Stable Isotopes Analysis of Caribou Antlers as Ecological Indicators

By

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Abstract

This study aims to determine whether *Rangifer tarandus* (caribou) antlers provide a unique isotopic signal relative to other hard tissues such as bone and teeth using stable isotopes of oxygen, carbon, and nitrogen. Variation in the rate and timing of tissue development should create different stable isotope profiles for each tissue. Tissue from fifteen male specimens housed at the Canadian Museum of Nature were sampled. Stable isotope analysis was conducted along the length of the antler, on the third molar, and the mandible. Isotopic differences were found between the three tissues, with the carbonate carbon ($\delta^{13}C$) and collagen nitrogen ($\delta^{15}N$) isotopes showing significant patterns of variation along the length of the antlers. Isotopic variation along the antler length could potentially reflect ecological or physiological changes within the male caribou. Addition testing with plant samples, including mixing models with antler values, may provide deeper insight into this isotopic variation.

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1.0 Introduction

Rangifer tarandus (caribou) are of both ecologic and socio-economic importance to the North. They are a keystone species – a species that defines the structure of an ecosystem through regulation of fundamental ecosystem processes – of the polar food web, providing a food source for Arctic carnivores (e.g. wolves and bears) and many Indigenous societies (Wolfe, 2004; Barber *et al.* 2018; Latham *et al.* 2013; DeMars & Boutin, 2017; Dickie *et al.* 2017; McLoughlin *et al.* 2003). Caribou in the North play an important ecological role, as they help to maintain the Arctic ecosystem through plant-herbivore interactions (Mallory & Boyce, 2018; Van der Wal, 2006) and distribute nutrients over long distances (Post *et al.* 2009; Van der Wal *et al.* 2007). For some indigenous groups, caribou represent an important economic resource and are of cultural significance (Wolfe, 2004; Mallory & Boyce, 2018; Uboni *et al.* 2016). However, climate change in the Arctic region has resulted in the decline of many of Canada's major caribou populations (Mallory & Boyce, 2018; Vors & Boyce, 2009; Albon *et al*. 2017; Fauchald *et al*. 2017) and it is unclear the degree to which their ecology has changed during these decades of accelerated anthropogenic climate change (i.e. since the 1950's). Studying caribou ecology through time requires analysis of both modern and Pleistocene caribou. Antlers would provide a non-lethal approach to field work study of modern caribou and make use of existing museum collections of Pleistocene caribou. Using stable isotope analysis, this study aims to determine whether antlers (as compared to other hard tissue of *Rangifer tarandus*) provide a unique and informative

isotopic signature with the long term goal of determining whether caribou antler isotopes can help us better understand the ecology of caribou in Canada.

1.1 Environmental Impacts of Climate Change

Ongoing climate change is having profound global impacts, affecting animals, plants, and people. Scientists are making a global effort to better understand climate change and its implications, from studies on industrial development and human utilization (i.e. land use) to ecological state shifts and animal welfare (Parmesan, 2006; Dirzo *et al.* 2015). Our understanding of climate change and its ecological impacts is still evolving. An increase in global temperatures is driving global shifts in weather systems, vegetation dynamics, and the loss of animal habitat (Barnosky *et al.* 2012; Ceballos *et al.* 2015; Parmesan, 2006). Currently an estimated one million species are threatened with extinction due to climate change and anthropogenic impacts (IPBES, 2020). However, no region is experiencing larger perturbations in climate and ecology than the poles. Both the Arctic and Antarctic now have longer summers, shorter winters, and are experiencing increased ice and snow melting and precipitation (Post *et al.* 2009; Trenberth *et al.* 2007). The poles have not experienced a comparable degree of warming for thousands to millions of years. In the Canadian Arctic, for example, the temperature has increased roughly 0.8 – 1.2 °C over the past 150 years, the highest is has been for the last two million years (Trenberth *et al.* 2007; Ballantye *et al.* 2006; Post *et al.* 2009).

Temperature increases are amplified at the poles more than anywhere else on Earth due to the system of feedbacks between sea ice and snow extent, and solar

energy (Graversen & Wang, 2009). Albedo is a measure for solar energy reflected off the surface of the Earth. Ordinarily, the poles have a high albedo, as white snow and sea ice reflect solar rays away from the surface. However, climate change has critically altered this system. As temperatures rise, snow and sea ice extent have decreased significantly, causing a decrease in the overall albedo in the Polar Regions, allowing solar energy to reach the surface and further promoting snow and sea ice melt (Hall, 2004). Due to the decreased albedo amplifying the effects of climate change, the polar ecosystems have warmed at over twice the rate of the global average (NSIDC, 2020). The pronounced climate change experienced in the Arctic has resulted in alterations of vegetation dynamics and ecological landscape, with many species now endangered – such as the beluga whale, barren-ground caribou, eskimo curlew, and ivory gull (COSEWIC, 2019).

Polar Regions have comparatively low species diversity (i.e. are depauperate) and polar species are highly specialized, having adapted to their low productivity, highly seasonal environments (Anisimov *et al.* 2007; Mendoza & Araújo, 2019). Such highly specialized species may fail to adapt to the amplified rate of the ecological change at the poles, leading to their extinction (Anisimov *et al.* 2007; Matveyeva & Chernov, 2000). Due to the limited biocapacity of polar food webs, the decrease or possible extinction of one species may have substantial impacts on the remaining biota. The loss of keystone species (e.g. caribou, lemmings), in particular, can have cascading effects that deeply impact the remaining species. In Greenland, collared lemmings – a keystone species in the tundra ecosystem – are on the decline due to climate change (Schmidt *et al.* 2012; Gilg *et al.* 2012; Ims & Fuglei, 2005). The collapse of this lemming population has led to

the reduction of high-arctic predators like the snowy owl and stoat (Schmidt *et al.* 2012; Gilg *et al.* 2009; Ims & Steen, 1990). Thus, of all the global species, polar species are particularly vulnerable to climate change (Anisimov *et al.* 2007).

Warmer temperatures have also allowed the invasion of new species into polar environments. In areas like the Arctic, extended warm growing periods have allowed the encroachment of southern plant species (Post *et al.* 2009; Jepsen *et al.* 2008; Christensen *et al.* 2007). In some cases, the specialized polar species struggle to compete with the invading species (Kutz *et al.* 2009; Polley & Thompson, 2009; Davidson *et al.* 2011). As southern shrub and tree populations expand northward, they alter the Arctic soil through increased microbial activity, higher winter soil temperatures, and increased nutrient mineralization rates (Post *et al.* 2009; Sturm, 2005). Soil alteration has created a positive feedback loop, promoting the further growth of southern shrubs and trees at the expense of native plant populations (Strum, 2005; Post *et al.* 2009). As southern shrub and tree populations expand northward, they also bring foreign parasites and pests that can cause significant disruption to native plant communities (Callaghan & Johansson, 2009; Post *et al.* 2009). This “shrubification” of the Arctic is also having bottom up effects that impact Arctic megafauna like caribou and musk oxen, as the southern plants provide comparatively poor nutrient sources (Mallory & Boyce, 2018; Thompson & Barboza 2014; Post *et al.* 2009). Alteration of the plant growth season in the Arctic has created a trophic mismatch, leading to peak demand for resources by reproductive megafauna females falling later than seasonal

peak resource availability. This has contributed to a reduction in survival of offspring (Post *et al.* 2009; Post & Forchhammer, 2008).

The impact of climate change on megafauna is particularly important within the Arctic ecosystem as Arctic megafauna (e.g. megaherbivores) are essential to the maintenance of Arctic ecosystems because they are ecological engineers (Macias-Fauria *et al.* 2020). Species like caribou, muskoxen, bison, and Yakutian horse are important for maintaining the Arctic tundra ecosystem. Arctic megaherbivores reduce the shrubification of the tundra ecosystem through grazing, trampling and nutrient recycling – i.e. fecal deposition (Post *et al.* 2009; Van der Wal *et al.* 2007). The tundra ecosystem has more reflective surfaces than ecosystems dominated by shrub or forest. Forest and shrub ecosystems have limited exposed snow cover, which creates a smaller reflective surface than the tundra ecosystem. Megaherbivores within the Arctic help increase exposed snow cover by reducing the shrubification of the tundra and negating some of the loss of albedo (Zimov *et al.* 2012). Migration of the Arctic megaherbivores also causes the trampling of snow cover, which leads to colder, deeper freezing in the winter soil – increasing natural permafrost (Macias-Fauria *et al.* 2020; Zimov *et al.* 2012). Arctic megaherbivores are a natural solution to mitigate the rapid Arctic warming (Macias-Fauria *et al.* 2020; Griscom *et al.* 2017). Their loss would further increase the already rapid warming of the Arctic (Mallory & Boyce, 2018; Macias-Fauria *et al.* 2020). Macias-Fauria *et al.* (2020) proposed a climate change mitigation plan involving the reintroduction or expansion of bison, Yakutian horse, and caribou populations to Arctic ecosystems.

For plans like those proposed by Macias-Fauria *et al.* (2020) to be viable, we need to understand how Arctic species have and will likely respond to climate change. However, this requires access to appropriate, widely available, and informative ecological baseline data that can be used to compare the historical and paleontological ecology of Arctic megaherbivores and their modern counterparts. In this regard, the long term goal of my research is to use stable isotopes from antlers to understand how the ecology of caribou has or has not changed over long (thousands of years) and short (tens of years) timescales.

1.2 Canadian *Rangifer tarandus*

Caribou are found across the northern half of Canada. The majority of Canadian caribou are classified as a migratory species that are divided into three distinct subspecies. The division of subspecies is based on overall body size, antler shape, fur colour, and habitat. They include the Boreal Woodland Caribou, the Mountain Caribou, and the Barren-Ground Caribou. The Barren-Ground Caribou (*Rangifer tarandus groenlandicus*) is the smallest of the three distinct subspecies, distinguishable by their large curvy antlers, lighter pelage, and common habitat range of the Northwest Territories and Nunavut (COSEWIC, 2019). The Barren-Ground Caribou are the subject of my thesis, selected for their northern population distributions throughout Canada (Fig. 1) (Parlee *et al.* 2018).

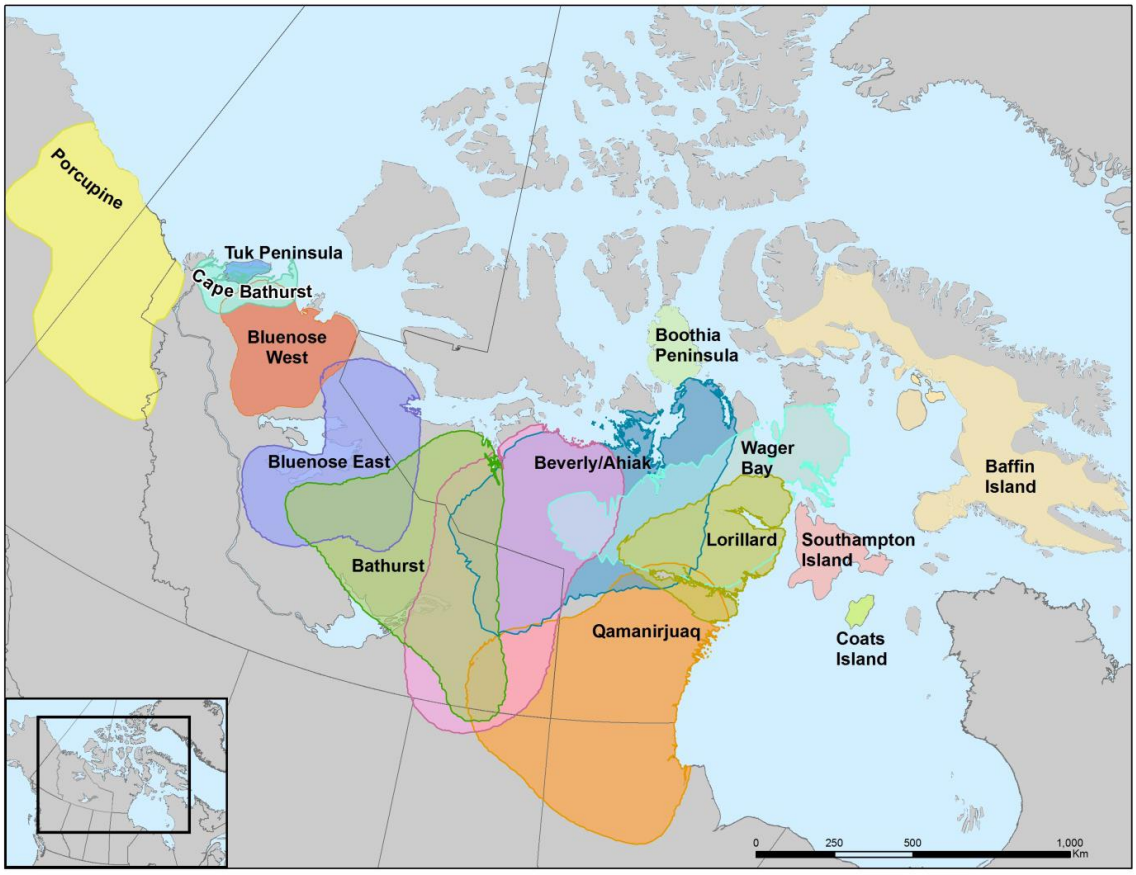


Figure 1. Barren-Ground Caribou populations within Canada (stars mark sampling location from Canadian Wildlife Services study in 1966-68) (COSEWIC, 2011; Parlee et al. 2018).

The Barren-Ground Caribou populations in Canada fluctuate in size on a decadal scale, with the most recent peak being in the late 1980s and early 1990s (BQCMB, 2020; Evans, 2019). However, they have since been on a steady decline, with some populations experiencing more drastic declines than others (Mallory & Boyce, 2018; Post *et al.* 2009). While the significance of the decline varies, Canadian science groups have been working to better understand Barren-Ground Caribou population dynamics. They have shown that changing Arctic climate and ecology has, overall, had negative consequences for Barren-Ground Caribou populations. Several Canadian populations of

this subspecies are expected to decrease by as much as 50% in the next 8 – 15 years (Barber *et al.* 2018). In addition, many are now classified as populations of special concern, threatened, or endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2014).

Many environmental factors caused by climate change are impacting the Barren-Ground Caribou populations of Canada. Increased periods of warming have resulted in longer summers allowing a longer grazing period, but it has also resulted in changing plant communities which has negative nutritional consequences (Mallory & Boyce, 2018; Thompson & Barboza, 2014). The plant communities are becoming more abundant in woody plants, which have a greater chemical and structural defense against herbivory, in addition to low quantities of protein (Thompson & Barboza, 2014). Warmer periods also increase the number of parasitic flies, which can result in decreased body condition and reproduction of the Barren-Ground Caribou (Mallory & Boyce, 2018; Thomas & Kiliaan, 1990; Albon *et al*. 2002; Hughes *et al.* 2009; Ballesteros *et al.* 2012; Cuyler *et al.* 2012; Pachkowski *et al.* 2013). Finally, warming is associated with an increase in icing events during the winter and an increase in wildfires during the summer. Both can cause catastrophic starvation periods for Barren-Ground Caribou populations, greatly altering their mortality as well as winter and summer ranges (Mallory & Boyce, 2018; Tyler, 2010; Joly *et al.* 2012; Anderson & Johnson, 2014).

1.2.1 Qamanirjuaq Population

The largest population of Barren-Ground Caribou is the Qamanirjuaq population, identified in Figure 1 in orange (COSEWIC, 2014; BQCMB, 2020). The herd is located primarily in Nunavut but has a migratory range that expands west into the Northwest Territories and south into northern Saskatchewan and Manitoba. The Qamanirjuaq herd, along with another population, the Beverly herd, have been harvest by the Inuit, Dene, Cree, and Métis indigenous groups for decades (Evans, 2019). In 1982, the Beverly and Qamanirjuaq Caribou Management Board (BQCMB) was established to safeguard the Qamanirjuaq and Beverly herds of Barren-Ground Caribou. Among other activities, BQCMB has studied the population dynamics of these two populations. In 1994, BQCMB estimated the Qamanirjuaq caribou population at 496,000. However, the herd has been on a steady decline (about 2% per year) and as of 2017 its population was estimated at 288,000. BQCMB has rated the Qamanirjuaq population as being of a medium to high vulnerability of decline and has advised that if the population continues to decline it may pass the point of recovery (BQCMB, 2020).

Given its size and socio-economic importance, the Qamanirjuaq population has been the subject of numerous scientific studies over the years (Parker, 1973; Miller F.L., 1973; Dauphine, 1973; Miller D.R., 1973; Drucker *et al.* 2010; 2012). These studies, along with the work of BQCMB, are the reason I have chosen the Qamanirjuaq population for my research as the previous work has provided the ecological information necessary to contextualize the stable isotope analyses performed as part of my thesis.

1.3 Canadian Wildlife Services Study

A Canadian Wildlife Services (CWS) study was conducted between 1966 and 1968 with a focus on creating a better understanding of caribou population dynamics, human utilization, and range conditions of the Qamanirjuaq and Beverly populations. The project was developed in response to conservation concerns related to the decreasing number of caribou during the 1940s. During the study, 999 Caribou were culled, 943 were from the Qamanirjuaq population and 56 from the Beverly population.

The CWS study was divided into four areas of review, each with its own set of goals (Parker, 1973; Miller, F.L. 1973; Dauphine, 1973; Millar, D. R. 1973). The areas of study included:

1. Total numbers, mortality, recruitment, and seasonal distribution
2. Sex and age composition
3. Seasonal physical and reproductive condition
4. Range evaluation

My thesis was conducted using caribou tissue gathered during the CWS study. Information on the range and habitat (including migration patterns) and the dietary preferences of the Qamanirjuaq population gathered during the study is summarized below. This information is relevant to the stable isotope analysis that was conducted as part of my thesis on the caribou tissue.

1.3.1 Range and Habitat

The range of the Qamanirjuaq population is limited by climatic conditions during both the summer and winter. The population's range is approximately 282,310 km^2, incorporating portions of two provinces, Manitoba (102,020 km^2) and Saskatchewan (16,680 km^2), and the districts of Mackenzie (12,690 km^2) and Keewatin (147,890 km^2) within Nunavut. The herd ranges above the tree line during the summer season in the tundra ecosystem, then retreats below it during the winter months to the boreal forest ecosystem – see Figure 2 (Parker, 1973).

The range of the Qamanirjuaq population includes several ecozones: true tundra, a transition zone and boreal forest. True tundra is characterized by frequent outcrops, glacial drifts, muskegs, and low flat *Carex* (Sedge) meadows. South of the true tundra environment is a forest-tundra transition zone marked by intermixed vegetation of *Carex* meadows and a few stunted trees (i.e. black spruce, willow, birch, and tamarack). South of the tree line is reached the area is dominated by boreal forest, which is primarily black and white spruce, with the infrequent growth of other trees such as white birch, tamarack, jack pine, stunted aspen, and balsam poplar (Larsen, 1965; Parker 1973). The temperatures of the true tundra range with mean monthly temperatures of -25.5°C to 9°C, while the temperature range in the southern part of the Qamanirjuaq distribution (i.e. in northern Manitoba and Saskatchewan) is warmer with mean monthly temperatures of 15°C and minimum temperatures of -20°C (Parker, 1973; Kendrew & Currie, 1955). The entire range of the Qamanirjuaq population

experiences a mean annual snowfall of roughly 120 to 150 cm, while total annual precipitation rarely exceeds 400 mm (Parker, 1973).

Figure 2. Ecozones and ecoregions within the Beverly and Qamanirjuaq caribou ranges taken from the Government of Northwest Territories, Government of Nunavut, Timoney et al. (1992), arrows showing spring migratory pattern. Star indicates sample collection site.

1.3.2 Migration Pattern

The migration pattern of the Qamanirjuaq population is largely unchanged since the CWS study (as illustrated in Fig. 3). The majority of the herd, excluding adult males, winter in northern Saskatchewan and Manitoba. Adult males tend to scatter throughout the forest region during the winter as either individuals or small bands. Spring migration begins in April, with cows, calves, and yearlings moving north/northeast (Fig. 3). The herd moves in bands that vary in size from a few individuals to thousands. While cows, calves, and yearlings move north, the majority of males older than 23 months do not leave the tree line until June. Throughout June and July, adult males remain scattered and only begin migrating north towards the end of July. After August, caribou retreat southward and congregate into three main groups: the Churchill, Duck Lake, and Windy Bay herds (Fig. 3). By late September, the groups begin to merge in the west near North Henik Lake in preparation for the rut (Fig. 3). After the rut (i.e. mating season) in early November, the fall migration occurs as the caribou head towards the tree line. Throughout this migration the CWS study observed that caribou of the Qamanirjuaq population travel roughly 13 miles per day (Parker, 1973).

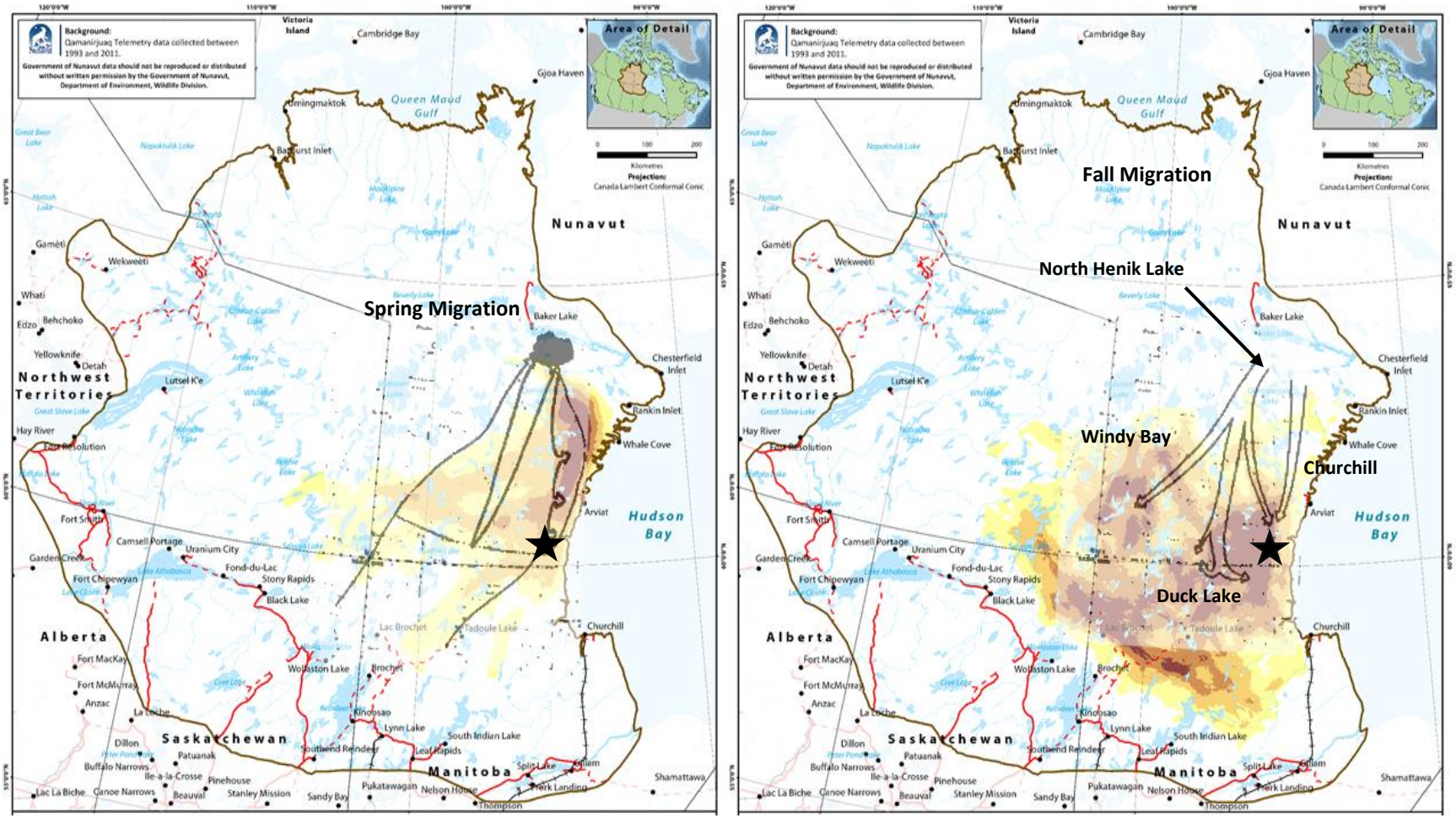


Figure 3. Spring migration pattern (left) and Fall migration pattern (right) for Qamanirjuaq caribou population. The colouration indicates the intensity of land use by the Qamanirjuaq population throughout the spring migration collected between 1996 – 2012 by the Government of Northwest Territories, Government of Nunavut, and Natural Resources Canada. Overlaid are migratory patterns identified in the CWS study for 1967 from images made by F.L. Miller in 1973 (BQCMB, 2020; Miller F.L., 1973). Star indicates the sample collection site.

1.3.3 Seasonal Dietary Changes

Seasonal dietary changes were examined as part of the CWS study. Rumen samples (i.e. stomach content) were collected throughout the 1966 to 1968 collection period. The analysis found significant differences in seasonal diets (Fig. 4). In the winter season, caribou consumed more lichen than any other plant species (Table 1), aligning with the known migration patterns (Fig. 3). The winter diet in the boreal forest was primarily made up of terrestrial lichens, *Cladina* spp. and *Cladonia* spp. *Stereocaulon*, an important food source prior to snow melt (Miller F.L., 1973).

