$$\delta X = (R\ sample/R\ standard - 1) \times 1000$$

In this equation X is ^{13}C, and R is the ratio of heavy to light isotopes ($^{13}C/^{12}C$).

Using known $\delta^{13}C$ values end-members from Larsen et al.,[21-22] we used a one-sample proportion z test (α =0.05) in JMP Pro 12 Statistical Analysis Software (SAS Institute, Cary, NC, USA) to compare which of our terrestrial C_3 plant tissue (leaf and stems combined) samples actually mapped in the LDA as terrestrial C_3 plant end-members compared to terrestrial C_3 plant data from interior Alaskan boreal forests.[21-22] We used JMP Pro 12 for all statistical analysis. The limited number of samples per forage type prohibited the use of a chi-square test to compare how different plant forage types mapped in the LDA.

3.4 Results

The bulk stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) per mil (‰) isotope values of forage samples (Fig. 3.2) along with the average CSIA-AA values from each sample are provided in Table 3.2. The bulk $\delta^{15}N$ values for plant forage types varied from -9.1 to 0.9 ‰ with means

(±1SD) of deciduous shrubs -4.1 ± 2.4 ‰, evergreen shrubs -7.0 ± 2.4 ‰, forbs -2.3 ± 1.9 ‰, and graminoids -2.4 ± 3.0 ‰, respectively (Table 3.2). Bulk $\delta^{13}C$ values for these forage types were less variable with a range -30.2 to -25.6 ‰ and respective means (±1SD) of deciduous shrubs -28.9 ± 0.5 ‰, evergreen shrubs -27.8 ± 0.6 ‰, forbs -27.9 ± 1.2 ‰, and graminoids -27.8 ± 0.8 ‰ (Table 3.2). We analyzed only two mushroom samples, which by comparison were more variable in both bulk $\delta^{15}N$ and $\delta^{13}C$ values (Table 3.2). Our single lichen sample had a bulk $\delta^{15}N$ value of -2.0 ‰ and bulk $\delta^{13}C$ value of -21.8 ‰.

Of 40 selected forage samples that were analyzed for $\square^{13}C_{AA}$ via GC-C-IRMS, only 35 (32 plants, 2 mushrooms, and 1 lichen) had sufficient peak resolution in the subsequent chromatogram (an example chromatogram, Fig. 3.3). We successfully resolved the AAs: Ile, Leu, Phe, Thr, Ala, Asp, Glu, Pro, Ser, and Val. We only considered Ile, Leu, Phe, Thr, and Val as these are synthesized by primary producers and are therefore essential to animals (EAAs). Stable isotope values of EAAs stay relatively constant through the food chain and therefore can be used in nutrient tracing.[21-22] When run in triplicates these EAAs had distinct peaks in their resulting chromatograms (Fig 3.2), from which isotopic ratios could be calculated (Table 3.2).

The $\square^{13}C_{EAA}$ data of forage samples (n = 35) were then compiled into a multivariate end-member canonical plot with Linear Discriminant Analysis (LDA) (Fig. 3.4). We did not observe a discernable visual separation among $\delta^{13}C_{EAA}$ of terrestrial C_3 plants that make up female Dall's sheep summer forage types (i.e., deciduous shrubs, evergreen shrubs, forbs, and graminoids) (Fig. 3.4). Terrestrial C_3 plants comprised 91 % of our final sample set (32 of the 35 samples), but when classified by the LDA in comparison to previously mapped terrestrial C_3 plants, these forage samples did not all map as terrestrial C_3 plant end-members as reported by Larsen et al.[21-22] Instead, a portion (16, 50 %) of our terrestrial C_3 plant forage samples, when compared to previously mapped end-members, were classified as fungi (Fig. 3.4).[21-22] Of the terrestrial C_3 plant forage groups, forbs and graminoids had the least amount of observed variability and deciduous shrubs had the most (Fig. 3.4).

Our terrestrial C_3 plants were statistically different from the previously-published terrestrial C_3 plant end-members (z test, $\alpha = 0.05$, $P < 0.0001$) (Table 3.3). The two mushrooms in our sample both mapped as fungi end-members. Among our terrestrial C_3 plant forage types, 5 of the 8 (63 %) deciduous shrubs, 3 of the 4 (75 %) evergreen shrubs, 6 of the 12 (50 %) forbs, and 2 of the 8 (25 %) graminoids were classified by the LDA as terrestrial C_3 plant end-members (Fig.

3.4). Within the graminoids, both grasses and sedges were classified in the LDA as fungi and terrestrial C_3 plant end-members, thus showing no obvious pattern. Of our 35 analyzed forage samples (including 2 mushrooms), 16 samples were classified as terrestrial C_3 plant LDA values of Larsen et al.,[21-22] while the remaining 19 samples (including 16 terrestrial C_3 plants, 1 lichen, and 2 mushrooms) mapped as fungi in the LDA.

3.5 Discussion

Ecological applications of compound specific analysis of unique $\delta^{13}C$ patterns (fingerprints) of essential amino acids (EAAs) (CSIA-EAA) have proven to be an effective tool to map nutrient and energy flow with regards to both individual organisms and ecosystems.[14] However, our particular assessment of this technique yielded unexpected results. We rejected our initial hypothesis that Dall's sheep forage types would distinctly cluster in a Linear Discriminant Analysis (LDA). The patterns of the stable carbon isotope values of EAAs ($\delta^{13}C_{EAA}$) of our forage samples, particularly the terrestrial C_3 plants, did not group distinctly by their respective forage types (i.e., evergreen shrubs, deciduous shrubs, forbs, graminoids, lichen, and mushrooms). One possible explanation is that the bulk of free AAs in arctic soils are derived from fungi, which are known to comprise the bulk of the microbial biomass in boreal and arctic soils.[39-40] If this were the case, then mycorrhizal, non-mycorrhizal and fungal would ultimately be deriving EAAs from the same taxonomic end member. Because a majority of Dall's sheep summer diet consists of terrestrial C_3 plants, our findings suggest that the use of CSIA-EAA may not be effective in tracing and mapping their diet's nutrient sources.