Table 1. Percentage of occurrence of plants in caribou rumen samples. Numbers in bold indicate the number of rumens sampled. The columns are broken into the various collection periods conducted during the Canadian Wildlife Services study (Miller F.L. 1973).

	Collection Period					
	Jan.-Feb.	**April**			**November**	
Plants	**1967**	**1966**	**1967**	**1968**	**1966**	**1967**
Bryophyta	**14**	**19**	**20**	**20**	**20**	**20**
Polytrichium commune	43	21	40	10	45	25
P. junipernum	50	16	85	70	70	35
P. piliferum	36	0	66	50	55	20
***Dicranum* spp.**	50	63	95	15	20	35
Drepanocladus uncinatus	14	0	25	5	0	5
Pleurozium schreberi	57	58	85	68	20	20
Hylocomium splendens	0	0	15	0	0	0
Ptilidium ciliare	64	84	100	75	25	50
Lichens	**7**	**26**	**80**	**72**	**85**	**57**
***Cladina* spp.**	100	96	90	100	100	100
***Cladonia* spp.**	43	65	20	54	62	42
***Stereocaulon* spp.**	86	62	96	19	35	53
***Cetraria* spp.**	0	0	0	0	4	0
Podetial	100	89	97	100	100	100
5% Podetial	86	75	82	54	28	30
Conifer Needles	**11**	**10**	**17**	**16**	**23**	**33**

Larix laricina	73	50	24	31	87	52
***Picea* spp.**	100	100	100	100	78	100
Pinus banksiana	55	80	18	25	9	100
Woody Angiosperms	**12**	**16**	**16**	**16**	**15**	**16**
***Salix* spp.**	0	0	0	0	0	0
***Betula* spp.**	17	19	63	50	13	38
Rubus chamaemorus	8	0	0	19	0	0
Empetrum nigrum	0	0	6	0	0	6
***Ledum* spp.**	67	75	94	94	100	81
Loiseleuria procumbans	0	6	0	0	0	0
Kalmia polifolia	42	31	19	31	47	50
Andromeda polifolia	25	6	19	44	80	50
Chamaedaphne calyculata	0	6	0	50	40	6
Arctostaphylos rubra	0	6	0	0	0	0
Vaccinium uliginosum	92	81	75	19	67	81
V. myrtilloides	92	94	38	44	100	88
V. vitis-idaea	100	100	100	100	100	100
Oxycoccus microcarpus	0	0	6	0	20	0
Grasslike Plants	**10**	**15**	**8**	**0**	**15**	**9**
***Equisetum* spp.**	60	20	13	0	100	89

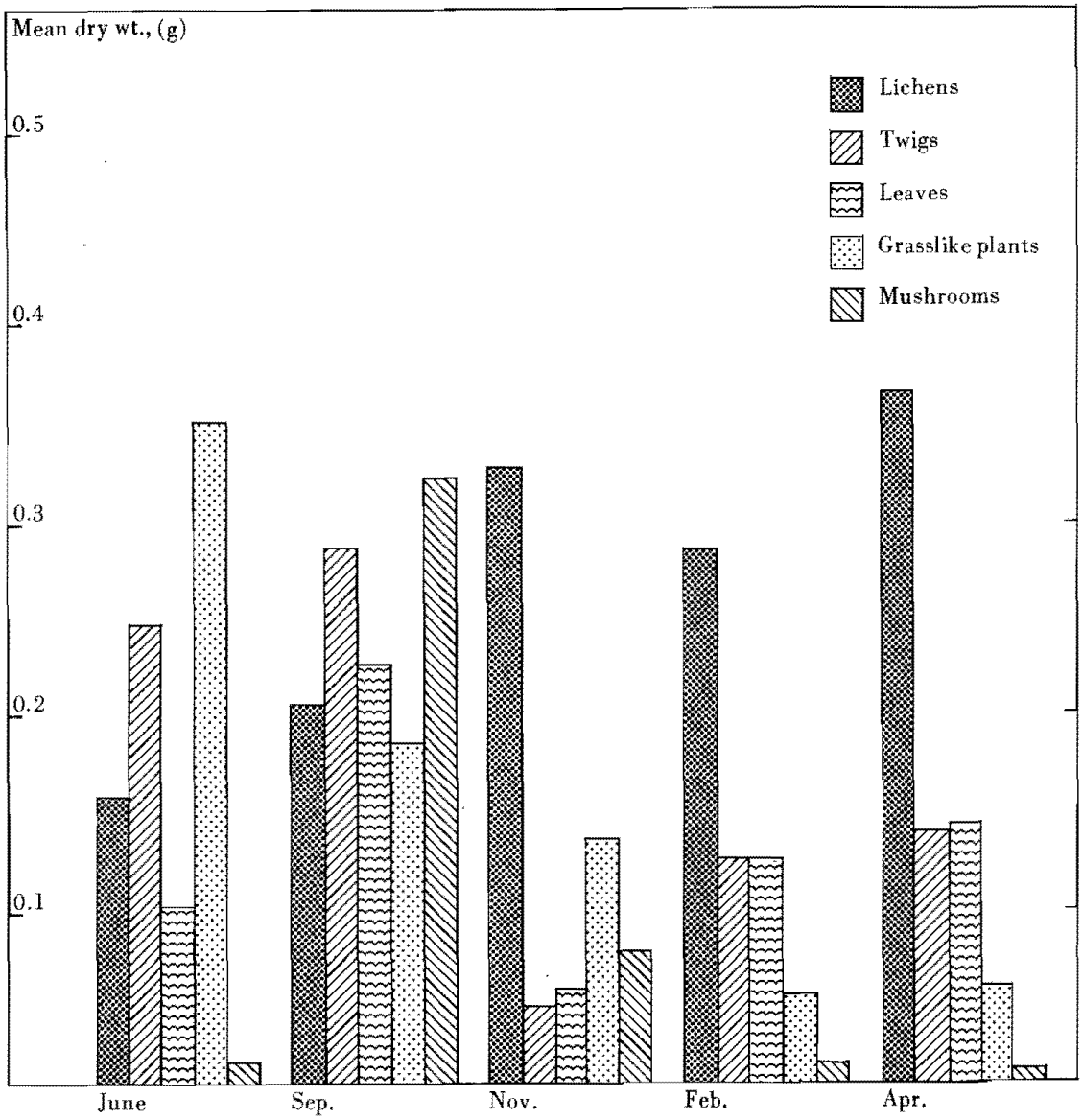


Figure 4. Weight of five major forage items found in caribou rumen samples collected between 1966-1968, months represented on the graph are additive from the three-year collection period (Miller D.R. 1973).

1.4 Stable Isotopes

Stable isotopes analysis represents an integral part of my research, stable isotopes can provide information on the ecology of *Rangifer tarandus*. My long-term goal is to determine if isotopes within caribou antlers can be used to provide that ecological information. The first step toward this goal is determining if and to what degree stable isotopes vary along the antler and vary in pattern among bone, tooth, and antler. The term 'isotope' refers to an element that has two or more forms that differ in number of neutrons in their nuclei (Hoefs, 2009). Variation in the number of neutrons changes an element's atomic mass. The variants are commonly referred to as 'heavy' or 'light', depending on whether they possess greater or fewer neutrons. Atomic mass differences create variation between the rate at which an isotope reacts creating fractionation between the isotopes. Differences in the number of neutrons can also result in isotopes that are either stable or radioactive, the latter meaning they decay over time (Fry, 2008). Stable isotopes, the focus of my thesis, are those that do not undergo radioactive decay.

Stable isotopes differ significantly in abundance within the natural environment. There is typically a 'common' isotope, which makes up usually 90-99% of the molecules of an element, and then one or more 'uncommon' isotopes, which in some isotopes can be no greater than 1% of the molecules. Within a natural system, whether as a result of abiotic or biotic factors, the ratios of these different isotopes can vary due to a process called fractionation (Koch, 2007). Fractionation results from biases in physical and chemical processes that occur due to variations in isotopic mass (i.e. of the 'heavy' and

'light' isotopes) and differences in their bonds. These physical and chemical processes are typically biased against heavier stable isotopes. In highly kinetic environments (e.g. warm environments), for example, the fractionation between the heavy and light isotopes becomes less significant as energy becomes more readily available making it easier to process heavy isotopes (Fry, 2008). In contrast, low energy can cause the preferential reaction of lighter isotopes, creating a large fractionation between heavy and light isotopes (Farquhar *et al.* 1968). Variation in the abundance of stable isotopes is therefore measured as the fractionation difference, the difference between the heavy and light isotopes in the measured substance or tissue (Fry, 2008).

Stable isotope composition is expressed in δ notation. Here the ratio of heavy to light isotope from a sample are compared to an international set standard (standards are maintained by the International Atomic Energy Association). After the two ratios are compared, they are subtracted by one then multiplied by 1000 to create a stable isotope value that is in parts per thousand (Fry, 2008).

$$\delta R^{Heavy/Light} in \text{‰} = \left\{ \frac{R^{Heavy/Light} Sample}{R^{Heavy/Light} Standard} - 1 \right\} \times 1000$$

To determine the value of a sample stable isotope analysis is used to analyze precise isotopic abundances. From these values we can begin to attempt to understand where isotopic fractionation between the isotopes has occurred. The first discovery of stable isotopes was developed roughly 100 years ago when Francis W. Aston first saw three isotopes of neon (Fry, 2008). Since then, stable isotope analysis has become increasingly valuable to the Earth and Biological Sciences. High-sensitivity mass

spectrometers have allowed a deeper understanding of stable isotope fractionation. Through the collaboration of research groups, we now have developed known global isotopic trends and a better understanding of the source of isotopic fractionation (Koch, 2007; Fry, 2008).

The application of stable isotope analysis in modern ecology has advanced greatly in the past 40 years. Scientific groups have found unique and revolutionary ways to use different stable isotopes. Global patterns and previous scientific studies have allowed more accurate predictions for the source of fractionation in modern ecology. Ecologists now use elements, such as hydrogen, oxygen, carbon, nitrogen, and sulfur to study unique components of biological and geological systems (Koch, 2007; Fry, 2008). Hydrogen and oxygen isotopes have been used to determine hydrological sources within an ecosystem, whether water is from rainfall or groundwater. Carbon and nitrogen isotopes are often used to determine dietary and trophic information about an organism. Sulfur, a component of many rocks, can be used to determine geographical changes or differences between terrestrial and marine food webs (Hobson & Wassenaar, 1999; Ehleringer & Rundel, 1989). These many studies have led to our modern understanding of stable isotope variation in ecological context and it is important we continue to work to expand this knowledge.

In completing my research, stable isotopes were examined in relation to three elements – carbon, nitrogen, and oxygen – in order to changes along the antler and differences in pattern among tissues. Below is a summary of the each of these isotopes and how they relate to terrestrial ecology.

1.4.1 Carbon: $\delta^{13}C$

Carbon has two naturally occurring stable isotopes: ^{12}C, which makes up 98.9% of all stable carbon, and ^{13}C contributing to the remainder (Farquhar *et al.* 1968). Carbon fractionation – notated as $\delta^{13}C$ – is the ratio between ^{13}C / ^{12}C isotope abundances. The $\delta^{13}C$ value can change among habitats, plant types, and within the atmosphere (Terri & Stowe, 1976; Ehleringer *et al.* 1997). In the atmosphere, carbon is found primarily within atmospheric CO_2. The $\delta^{13}C$ of atmospheric CO_2 can be affected by several different factors, including volcanism, melting permafrost, and the anthropogenic burning of fossil fuels (Hoefs, 2009; Freyer, 1979; Freyer & Belacy, 1983; Trudinger *et al.* 1999, Heimann & Maier-Reimer, 1996). However, variations in the isotopic composition of atmospheric carbon have only a slight influence on the $\delta^{13}C$ values of ecosystems (. It is only a major concern when comparing different time periods (e.g. comparing thousands of years ago to today) (Long *et al.* 2005; Iacumin *et al.* 1997; Iacumin *et al.* 2000; Richards & Hedges, 2003).

When considering terrestrial environments, atmospheric CO_2 is incorporated into plant tissues through the stomata of leaves (Hoefs, 2009). Significant differences in $\delta^{13}C$ do occur among terrestrial C_3 plants and C_4 plants, due to differences in CO_2 uptake and resulting fractionation (Kelly, 2000; Sternberg, 1989; Ehleringer, 1991; Lajtha & Marshall, 1994). Terrestrial C_3 plants (mean value of $\delta^{13}C$=–27‰, range= –35 to –21‰) use a primary CO_2-fixing enzyme called RuBP (Ribulose 1,5 Bisphosphate) that will discriminate against the heavy isotope, ^{13}C. In contrast, C_4 plants (mean value of $\delta^{13}C$ = –

13‰, range = –14 to –10‰) have an additional step where PEP carboxylase acts as a pump, which directly transports CO_2 to the CO_2-fixing enzyme RuBP. The additional step reduces the discrimination against the heavier carbon isotope found in C_3 plants (Kelly, 2000; O'Leary, 1981, 1988; Farquhar *et al*. 1989; Boutton, 1991; Ehleringer, 1991; Fry, 2008). As a result, C_3 and C_4 plants are isotopically differentiable.

Differences in the temperature and rainfall preferences for C_3 and C_4 plant species result in distributional differences and, thus, variation in $\delta^{13}C$ values among different habitats. C_4 plants thrive in warm dry (xeric) climates (Ehleringer, 1991; Kelly, 2000) (Fig. 5). C_3 plants dominate mesic environments – an environment containing a moderate amount of moisture (Ehleringer, 1991; Kelly, 2000) (Fig. 5).

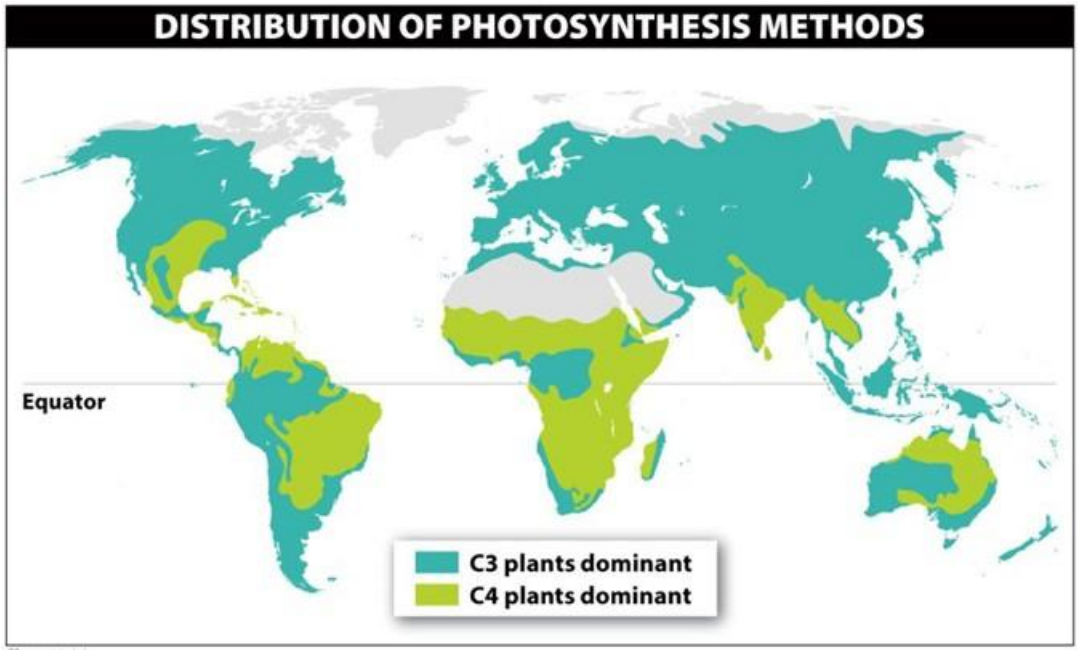


Figure 5. Distribution of C_3 and C_4 plants throughout the globe (Phelan, 2014).

C_3 and C_4 plants are unevenly distributed around the globe, C_3 plants are predominant at high-latitude whereas C_4 plants are predominant at low-latitudes (Terri

& Stowe, 1976). The variation among C_3 and C_4 plant distribution is caused by temperature, moisture content, and atmospheric CO_2 levels (Terri & Stowe, 1976; Ehleringer *et al.* 1997). For C_3 plants, photosynthesis becomes increasingly inefficient with high temperatures, low moisture, and low CO_2 levels (Ehleringer *et al.* 1997; MacFadden *et al.* 1999). The Arctic ecosystem is dominated exclusively by C_3 plants but variation in $\delta^{13}C$ values can still occur within a C_3 plant ecosystem (Kristensen *et al.* 2011).

Environmental factors that can affect $\delta^{13}C$ in C_3 plants include temperature, water stress, and light level (Kohn, 2010). Temperature changes can inhibit the effectiveness of C_3 plant photosynthesis. At higher temperatures, C_3 plant photosynthesis becomes less effective, primarily due to photorespiration. In these scenarios, Rubisco will preferentially select O_2 instead of CO_2, resulting in higher $\delta^{13}C$ fractionation values in the leaves (MacFadden *et al.* 1999). Similarly, if experiencing water stress, whether through a decrease in rainfall, relative humidity, or soil moisture, the stomata of C_3 plants will close, preventing CO_2 exchange creating higher $\delta^{13}C$ values in the leaves (Kohn, 2010). Lastly, light availability will cause a similar reaction as stomata will also close during low light causing increased fractionation (Van der Merwe & Medina, 1989).

For terrestrial mammals, food is the primary source of carbon. Their tissue reflects the plants that are consumed during their tissue growth with a physiological and trophic level offset (Koch *et al.* 1992). The stable isotopes of a mammal's tissue are differentiable depending on the plants consumed and the environment within which the

plants are grown (Cerling *et al.* 1997). Most herbivorous mammals will have 12-14‰ higher $\delta^{13}C$ value in their tissue over their preferred plant group (i.e. C3 or C4 plants) (Koch et al. 1992; Cerling et al. 1997; Cerling & Harris 1998; MacFadden *et al.* 1999). Between trophic levels most mammals have a ~1-2‰ enrichment in $\delta^{13}C$ values with higher degrees of variation in tertiary and quaternary diets (specifically in collagen tissue) (Codron *et al.* 2017; Kelly, 2000; DeNiro & Epstein, 1981; Tieszen *et al.* 1983; Tieszen & Boutton, 1989; Hobson *et al.* 1996). From our understanding of trophic levels, $\delta^{13}C$ values within plants and physiological effects, we can infer their dietary and habitat preferences by studying the $\delta^{13}C$ values of their various tissues. (DeNiro & Epstein, 1981; Tieszen *et al.* 1983; Tieszen & Boutton, 1989; Hobson *et al.* 1996).

1.4.2 Nitrogen: $\delta^{15}N$

Like carbon, nitrogen has two naturally occurring stable isotopes: ^{14}N, which makes up 99.6% of nitrogen, and ^{15}N comprising the remaining 0.4% (Koch, 2007; Bleam, 2017). Unlike carbon, the variation in $\delta^{15}N$ within a terrestrial ecosystem is not defined by a single source, like photosynthesis (DeNiro & Hastof, 1985; Mizutani & Wada, 1988; Ehleringer & Rundel, 1989; Kelly, 2000). Therefore, it can be complicated to compare $\delta^{15}N$ values across terrestrial food webs, even for individuals of the same species from different geographic regions. Despite this challenge, $\delta^{15}N$ values have been shown to be useful, for example, identifying the dietary contributions of different plant types to the terrestrial food chain (Schoeninger & DeNiro, 1984; Sealy *et al.* 1987; Ambrose, 1993; Kelly, 2000).

Many factors can affect nitrogen isotope values in plants, including soil, moisture, temperature, altitude, latitude, atmospheric nitrogen deposition, and the nitrogen fixing bacteria within the plants (Koch, 2007; Nadelhoffer & Fry, 1994; Högberg, 1997; Handley *et al.* 1999; Martinelli *et al.* 1999). The moisture content and temperature of a soil impacts how nitrogen is processed within it altering the degree of which nitrification, ammonification, and leaching occur (Martinelli *et al.* 1999). From this relationship, $\delta^{15}N$, in soil and subsequently plants, has a negative correlation with moisture and positive correlation with temperature (Rabanus-Wallace *et al.* 2017; Ambrose, 1991; Shearer *et al*. 1978; Mariotti *et al*. 1980). In the cold wet ecosystem of the Arctic, $\delta^{15}N$ within plants and soil is relatively low. This is particularly true in the permafrost regions where low soil temperatures cause low mineralization rates thus limiting the availability of inorganic nitrogen (Handley *et al.* 1999; Stevens *et al.* 2008). Therefore, the plants that grow in this region are generally poor nitrogen-fixing plants that are not inhibited by this limited resource, plants such as mosses, lichen, graminoid shrubs and a few tree species (Rabanus-Wallace *et al*. 2017; Mann *et al*. 2013). $\delta^{15}N$ values can vary within the tundra ecosystems through seasonal changes in temperature and moisture levels. Increases in temperature from a season can causes a ~2-3‰ enrichment within soil while increased precipitation by a few 100 mm can cause an inverse affect, a ~2-3‰ depletion (Craine *et al.* 2015).

For terrestrial mammals, nitrogen is sourced from consumed foods (primarily dietary protein). The $\delta^{15}N$ values of their tissue reflect the consumed foods with a~3-5‰ enrichment in $\delta^{15}N$ between each trophic level (DeNiro & Epstein, 1981; Minagwa

& Wada, 1984; Kelly, 2000). The ~3‰ enrichment between trophic levels is created as a byproduct of metabolism. As an animal metabolizes the nitrogen within their body, ^{14}N is preferentially used and excreted in the form of nitrogenous waste, creating an enrichment of ~3-5‰ in $\delta^{15}N$ values (DeNiro & Epstein, 1981; Kelly, 2000). This fractionation of ^{15}N in consumer tissues makes $\delta^{15}N$ values one of the most important tools for understanding interactions in paleoecosystems (Kelly, 2000; Minagwa & Wada, 1984; Peterson & Fry, 1987).

$\delta^{15}N$ values are not limited to trophic level reconstruction, they also reflect nutrient and water stress in consumers (Ambrose, 1991; Kelly, 2000; Hobson & Clark, 1992; Cormie & Schwarcz, 1996). For example, ^{15}N enrichment in consumer tissue can be a result of urea-recycling during water stress, such as occurs in ruminants including caribou and other cervids (Kelly, 2000; Sealy *et al*. 1987).

1.4.3 Nitrogen: $\delta^{15}N$ - Carbon: $\delta^{13}C$

Nitrogen and carbon isotope values have been shown to covary positively (Fig. 6). Though this relationship may not be universal (Kelly, 2000; Mizutani *et al.* 1991), many studies still use it to determine dietary sources. The positive relationship between $\delta^{15}N$ and $\delta^{13}C$ relates to the relative moisture in the environment (Heaton *et al.* 1986; Sealy *et al.* 1987; Kelly, 2000). Differences in temperature, moisture content, and atmospheric CO_2 levels cause changes to the dominance of either C_3 or C_4 plants but also affect the $\delta^{13}C$ values within the plants (Terri & Stowe, 1976; Ehleringer *et al.* 1997; Kelly, 2000; Phelan, 2014) Similarly, temperature and humidity affect nitrogen fixation which also

impacts the $\delta^{15}N$ within plants (Rabanus-Wallace *et al.* 2017; Ambrose, 1991; Shearer *et al*. 1978; Mariotti *et al*. 1980). From our understanding of isotopic fractionation of $\delta^{13}C$ and $\delta^{15}N$ within plants we can make inferences about the environment in which they were formed. For example, throughout Northern Canada, the cold wet ecosystem of the Arctic is dominated by only C_3 plants (Kristensen *et al.* 2011). Here $\delta^{13}C$ and $\delta^{15}N$ values are relatively low, however, the values can change with environmental changes (i.e. seasonal changes), such as increased temperature, moisture, and light level (Heaton *et al.* 1986; Sealy *et al.* 1987; Ambrose, 1991; Kelly, 2000; Kohn, 2010). Using animal tissue we look at $\delta^{13}C$ and $\delta^{15}N$ values to infer characteristics of their environment including seasonal changes and habitat (i.e. forests, deserts, tundra, and nearshore/coastal environments) (Hobson & Welch, 1992; Kelly 2000; Ambrose, 1993; Peterson & Fry, 1987).

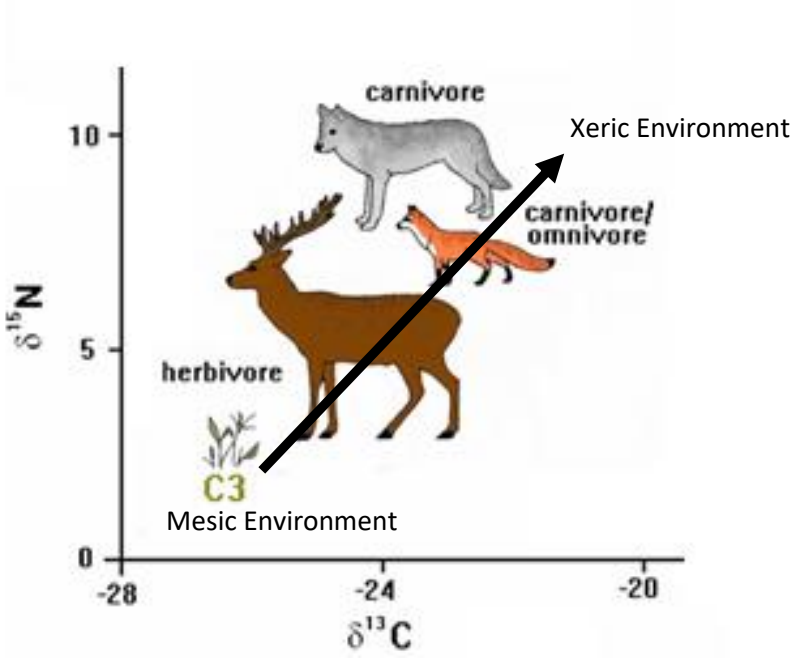


Figure 6. The trophic level and base plant diet impact on nitrogen and carbon stable isotopes (Schulting 1998, modified by S. Svyatko).