We secondarily predicted that our terrestrial C_3 plant samples would map differently from known terrestrial C_3 plant end-members[21-22] in an LDA because of their unique habitat of origin and that hypothesis was partially supported. Fifty percent of our successfully run terrestrial C_3 plant forage samples, when compared to previously mapped end-members were classified as fungi.[21-22] These differences could be explained by differences in the habitat type of the collection locations of our samples versus the end-members from Larsen et al.[21-22] All of our terrestrial C_3 plant samples came from alpine habitats in south central Alaska, while all the terrestrial C_3 plant samples from Larsen et al.[22] were collected from greenhouses or boreal forests in interior Alaska. All but two of the terrestrial C_3 plant end-members from Larsen et al.[21] originated from lacustrine ecosystems (adjacent to lakes) on Alaska's North Slope. Both collection sites, where the previous end-member samples were collected, are geographically and ecologically different than our sample collection sites. Therefore, 'local scale' differences could affect CSIA-EAA results and interpretation, making 'local scale' sourcing of samples in future CSIA-EAA studies an important consideration. In this instance, 'local scale' is defined in the ecological sense, as a phenomenological community, an identifiable assemblage of organisms in a local habitat patch.[41] In previous CSIA-EAA studies, investigators have utilized $\delta^{13}C_{EAAs}$ from global compilations of EAA synthesizers.[14] To our knowledge, only one study has examined the $\delta^{13}C_{EAA}$ values of plants at the local scale.[42] Currently there may not be enough information to assess how time and space may affect $\delta^{13}C_{EAA}$ fingerprints,[14] but our results suggest that perhaps more investigation into this topic – particularly with regard to mycorrhizal vs. non-mycorrhizal plants across ecosystems with variation in bacteria vs. fungi in soil microbial biomass could present an opportunity to refine and expand the study of CSIA-EAA making an already useful scientific tool even better.

The fact that some of our terrestrial C_3 plant samples mapped differently compared to known end-members is curious in itself, but where these plants mapped in the LDA is also interesting. Of our terrestrial C_3 plant forage samples, 50 % were classified as terrestrial C_3 plants according to known end-member terrestrial C_3 plant data, but the other 50% classified as fungi, based on Larsen et al.[21-22] This may indicate some of these plants obtained their AAs from an outside source in or around the soil: fungi. Terrestrial C_3 plants and fungi show different $\delta^{13}C_{EAA}$ values, because fungi typically source many of their AAs from soil organic matter,[43] while terrestrial C_3 plants synthesize their own AAs from inorganic nitrogen.[44] But certain terrestrial C_3 plants, often in resource limited environments, supplement AAs and other resources from symbiotic mycorrhizal relations with fungi.[45] In this scenario, belowground structures of both organisms shuttle nutrients and water and photosynthesis from the plant drives primary productivity of both.[46]

Many arctic and alpine terrestrial C_3 plants are known to obtain and supplement nutrients from soil-based free AA sources. This soil-based free AA pool is typically a result of bacterial and fungal synthesis. For many arctic and boreal plants, a large proportion of total plant nitrogen is derived from the uptake of free AA, which can be at high concentrations in soils.[47] A study examining nitrogen uptake and kinetics of three AAs (glycine, aspartic acid, and glutamic acid) by arctic terrestrial C_3 plants found that mycorrhizal plants showed a higher AA uptake than non-mycorrhizal species.[26] Terrestrial C_3 plant taxa common to other northern alpine habitats utilize mycorrhizal relationships for AA supplementation as a means of survival in their severe environments.[48-50] There are examples of this in the Beartooth Plateau in the Rocky Mountains, while C_3 terrestrial plant genera like *Betula* and *Dryas* form ectomycorrhizae with basidiomycete fungi, while the genus *Vaccinium* forms ericoid mycorrhizae with ascomycete fungi.[50] This fungal influence on the free AA pool that these arctic plants are sourcing could be influencing their CSIA-AA fingerprints. The resource limiting conditions of the Dall's sheep habitat we collected our samples from may have influenced some of the terrestrial C_3 plants to rely on other nutrients sourced through soil or mycorrhizal channels. This in turn may potentially change their $\delta^{13}C_{EAA}$ fingerprints making them to be classified as fungi when compared to previously mapped fungi end-members.

How could this information be used to better understand the effects of climate change on alpine plant communities and the animals that depend on those plants, like Dall's sheep? Climate change has the potential to affect alpine plant community species composition,[51] as well as the functional dynamics of plants[52] within communities. Dall's sheep and other alpine species exist in a balance of community-dependent relationships that have evolved over thousands of years; however, climate related changes to the composition and dynamics of these alpine habitats could threaten that balance. One of the most direct climate-induced changes on alpine plant community compositions is the encroachment of shrub- and tree lines into alpine habitats.[51] In the Rocky Mountains (USA), over the course of 20 years, researchers found that experimental warming had effects on both community composition and function of individual plants.[52] These authors described changes in community plant assemblages, shifting from primarily mycorrhizal grasses to predominantly non-mycorrhizal sedges.[52] The mycorrhizal grasses that remained showed an increased colonization of arbuscular mycorrhizal (AM) fungi in response to resource stress brought on by the warming climate.[52] This suggests that climate warming can reduce the number of mycorrhizal plants in alpine environments. Additionally, the mycorrhizal plants remaining on the landscape have an increased reliance on their symbiotic fungi partners.

Dall's sheep, an herbivore with longer generation times that have evolved alongside the alpine plant communities they depend on for food sources, and as such are vulnerable to any climate induced changes within those plant communities.[53] Because climate change affects mycorrhizal plant presence and activity, and if the $\delta^{13}C_{EAA}$ patterns of terrestrial C_3 plants are influenced by mycorrhizal relationships, CSIA-EAA could potentially be a tool to monitor such changes. However, this may be problematic in high-latitude ecosystems, where fungi dominate the soil microbial biomass as discussed above. The more direct impacts on alpine plant communities most likely would indirectly impact Dall's sheep by reshaping plant-herbivore interactions.[54] Climate change impact on arctic alpine plant species distribution and influences on plant habitat and nutrient sources, could lead to altered herbivore resource availability and plant-herbivore interactions.[55] Increases in mycorrhizal plant activity brought on by climate warming could have a mixed impact on Dall's sheep plant forage species.[56] Increased mycorrhizal plant activity has been linked to improved plant mineral nutrition, faster plant growth, and regrowth, but also has been associated with altered levels of plant herbivore defense mechanisms, like the production of secondary metabolites.[56] Fully quantifying these effects remains a challenge, but climate related changes in the arctic alpine plant communities within Dall's sheep habitat would irrevocably impact the established balance that Dall's sheep have developed with those plants influencing the wellbeing of both plant and herbivore.

3.6 Conclusion

Alpine environments inside and outside of the arctic are complex systems that rely on the connectivity of conditions, habitat, and organisms within that system. Although plants and animals have behaviorally and physiologically adapted to live in these harsh environments, these adaptations often rely on seasonal and trophic balances that can be disrupted by climate change.[57] This level of connectivity creates the potential for translatory effects through the trophic chains of these particularly vulnerable ecosystems when experiencing high rates of warming due to climate change. Compound specific analysis of unique $\delta^{13}C$ patterns (fingerprints) of amino acids (CSIA-AA) has already proven to be a powerful scientific tool in ecological and physiological research in the past and could be an effective means to monitor some of the climate change related effects to arctic and alpine communities. This includes the impacts of climate change to arctic alpine terrestrial C_3 plant communities within Dall's sheep habitat that have the potential to directly and indirectly impact the Dall's sheep populations that rely on them as forage. Further research is necessary to understand if and how mycorrhizal activity influences CSIA-AA values and what, if any, mycorrhizal plant activity and the plants themselves play a role in Dall's sheep forage. Regardless, a better understanding of the undercurrents of change in arctic alpine habitats as a whole driven by climate change through the lens of CSIA-AA, shows promise in bottom-up approaches evaluating ecosystem-wide effects.

3.7 Literature Cited

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3.8 Tables

Table 3.1: *Unique $\delta^{13}C$ (‰) fingerprint mean-centered values of different essential amino acids (EAAs) of bacteria, fungi, and terrestrial C_3 plants from Larsen et al. (2009, 2013). The listed $\delta^{13}C$ (‰) fingerprint values are for the following EAA's: threonine (Thr), valine (Val), leucine (Leu), isoleucine (Ile) and phenylalanine (Phe).*

Reference	***Type***	***Thr***	***Val***	***leu***	***Ile***	***Phe***
Larsen et al. (2013)	*Bacteria*	*-7.28*	*1.12*	*0.32*	*-0.48*	*6.32*
	Bacteria	*-4.84*	*1.06*	*-0.04*	*-0.14*	*3.96*
	Bacteria	*-4.84*	*0.96*	*0.06*	*-0.94*	*4.76*
	Bacteria	*-8.76*	*2.54*	*1.34*	*-0.16*	*5.04*
	Bacteria	*-7.34*	*2.16*	*2.06*	*0.46*	*2.66*
	Bacteria	*-8.62*	*3.88*	*1.98*	*-0.12*	*2.88*
	Bacteria	*-8.18*	*3.42*	*1.82*	*0.02*	*2.92*
	Bacteria	*-7.24*	*2.66*	*0.86*	*1.06*	*2.66*
	Bacteria	*-6.04*	*2.26*	*1.26*	*-1.64*	*4.16*
	Bacteria	*-5.9*	*3.3*	*-0.7*	*0.5*	*2.8*
	Bacteria	*-7.04*	*3.46*	*0.36*	*-0.04*	*3.26*
	Bacteria	*-6.26*	*1.84*	*0.04*	*-0.16*	*4.54*
Larsen et al. (2013)	*C3 Plant*	*-14.88*	*6.52*	*7.82*	*-1.38*	*1.92*
	C3 Plant	*-15.2*	*4.8*	*8.4*	*-0.6*	*2.6*
	C3 Plant	*-13.32*	*5.48*	*8.48*	*-1.72*	*1.08*
	C3 Plant	*-12.32*	*5.18*	*7.18*	*-0.72*	*0.68*
	C3 Plant	*-11.68*	*4.62*	*6.52*	*-0.18*	*0.72*
	C3 Plant	*-16.56*	*5.54*	*7.94*	*0.64*	*2.44*
	C3 Plant	*-14.32*	*5.28*	*6.68*	*0.58*	*1.78*
	C3 Plant	*-16.88*	*5.12*	*9.72*	*-0.28*	*2.32*
	C3 Plant	*-12.62*	*4.58*	*7.38*	*-0.22*	*0.88*
	C3 Plant	*-14.1*	*4.9*	*8.9*	*-0.5*	*0.8*
	C3 Plant	*-19.12*	*6.48*	*9.48*	*-0.32*	*3.48*
	C3 Plant	*-11.28*	*4.72*	*7.82*	*-1.78*	*0.52*