1.4.4 Oxygen: $\delta^{18}O$

Unlike carbon and nitrogen, oxygen has three naturally occurring stable isotopes: ^{16}O being the most abundant at 99.76%, ^{17}O the least abundant at 0.04%, and ^{18}O at 0.2% (Taylor, 1948). Concentrations of ^{17}O are very low and most studies focus on $\delta^{18}O$ (Taylor, 1948). Atmospheric oxygen has relatively little effect on isotope values within terrestrial ecosystem (Fry, 2003; Koch, 2007). Water $\delta^{18}O$ values vary both geographically and temporally, providing an indicator for precipitation and evaporation within an ecosystem (Koch, 2007). Variation occurs geographically as water vapour travels inland from the coast; Rayleigh distillation occurs, ^{18}O in water vapour is lost through rain-out, leaving lower $\delta^{18}O$ values within the water vapour (Clark & Fritz, 1997; Hoefs, 2009). The results are a geographical gradient with high-latitudes and coastal

regions having higher $\delta^{18}O$ values, a similar effect occurs in regions of high precipitation as more ^{18}O is release through rain-out (Rindsberger *et al.* 1990; Hoefs, 2009).

$\delta^{18}O$ values are also broadly correlated with temperature; waters become ^{18}O enriched in warmer temperatures, and depleted in cold (Higgins & MacFadden, 2004; McCrea, 1950; Bryant *et al.* 1996). This is largely due to evaporation rates, the lighter ^{16}O is preferentially released into the atmosphere, creating an enrichment in ^{18}O within water during warmer temperatures (Koch, 2007). Evaporation has a greater effect on $\delta^{18}O$ values in smaller water sources. The effect of evaporation on $\delta^{18}O$ values is particularly important for the leaves of plants. Increases in temperature cause the evaporation of water within plant leaves, resulting in enrichment in ^{18}O. That enrichment then transports throughout the phloem of the plant, affecting $\delta^{18}O$ values in plant organs (i.e. roots, stems, and branches) (Koch, 2007; Helliker & Ehleringer, 2000). Evaporation rates can be affected by more than just temperature, increases in relative humidity, precipitation and even wind can alter the amount of evaporation within the environment and the $\delta^{18}O$ values (Clark & Fritz, 1997).

If temperatures are above 20^{o}C, a phenomenon known as the "Amount Effect" occurs. The "Amount Effect" is when $\delta^{18}O$ values decrease despite increases in temperature. This is a result of a significant increase in precipitation and/or humidity (e.g. the summer rainy season). The increase of precipitation and/or humidity cause a decrease in evaporation within the ecosystem, resulting in a decrease of $\delta^{18}O$ values in the environment (i.e. within the meteoric water and plants) (Higgins & MacFadden, 2004; Rozanski *et al.* 1993; Bard *et al.* 2002; Straight *et al.* 2004). In the high Arctic,

temperatures do not often reach the 20 ^{o}C threshold, however, with climate change it is anticipated that the threshold would be reached more frequently (Higgins & MacFadden, 2004; Dansgaard, 1964; Rozanski *et al.* 1993; Straight *et al.* 2004).

Oxygen isotopes are introduced into the tissues of animals primarily through water intake supplied to the body by diet, drinking, and inhalation, though the latter is negligible (Podlesak *et al.* 2008). Oxygen isotopes are exchanged with the animal tissue at equilibrium with the body water, in homeotherms (mammals and birds) there is a constant fractionation caused by internal body temperature (Kohn, 1996; Podlesak *et al.* 2008). Therefore, in terrestrial mammals' variation in $\delta^{18}O$ values of consumed waters is reflected in vertebrate tissue and can be used to infer seasonal/climatic patterns, migration routes and altitude changes (Koch, 2007).

1.5 Tissue Development

As part of my research, stable isotope analysis of oxygen, carbon, and nitrogen was conducted on various tissue types of *Rangifer tarandus* (caribou). During tissue development, materials consumed through drinking, eating, and breathing are incorporated into new tissues. In principle, the stable isotope values occurring in the environment will be captured in that tissue with little to no additional fractionation, enabling the inference of habitat, diet, and drinking water source. However, skin, bone, teeth, muscle, and hair incorporate stable isotopes from the environment differently due to differences in rates of tissue formation and the physical and chemical processes involved in tissue synthesis (Hedges, 2007; Drucker *et al.* 2010; Gannes *et al.* 1997).

Different types of tissue have different rates of formation and as such reflect different periods of time. Some tissues can also have turnover, meaning new growth and/or degradation (e.g. bone absorption and remodeling). Diagenesis – the physical and chemical change of material – can also alter stable isotope values after prolonged environmental exposure (Macko *et al.* 1990). As my research involved well-preserved modern tissues, it was not necessary to consider this phenomenon further.

Teeth have been heavily studied and have proven to be particularly useful given that, once fully mineralized, stable isotopes from the environment are no longer incorporated in the tissue (Passey & Cerling, 2002; Fricke & O'Niel, 1996; Fricke *et al.* 1998). In the case of caribou, teeth develop over roughly 6 months and reflect only food consumed during this growth period (Drucker *et al.* 2012; Kohn, 2004). Bone remodels (i.e. new bone is developed while old bone is absorbed) slowly over time, providing an average of the stable isotopic values of consumed foods over years (Eriksen, 2010; Eriksen *et al.* 1990; Matsubayashi & Tayasu, 2019). Antler tissue has not been thoroughly studied but stable isotope values from this tissue may provide unique ecological information, as antler is one of the fastest growing bone (Chen *et al.* 2009; Goss, 1983; Lincoln, 1992). Antler may provide a high-resolution record of ecology during their relatively brief growth period (months). Yearly re-growth and shedding also make antler a useful and humane tool in studying yearly variations in cervid ecology using stable isotopes.

1.5.1 Bone

Antler and bone tissues are largely comprised of the same material, being a composite of protein (mostly collagen) and calcium hydroxylapatite (which binds carbonate and phosphate groups) (Stevens & O'Connell, 2016). However, there are several distinct differences. The first and primary difference is the speed of growth, with antler tissue undergoing rapid development over a few months in the spring and summer whereas bone can take 3-5 years to fully form (Heckeberg, 2017; Goss, 1983; Pasda, 2009; Skogland, 1985). A second major difference is that long bones contain more spaces to accommodate structures and tissues like blood vessels and marrow, which produce necessary materials for body growth and function. Antlers pull materials out of the body, like calcium and phosphate, but do not provide materials back to the body (Chen *et al.* 2009). A third difference is that bone, remodels over a period of ~10 years within large mammals. In contrast, antlers never remodel (Stevens & O'Connell, 2016; Hedges *et al.* 2007; Ambrose & Norr, 1993; Tieszen & Fagre, 1993).

Bone is remodeled through a two-step process, bone resorption and ossification (Eriksen, 2010; Eriksen *et al.* 1990). Bone resorption occurs when osteoclasts break down bone tissue into its base minerals and allow the blood stream to transport the material to where it is needed. Ossification is essentially the reverse. Osteoblasts replace cartilage and mesenchyme tissue directly on to the bone where it ossifies to bone tissue (Eriksen, 2010; Komori *et al.* 1997). For both bone and antler, isotopes will vary during the formation of collagen, carbonates, and phosphates due to an individual's diet (Stevens & O'Connell, 2016). For bone, the stable isotope values will reflect an individual's intake average over a period of ~10 years within bone tissue.

1.5.2 Teeth

Teeth grow over a finite period and do not remodel; in this way they are comparable to antlers. The growth rate of a tooth varies from species to species but usually occurs over a 6 – 12 month period during the early years of the species life, for caribou teeth development occurs over 6 months (Passey & Cerling, 2002; Drucker *et al.* 2012; Kohn, 2004). Teeth are comprised of two major parts, the enamel, which is the hard-outer layer, and the dentin, a connective tissue under the enamel. Enamel is mainly comprised of hydroxylapatite. Isotopic variation can be found in carbonate and phosphate substitutions made during enamel formation (Chritz *et al.* 2009; Koch, 1998; Kohn & Cerling, 2002). Enamel forms through a process called amelogenesis, this occurs during the Bell stage of tooth development (Fig. 8) (Passey & Cerling, 2002; Eisenmann, 1985). Amelogenesis occurs in two stages, the secretory stage and maturation. During the secretory stage, ameloblasts surround the forming tooth and secrete an enamel matrix in sequential layers, building the enamel wall. Maturation occurs after the formation of the enamel, starting at the crown of the tooth and moving down towards the root. For isotopic analysis, this maturation stage is the most important. The enamel matrix is slowly mineralized, followed shortly by the tooth eruption (Bronckers, 2017; Josephsen & Fejerskov, 1977; Takano & Ozawa, 1980).

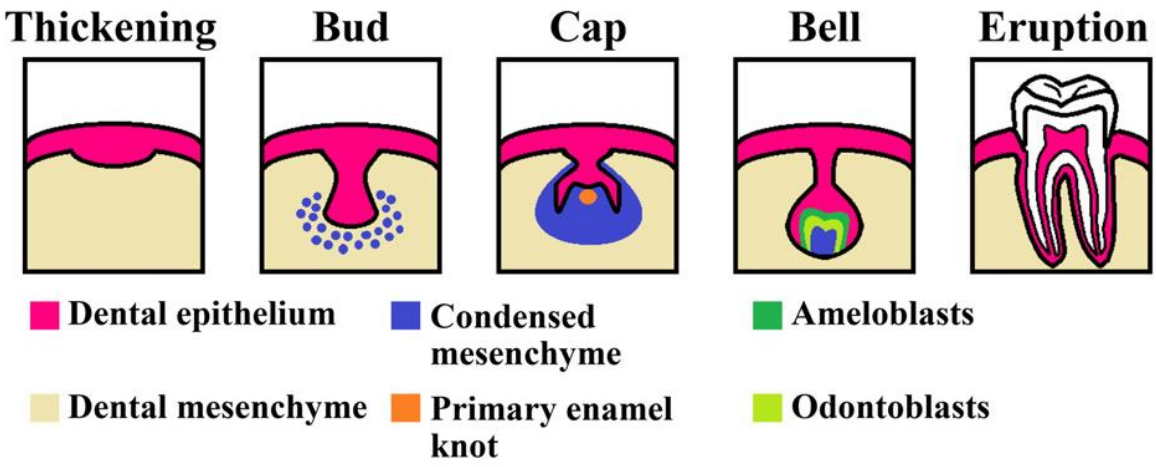


Figure 7. Tooth development and process (Amrollahi et al. 2016).

Once the tooth mineralizes, it will no longer have isotopic exchange with the body fluids (Passey & Cerling, 2002; Lee-Thorpe & Van der Merwe, 1987; Quade *et al.* 1992; Bocherens *et al*. 1996; Zazzo *et al.* 2000; Cerling *et al*. 2001). The isotopes within the enamel will reflect the diet consumed during tooth formation, often reflecting the first 6 – 12 months of the individual's life (Fricke & O'Niel, 1996; Fricke *et al.* 1998).

1.5.3 Antler

Antlers occur in members of the deer family Cervidae and, of the 40 extant deer species, 36 grow antlers annually. Cervid antlers vary among species, taking on a wide variety of shapes and sizes, from a simple spike to the large palmate antler of a moose (Goss, 1983). *Rangifer tarandus* are unique among these 36 extant species as they are the only species where both males and females develop antlers, though female antler growth is not uncommon in the history of mammal evolution (Lincoln, 1992; Schaeffer & Mahoney, 2001; Roberts, 1996).

For female caribou, the functional advantages of antler development outweigh the costs (Schaeffer & Mahoney, 2001; Clutton-Brock, 1982; Kiltie, 1985). Antlers in both male and female caribou, are primarily used for resource competition and intraspecific combat (Schaeffer & Mahoney, 2001; Clutton-Brock, 1982; Markusson & Folstad, 1997). Females are unique to males as they carry their antlers through the winter to remove snow cover from subnivean food and to defend food patches (Schaeffer & Mahoney, 2001; Espmark, 1964; Barrette & Vandal, 1986, 1990).

Antler growth varies between male and female caribou. Antler growth is triggered by hormonal release that is aligned with the seasonal reproductive cycle. For the males, the gonads release concentrations of testosterone to initiate development, while females have a similar hormonal release during the onset of ovarian activity (Lincoln, 1992). Males begin antler formation in February and March, while females grow their antlers beginning in May and June (Baski & Newbery, 1987, 1989). Males shed their antlers at the end of the rutting season, which occurs in early winter, while females often shed their antlers when calving in late spring the following year (Shah *et al.* 2008).

Antler growth is a very fast development process in which hydroxylapatite (i.e. calcium phosphate hydroxylapatite $Ca_{10}(PO_4)_6(OH)_2$), and collagen form a compact bone surrounding a core of cancellous bone. An antler cross-section consists of concentric rings of collagen protecting a series of blood vessels that pull fluid and minerals from the body to sustain fast growth (Chen *et al.* 2009). Their rapid development and lack of

remodelling has the potential to result in unique, high resolution stable isotopic records of caribou ecology.

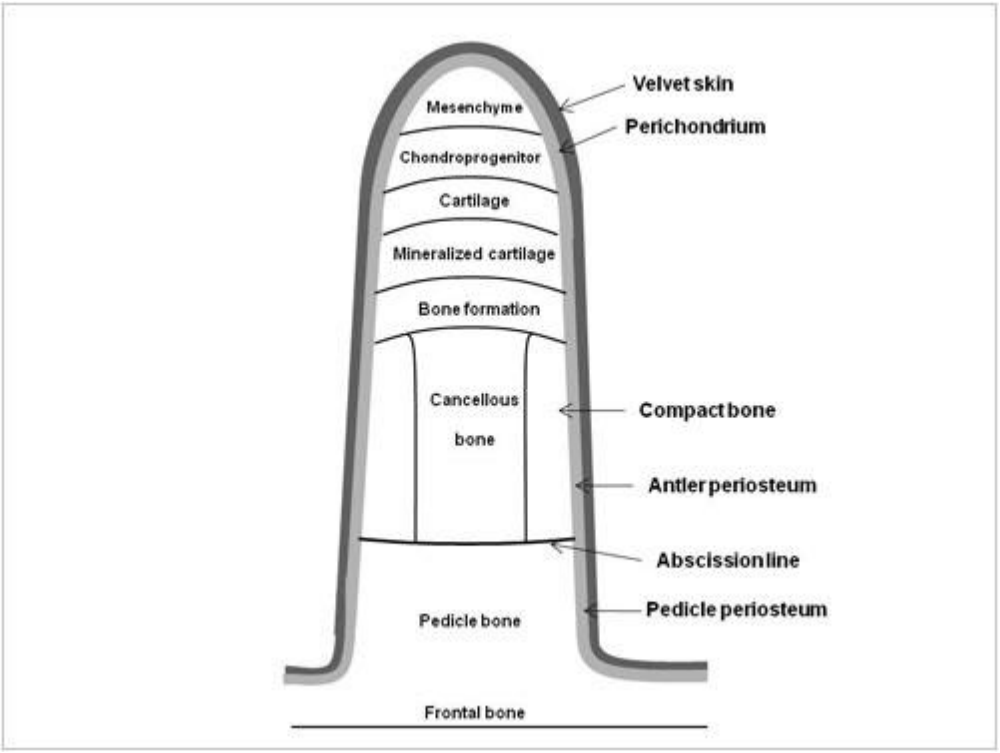


Figure 8. Pedicle bone of initiating the start of antler formation. Upper layers of cartilage mineralize to form antler bone (MSU, 2020 – adapted from Price et al. 2005).

Rangifer tarandus antler development is one of the fastest growing hard tissue known, developing at a rate between 1.7cm/day up to 10cm/day (Heckeberg, 2017; Goss, 1983). Antler growth begins on the pedicle, a bony platform found in the frontal bone of the skull. A velvet membrane is formed on the surface of the pedicle during antler growth (Fig. 7). This smooth-haired skin has a high density of blood vessels, which aids in rapid growth by supplying a large quantity of nutrients (Krauss *et al.* 2011; Li *et al.* 2004). On the tip of the antler, under the velvet membrane, a cap-like area of mesenchymal cells continuously forms new bone tissue. Thus, the tip of the antler is

made of the youngest material (Krauss *et al.* 2011; Banks, 1974; Kierdorf *et al*. 2003; Li *et al*. 2005; Wislocki, 1942). Once the antler is fully developed, the velvet skin is shed, and the bone has completely developed and will not undergo remodelling (Krauss *et al*. 2011; Stevens & O'Connell, 2016).

Antlers will incorporate isotopes during the formation of the compact and cancellous bone (i.e. the collagen, carbonates, and phosphates) through the individual's diet (Stevens & O'Connell, 2016). This tissue will indicate a dietary time series of their growth period.

1.6 Thesis Objective

As highlighted above, caribou are an important species for maintaining the Arctic ecosystem. However, they are being greatly impacted by climate change. My future research goal is to determine if *Rangifer tarandus* antlers can be used as effective ecological indicators relative to other hard tissues such as bone and teeth using stable isotopes of oxygen, carbon, and nitrogen. Antlers are large, fast growing tissue, that grow and shed annually. They could provide a high-resolution record of the ecological changes that occur during their growth period from the early spring to late summer. In comparison, other tissue such as bone only provides decadal averages. Teeth are excellent resources but only reflect the early stages of life.

My Master's thesis aims to address two main questions: 1) How do isotopic signals on antlers differ from two well-studied hard tissues, teeth and bone and 2) Do stable isotopes vary along the length of the antler? Due to differences in the timing of

tissue development, it is predicted that isotopic variation will occur among the three tissues. Further, it is predicted that variation will also occur along the growth axis of the antler. If variation along the length of the antler occurs, possible explications for isotopic change could be the seasonal migration of caribou and associated ecological changes or physiological isotopic fractionation.

2.0 Methods

This study was conducted using caribou tissue (i.e. antlers, teeth, and bones) gathered during a Canadian Wildlife Services (CWS) study conducted in the late 1960s (Parker, 1973; Miller D.R., 1973; Miller F.L., 1973; Dauphine, 1973). During the CWS study, 999 caribou were culled, 943 were from the Qamanirjuaq population and 56 from the Beverly population. Culling was conducted to better understand the biology of the caribou population with specific interest in determining age, sex, foraging relationships, growth, and physical and pathological conditions. The project collected caribou over two years between April 1966 to July 1968 to determine seasonal and annual changes to the biology of the caribou. The caribou collected were weighed and their body size measured. A portion of the CWS project studied their rumen contents and mandibles. All samples of skulls, including teeth, antler and bone were carefully scrubbed clean with a stiff-bristle brush after being soaked in hot water for several days. The age and sex of each specimen was determined through dental measurements of the right mandible, with the left being archived (Parker, 1973). The Canadian Museum of Nature housed the samples after the data collected.

The CWS study has allowed me to minimize the variables between my selected samples. A total of 15 caribou were selected from the Qamanirjuaq population, all available caribou culled during the September 1967 collection period, specifically between September 15th and the 21st. The 15 individual sample size was selected to ensure that antlers could be thoroughly analyzed with samples taken every 3 cm along

the length. The selected caribou were all male, approximately 50 months in age, and of similar weight and body size. To minimize migration differences, all selected caribou were understood to be from the same herd (i.e. the Churchill herd) (Fig. 3). A detailed description of the population, range, habitat, migration routes, and diet are provided in section 1.3.

2.1 Samples

All specimens selected were housed at the Natural Heritage Campus of the Canadian Museum of Nature. Table 2 provides summary information on the selected caribou that were included in my study.

Table 2: List of samples containing collection date, location and specimen size as recorded by Canadian Wildlife Services.

CMN Catalogue Number	Date Collected	Latitude	Longitude	Age (M)	Weight (lbs)	Total Length (cm)	Tail (cm)	Hind Foot (cm)	Ear (cm)
CMNMA 39079	1967-09-17	61.15	-95.4167	39	218	172	12	49	12
CMNMA 39090	1967-09-18	61.25	-95.35	59	283	188	14.5	56	12.5
CMNMA 39102	1967-09-18	61.25	-95.35	51	258	180	14.5	53	12
CMNMA 39104	1967-09-18	61.25	-95.35	54	277	178	12.5	57	11.5
CMNMA 39120	1967-09-19	61.25	-95.3167	39	241	168	12	53	12
CMNMA 39121	1967-09-19	61.25	-95.3167	51	221	169	17	55	13.5
CMNMA 39132	1967-09-19	61.25	-95.3167	51	260	185	16	54.5	11
CMNMA 39145	1967-09-21	61.2	-95.25	51	288	187	14	56.5	13
CMNMA 39148	1967-09-21	61.2	-95.25	51	274	182.5	14	56.5	12
CMNMA 39149	1967-09-21	61.2	-95.25	51	283	175	14.5	53	13
CMNMA 39151	1967-09-21	61.2	-95.25	51	281	182	16	56.5	13
CMNMA 39107	1967-09-19	61.21667	-95.0833	51	268	176	10.5	53.5	12

CMNMA 39108	1967-09-19	61.21667	-95.0833	51	270	180	12	53	12
CMNMA 39110	1967-09-19	61.21667	-95.0833	39	284	182	10	52	11
CMNMA 39136	1967-09-21	61.21667	-95.0833	51	342	182	11	54	13

2.2 Isotope Sampling

Samples for stable isotope analysis were taken along the length of one antler as well as from the mandible (jawbone) and third lower molar of each specimen (for a total of 15 specimens). The third lower molar was selected for this study as it is the final tooth to erupt, indicating a fully mature adult. For each of the 15 antlers, samples were taken at the burr of the antler and then sequentially every 3 cm to the tip of the antler. Samples were also collected at approximately the middle of each tine (Fig. 9).

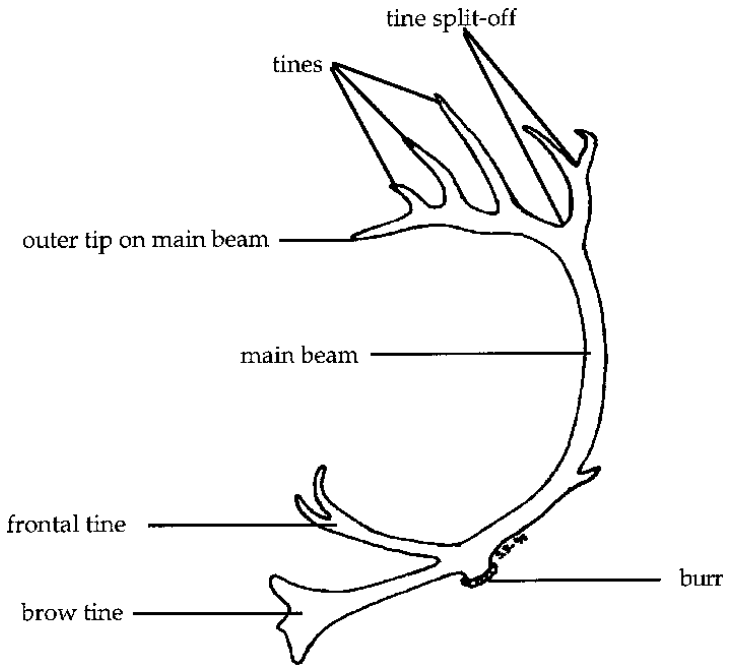


Figure 9: Antler structure and anatomical nomenclature (Markusson & Folstad, 1997).

One mandible sample was taken from the middle of the jawbone for each specimen. Three samples of tooth enamel were collected along the third lower molar from root to crown, with approximately 3 mm between each sample. Collecting enamel was done meticulously to avoid the addition of dentin to the sample. A Dremel © tool was used to collect the samples (8050 Micro and 8220 12Vmax High Performance Cordless). Prior to sampling, the Dremel © tool was used to remove a surface layer (a few microns) to eliminate potential contaminants. It should be noted that all specimens have been stored at the museum since they were collected, and no environmental contamination is expected. Six (6) mg of powder was collected for antler and bone sampling, while approximately 1 – 2 mg was collected for tooth enamel sampling.

2.2.1 Carbonate chemical pre-preparation

Isotopic chemical pre-preparation procedures followed the standard methods for isolating carbonate (O'Connell *et al.* 2001; Longin, 1971). 1 ml of 2-3% NaOCl (bleach) was added to 3 mg of bone powder to isolate carbonate tissue. Samples were agitated then left to sit in a refrigerator for 24 hours. Samples were then placed in a centrifuge to separate powdered bone/enamel/antler from bleach. The separated liquid was then aspirated, and new NaOCl solution was added. After another 24 hours of refrigeration, the samples were centrifuged and the NaOCl solution aspirated. To ensure no bleach remained, the powder samples were washed with deionized water. The sample wash was conducted by adding approximately 1 ml of deionized water, agitating the sample, centrifuging, and aspirating the water. The whole process was repeated five times

adding 1 ml of deionized water each time. Standard sampling procedure suggests adding 0.5 ml of 1 M acetic acid buffered with calcium acetate to a pH of 5 (Longin, 1971; Koch *et al.* 1997). The acetic acid protocol is designed for fossilized bone and teeth, as a necessary step to eliminate exogenous carbonate contamination (i.e. weathering). However, antler tissue is made of fine crystals smaller than bone (Chen *et al.* 2009). The acetic acid was too strong and dissolved all powdered antler material. Since the current samples are modern, I did not expect exogenous carbonate contamination. Therefore, acetic acid treatment was unnecessary.