Table 3.1 Continued

Reference	*Type*	*Thr*	*Val*	*leu*	*Ile*	*Phe*
Larsen et al. (2009)	*Bacteria*	*-7.8*	*1.8*	*0.5*	*2*	*3.5*
	Bacteria	*-7.18*	*1.22*	*0.62*	*1.72*	*3.62*
	Bacteria	*-2.54*	*0.96*	*-0.14*	*0.16*	*1.56*
	Bacteria	*-2.2*	*3.1*	*-0.9*	*0.8*	*-0.8*
	Bacteria	*2.26*	*-1.34*	*-1.84*	*-0.54*	*1.46*
	Bacteria	*-4.34*	*1.56*	*-1.54*	*-0.24*	*4.56*
	Bacteria	*-1.92*	*0.18*	*0.58*	*-0.92*	*2.08*
	Bacteria	*2.66*	*-0.34*	*-0.64*	*-2.84*	*1.16*
	Bacteria	*0.66*	*-0.84*	*-0.74*	*-1.94*	*2.86*
	Bacteria	*-2.18*	*-1.18*	*-0.48*	*2.12*	*1.72*
Larsen et al. (2009)	*C_3 Plant*	*-11.18*	*4.22*	*7.02*	*0.52*	*-0.58*
	C_3 Plant	*-15.56*	*3.74*	*8.84*	*2.14*	*0.84*
	C_3 Plant	*-14.98*	*4.82*	*10.32*	*1.02*	*-1.18*
	C_3 Plant	*-11.7*	*4.4*	*6.2*	*-0.6*	*1.7*
	C_3 Plant	*-14.5*	*6.8*	*8.3*	*0.5*	*-1.1*
	C_3 Plant	*-12.46*	*4.74*	*6.84*	*0.24*	*0.64*
	C_3 Plant	*-15.98*	*5.82*	*8.32*	*0.22*	*1.62*
	C_3 Plant	*-11.8*	*5*	*6.4*	*0.9*	*-0.5*
	C_3 Plant	*-11.08*	*5.22*	*6.62*	*0.32*	*-1.08*
	C_3 Plant	*-13.52*	*4.98*	*8.08*	*-0.52*	*0.98*
Larsen et al. (2009)	*Fungi*	*-3.96*	*1.14*	*5.14*	*-4.86*	*2.54*
	Fungi	*-5.8*	*1.4*	*4.4*	*-2.1*	*2.1*
	Fungi	*-7*	*3.1*	*4.9*	*-2.4*	*1.4*
	Fungi	*-9.38*	*4.42*	*5.42*	*-4.18*	*3.72*
	Fungi	*-5.92*	*0.38*	*5.48*	*-2.62*	*2.68*
	Fungi	*-8.32*	*5.18*	*5.38*	*-3.72*	*1.48*
	Fungi	*-3.92*	*0.18*	*5.88*	*-4.12*	*1.98*
	Fungi	*-7.14*	*4.56*	*5.76*	*-4.44*	*1.26*
	Fungi	*-5.92*	*3.48*	*6.18*	*-5.52*	*1.78*
	Fungi	*-9.56*	*5.04*	*5.84*	*-3.66*	*2.34*
	Fungi	*-7*	*2.7*	*6.2*	*-4.3*	*2.4*
	Fungi	*-6.78*	*1.82*	*4.42*	*-3.58*	*4.12*
	Fungi	*-4.76*	*0.44*	*2.94*	*-2.96*	*4.34*

Table 3.1 Continued

Reference	***Type***	***Thr***	***Val***	***leu***	***Ile***	***Phe***
Larsen et al. (2013)	*Fungi*	*-10.12*	*1.18*	*6.08*	*-0.52*	*3.38*
	Fungi	*-10.02*	*-0.22*	*5.28*	*0.38*	*4.58*
	Fungi	*-11*	*0.8*	*5.8*	*-0.5*	*4.9*
	Fungi	*-11.62*	*2.98*	*5.68*	*-1.12*	*4.08*
	Fungi	*-7.12*	*0.38*	*4.08*	*-2.32*	*4.98*
	Fungi	*-8.08*	*0.32*	*4.72*	*-1.48*	*4.52*
	Fungi	*-7.84*	*-1.14*	*4.36*	*-1.24*	*5.86*
	Fungi	*-7.82*	*-1.12*	*6.18*	*-1.62*	*4.38*
	Fungi	*-7.04*	*0.06*	*5.26*	*-2.14*	*3.86*

Table 3.2: *Table of unique $\delta^{13}C$ patterns (fingerprints) of different essential amino acids (EAAs) from compound specific isotope analysis (CSIA-AA) and bulk stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values (in ‰)) of female Dall's sheep forage items (n=35) from the Chugach Mountains (Note: 35 of 40 forage samples produced quantifiable peaks). All values represent means of triplicate analysis for bulk stable isotopes and the following EAA's: threonine (Thr), valine (Val), leucine (Leu), isoleucine (Ile) and phenylalanine (Phe). The 'Fingerprint Assignment' column identifies how the terrestrial C_3 plants, lichens, and mushrooms collected in this study mapped in a Linear Discriminant Analysis when using previously published primary producer end-members (Table 3.1, Larsen et al., 2009, 2013)*

Scientific Name	Common Name	Forage Type	Thr	Val	leu	Ile	Phe	Bulk $\delta^{15}N$ (‰)	Bulk $\delta^{13}C$ (‰)	Fingerprint Assignment
Salix glauca	Grayleaf Willow	Deciduous shrub	-14.2	4.2	8	-0.7	2.7	-4.2	-28.2	Plant
Diapensia lapponica	Lapland Diapensia	Deciduous Shrub	-9	3.5	5.8	-1.9	1.6	-3.2	-28.7	Mushroom
Potentilla fruticosa	Shrubby Cinquefoil	Deciduous Shrub	-12.8	4.1	7.6	-2.3	3.4	-3.1	-29.1	Mushroom
Arctostaphylos alpina	Alpine Bearberry	Deciduous Shrub	-11.7	4.6	7.4	-1.6	1.4	-9.1	-28	Plant
Salix alaxensis	Felt-leaf Willow	Deciduous Shrub	-13.1	4.8	6.1	-1	3.2	-5.1	-29.3	Plant
Betula nana	Dwarf Birch	Deciduous Shrub	-14	5	4.5	1.5	2.9	-0.6	-29.4	Plant
Vaccinium uliginosum	Alpine Blueberry	Deciduous Shrub	-11.5	4.5	7.1	-2	2	-3.7	-29.4	Plant
Salix monticola	Mountain Willow	Deciduous Shrub	-6.7	2.8	5	-1.9	0.8	-3.8	-28.7	Mushroom
Dryas octopetula	White Mountain Avens	Evergreen shrub	-12.1	4.1	7.4	-1.4	2.1	-3.7	-28.4	Plant