Finally, samples were placed in a dry oven overnight to remove remaining water. The isolated carbonate tissue was sent to the University of Western Ontario to be placed in a a Thermo Finnigan Delta^{plus} XL Isotope Ratio Mass Spectrometer interfaced with GasBench, Costech EA , GC/C, and PreCon peripherals to determine $\delta^{13}C$ and $\delta^{18}O$ isotope values.

2.2.2 Collagen chemical pre-preparation

The standard isotopic sampling method was followed for collagen with slight modifications (Longin, 1971; O'Connell *et al.* 2001). 1 – 2 mg of bone and antler were weighed out and placed into separate plastic microcentrifuge tubes with 1.5 ml of 0.1 M HCl solution (initial procedure suggested 1 M HCl but was reduced to avoid the rapid breakdown of antler powder). Tubes were given aluminum foil caps and placed into a refrigerator for 30 minutes to allow the samples to decalcify. Published research procedures suggested a two-day decalcification period but caused the complete

degradation of antler powder material. The resulting solution was aspirated then rinsed using the same deionized water washing technique as the carbonate sample preparation. Samples were placed in the freeze dryer overnight to remove remaining water. Alterations to the typical collagen preparation procedure were made as studies initially suggested the addition of a petroleum ether wash to defat bone material. From research by Chen *et al.* (2009), I deemed that antlers did not contain enough fatty tissue for this procedure. Defatting can also affect the nitrogen isotopes (Logan & Lutcavage, 2008; Elliott & Elliott, 2016). As such, I opted to remove the defatting process for both bone and antler. Samples were then placed in a freeze dryer overnight.

The collagen was isolated for each sample the tissue was sent to the University of Western Ontario to be placed into a Thermo Finnigan Delta^{plus} XL Isotope Ratio Mass Spectrometer interfaced with GasBench, Costech EA , GC/C, and PreCon peripherals to determine $\delta^{13}C$ and $\delta^{15}N$ isotope values.

2.2.3 Statistical analysis

Stable isotope values of carbonate tissue in antler, bone, and teeth were compared within an individual to determine tissue difference. A linear regression statistical analysis was then used to determine if there was linear relationship between the stable isotope values and position along the length of the antler. Analysis was conducted comparing carbon and oxygen isotope values to the length of the antler (from carbonate results). Similarly, nitrogen and carbon isotopes were compared over the length of the antler (from collagen results).

3.0 Results

I found significant evidence of change in both carbonate carbon and collagen nitrogen isotope values along the lengths of *Rangifer tarandus* antlers. While the isotope values along the antlers demonstrated a high degree of variability, this variability provides fascinating insight into the dietary changes known to occur in caribou.

3.1 Carbonate Isotope Results

For the carbonate isotopes, I only completed sampling for five individuals for $\delta^{13}C$ and $\delta^{18}O$ isotope values (a sixth was started but never finished). As discussed below, stable isotope analysis of carbonate oxygen showed low repeatability. The COVID-19 crisis, which resulted in the shutdown of the stable isotope laboratory, also eliminated the opportunity to consider other possible solutions to produce reliable oxygen values, as such, further sampling focused on the collagen component.

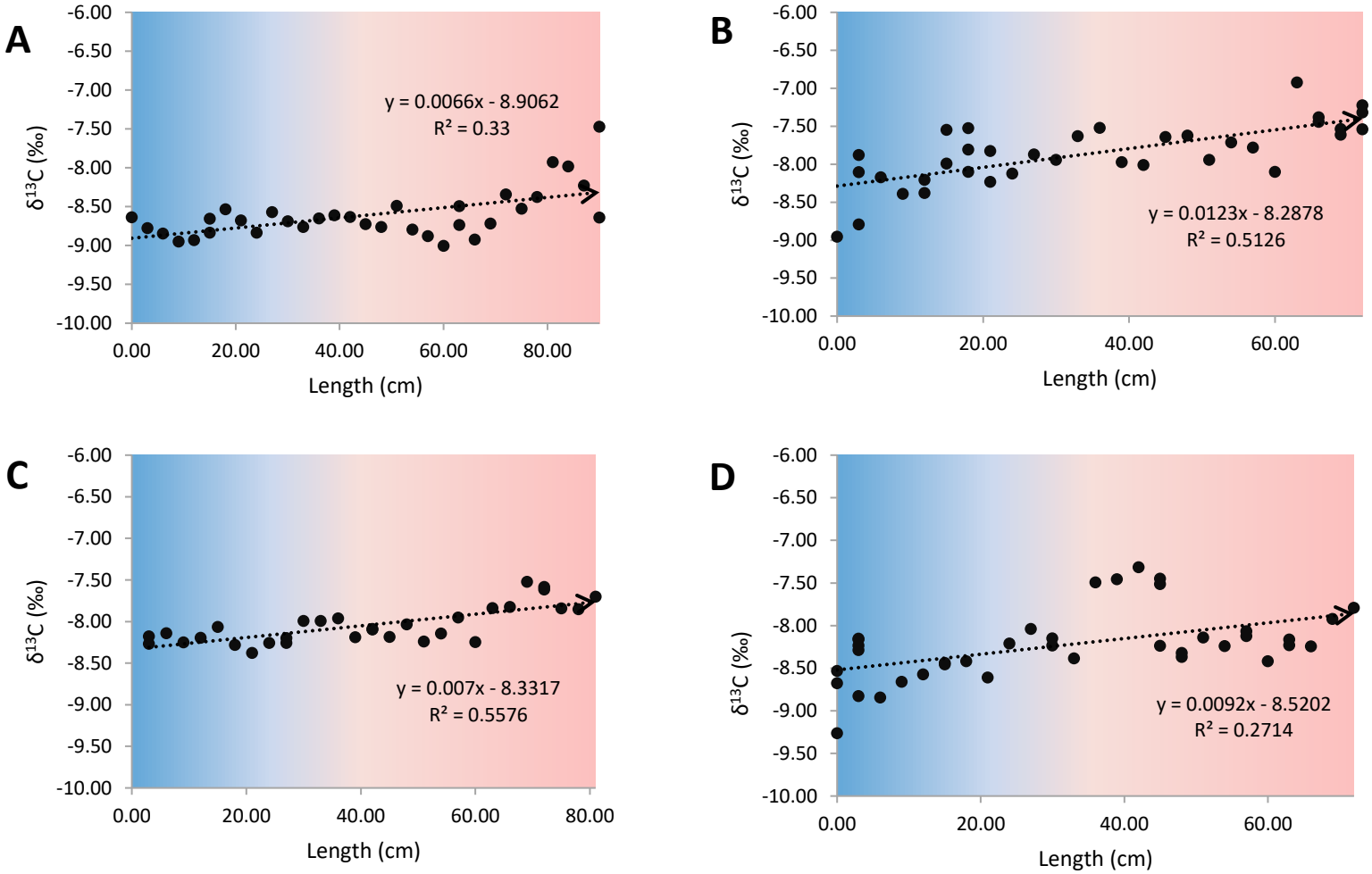
Carbonate $\delta^{13}C$ values from the three hard tissues tested were as follows. The average $\delta^{13}C$ value for bone samples was -9.16 ± 0.29‰; 1‰, lower than the overall average $\delta^{13}C$ value for antler samples (Table 3). The average $\delta^{13}C$ value for teeth was -8.79 ± 0.28‰, in between the values for antler and bone samples (Table 3). Tooth enamel $\delta^{13}C$ values shows a trend opposite to antler, decreasing throughout their growth by 1‰ from -9.33 ± 0.33‰ to -8.36 ± 0.14‰ (Fig. 13).

The carbonate $\delta^{13}C$ values had a range of 3‰ across all five samples; from -9.26‰ (found at the base of Antler D; Fig. 10) to -6.92‰ (found near the tip of Antler B; Fig. 10). The carbonate $\delta^{13}C$ values increased significantly along the length of the

antlers for all five individuals (ordinary least squares regression, $p < 0.05$; Fig. 10). $\delta^{13}C$ values started at an average of -8.76 ± 0.23‰ in the antler burr and increased by 1‰ to an average of -7.76 ± 0.51‰ at the tip of the antler (Fig 10). Duplicates did not show a significant difference in $\delta^{13}C$ between samples (averaging 0.22 ± 0.31‰; Fig. 12).

Carbonate $\delta^{18}O$ values from the three hard tissues experienced a wide degree of variability. The average $\delta^{18}O$ value for bone samples was 15.21 ± 1.57‰, the oxygen values within bone samples had greater variability than carbon values, ranging from 13.86‰ (on Specimen A; Fig. 14) to 17.59‰ (on Specimen E; Fig. 14). The average $\delta^{18}O$ value for teeth was 16.33 ± 0.72‰, teeth varied roughly 0.96 ± 0.68‰ between the crown and root values. Much like antler values, tooth $\delta^{18}O$ values did not show a consistent trend across all 5 samples.

Across all sampled antlers, carbonate $\delta^{18}O$ values ranged ~8‰, from 12 to 21‰ (on Antler B & F respectively; Fig. 11). The carbonate $\delta^{18}O$ values did not change significantly along the length of the antlers (ordinary least squares regression, $p > 0.05$; Fig. 11). $\delta^{18}O$ values averaged 16.44 ± 2.23‰ throughout all the antlers. However, there was a high degree of variation in $\delta^{18}O$ values between duplicate samples (averaging 1.69 ± 1.82‰), suggesting poor reproducibility (Fig. 12).


A
B
C
D
δ13C (‰)
Length (cm)
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R² = 0.33
y = 0.0123x - 8.2878
R² = 0.5126
y = 0.007x - 8.3317
R² = 0.5576
y = 0.0092x - 8.5202
R² = 0.2714

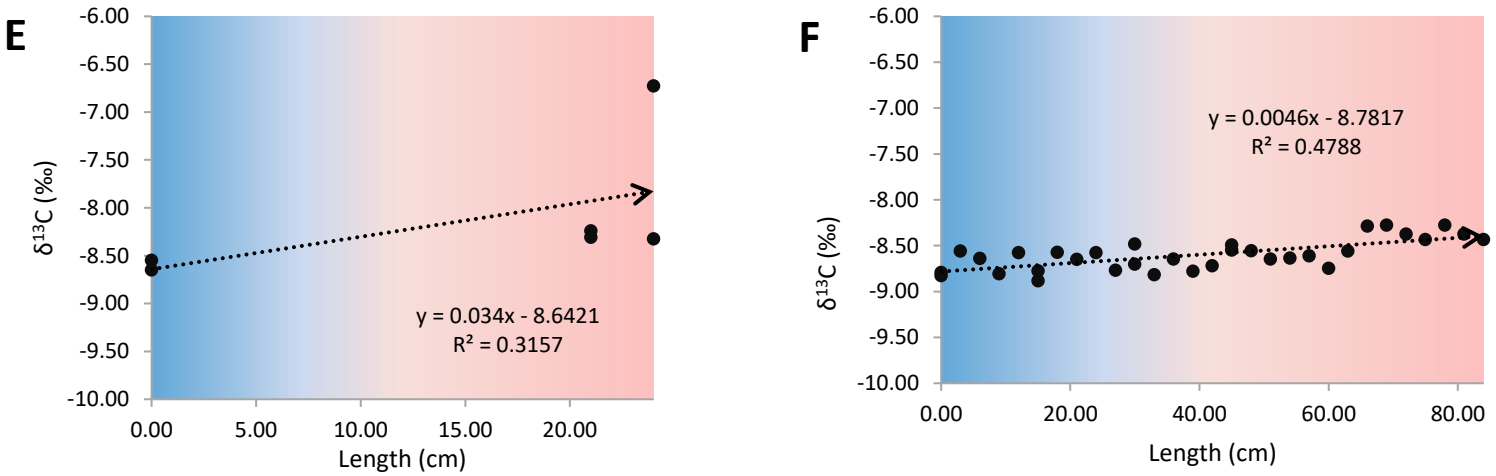
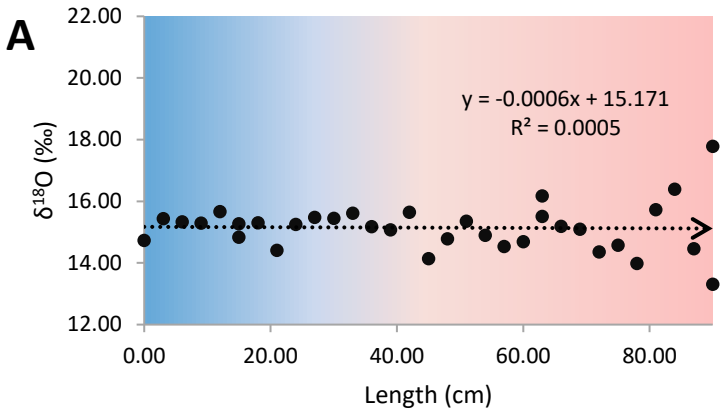


Figure 10. The X-axis represents the length of each antler, 0cm representing the burr. Linear relationship between antler length and $\delta^{13}C$ values for carbonate stable isotopes. Linear regression analysis indicate all antlers (with the exception of E due to lack of data) have significant p-values (A, p = 0.000381; B, p = 9.16E-07; C, p = 1.4E-06; D, p = 0.000798; and F, p = 8.18E-06). The $\delta^{13}C$ values ranged from -9.5 ‰ to -7.5 ‰ an almost consistent 1‰ increase $\delta^{13}C$ from the base of the antler to the tip. Antlers analyzed: A = 39107, B = 39108, C = 39145, D = 39148, E = 39149, F = 39151. Colour change representing seasonal temperature changes that occur during antler growth, blue representing the winter season while red represents the summer.


A
22.00
20.00
18.00
16.00
14.00
12.00
δ18O (‰)
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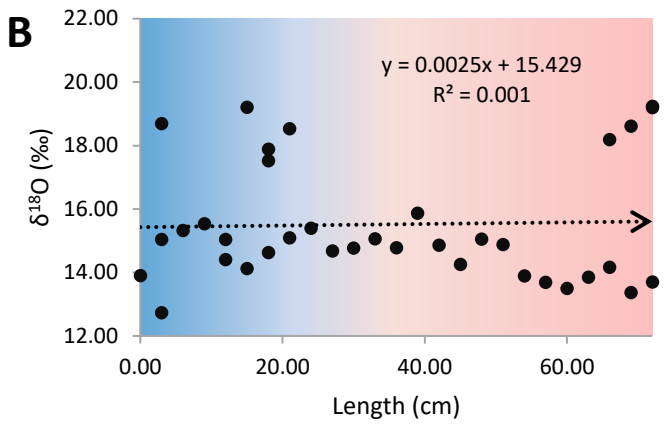
R² = 0.0005
0.00
20.00
40.00
60.00
80.00
Length (cm)


B
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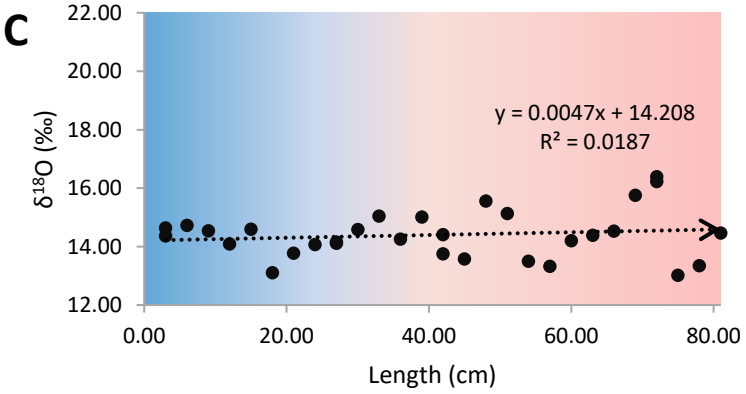
20.00
18.00
16.00
14.00
12.00
δ18O (‰)
y = 0.0025x + 15.429
R² = 0.001
0.00
20.00
40.00
60.00
Length (cm)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18.00
16.00
14.00
12.00
δ18O (‰)
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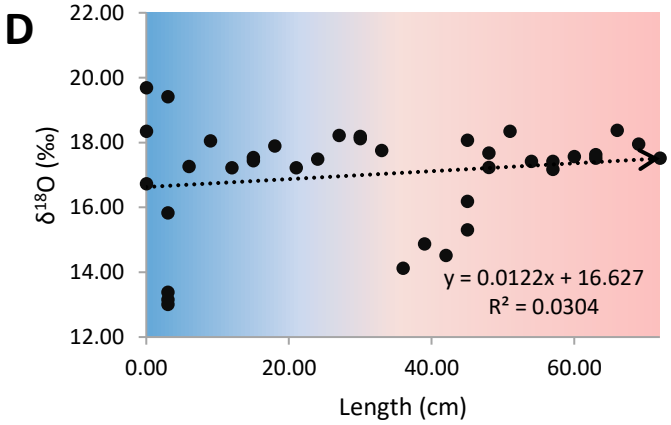
R² = 0.0187
0.00
20.00
40.00
60.00
80.00
Length (cm)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18.00
16.00
14.00
12.00
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R² = 0.0304
0.00
20.00
40.00
60.00
Length (cm)

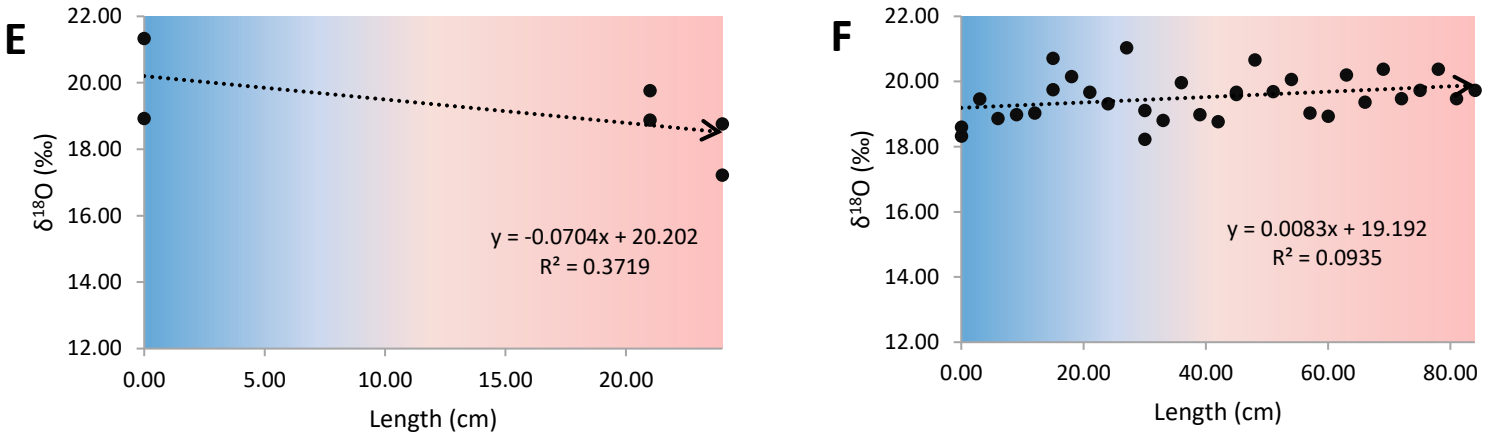


Figure 11. Linear relationship between antler length and $\delta^{18}O$ values for carbonate stable isotopes. Linear regression analysis for all antlers indicate no significant relationship between antler length and $\delta^{18}O$ values. The most significant result was sample 39151 (F) with a p-value of 0.083592. Antlers analyzed: A = 39107, B = 39108, C = 39145, D = 39148, E= 39149, F = 39151. Colour change representing seasonal temperature changes that occur during antler growth, blue representing the winter season while red represents the summer.

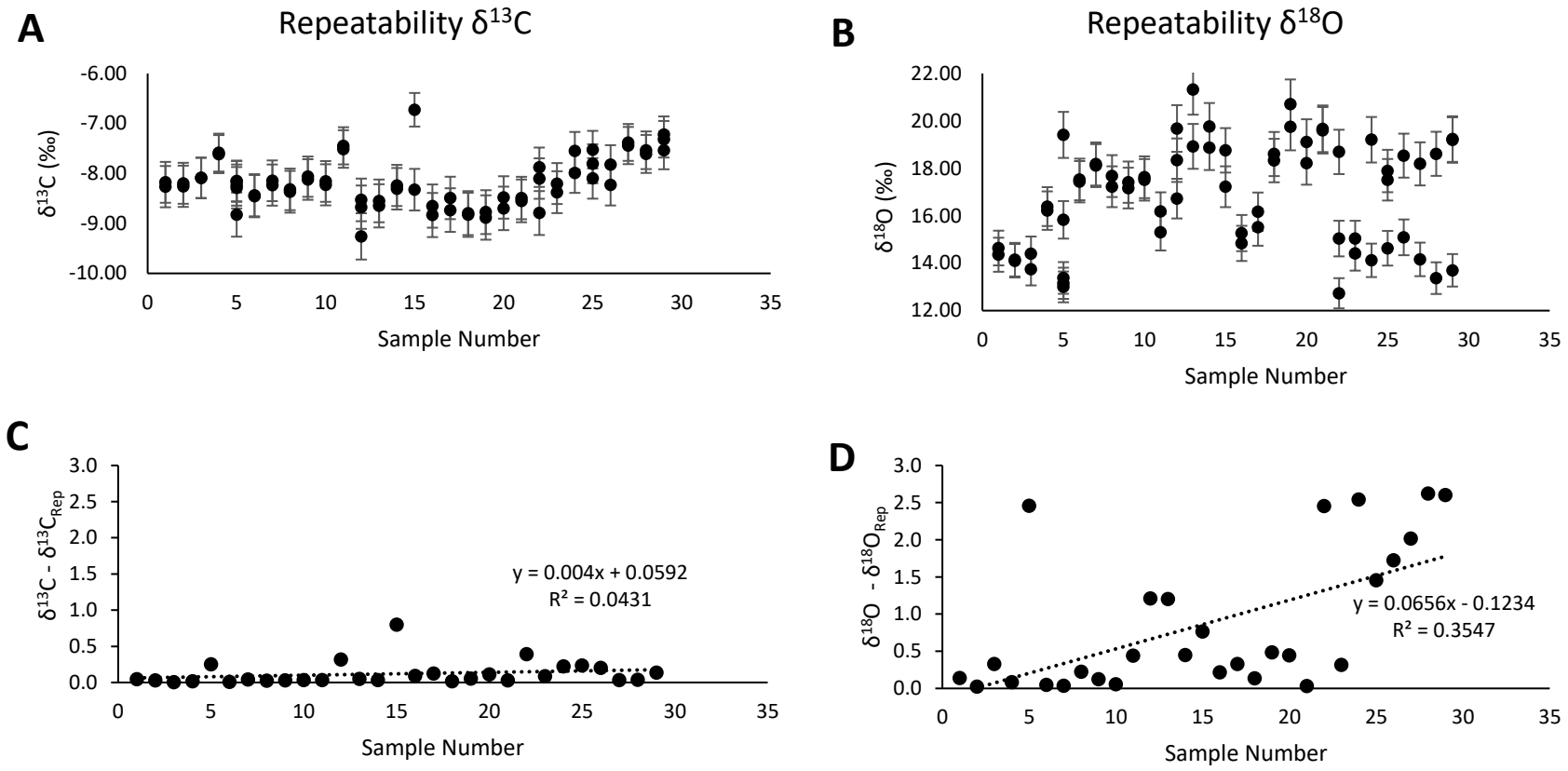
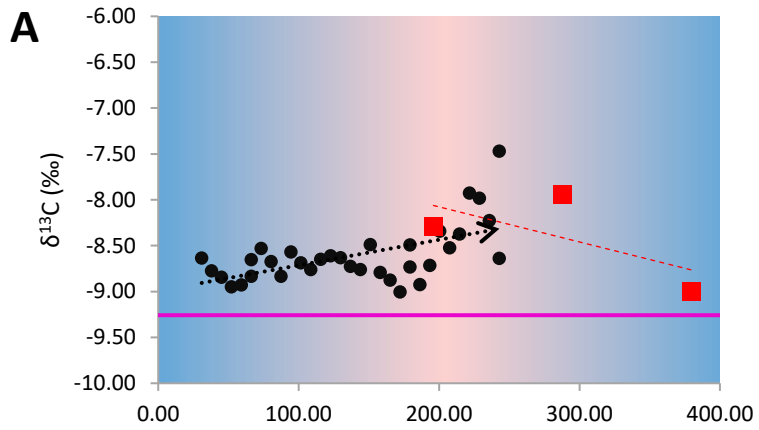
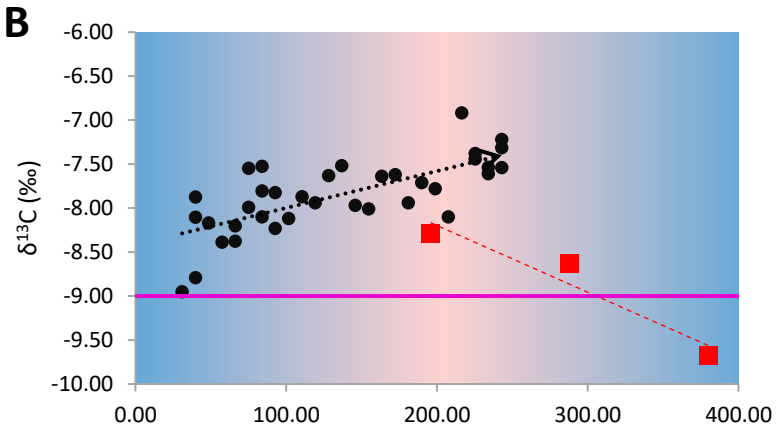
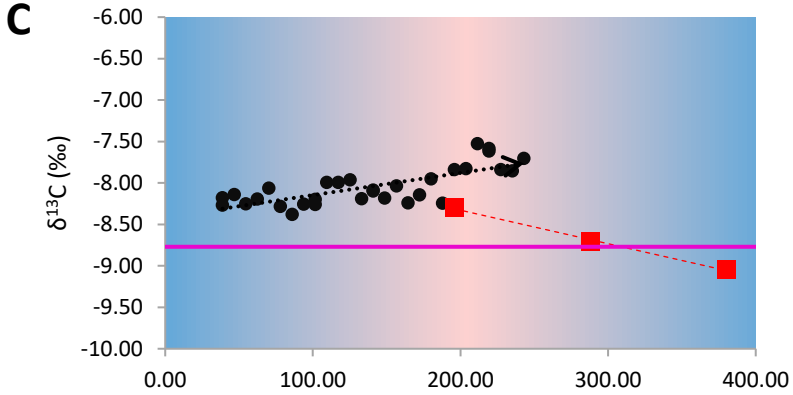
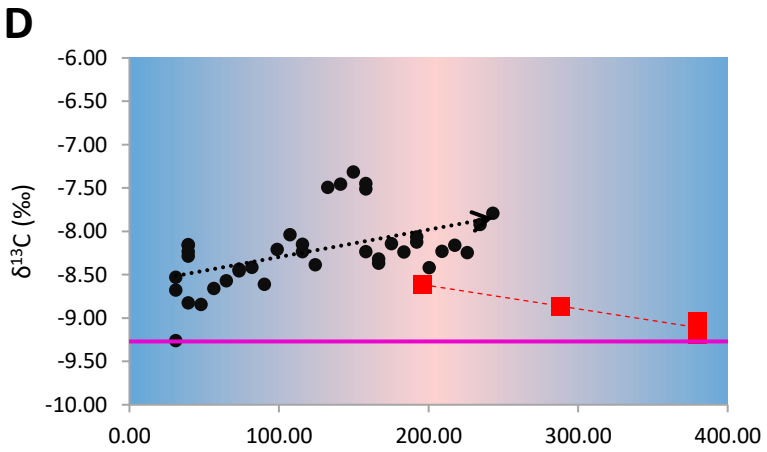


Figure 12. During stable isotope analysis every tenth sample was subject to repeat testing. A and B above are the values of the repeat testing, each sample number represents a different sample tested. C (carbon) and D (oxygen) show the difference between repeated samples for each sample duplicated. There are no correlations between sample numbers as the graph above shows multiple individuals. The only values compared are the repeated stable isotope analysis on one sample. $\delta^{13}C$ and $\delta^{18}O$ values of the repeat sampling are indicated strong repeatability, however, $\delta^{18}O$ values showed poor correlation upon repeat testing.


A
-6.00
-6.50
-7.00
-7.50
-8.00
-8.50
-9.00
-9.50
-10.00
δ13C (‰)
0.00
100.00
200.00
300.00
400.00


B
-6.00
-6.50
-7.00
-7.50
-8.00
-8.50
-9.00
-9.50
-10.00
δ13C (‰)
0.00
100.00
200.00
300.00
400.00


C
-6.00
-6.50
-7.00
-7.50
-8.00
-8.50
-9.00
-9.50
-10.00
δ13C (‰)
0.00
100.00
200.00
300.00
400.00


D
-6.00
-6.50
-7.00
-7.50
-8.00
-8.50
-9.00
-9.50
-10.00
δ13C (‰)
0.00
100.00
200.00
300.00
400.00

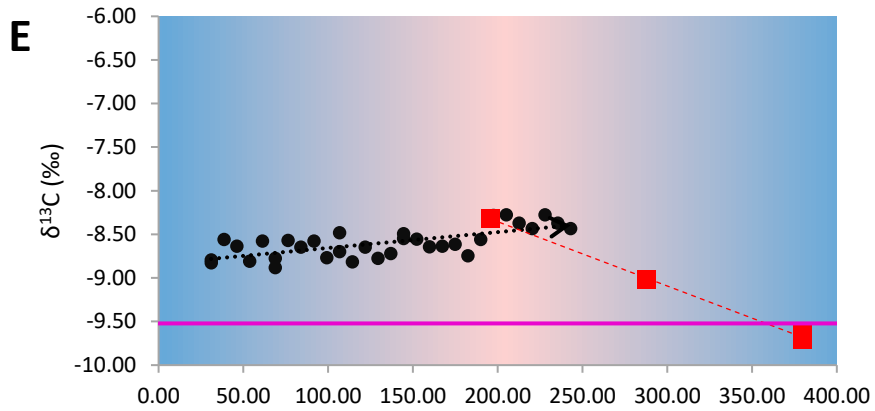
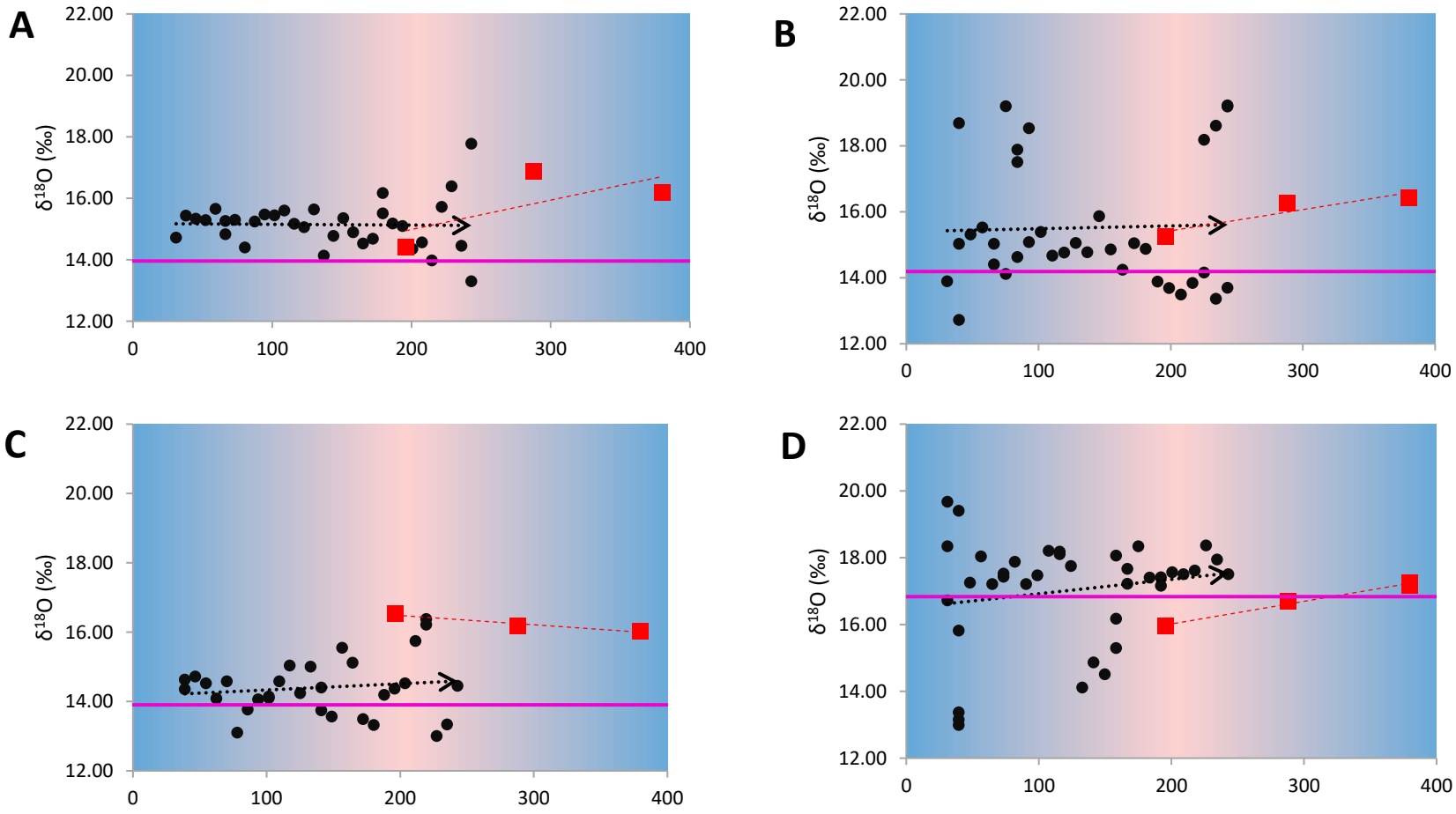


Figure 13. Comparison graphs between the antler $\delta^{13}C$ isotopes (indicated in black circles, dotted line representing line of best fit), tooth enamel $\delta^{13}C$ isotopes (indicated in red squares, dotted line representing line of best fit), and mandible (bone) $\delta^{13}C$ isotopes (indicated by the purple line). For the x-axis 0 is set as January 1st the x-axis represents just over a year of growth. Each tissue begins formation at a different time of year, antlers begin in the winter, teeth begin in the summer, and bone continuously grows. The colour gradient is used to demonstrate temperature changes during an entire year. Antlers analyzed: A = 39107, B = 39108, C = 39145, D = 39148, E = 39151.


A
B
C
D
22.00
20.00
18.00
16.00
14.00
12.00
δ18O (‰)
0
100
200
300
400

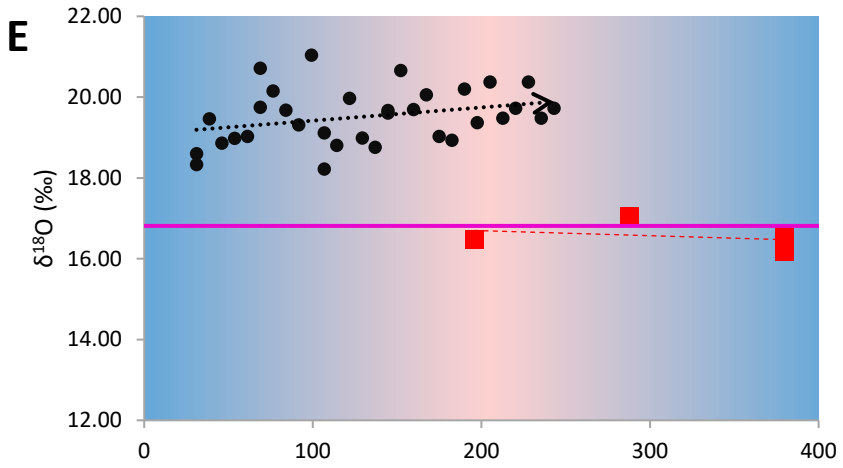


Figure 14. Comparison graphs between the antler $\delta^{18}O$ isotopes (indicated in black circles, dotted line representing line of best fit), tooth enamel $\delta^{18}O$ isotopes (indicated in red squares, dotted line representing line of best fit), and mandible (bone) $\delta^{13}O$ isotopes (indicated by the purple line). For the x-axis 0 is set as January 1st the x-axis represents just over a year of growth. Each tissue begins formation at a different time of year, antlers begin in the winter, teeth begin in the summer, and bone continuously grows. The colour gradient is used to demonstrate temperature changes during an entire year. The colour gradient is used to demonstrate temperature changes from February (0) to January 15th (350). Antlers analyzed: A = 39107, B = 39108, C = 39145, D = 39148, E = 39151.

Table 3: Average isotopic values for carbonate $\delta^{13}C$ & $\delta^{18}O$ for mandible (bone), tooth enamel, and antler, including $\delta^{13}C$ & $\delta^{18}O$ values from the antler burr and antler tip, complete table of samples in Appendix 1: Carbonate Stable Isotope Data.

Sample		Carbonate									
		Antler Tip	Antler Burr	Antler Average	Bone	Tooth Average	Antler Tip	Antler Burr	Antler Average	Bone	Tooth Average
		Carbon ($\delta^{13}C$ ‰)					Oxygen ($\delta^{18}O$ ‰)				
A	**39107**	-8.05	-8.63	-8.6	-9.26	-8.41	15.54	14.72	15.14	13.96	15.83
B	**39108**	-7.22	-8.95	-7.85	-9.00	-8.87	19.19	13.90	15.52	14.19	15.99
C	**39145**	-7.70	-8.22	-8.04	-8.77	-8.68	14.46	14.50	14.40	13.90	16.25
D	**39148**	-7.79	-8.82	-8.23	-9.27	-8.92	17.51	18.25	17.02	16.84	16.77
E	**39151**	-8.43	-8.81	-8.60	-9.52	-9.17	19.72	18.47	19.52	16.81	16.56

3.2 Collagen Isotope Results

For the collagen isotopes, I was only able to sample 12 of the 15 individuals. Unfortunately, the COVID-19 crisis led to the shutdown of the stable isotope laboratory and the remaining samples could not be analyzed.

Collagen $\delta^{13}C$ values did not differ between hard tissue of antler and bone (Table 4). The average $\delta^{13}C$ value for bone samples was -19.08 ± 0.34‰, only a 0.2‰ difference than antler (Table 4).

The collagen $\delta^{13}C$ values ranged approximately 1‰ from -19.68‰ (on Antler H; Fig. 15) to -18.37‰ (on Antler B; Fig. 15). The collagen carbon ($\delta^{13}C$) values did not show consistent trends along the length of the antler. Further, the samples from 12 individuals showed a high degree of variability (Fig. 15; Table 4). Only six antlers showed a significant linear relationship between $\delta^{13}C$ values and the antler length, though the slopes varied between both positive and negative values (ordinary least squares regression, $p < 0.05$; Fig. 15). The average $\delta^{13}C$ value for antler samples was -18.88 ± 0.26‰ (Table 4) and the average difference between burr and antler tip was 0.43 ± 0.29‰. Duplicate testing did not show a difference in $\delta^{13}C$ between samples (averaging 0.14 ± 0.13‰; Fig. 15).

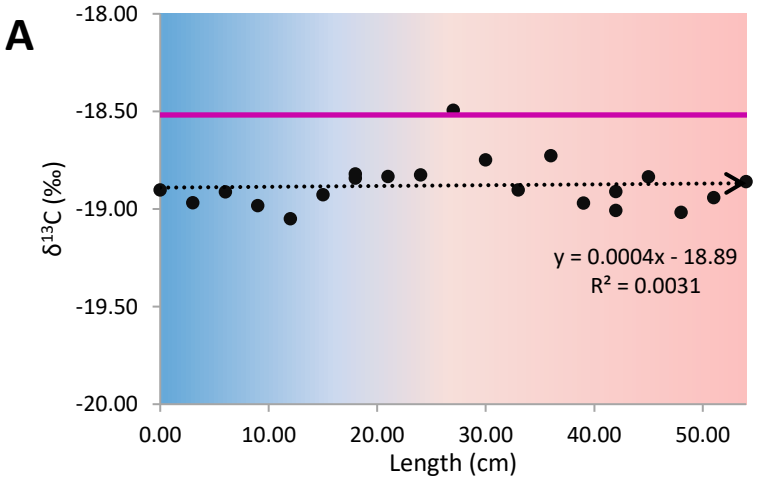
Bone $\delta^{15}N$ values ranged from 2.29‰ (on Specimen C; Fig. 16) to 7.00‰ (on Specimen G; Fig. 16). There was an average difference of 1.37 ± 0.84‰ between bone and antler $\delta^{15}N$ values (Table 4).

The collagen $\delta^{15}N$ values ranged ~5‰ from 2.26‰ (on Antler K; Fig. 16) to 6.91‰ (on Antler B; Fig. 16). The collagen nitrogen isotope ($\delta^{15}N$) values showed significant change along the length of the antlers (ordinary least squares regression, $p < 0.05$; Fig. 16). With the except Antler K, all antlers showed an increase in $\delta^{15}N$ values along their length (Table 4). The average difference in $\delta^{15}N$ values from the burr to the tip was 1.26 ± 0.91‰ (Fig. 16). $\delta^{15}N$ values started at an average of 4.36 ± 0.72‰ in the antler burr and increased by 1‰ to an average of 5.62 ± 0.85‰ in the tip of the antler (Fig. 16). However, there was a high degree of variation in the $\delta^{15}N$ values among individuals. The average $\delta^{15}N$ value for antler samples was 5.08 ± 0.81‰ (Table 4). Duplicate testing did not show a significant difference in $\delta^{13}C$ between samples (averaging 0.30 ± 0.36‰; Fig. 16).

Collagen $\delta^{13}C$ values were compared to $\delta^{15}N$ values throughout the length of the antler (Fig. 17). No consistent observable trend was seen across all 12 specimens, although certain individuals (i.e. Antlers B, H, I, J, and L; Fig. 17) did demonstrate slight variation between summer and winter values. Summer nitrogen values were higher than winter (Fig. 16) but carbon values did not have a consistent pattern (Fig. 15), as a result, individuals did not display a consistent shift in their $\delta^{13}C/\delta^{15}N$ ratios.

Collagen $\delta^{13}C$ values were also compared to carbonate $\delta^{13}C$ values throughout the length of the antlers (Fig. 18). Five antlers were sampled for both collagen and carbonate values and only two had similar observable trends between the two materials (Antler D & E, Fig. 18). The average difference between collagen $\delta^{13}C$ values and carbonate $\delta^{13}C$ values was 10.73 ± 0.35‰. The differences between collagen $\delta^{13}C$ values

and carbonate $\delta^{13}C$ values ranged by ~2‰ across all samples with the maximum difference at 11.98‰ and the minimum difference at 9.88‰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-18.00
-18.50
-19.00
-19.50
-20.00
δ13C (‰)
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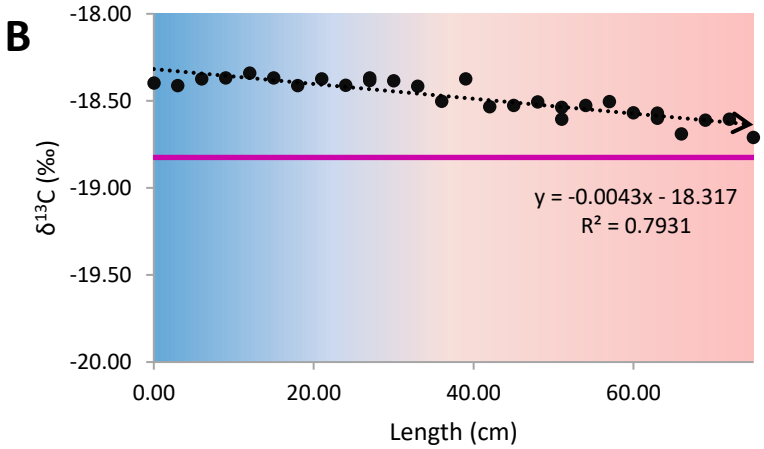
R² = 0.0031
0.00
10.00
20.00
30.00
40.00
50.00
Length (cm)


B
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-18.50
-19.00
-19.50
-20.00
δ13C (‰)
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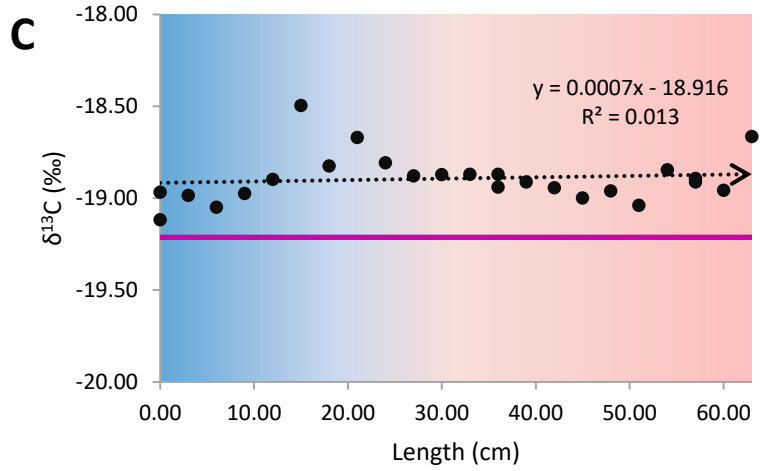
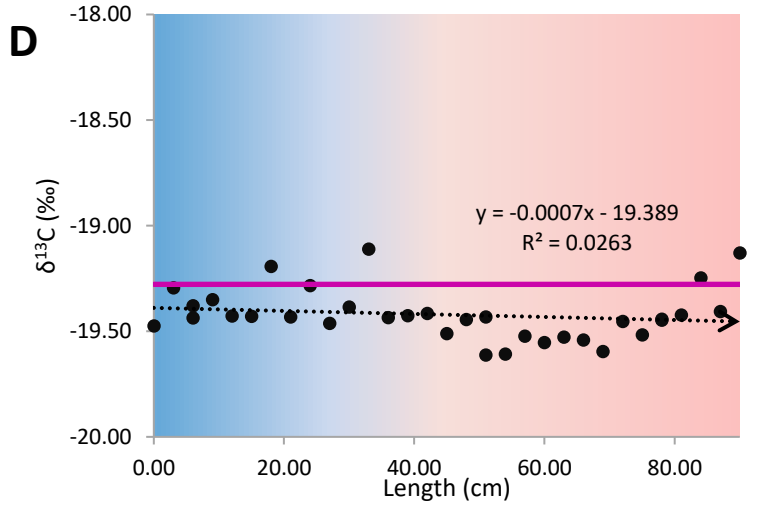
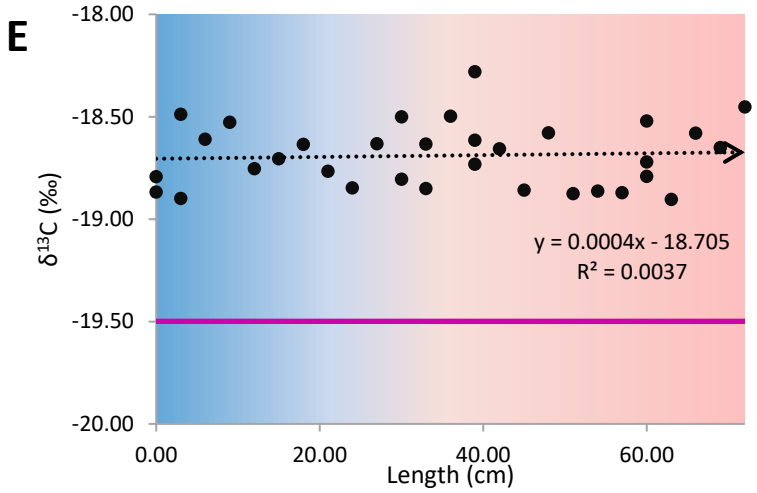
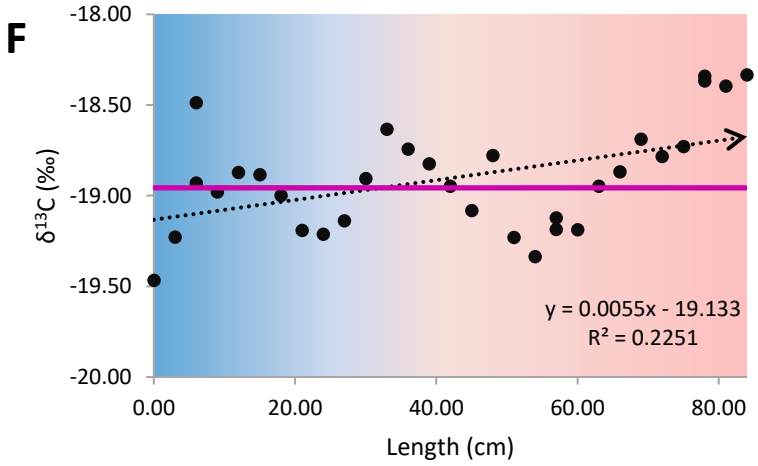
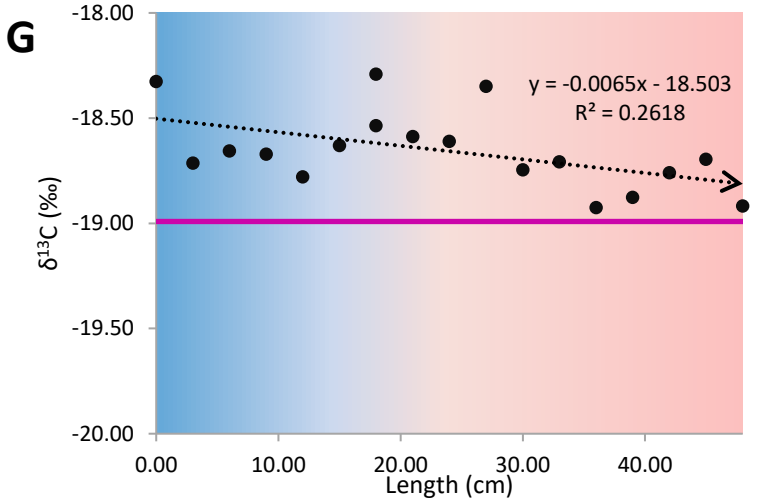
R² = 0.7931
0.00
20.00
40.00
60.00
Length (cm)