Table 3.2 Continued

Scientific Name	**Common Name**	**Forage Type**	**Thr**	**Val**	**leu**	**Ile**	**Phe**	**Bulk $\delta^{15}N$ (‰)**	**Bulk $\delta^{13}C$ (‰)**	**Fingerprint Assignment**
Harrimanella stelleriana	Alaskan Mountain-Heather	Evergreen Shrub	-8.8	4.1	6.6	-2.2	0.3	-8.7	-27.8	Plant
Empetrum nigrum	Crowberry	Evergreen Shrub	-10.8	4	7.2	-2.4	2	-8.7	-27	Mushroom
Loiseleuria procumbens	Alpine Azalea	Evergreen Shrub	-15.3	5.5	8.1	-0.7	2.4	-6.7	-28.1	Plant
Epilobium latifolium	Dwarf Fireweed	Forb	-12.4	3.7	6.3	-0.4	2.8	-1.1	-28.5	Plant
Delphinium glaucum	Larkspur	Forb	-12	3	6.1	-0.4	3.2	0.8	-26.5	Mushroom
Geum rossii	Ross Avens	Forb	-12	3.3	6.5	-0.3	2.5	-2.1	-27.6	Plant
Polemonium pulcherrimum	Jacob's Ladder	Forb	-13	4	6.4	-0.4	3.1	-0.7	-29.1	Plant
Lupinus arcticus	Arctic Lupine	Forb	-10.6	4	5.4	-0.5	1.8	0.9	-28.3	Plant
Saxifraga bronchialis	Yellow Spotted Saxifrage	Forb	-10.7	3.5	5.2	-0.4	2.4	-3.8	-27.9	Mushroom
Aconitum delphiniifolium	Monkhood	Forb	-11.4	3.6	6.2	-1.3	2.9	-4.9	-26.8	Mushroom

Table 3.2 Continued

Scientific Name	**Common Name**	**Forage Type**	**Thr**	**Val**	**leu**	**Ile**	**Phe**	**Bulk $\delta^{15}N$ (‰)**	**Bulk $\delta^{13}C$ (‰)**	**Fingerprint Assignment**
Geranium erianthum	Woolly Geranium	Forb	-14.6	5	6.4	0	3.2	-4.4	-28.7	Plant
Castilleja unalaschcensis	Yellow Paintbrush	Forb	-13.4	3.8	6.7	0	2.9	-3.3	-27.9	Plant
Epilobium angustifolium	Common Fireweed	Forb	-5.4	3	5.3	-3.6	0.7	-2	-30.2	Mushroom
Anemone narcissiflora	Narcissus Anemone	Forb	-6.6	2.9	5.7	-2.1	0.1	-3.3	-25.6	Mushroom
Campanula lasiocarpa	Mountain Harebell	Forb	-11.7	5.1	5.8	-2.4	3.2	-3.2	-27.9	Mushroom
Bromus pumpellianus	Arctic Brome	Graminoid	-12.7	3	6.4	-0.3	3.5	-2.2	-28	Mushroom
Calamagrostis canadensis	Bluejoint Reedgrass	Graminoid	-10.6	4	6	-2.2	2.7	0.5	-28.5	Mushroom
Carex microchaeta	Short Stalk Sedge	Graminoid	-12.3	4.1	6.4	-2.1	3.8	0.9	-27.6	Mushroom
Poa alpina	Alpine Bluegrass	Graminoid	-17.8	5.4	7.1	0.4	4.9	-3.3	-29	Plant
Carex bigelowii	Bigelow's sedge	Graminoid	-10.6	3.5	5	-0.6	2.7	0	-27.9	Mushroom
Vahlodea atropurpurea	Mountain Hairgrass	Graminoid	-14	3.9	5.6	-0.1	4.6	-3.9	-27.4	Mushroom

Table 3.2 Continued

Scientific Name	**Common Name**	**Forage Type**	**Thr**	**Val**	**leu**	**Ile**	**Phe**	**Bulk $\delta^{15}N$ (‰)**	**Bulk $\delta^{13}C$ (‰)**	**Fingerprint Assignment**
Festuca ovina	Rocky Mountain Fescue	Graminoid	-8.2	4.2	3.6	-1.4	1.8	-2.8	-27.1	Mushroom
Carex aenea	Bronze Sedge	Graminoid	-9.1	3.9	4.8	-0.1	0.5	-8.3	-26.6	Plant
Nephroma arcticum	Arctic Kidney Lichen	Lichen	-8.7	2.8	3.7	-1.9	4	-2	-21.8	Mushroom
Boletus edulis	King Bolete	Mushroom	-8.2	3.6	5.3	-2.8	2	-2.1	-24.8	Mushroom
Russula xerampelina	Shrimp Russula	Mushroom	-6	5.3	2.8	-4.9	2.8	3.8	-27.6	Mushroom

Table 3.3: *Comparison, using a one-sample z test of a set of terrestrial C_3 plant forage items (n=32 of 35 or 94%) in female Dall's sheep summer diet in the Chugach Mountains and previously published end-members (Table 3.1., Larsen et al., 2009, 2013). Based on a Linear Discriminant Analysis of different unique $\delta^{13}C$ patterns (fingerprints) of the essential amino acids (EAAs): threonine (Thr), valine (Val), leucine (Leu), isoleucine (Ile), and phenylalanine (Phe).*

One sample proportion z test	
Test Proportion (H_0)	*0.94*
Sample Size (n)	*32*
Frequency	*16*
Sample Proportion	*0.50*
Test Statistic	*-10.79*
P =	*3.72E-27*
α =	*0.05*
Percent of C_3 Plants that Mapped as C_3 Plants	*50 %*