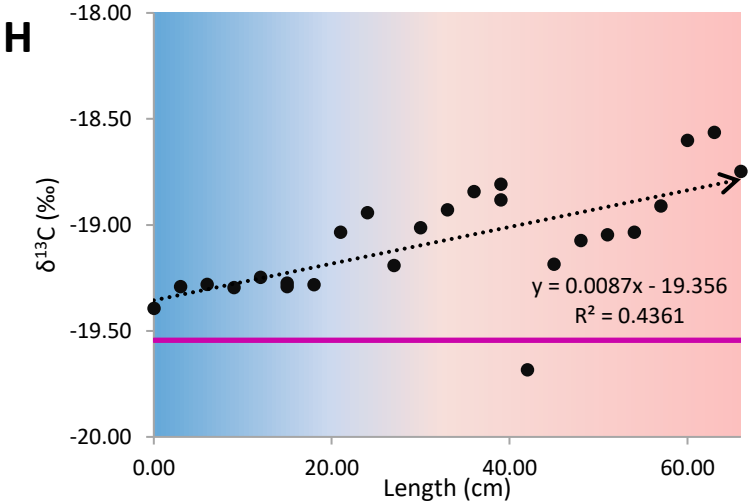

C
-18.00
-18.50
-19.00
-19.50
-20.00
δ13C (‰)
y = 0.0007x - 18.916
R² = 0.013
0.00
10.00
20.00
30.00
40.00
50.00
60.00
Length (cm)


D
-18.00
-18.50
-19.00
-19.50
-20.00
δ13C (‰)
y = -0.0007x - 19.389
R² = 0.0263
0.00
20.00
40.00
60.00
80.00
Length (cm)


E
-18.00
-18.50
-19.00
-19.50
-20.00
δ13C (‰)
y = 0.0004x - 18.705
R² = 0.0037
0.00
20.00
40.00
60.00
Length (cm)


F
-18.00
-18.50
-19.00
-19.50
-20.00
δ13C (‰)
y = 0.0055x - 19.133
R² = 0.2251
0.00
20.00
40.00
60.00
80.00
Length (cm)


G
-18.00
-18.50
-19.00
-19.50
-20.00
δ13C (‰)
y = -0.0065x - 18.503
R² = 0.2618
0.00
10.00
20.00
30.00
40.00
Length (cm)


H
-18.00
-18.50
-19.00
-19.50
-20.00
δ13C (‰)
y = 0.0087x - 19.356
R² = 0.4361
0.00
20.00
40.00
60.00
Length (cm)

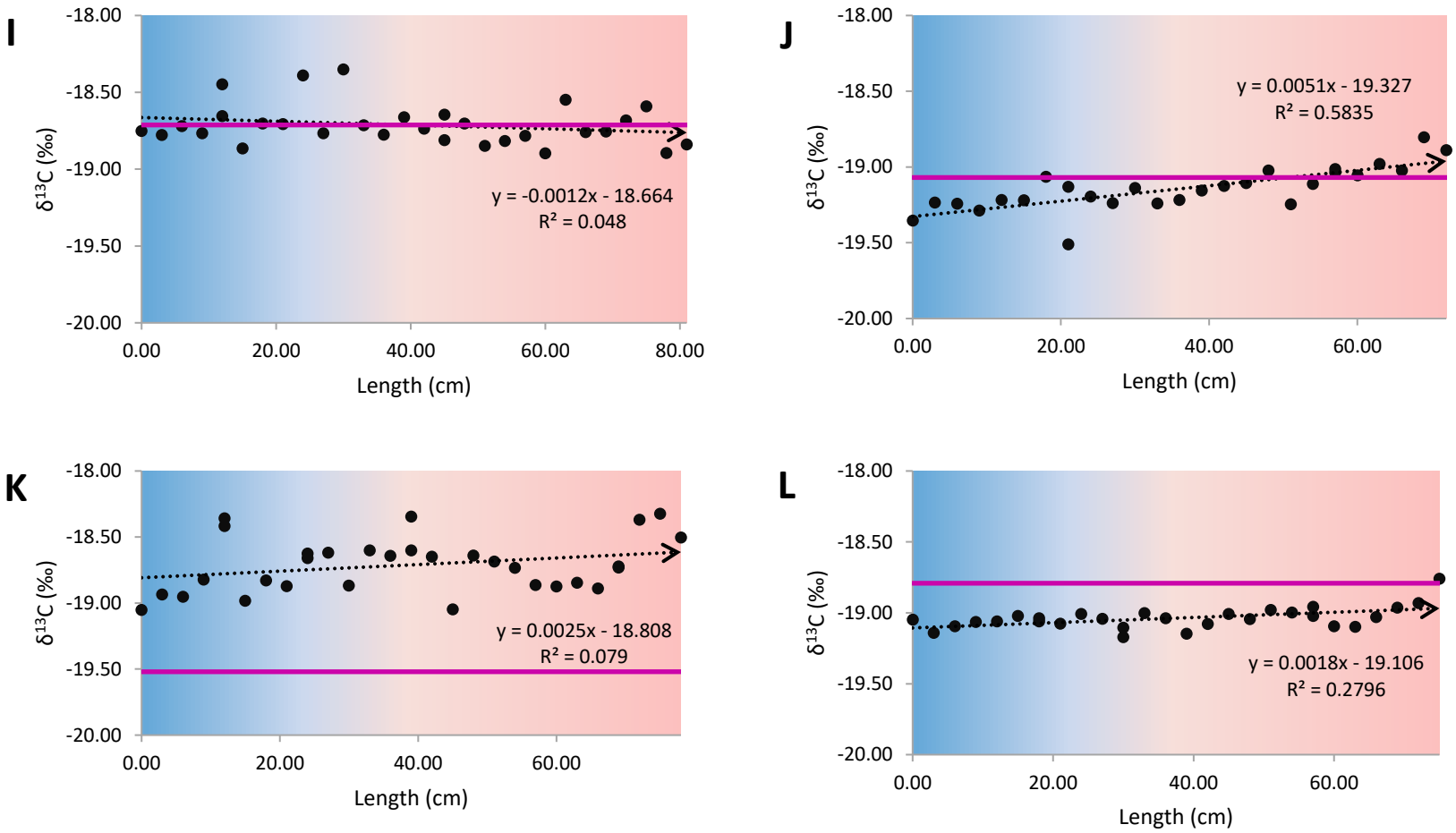
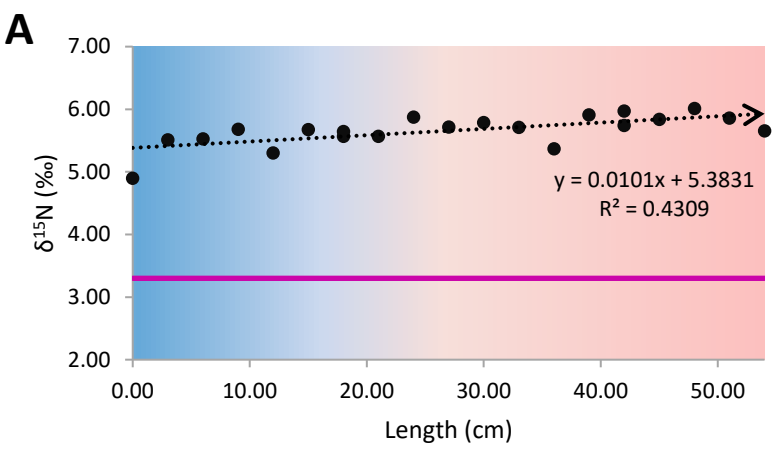
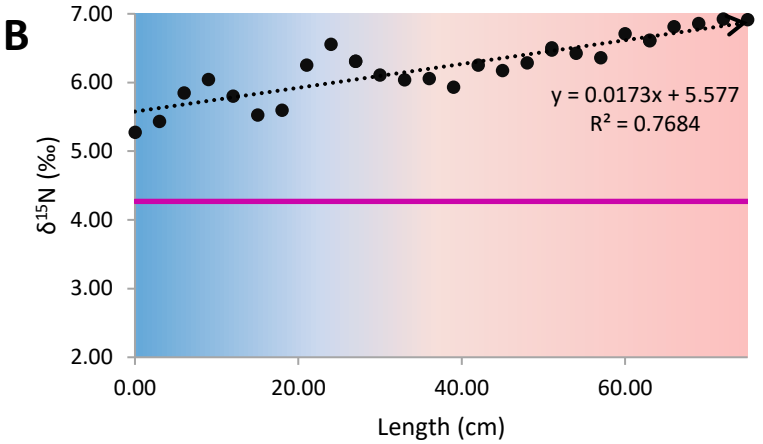
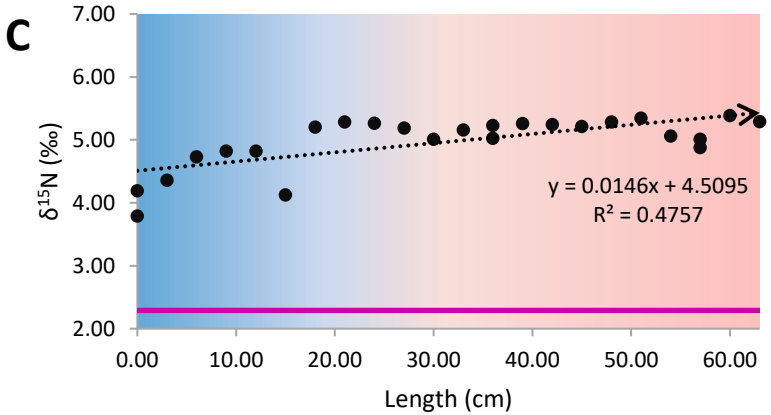
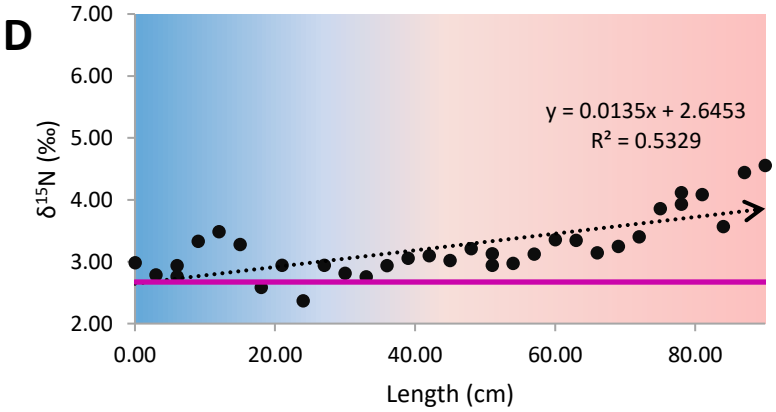


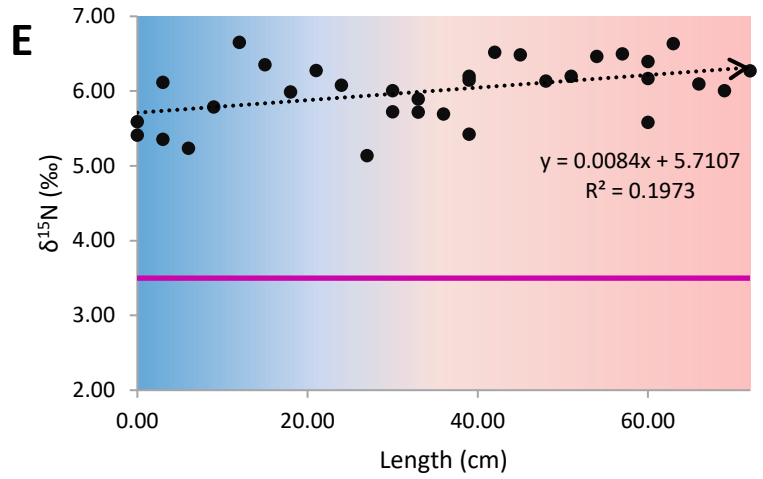
Figure 15. Linear relationship between antler length and $\delta^{13}C$ values for collagen stable isotopes. Linear regression analysis indicates some antlers follow a linear relationship with significant p-values. Antlers following a linear relationship are as follows: B, p = 9.8E-11; F, p = 0.006084; G, p = 0.029982; H, p = 0.000328; J, p = 3.55E-6; L, p = 0.003191. Antlers analyzed: A = 39079, B =39090, C = 30102, D = 39107, E = 39108, F = 39110, G = 39120, H = 39132, I = 39145, J = 39148, K = 39149, L = 39151. The colour gradient along the graph represents temporal change that occurs during antler growth, blue representing the winter and red the summer. Purple line represents the average bone isotope value.

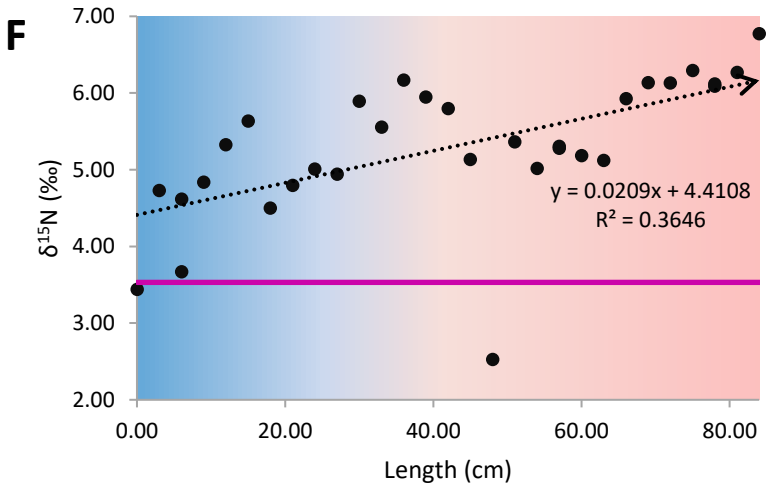

A
7.00
6.00
5.00
4.00
3.00
2.00
δ15N (‰)
y = 0.0101x + 5.3831
R² = 0.4309
0.00
10.00
20.00
30.00
40.00
50.00
Length (cm)

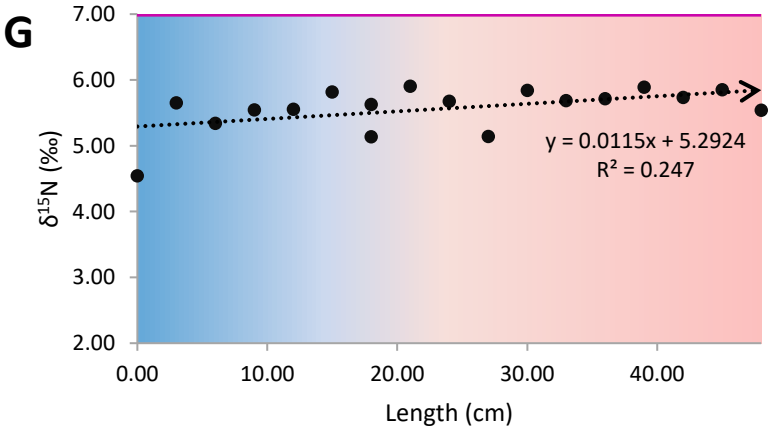

B
7.00
6.00
5.00
4.00
3.00
2.00
δ15N (‰)
y = 0.0173x + 5.577
R² = 0.7684
0.00
20.00
40.00
60.00
Length (cm)

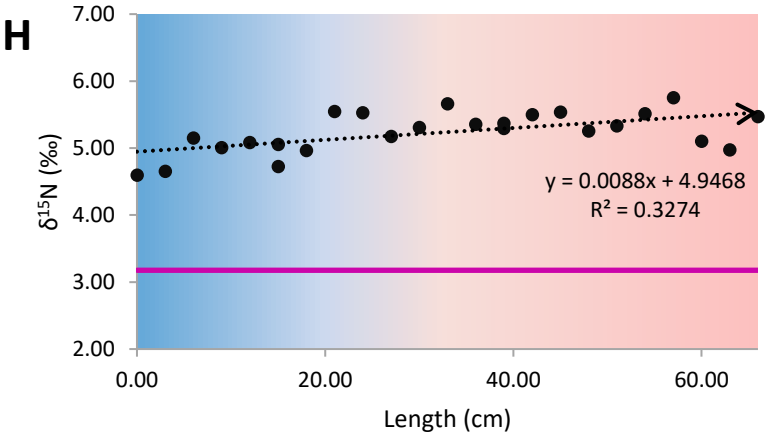

C
7.00
6.00
5.00
4.00
3.00
2.00
δ15N (‰)
y = 0.0146x + 4.5095
R² = 0.4757
0.00
10.00
20.00
30.00
40.00
50.00
60.00
Length (cm)


D
7.00
6.00
5.00
4.00
3.00
2.00
δ15N (‰)
y = 0.0135x + 2.6453
R² = 0.5329
0.00
20.00
40.00
60.00
80.00
Length (cm)


E
7.00
6.00
5.00
4.00
3.00
2.00
δ15N (‰)
y = 0.0084x + 5.7107
R² = 0.1973
0.00
20.00
40.00
60.00
Length (cm)


F
7.00
6.00
5.00
4.00
3.00
2.00
δ15N (‰)
y = 0.0209x + 4.4108
R² = 0.3646
0.00
20.00
40.00
60.00
80.00
Length (cm)


G
7.00
6.00
5.00
4.00
3.00
2.00
δ15N (‰)
y = 0.0115x + 5.2924
R² = 0.247
0.00
10.00
20.00
30.00
40.00
Length (cm)


H
7.00
6.00
5.00
4.00
3.00
2.00
δ15N (‰)
y = 0.0088x + 4.9468
R² = 0.3274
0.00
20.00
40.00
60.00
Length (cm)

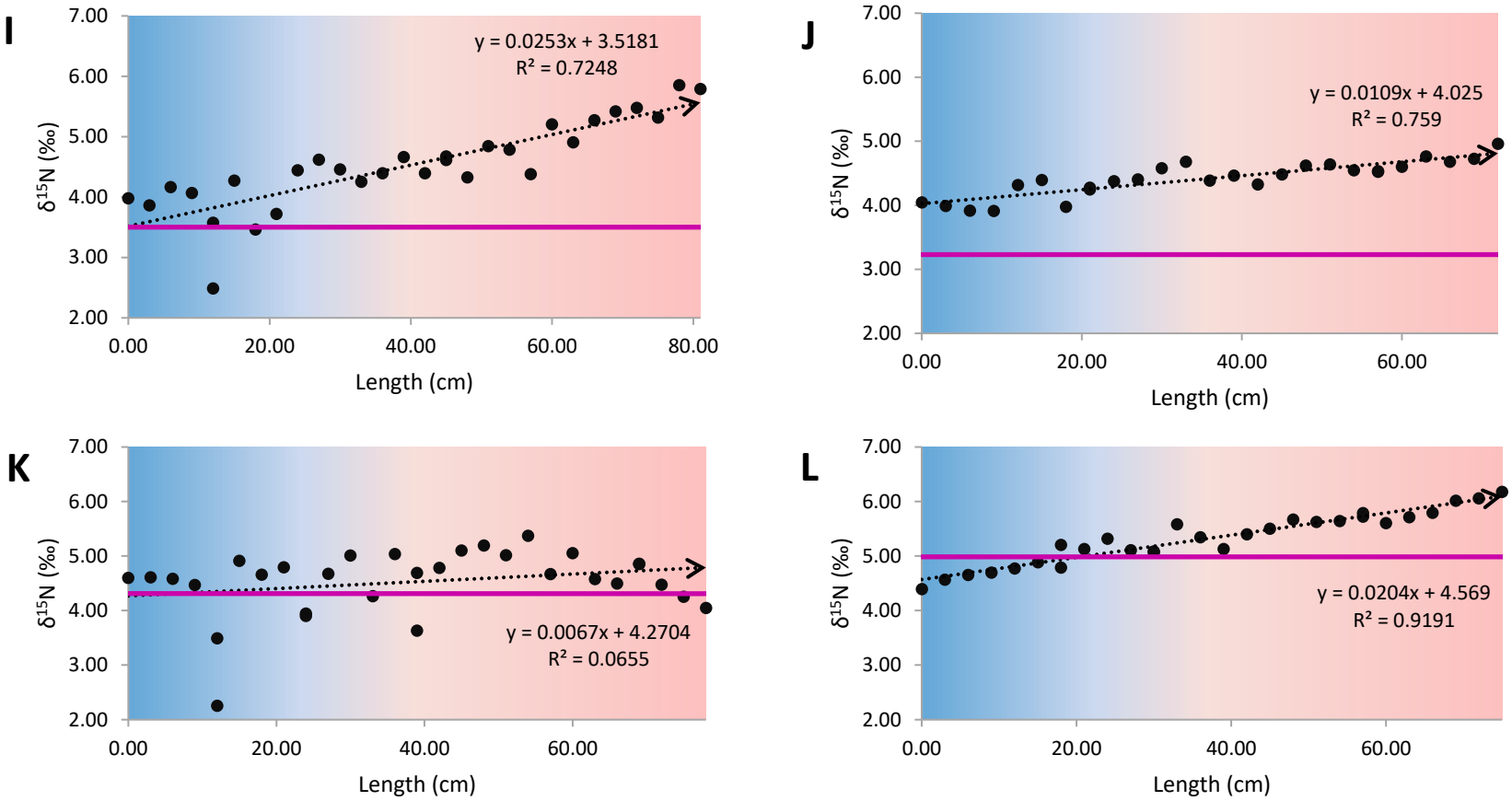
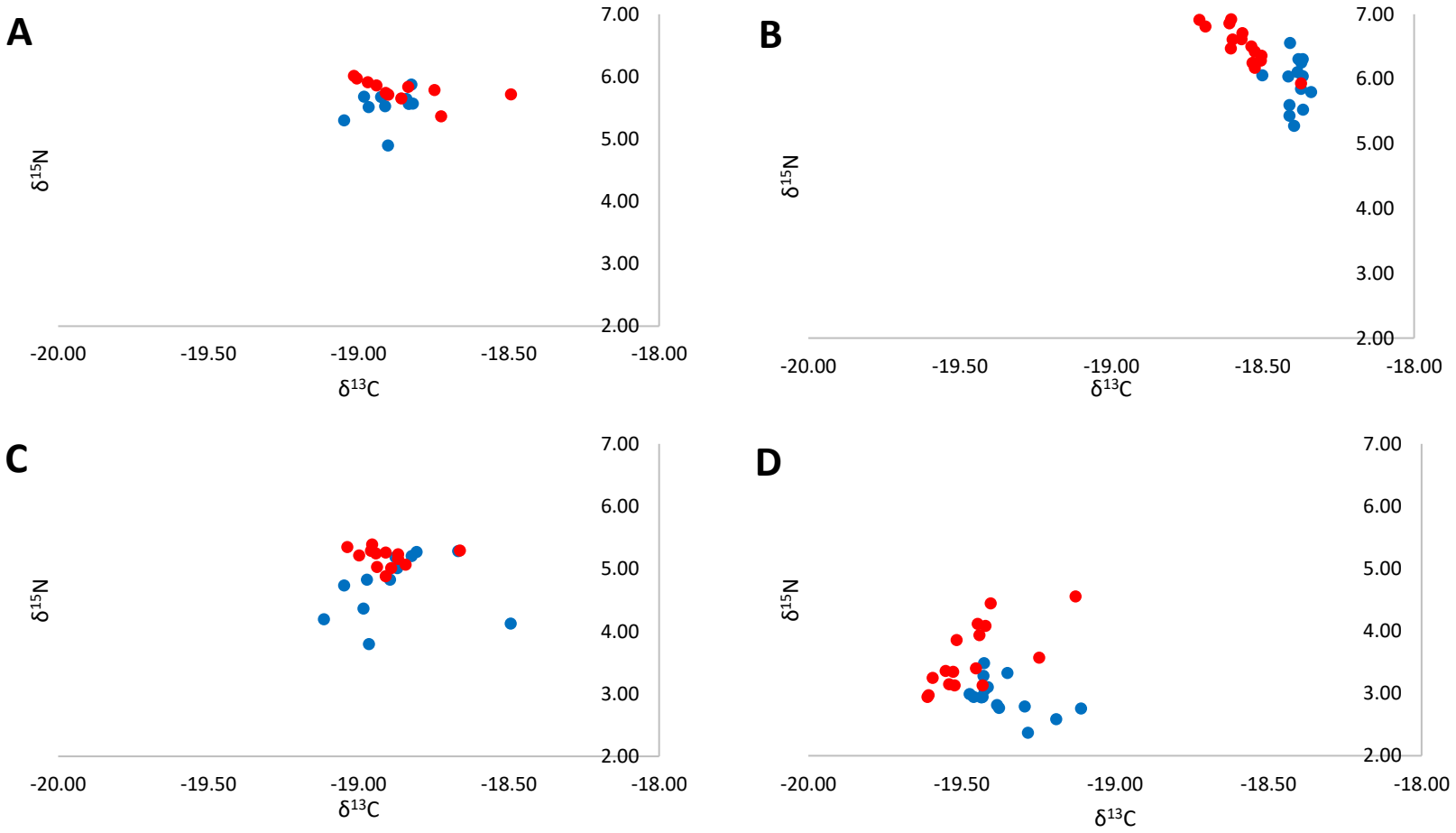
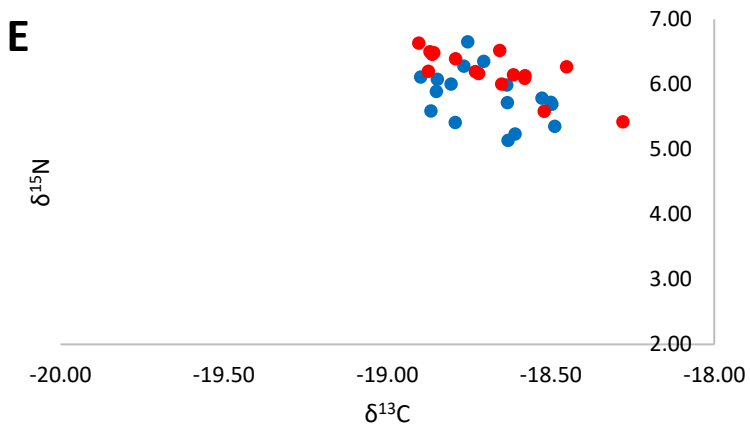
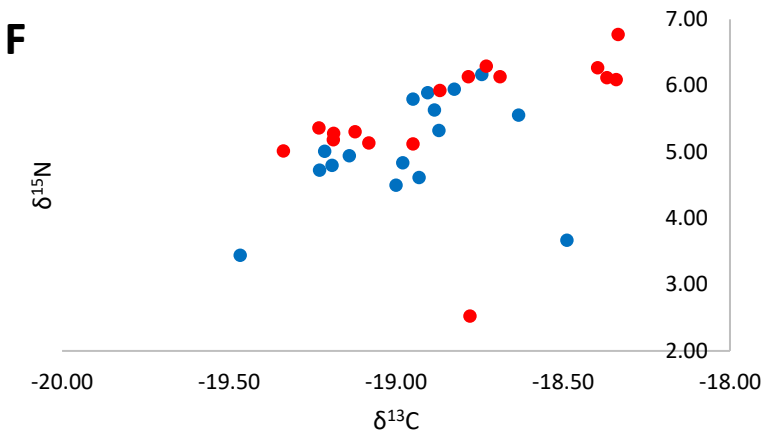
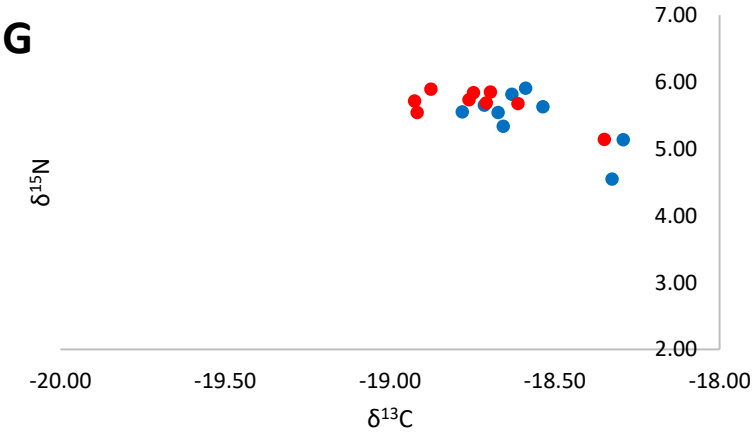


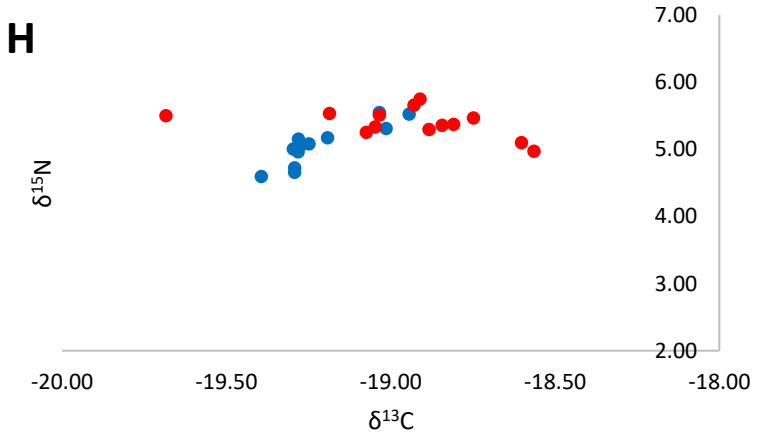
Figure 16. Linear relationship between antler length and $\delta^{15}N$ values for collagen stable isotopes. Linear regression analysis indicates all antlers, except (K) 39150, follow a linear relationship with significant p-values. The p-values for all significant linear relationships are: A, p = 0.001229; B, p = 4.57E-10; C, p = 0.000137; D, p = 9.59E-07; E, p = 0.009617; F, p = 0.000253; G, p = 0.035858; H, p = 0.0028; I, p = 2.48E-09; J, p = 3.37E-09; L, p = 2.85E-16. K has a linear regression p-value of 0.164788. Antlers analyzed: A = 39079, B =39090, C = 30102, D = 39107, E = 39108, F = 39110, G = 39120, H = 39132, I = 39145, J = 39148, K = 39149, L = 39151. The colour gradient along the graph represents temporal change that occurs during antler growth, blue representing the winter and red the summer. Purple line represents the average bone isotope value.


A
B
C
D
7.00
6.00
5.00
4.00
3.00
2.00
δ15N
-20.00
-19.50
-19.00
-18.50
-18.00
δ13C


E
7.00
6.00
5.00
4.00
3.00
2.00
δ15N
-20.00
-19.50
-19.00
-18.50
-18.00
δ13C


F
7.00
6.00
5.00
4.00
3.00
2.00
δ15N
-20.00
-19.50
-19.00
-18.50
-18.00
δ13C


G
7.00
6.00
5.00
4.00
3.00
2.00
δ15N
-20.00
-19.50
-19.00
-18.50
-18.00
δ13C


H
7.00
6.00
5.00
4.00
3.00
2.00
δ15N
-20.00
-19.50
-19.00
-18.50
-18.00
δ13C

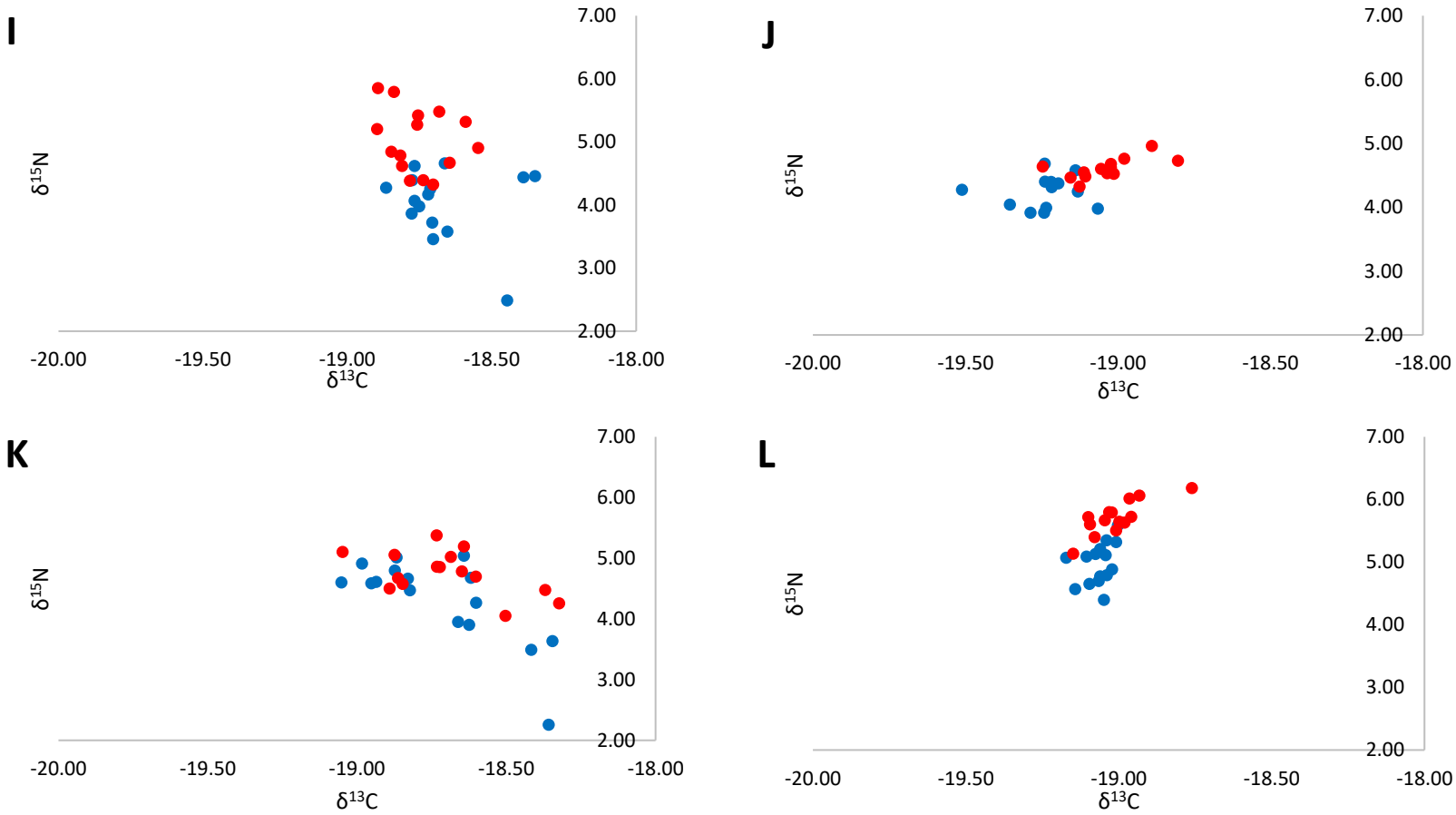
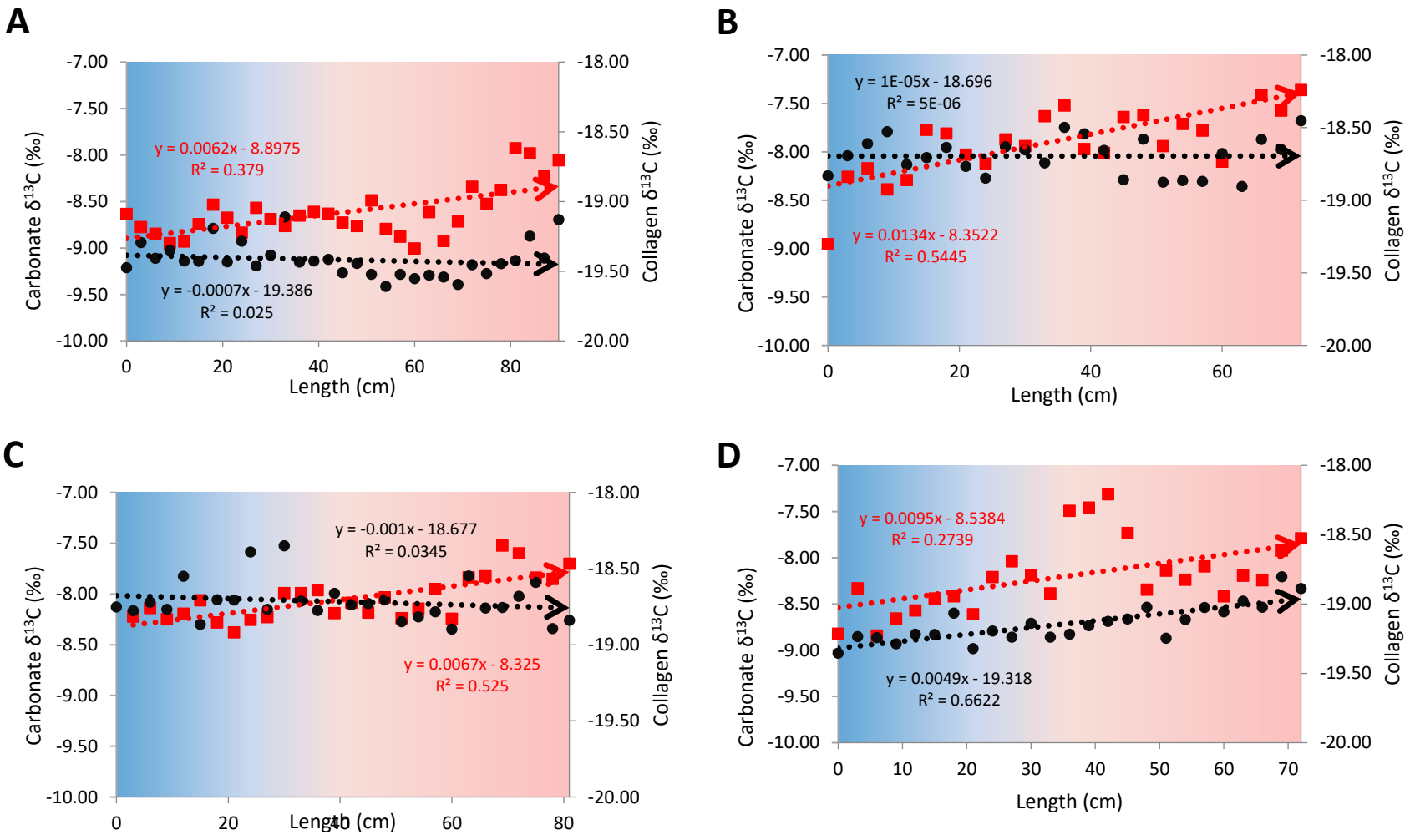


Figure 17. $\delta^{13}C/\delta^{15}N$ values for collagen data for each individual antler. Values are shown in two separate groups, summer (red) values and winter (blue) values. The degree of variation within the antler is quite unique with some individuals showing little change throughout the antler with others containing a high degree of variation. Blue points represent the half of the antler formed between February and April while the red represent April to August. Antlers analyzed: A = 39079, B =39090, C = 30102, D = 39107, E = 39108, F = 39110, G = 39120, H = 39132, I = 39145, J = 39148, K = 39149, L = 39151.


A
y = 0.0062x - 8.8975
R² = 0.379
y = -0.0007x - 19.386
R² = 0.025
Carbonate δ13C (‰)
Collagen δ13C (‰)
Length (cm)
B
y = 1E-05x - 18.696
R² = 5E-06
y = 0.0134x - 8.3522
R² = 0.5445
Carbonate δ13C (‰)
Collagen δ13C (‰)
Length (cm)
C
y = -0.001x - 18.677
R² = 0.0345
y = 0.0067x - 8.325
R² = 0.525
Carbonate δ13C (‰)
Collagen δ13C (‰)
Length (cm)
D
y = 0.0095x - 8.5384
R² = 0.2739
y = 0.0049x - 19.318
R² = 0.6622
Carbonate δ13C (‰)
Collagen δ13C (‰)
Length (cm)

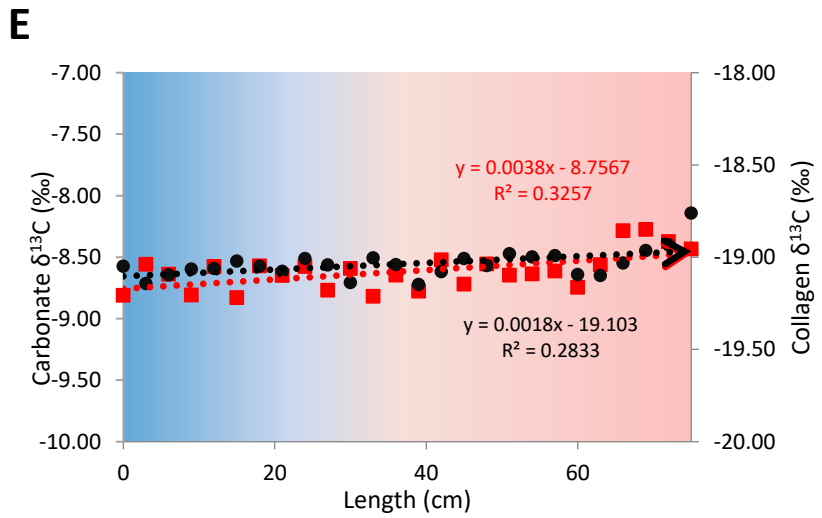


Figure 18. Comparison graphs between the collagen $\delta^{13}C$ isotopes (indicated in black circles) and carbonate $\delta^{13}C$ isotopes (indicated in red squares) along the length of the antler. Collagen and carbonate values followed different trends 3 of the 5 samples with similarities only in Antlers D & E. Antlers analyzed: A = 39107, B = 39108, C = 39145, D = 39148, E = 39151.

Table 4: Average isotopic values for collagen $\delta^{13}C$ & $\delta^{15}N$ for mandible (bone) and antler, including $\delta^{13}C$ & $\delta^{15}N$ values from the antler burr and antler tip. Complete table of all collagen isotope data see Appendix 2: Collagen Stable Isotope Data.

Sample		Collagen							
		Antler Tip	Antler Burr	Antler Average	Bone	Antler Tip	Antler Burr	Antler Average	Bone
		Carbon ($\delta^{13}C$ ‰)				Nitrogen ($\delta^{15}N$ ‰)			
A	39079	-18.86	-18.90	-18.88	-18.52	5.65	4.90	5.66	3.30
B	39090	-18.71	-18.40	-18.48	-18.82	6.91	5.28	6.24	4.27
C	39102	-18.66	-19.04	-18.89	-19.21	5.29	3.99	4.97	2.29
D	39107	-19.13	-19.47	-19.42	-19.28	4.55	2.99	3.25	2.67
E	39108	-18.45	-18.83	-18.69	-19.50	6.27	5.50	6.00	5.13
F	39110	-18.33	-19.47	-18.90	-18.96	6.77	3.44	5.30	3.53
G	39120	-18.92	-18.33	-18.66	-18.99	5.54	4.54	5.56	7.00
H	39132	-18.75	-19.39	-19.07	-19.54	5.47	4.60	5.23	3.18
I	39145	-18.84	-18.75	-18.71	-18.71	5.79	3.98	4.52	3.50
J	39148	-18.89	-19.35	-19.14	-19.07	4.96	4.04	4.42	3.23
K	39149	-18.50	-19.05	-18.71	-19.52	4.05	4.60	4.53	4.31
L	39151	-18.76	-19.05	-19.04	-18.79	6.18	4.40	5.33	4.99

4.0 Discussion

The objective of this study was to determine whether *Rangifer tarandus* (caribou) antlers demonstrate a different isotopic signal in comparison to other hard tissues such as bone and teeth using stable isotopes of oxygen, carbon, and nitrogen. It also aimed to determine whether isotope values varied along the length of the antler growth axis.

4.1 Antler Trends

The following discussion of antler trends is presented in the same order as the results. It was predicted that isotopic variation would occur along the growth axis of the antler. Predictions for the cause of this variation were seasonal migration and physiological fractionation.

4.1.1 Carbonate – Carbon isotopes

Fifteen specimens were selected for analysis. However, after initial complications with oxygen isotope repeatability, remaining carbonate samples were set aside until a solution could be found to produce reliable oxygen values. Therefore, only five of the 15 were analyzed for carbonate carbon. All specimens showed significantly increasing $\delta^{13}C$ values of ~1‰ along the length of the antler (Fig. 10). Increasing $\delta^{13}C$ values during antler growth are likely a result of dietary changes during the spring migration (Fig. 3). The spring migration of the Qamanirjuaq population occurs during the first snow melt when the antlers have begun to form (February to March). The antlers continue to grow

as the population moves through the entirety of the spring migration, from northern Saskatchewan and Manitoba to North Henik Lake in central Nunavut (Miller F.L., 1973).

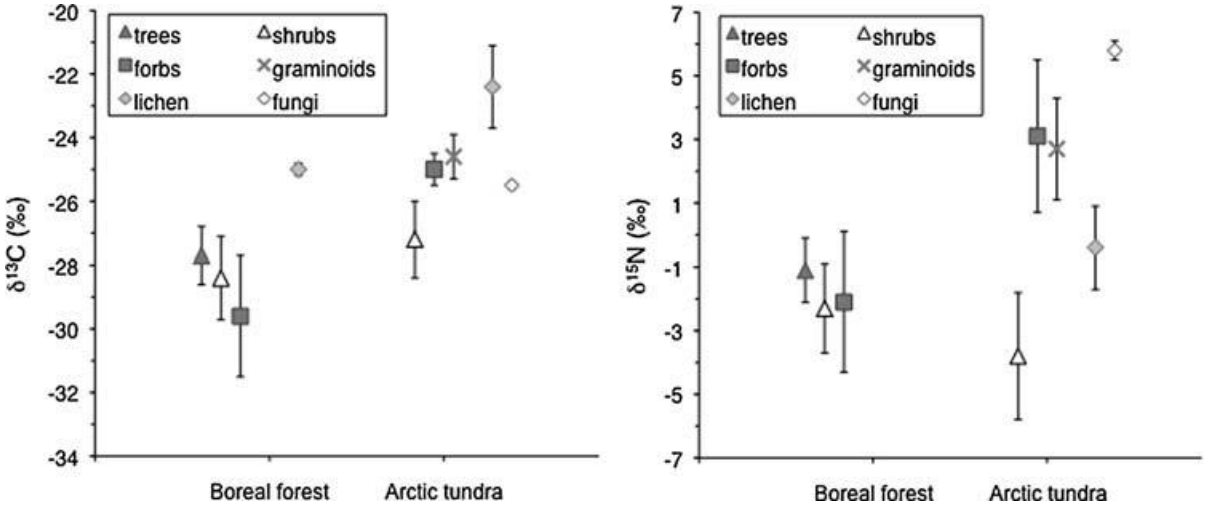


Figure 19. From paper Drucker et al. (2012) a review of the plant isotopes within the boreal forest and Arctic tundra from the following papers Brooks et al. 1997; Milligan, 2008; Griffith et al. 2002; McLeman, 2006; and Barrett, 1994.

The caribou tend to winter in the boreal forest of northern Saskatchewan and Manitoba, where they have access to various food sources, including lichen, a few shrubs, and sedges – primarily *Carex aquatilis* and *Equisetum fluviatile* (Table 1; Fig. 4) (Parker, 1973; Baldwin, 1953). During the spring, the caribou begin to migrate northward toward their calving ground near North Henik Lake (Fig. 3), an area characterized by tundra. Various plants and lichens have been shown to increase by 3-5‰ in $\delta^{13}C$ between the boreal forest and tundra (Brooks *et al.* 1997; Milligan, 2008; Griffith *et al.* 2002; McLeman, 2006; Barrett, 1994), potentially accounting for the increases in $\delta^{13}C$ within antlers. Previous studies suggest that caribou show a shift in $\delta^{13}C$ due to greater consumption of lichen during the winter season (Drucker *et al.* 2010, 2012). However, lichen is consumed continuously throughout the year and is a staple of

the Qamanirjuaq caribou diet (Fig. 8), suggesting that a dietary shift away from lichens in the spring may not be a contributor to $\delta^{13}C$ along antlers.

Dietary shifts are, however, apparent from rumen samples (Table 1). The winter collection period (for 1967) found a higher percentage of occurrence for flora species of the genus *Equisetum*, and a higher occurrence for select species within the conifer and woody angiosperm groups (i.e. *Larix laricina*, *Andromeda polifolia, Kalmia polifolia, Pinus banksiana* and *Vaccinium myrtilloides*) in the rumen content of the Qamanirjuaq caribou. In comparison, the summer collection period had much higher *Betula* spp., *Pleurozium schreberi, Dicranum* spp., *Ptilidium ciliare*. Kristensen *et al.* (2011) found that, despite a lack of photosynthetic difference between Arctic plant groups, plants with higher drought tolerance – higher water-use efficiency – have a higher $\delta^{13}C$ value. In general, there is a 1‰ enriched in ^{13}C in more drought-tolerant plants compared to other graminoids at a similar latitude (Kristensen *et al.* 2011, Marshall *et al.* 2007). Higher drought-tolerant plants (e.g. *Pleurozium schreberi*) are commonly found in the Arctic tundra (Teeri, 1973; Ellis, 2020) and may have a large impact on the shift in $\delta^{13}C$ found throughout the length of the antler.

As part of future work in this area, I plan to test whether the $\delta^{13}C$ values in plants collected from rumen samples, during the CWS study, align with the shifts in $\delta^{13}C$ found throughout the antler length. I hope to test the isotopic composition of several Arctic plant species, including lichens (i.e. *Cladina* spp., *Cladonia* spp. and *Stereocaulon* spp.), Bryophyta (i.e. *Pleurozium schreberi* and *Ptilidium ciliare*), Angiosperms (i.e. *Kalmia*

polifolia, Andromeda polifolia, Vaccinium myrtilloides, and *Betula* spp.), and *Equisetum* spp.

4.1.2 Carbonate – Oxygen isotopes

Oxygen isotope analysis from carbonates of antler tissue proved to be challenging because the second pre-treatment step (1 M acetic acid treatment for 24 hours) dissolved all the antler material. The acetic acid pre-treatment is designed to remove exogenous carbonates – carbonates formed through post-mortem weathering – however, the antlers studied were collected immediately after death, thus the acetic acid pre-treatment was deemed inconsequential. After I forwent the acid treatment, oxygen isotope analysis of the carbonates did not produce repeatable results (Fig. 12). There are several potential reasons for these complications.

Firstly, the pre-treatment used for this study was one designed for bone and tooth enamel and, while antler has a similar make up of protein and calcium hydroxylapatite, the crystal structure of antlers is different. Chen *et al.* (2009) studied the structure and mechanical properties of bone and antler in North American Elk (*Cervus elaphus canadensis*). Caribou, elk, and moose have the fastest growing antlers of the 40 cervid species that grow antlers. Chen *et al.* (2009) found that antlers have a platelet crystalized structure that range from 20 – 70 nm in length and have a thickness of 4 nm. Bone has a similar structure and the crystals vary in size from 25 – 50 nm with a thickness of 3 nm. The different crystal structure of antlers provides a higher surface area for the transportation of nutrients within the antlers vascular system (Chen *et al.*

2009). The high surface area could also have caused a dissolution of the antler material during the 1 M acetic acid treatment, resulting in the antler material being lost.