3.9 Figures

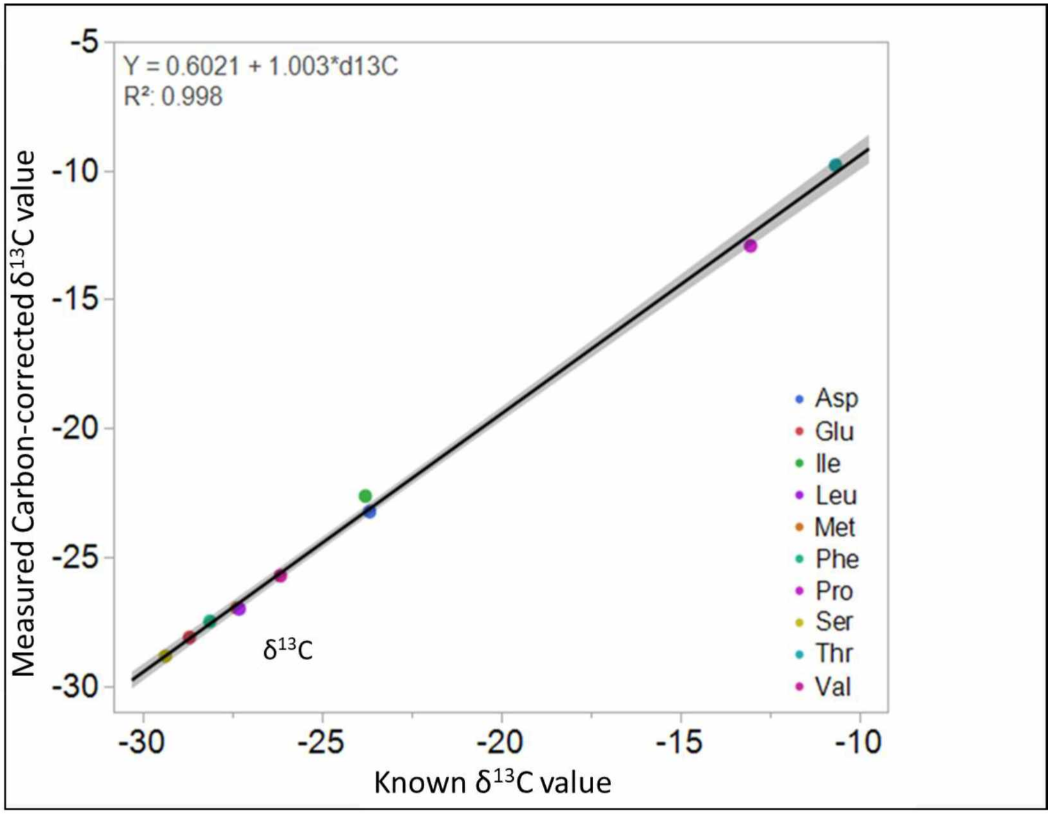

Figure 3.1: *Relationship between known $\delta^{13}C$ values of individual amino acids [aspartic acid (Asp), glutamic acid (Glu), isoleucine (Ile), leucine (Leu), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), and valine (Val)] represented by colored symbols in the mixed amino acid standard UAF AA2 and measured-carbon-corrected $\delta^{13}C$ values of UAF AA2 using a separate mixed amino acid standard (UAF AA1) from all derivatization batches (n = 99). Measured $\delta^{13}C$ values are based on means from triplicate injections of the same standard. The gray colored regions show the 95% confidence interval for the fitted linear regression line and the linear regression equation is provided in the graph.*

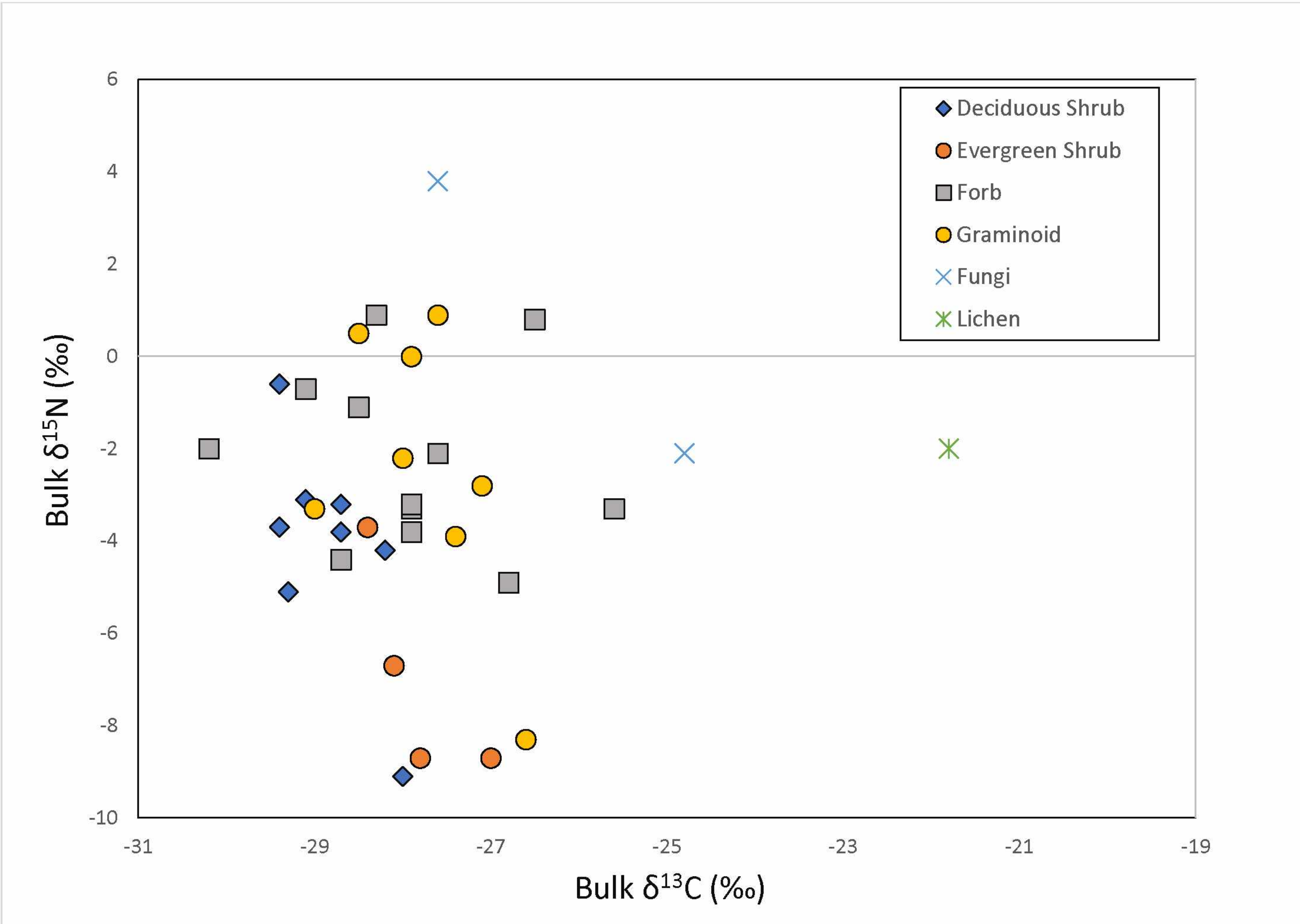

Figure 3.2: Bulk carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope per mil (‰) values for a subset (n = 35) of female Dall's sheep summer forage samples collected during 2016-2018 in the Chugach Mountains, Alaska. Different symbols and colors represent different forage types. The gray line represents 0, as there are $\delta^{15}N$ values that are both positive and negative.

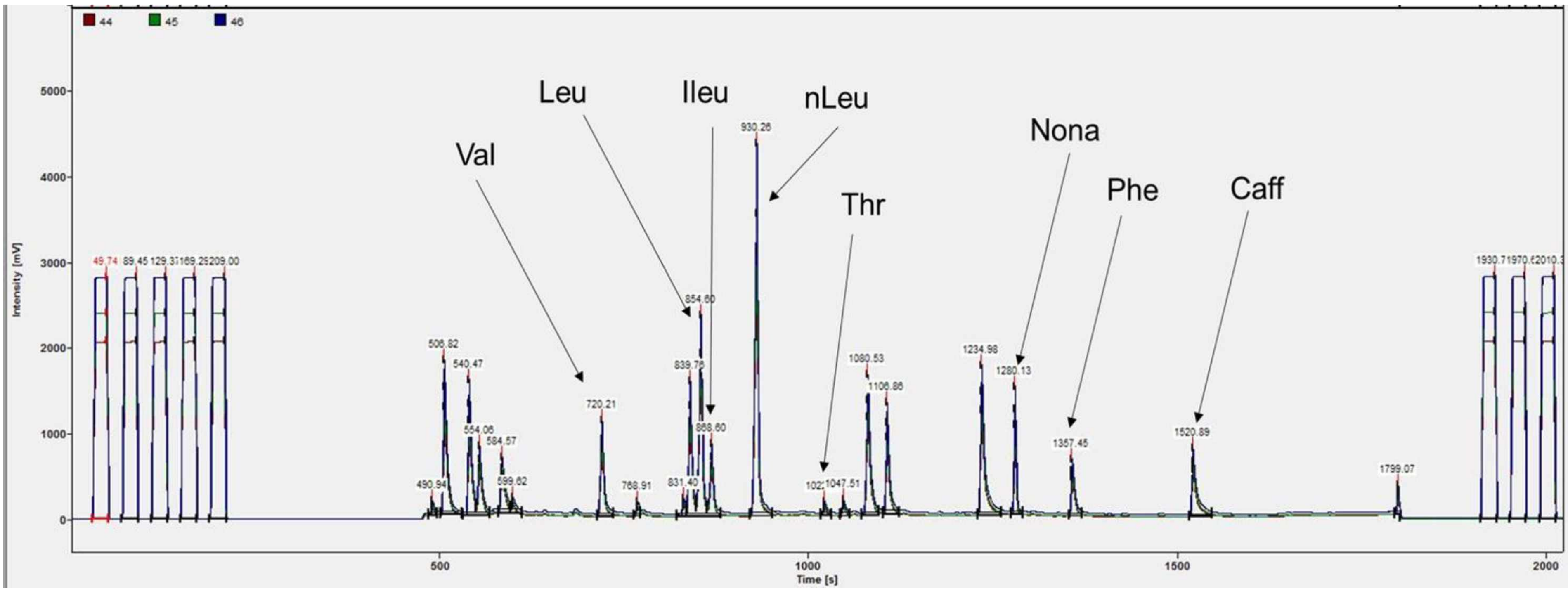

Figure 3.3: *A sample chromatogram produced by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) during the compound-specific stable isotope ($\delta^{13}C$) analysis of individual amino acids (AA) of a subset of female Dall's sheep summer forage samples. These forage samples were collected study in the Chugach Mountains of Alaska, during the summers (June 1-August 31) of 2016, 2017, and 2018. This particular chromatogram came from a triplicate run of the forb (herbaceous shrub) Larkspur (Delphinium glaucum). The labeled peaks are the internal standards used: norleucine (nLeu), nonadecane (Nona), caffeine (Caff), as well as the essential AAs: threonine (Thr), valine (Val), isoleucine (Ile), leucine (Leu), and phenylalanine (Phe).*

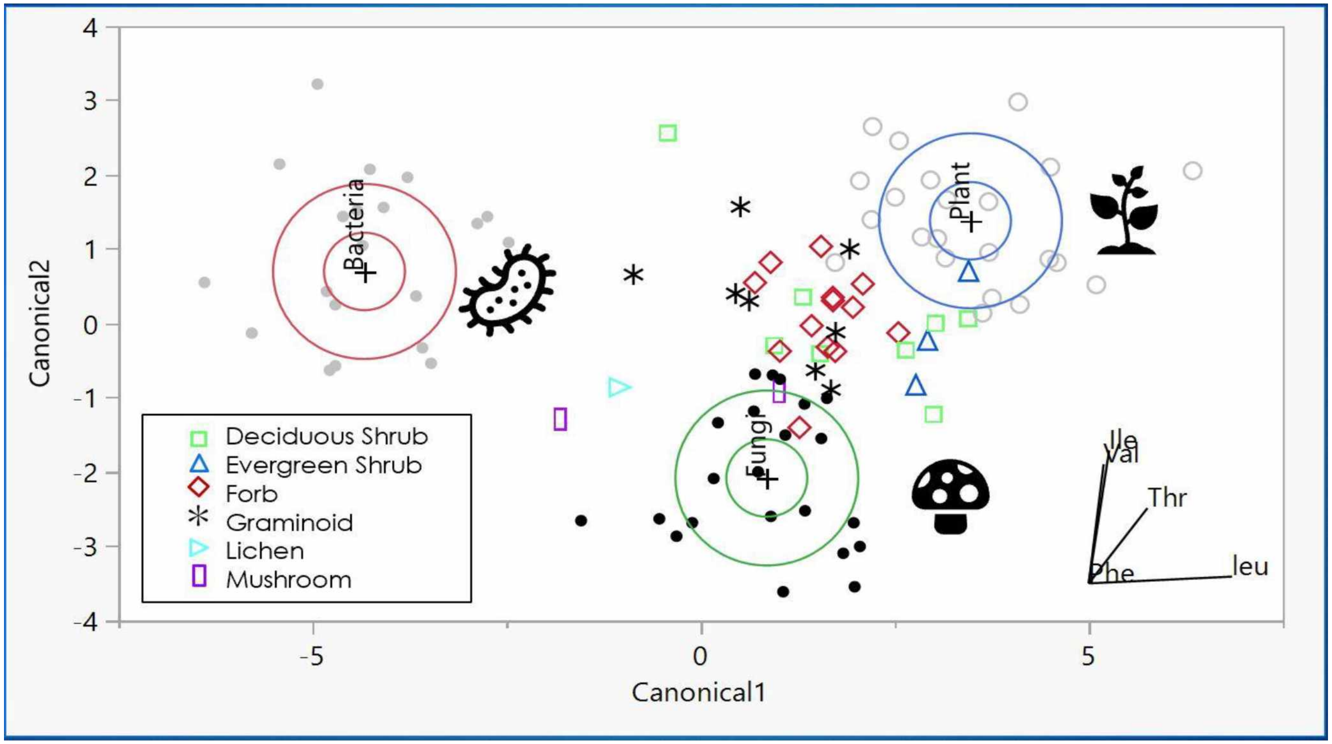

Figure 3.4: Results from the Linear Discriminant Analysis (LDA) of a compound-specific carbon stable isotope ($\delta^{13}C$) analysis of individual amino acids (CSIA-AA) of a subset (n=35) of female Dall's sheep summer forage samples (deciduous shrubs, evergreen shrubs, forbs, graminoids, lichen, and mushrooms) collected in the Chugach Mountains of Alaska during the summers (June 1-August 31) of 2016, 2017, and 2018. These samples are indicated as astricts and are mapped alongside of known CSIA-AA values for the end-member: bacteria, fungi, and plants from Larsen et al. (2009, 2013) represented by gray dots, black dots, and open circles. The circles represent the interquartile range of the values for the end-members. The vectors represent the five AA (Ile-isoleucine, leu-leucine, Phe-phenylalanine, Thr-threonine, and Val-valine) used in this study, as the differences between the $\delta^{13}C$ of these five proved to be most informative.

Chapter 4: General Conclusion

The first part (Chapter 2) of my research described the summer diet composition and forage quality for female Dall's sheep in the Chugach Mountains of Alaska. Dall's sheep summer diet composition has been assessed in several other mountain ranges across their native range, but never in the Chugach Mountains of south-central Alaska. Previous analysis suggests that Dall's sheep diet varies from one mountain range to another (Ayres, 1986; Hoefs & Cowan, 1979; Jones et al., 1963; Nichols, 1971; Valdez & Krausman, 1999; Whitten, 1975; Winters, 1980). These studies point to a high degree of variability in Dall's sheep diet based on location. Our findings may serve as a baseline for future research in the Chugach Mountains and a comparison to other mountain ranges throughout Alaska. We showed that summer diet composition changed annually and from early summer to late summer.

We examined forage quality in the form of crude protein and gross energy, components critical to vital physiological functions that affect survival and reproduction. Both varied significantly between some years and all years showed variability within the summer season (early to late). We also noted that there were similar changes in the apparent digestibility (D_a). We recommend that future research investigates factors driving these changes.

We also noted weather conditions [average daily temperature (°C) and total precipitation (cm)] during our study period and evaluated the association with forage quality. The Arctic is warming at a rapid rate, and the effects of this warming on Dall's sheep are not widely understood. Evidence strongly suggests that climate change is having an ecosystem wide effect in many northern latitude environments (Aycrigg et al., 2021; Cooper, 2014; Hassol & Corell, 2006; Juhasz et al., 2020). With this warming, some arctic summers have been hotter and drier. These conditions may have a negative effect on summer forage for arctic herbivores similar to Dall's sheep (Lenart et al., 2002). We did not observe any major annual differences in weather conditions (temperature and precipitation) during this study, it is therefore difficult to determine how growing conditions, forage availability, and nutrient composition of Dall's sheep forage may change under more extreme climate scenarios. From what we observed moderate weather conditions coincided with the highest Dall's sheep forage quality.

The second part (Chapter 3) of my research investigated the use of compound-specific stable carbon isotope analysis of amino acids (CSIA-AA) as a potential tool for sourcing Dall's sheep diet. We tested to see if we were able to distinguish clear clusters of CSIA-AA values for different forage types using a linear discriminant analysis (LDA). We did not observe clear clustering among our different terrestrial C_3 plant forage sample types, suggesting that CSIA-AA might not prove useful in tracing different Dall's sheep diet sources in the manner we originally predicted. We also tested to see if the CSIA-AA values of our alpine-derived, terrestrial C_3 plant Dall's sheep forage samples might be unique from previously mapped terrestrial C_3 plant end-members sourced from different habitats. Some of our terrestrial C_3 plant forage item CSIA-AA values mapped differently compared to known data values (Larsen et al., 2009 & 2013). We found that 50% of our terrestrial C_3 plant forage samples that we evaluated when classified in an LDA with known end-members (bacteria, fungi and terrestrial C_3 plants) mapped as fungi. This could be a result of alpine terrestrial C_3 plants relying on another avenue for amino acid uptake due to their unique environment, mycorrhizal relationships (Gardes & Dahlberg, 1996; Haselwandter & Read, 1987; Cripps & Eddington, 2005), or potentially another fungal based source in the soil. Previous studies have shown that climate change can affect mycorrhizal function and plant compositions. If through this we can understand how climate change is influencing plant composition and resource acquisition, this could lead to further investigation into the herbivore-plant interactions between those plants and the Dall's sheep consuming them.

Managing any major population of wildlife across large scales is a struggle even without the threat of rapidly changing conditions within their habitat. Our project provides a baseline of nutritional data for Dall's sheep to better assess future change. Collectively, Chapter 2 helps provide a snapshot of current nutrition and Chapter 3 gives us a tool to track nutrition across time. Based on the stable nature of isotopes, there is the potential to assess archived tissue samples stored at the Alaska Department of Fish and Game in conjunction with any future samples collected.

Future studies into how climate-related weather condition changes (e.g., warming, shifts in plant community composition, rain on snow events) are affecting Dall's sheep dynamics and their forage could further the understanding and management of their subpopulations across Alaska. If climate change continues to alter the Dall's sheep habitat at the current rate, the species may struggle to respond. Monitoring nutrition could be a key component to building management strategies that foster resiliency in future populations.

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