A second plausible explanation is a post-sampling re-crystallization of antler material. Re-crystallization can result in changes in the $\delta^{18}O$ values of carbonates. Koch *et al.* (1997) noted that acids can promote the recrystallization of hydroxylapatite into brushite during chemical pre-treatment. This recrystallization can be particularly potent with acids such as 1M acetic acid; a step – as previously mentioned – which was removed during pre-treatment. While Koch *et al.* (1997) did not determine any recrystallization when using 2% NaOCl, they did not conduct the experiment on antler crystals. The submersion of the antler crystals in 2% NaOCl for 24 hours, in conjunction with the naturally high surface area of antler crystals, could have potentially caused the disproportionate recrystallizing of hydroxylapatite into brushite or other minerals. This offset would have caused a change in the $\delta^{18}O$ samples but would not impact the $\delta^{13}C$ values because brushite does not contain carbon. Koch *et al*. (1997)'s study also provides a second possibility to explain the poor repeatability of the carbonate oxygen isotopes in *Rangifer tarandus* antlers. Structural carbonate within biological hydroxylapatite is chemically bound at phosphate and hydroxyl lattice sites. The variation between chemical bond strengths could lead to differences in atmospheric exchange rates at these two sites. If, during pre-treatment, the high surface area of antler crystals allowed partial dissolution of carbonate at these sites, the disproportional release of carbonate from the phosphate and hydroxyl lattice sites could have altered the apparent $\delta^{18}O$ values of samples. It is unlikely that the 2% NaOCl solution would

cause these complications presented by Koch *et al.* (1997), however, with relatively little understanding of antler isotope analysis I believe it is worth further investigation in the future.

Finally, the sample washing procedure, which is conducted to remove all traces of pre-treatment chemicals from the sample, could have led to the poor repeatability of stable oxygen isotopes. The high surface area of antler crystal structures may have led to the rehydration of antler crystals during the wash with deionized water. Oxygen isotopes from the water molecules may have bonded or replaced the oxygen within the carbonate. Figure 12 shows the comparison of repeat samples with most varying by 5‰, possibly indicating a contamination from a single source (i.e. the deionized water). The rehydration may have transpired when samples were heated in an oven to dry overnight. However, to test this assumption, samples were placed in a freeze drier overnight. Oxygen isotope values from duplicates of the same sample still showed poor repeatability. Rehydration in antlers has been shown to occur and improve antler elasticity and fracture toughness in comparison to dry antler (Chen *et al.* 2009). A high rehydration potential may be important for antler function but may have caused the isotope sampling issues within *Rangifer tarandus* antlers.

For future analysis, experimenting with different pre-treatment protocols will be critical. In combination with Fourier-transformed infrared spectroscopy (FTIR), which provides information on crystal structure, it will provide a means of understanding variability in carbonate oxygen isotopes. In addition to FTIR and pre-treatment experiments, use of phosphate oxygen isotope values could present an alternative that

may suffer less from oxygen exchange with the atmosphere and water due to greater bond strength.

4.1.3 Collagen – Carbon Isotopes

There is a debate among scientists about whether carbonate or collagen stable carbon isotopes offer a better isotopic representation of diet, with many studies advocating for the use of both (Clementz *et al*. 2009; Froehle *et al.* 2010; Codron *et al.* 2018). The utility of sampling the same tissue for carbon isotopes from both tissues relates to the difference in carbon sourcing. Carbonate carbon incorporates carbon from all nutrient consumption, thus reflecting the average diet, whereas collagen is incorporating carbon preferentially from dietary protein (Ambrose & Norr, 1993). Studying both tissues might therefore provide a more holistic understanding of diet. There is, however, much more ecological research utilizing collagen carbon isotopes, as finding the source of dietary protein can often be easier than observing the entire bulk diet. Herein, I was able to utilize both for a subset of specimens.

I expected that migration between the boreal forest and tundra ecozones during the spring migration would result in an increase in $\delta^{13}C$ values for antler collagen, as with the carbonates. Unlike the antler carbonates, however, collagen $\delta^{13}C$ values from 12 sampled antlers did not show increases (Fig. 15). Only six of 12 showed a statistically significant change in $\delta^{13}C$ (Fig. 15). In particular, the five antlers analyzed for both carbonate and collagen stable carbon isotopes did not show consistent change (Fig. 18). Of the five individuals sampled for both carbonate and collagen $\delta^{13}C$ values, only two

showed consistent increases along the length of the antler for both tissues (Fig 18). However, future study will need to increase the carbonate sampling to create a better comparison to collagen values.

Typically, carbonate and collagen carbon values show a linear relationship, with carbonate $\delta^{13}C$ showing a ~9‰ enrichment (Codron *et al.* 2018; Ambrose & Norr, 1993). Carbonate $\delta^{13}C$, however, has been found to be significantly less variable than collagen $\delta^{13}C$, particularly with regards to seasonal dietary changes involving high protein plants (Codron *et al.* 2012). Nutrients consumed by *Rangifer tarandus* of the Qamanirjuaq population are entirely derived from plant consumption. Within a plant, $\delta^{13}C$ can vary among the carbohydrates, lipids, and proteins (Dungait *et al.* 2008), potentially producing differences between carbon isotopes within collagen and carbonates. If plant protein is enriched in ^{13}C, then the caribou consumption of high protein plants during the summer months (Drucker *et al.* 2001) could result in $\delta^{13}C$ trend differences between collagen and carbonates.

High protein plant consumption could result in the different trends between collagen and carbonate results. Additionally, individualistic migratory and dietary behaviour might contribute to the variability among collagen findings. During the spring and summer, male caribou migratory patterns are inconsistent, many are scattered throughout the populations range, until they begin to migrate north to Henik Lake for the rut (Fig. 3) (Parker, 1973). Males may move in either small bands or independently (Parker, 1973; Miller F.L., 1973). To minimize potential variation due to differences in migration routes, samples were selected from similar localities, however, this does not

eliminate all possible effects of the scattered spring migration behavior. The lack of consistent migratory patterns among the males could lead to the consumption of different high protein plants. Caribou have very flexible foraging behaviour and can switch dietary items depending on biomass availability (Barboza, 2018; Van der Wal *et al.* 2000, Thompson *et al.* 2015, Denryter *et al.* 2017). It is possible that the male caribou selected for study had different foraging behaviours. If the high protein plants varied significantly in $\delta^{13}C$ values the result would cause a high degree of variation within my antler samples.

To address this hypothesis, I suggest two additional stable isotope analyses. Strontium isotope analysis along the antler growth axis could be used to measure different migratory patterns between the male caribou. Strontium isotopes have been, previously, used to measure migration patterns in *Rangifer tarandus* teeth (Britton *et al.* 2011) but not antlers. A second stable isotope analysis could be conducted on Arctic plants, studying the difference in protein $\delta^{13}C$ values. Arctic plants found in rumen contents (Table 1) may provide information as to the variability found within collagen carbon samples.

As mentioned, protein is a limiting nutrient for herbivores like caribou during the winter months (Gerhart *et al.* 1996), limited protein availability may also cause the variation seen with collagen carbon results (Fig. 15). Like many ruminants, caribou will recycle protein from reserves into urea though protein catabolism, when protein sources are scarce (Drucker *et al.* 2010; Soveri *et al.* 1992; & Westerling, 1970). Recycling of proteins occurs within the foregut and causes a substantial enrichment ^{13}C

(Parker *et al.* 2005). If both diet and protein recycling production vary throughout the antler growth season it may result in the observed changes. Furthermore, the rut, which occurs in November, may also lead to nutrient stress and increased protein recycling.

Finally, one other possible cause for the variation observed in collagen carbon $\delta^{13}C$ values is methane production. Herbivores produce methane through microbial fermentation in the gastrointestinal tract this causes the preferential loss of ^{12}C, resulting in higher $\delta^{13}C$ values within the body and newly synthesized tissue (Codron *et al.* 2018; Hedges, 2003; Passey *et al.* 2005). Seasonal changes in methane production could result in the $\delta^{13}C$ values found within the antler. However, this would affect all synthesized tissue and the results would be present in collagen and carbonate isotopes. Presently, the lack of variation among carbonate values indicates that this is an unlikely explanatory factor, more carbonate results are needed.

4.1.4 Collagen – Nitrogen Isotopes

For 11 out of the 12 *Rangifer tarandus* antlers sampled, there was significant increase in $\delta^{15}N$ values of 1-3‰ along the growth axis (Table 4). $\delta^{15}N$ values are often used to determine the trophic level of a species, with increases of ~3‰ indicating a shift from one trophic level to another (Minagwa & Wada, 1984; DeNiro & Epstein, 1981; Kelly, 2000). The consistent 1-3‰ for caribou antlers are therefore nearly the equivalent of an increase in one trophic level (Fig. 6). Though individualistic foraging behaviours may have driven the observed changes in $\delta^{13}C$ from collagen, this does not appear to be

the case for $\delta^{15}N$ despite my expectations (Fig. 6). This unexpected and consistent increase in $\delta^{15}N$ may have three plausible explanations.

The first possibility is that the increasing $\delta^{15}N$ values are reflective of increased mushroom consumption during the late summer and early winter (Table 1). Caribou show virtually no mushroom consumption during the months of June, February, and April but significant consumption in September (Fig. 4). Several other studies show that caribou actively seek to consume mushrooms (Launchbaugh & Urness, 1992; Karaev, 1968; Boertjie, 1981). Fungi (i.e. mushrooms) have a substantially higher $\delta^{15}N$ values relative to other Arctic plants (Fig. 19) (Drucker *et al.* 2012; Barnett, 1994). The dietary preference of mushrooms may therefore create the increase in $\delta^{15}N$ along the antler growth axis. Future analysis of isotopic composition of $\delta^{15}N$ in Arctic fungi from the same region where the antlers were harvested is, however, necessary.

The second possibility is nutrient stress (Ambrose, 1991; Kelly, 2000; Hobson & Clark, 1992; Cormie & Schwarcz, 1996). Enrichment of $\delta^{15}N$ values in tissue are shown to be caused by nutrient stress, due to metabolizing of the body's tissue, particularly nitrogen-rich fat, resulting in the recycling of nitrogen in the body (Kelly, 2000). This effect can be particularly strong in select ruminants (including *Rangifer tarandus*), where water stress result in recycling of urea in the gut as a source of nitrogen for microbial digestion (Ambrose, 1991). The 1966/1967 winter was associated with high snow accumulation and lower than average temperatures (Dauphine, 1973), which may have resulted in higher than average nutrient stress. Deep snow can inhibit the caribou's access to subnivean plant materials causing an increase in nutrient stress (Schaeffer &

Mahoney, 2001; Espmark, 1964; Barrette & Vandal, 1986, 1990). Furthermore, the rut occurs in October and males are usually starved prior to and during the rut (Drucker *et al*. 2010), further contributing to nutrient stress and $\delta^{15}N$ increases in the antler collagen.

Finally, consistent $\delta^{15}N$ increases in male Qamanirjuaq caribou antlers may be caused by nutrient and bone resorption during antler growth. Baksi and Newbrey (1989) found that bone ash and calcium percentage were significantly lower in July relative to January in caribou ribs while osteoid volume (collagen) was significantly higher. Antler growth is highly taxing and often nutrients will be repurposed from within the body (often from the rib cage) to aid with heavily nutrient dependent growth of antlers. The redistributing of nutrients may have an isotopic effect that has not been observed, it could be a potential cause of the persistent increase in $\delta^{15}N$ values.

4.2 Tissue Comparison

Bone and teeth have been thoroughly studied, their patterns of isotope incorporation are well known and commonly used for ecological studies (e.g. Passey & Cerling, 2002; Fricke & O'Niel, 1996; Fricke *et al.* 1998; Eriksen *et al.* 1990). Herein, I show that antlers are unique in that their patterns of isotopic variation are different from teeth and bone. I hypothesized that isotopic variation would occur between the three tissues due to differences in timing of tissue development and times over which the different tissues exchange isotopes with the available "pool" in the body (Hedges, 2007; Drucker *et al.* 2010; Gannes *et al.* 1997). Antler, bone, and tooth tissues form during different times of

the year and incorporate isotopes from the diet over different temporal scales. Antler forms from February to end of August, a roughly 200-day growth period (Miller F.L., 1973; Schaefer & Mahoney, 2001). In contrast, formation (amelogenesis) and mineralization of the third lower molar (M_3) takes approximately 6 months. Mineralization of the M_3 in *Cervus elaphus* (Elk) begins at roughly 13 months of age and continues until approximately 18 months of age, thus forming from the mid to late summer through winter of the second year of life (Miller F.L., 1973; Fricke *et al.* 1998; Kohn, 2004; Brown & Chapman, 1991). We can reasonably assume a similar developmental trajectory for caribou lower third molars. In contrast, bone continuously remodels and does not represent a single snapshot in time (Hedges *et al.* 2007). For example, Matsubayashi & Tayasu (2019) tested stable isotope turnover in bone collagen for sika deer and found that isotopic composition remained unchanged during the first 2.5 years of rapid bone growth and reflected the average diet.

I also expected that any difference in stable isotope values among the three tissues would be related to the differences in the temporal scale over which isotopes are incorporated from the diet into the tissue rather than differences in developmental pathways. The developmental pathway is similar for all three tissues used herein. The mineral components of antler, bone, and tooth enamel are all comprised of hydroxylapatite (Chen *et al.* 2009; Chritz *et al.* 2009; Koch, 1998; Kohn & Cerling, 2002), which is sourced from the bicarbonate pool in the body fluid that derives its isotopic signature from all dietary components – protein, carbohydrates and lipids (Codron *et al.* 2018). Similarity in the isotopic source for the three tissues suggests that timing of

development and duration of exchange with the body fluid should have a greater impact on their comparative isotopic signatures.

Bone and antler incorporate protein in the form of collagen (type 1) into the mineral matrix (Stevens & O'Connell, 2016). Both antler and bone are supplied the collagen proteins through the blood stream. Antlers have highly vascularized tissue with blood being supplied through the velvet membrane and the pedicle. Bone growth and remodelling is conducted by osteoblasts and osteoclasts; the bone forming cells (osteoblasts) are supplied material through extracellular fluid, primarily comprised of blood and plasma (Chen *et al.* 2009; Eriksen, 2010; Eriksen *et al.* 1990). It is therefore reasonable to presume that both tissues have a similar isotopic source.

I found that the $\delta^{13}C$ values of carbonates from bone are approximately 1‰ lower than antler tissue for all five individual caribou (Fig. 13), a likely result of bone remodeling, which leads to time averaging of isotopic signals from the diet over several years (Matsubayashi & Tayasu, 2019; Hedges *et al.* 2007). The Qamanirjuaq caribou show distinct seasonal shifts in diet between the summer and winter months (Miller F.L., 1973). Antler formation, occurring from early February to late August, largely reflects diet during the summer months, which is overall higher in $\delta^{13}C$ (Fig. 10) (Drucker *et al* 2010; 2012). In contrast, the time averaging of bone isotopic values creates a $\delta^{13}C$ signal that reflects both the summer and winter diets, which show overall lower $\delta^{13}C$ values (Fig. 13).

During the winter months, both antler and tooth isotopic values are at their lowest (Fig. 13; dark blue area of the plot). Antler $\delta^{13}C$ values then increase along the

axis of the antler growth (from pedicle to tip), representing the late winter to late summer (Fig. 13). The values for tooth and antler peak during the late summer months, the period when antler formation is completed (the tip of the antler) and tooth mineralization is beginning (the crown; Fig. 13). Contrary to antlers, tooth enamel $\delta^{13}C$ values decreased along from the crown to root (Fig. 13), representing their period of formation, late summer to mid-winter. Combining antler and tooth stable isotopes may therefore provide a record of nearly year-round dietary data for caribou, which would be unprecedented for cervids, but this requires further testing.

5.0 Conclusion

The Qamanirjuaq population of *Rangifer tarandus groenlandicus* (Barren-Ground Caribou) are of both ecologic and socio-economic importance to the North. Unfortunately, given pronounced Arctic climate change, the population is in decline. This study determined that male *Rangifer tarandus* antlers of the Qamanirjuaq population can provide a unique isotopic signature in comparison to other hard tissues such as bone and teeth. Antlers isotope signals may reflected the isotopic composition of food and water consumed during the spring and summer of a single year and could provide a high-resolution record of ecological conditions. In comparison to other hard tissues such as bone and teeth, antlers had different stable isotope profiles of carbon and nitrogen. Variation in the rate and timing of tissue development created the different stable isotope profiles for each tissue observed. Fifteen males were selected, from the Canadian Museum of Nature collection, based on geographic location, sex, maturity, and collection date. Samples were then processed using alterations to the standard procedures for both collagen and carbonate extraction. The carbon and nitrogen isotope results for collagen along the antlers showed a significant pattern of variation, demonstrating the potential of such data as ecological indicators during the antler growth period. Similarly, carbon and oxygen isotope results for carbonates showed patterns of variation for carbon, potentially providing another signal of ecological variation during antler growth. This study helps provide a tool for better understanding the ecology of caribou populations within Canada. Moving forward, additional stable isotopes should be measured to verify findings and to incorporate new

potential avenues for discovery. Pleistocene antlers will be sampled to help determine the adaptability of climate change in *Rangifer tarandus.*

5.1 Future Implications

This study lays the foundation for future research into the use of antlers as ecological indicators. My study has shown that antlers vary isotopically throughout the length of the antler in both $\delta^{13}C$ and $\delta^{15}N$. The variation along the length of the antler is most likely an indication of dietary changes between ecozones and nutritional stress. The implications of these findings provide us with the opportunity to use antlers as a tool to determine changes in regards to caribou ecology. Antlers are particularly beneficial as they provide a high-resolution image of the spring and summer period, a period of migration and growth for caribou. However, further research is required before more definitive conclusions can be made. Stable isotope analysis of Arctic plants will help support the conclusions drawn in the discussion section. To determine the isotopic variation within diet, the plants found in the rumen contents of caribou collected (see Table 1 and Fig. 4) will need to be analyzed. In addition, other isotopes need to be analyzed to create a greater understanding of the ecology an antler can represent. The completion of oxygen isotope analysis may potentially show temperature changes throughout the antler growth season and strontium isotopes may provide information about migratory patterns during the spring migration.

The implications of this research can also be applied to Pleistocene caribou. Pleistocene caribou survived dramatic climate change similar in many respects to the

climate change experienced by modern caribou. By researching the effect of climate change on Pleistocene caribou through antler fossils, we may be able to generate a better understanding of the impact modern climate change is having on the Qamanirjuaq and other caribou populations. Within the fossil record, antlers are more abundant as they grow and are shed annually, therefore representing an ideal fossil to study Pleistocene caribou ecology.

The benefits of antler isotope research are just beginning to be understood and this study helps further the research. Antlers yearly growth and shedding provide a humane means of studying dietary and potentially ecological changes around a caribou population.

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Appendix 1: Carbonate Stable Isotope Data

*Individual samples represented by their CMN number (i.e. 39***). Samples with A indicate powder collected along the length of the antler, B indicate samples collected along the mandible, M indicate samples taken long the third molar, and finally TA indicate samples collected on the tine of the antler.*

		13C	18O	18O
Num	**Sample**	**VPDB**	**VSMOW**	**VPDB**
1	39107-1A	**-8.63**	**14.72**	**-15.70**
2	39107-2A	**-8.77**	**15.44**	**-15.00**
3	39107-3A	**-8.84**	**15.34**	**-15.10**
4	39107-4A	**-8.95**	**15.29**	**-15.15**
5	39107-5A	**-8.93**	**15.66**	**-14.78**
6	39107-6A	**-8.83**	**15.27**	**-15.17**
7	39107-6A dup	**-8.65**	**14.83**	**-15.59**
8	39107-7A	**-8.53**	**15.31**	**-15.13**
9	39107-8A	**-8.67**	**14.40**	**-16.01**
10	39107-9A	**-8.83**	**15.25**	**-15.19**
11	39107-10A	**-8.57**	**15.48**	**-14.97**
12	39107-11A	**-8.69**	**15.45**	**-14.99**
13	39107-12A	**-8.76**	**15.61**	**-14.84**
14	39107-13A	**-8.65**	**15.18**	**-15.26**
15	39107-14A	**-8.61**	**15.07**	**-15.36**
16	39107-15A	**-8.63**	**15.64**	**-14.81**
17	39107-16A	**-8.72**	**14.14**	**-16.26**
18	39107-17A	**-8.76**	**14.78**	**-15.64**
19	39107-18A	**-8.49**	**15.35**	**-15.09**
20	39107-19A	**-8.79**	**14.90**	**-15.53**
21	39107-20A	**-8.88**	**14.53**	**-15.88**
22	39107-21A	**-9.00**	**14.69**	**-15.73**
23	39107-22A	**-8.73**	**16.17**	**-14.29**
24	39107-22A dup	**-8.49**	**15.51**	**-14.93**
25	39107-23A	**-8.92**	**15.19**	**-15.25**
26	39107-24A	**-8.71**	**15.10**	**-15.34**
27	39107-25A	**-8.34**	**14.36**	**-16.05**
28	39107-26A	**-8.52**	**14.57**	**-15.84**
29	39107-27A	**-8.37**	**13.98**	**-16.42**
30	39107-28A	**-7.93**	**15.72**	**-14.73**
31	39107-29A	**-7.98**	**16.39**	**-14.08**
32	39107-30A	**-8.23**	**14.46**	**-15.95**
33	39107-31A	**-8.64**	**13.31**	**-17.07**
34	39107-31A	**-7.47**	**17.78**	**-12.73**
35	39107-1TA	**-8.72**	**17.54**	**-12.97**
36	39107-2TA	**-8.38**	**15.48**	**-14.96**

37	39107-3TA	**-8.21**	**14.88**	**-15.55**
38	39107-4TA	**-7.85**	**15.29**	**-15.14**
39	39107-5TA	**-8.07**	**14.22**	**-16.19**
40	39107-6TA	**-7.57**	**15.12**	**-15.32**
41	39107-7TA	**-7.58**	**14.72**	**-15.71**
42	39107-7TA dup	**-7.70**	**15.42**	**-15.02**
43	39107-1B	**-9.28**	**13.86**	**-16.53**
44	39107-1B dup	**-9.24**	**14.05**	**-16.35**
45	39107-1M	**-8.30**	**14.42**	**-15.99**
46	39107-2M	**-7.95**	**16.88**	**-13.61**
47	39107-3M	**-9.00**	**16.18**	**-14.28**

		13C	**18O**	**18O**
Num	**Sample**	**VPDB**	**VSMOW**	**VPDB**
48	39108-1A	**-8.95**	**13.90**	**-16.50**
49	39108-2A	**-7.88**	**18.69**	**-11.85**
50	39108-2A	**-8.79**	**15.04**	**-15.39**
51	39108-2A dup	**-8.10**	**12.73**	**-17.63**
52	39108-4A	**-8.39**	**15.53**	**-14.91**
53	39108-5A	**-8.38**	**15.04**	**-15.39**
54	39108-5A dup	**-8.20**	**14.41**	**-16.01**
55	39108-6A	**-7.55**	**19.21**	**-11.35**
56	39108-7A	**-7.81**	**17.52**	**-12.99**
57	39108-7A dup	**-7.52**	**17.89**	**-12.63**
58	39108-8A	**-7.83**	**18.53**	**-12.00**
59	39108-23A	**-7.44**	**18.19**	**-12.34**
60	39108-24A	**-7.54**	**18.61**	**-11.93**
61	39108-25A	**-7.32**	**19.23**	**-11.32**
62	39108-25A dup	**-7.22**	**19.19**	**-11.36**
63	39108-2M	**-8.63**	**16.27**	**-14.19**
64	39108-3M	**-8.28**	**15.26**	**-15.18**
65	39108-1TA	**-8.05**	**13.40**	**-16.98**
66	39108-4TA	**-7.66**	**15.30**	**-15.14**

		13C	**18O**	**18O**
Num	**Sample**	**VPDB**	**VSMOW**	**VPDB**
67	38145-2A	**-8.26**	**14.36**	**-16.05**
68	38145-2A dup	**-8.18**	**14.64**	**-15.78**
69	38145-3A	**-8.14**	**14.72**	**-15.70**
70	38145-4A	**-8.25**	**14.53**	**-15.88**
71	38145-5A	**-8.19**	**14.09**	**-16.32**
72	38145-6A	**-8.06**	**14.59**	**-15.83**

73	38145-7A	**-8.28**	**13.10**	**-17.27**
74	38145-8A	**-8.38**	**13.77**	**-16.62**
75	38145-9A	**-8.25**	**14.06**	**-16.34**
76	38145-10A	**-8.20**	**14.15**	**-16.26**
77	38145-10A dup	**-8.26**	**14.10**	**-16.30**
78	38145-11A	**-7.99**	**14.58**	**-15.83**
79	38145-12A	**-7.99**	**15.04**	**-15.39**
80	38145-13A	**-7.96**	**14.25**	**-16.16**
81	38145-14A	**-8.19**	**15.01**	**-15.42**
82	38145-15A	**-8.09**	**14.40**	**-16.01**
83	38145-15A dup	**-8.09**	**13.75**	**-16.64**
84	38145-16A	**-8.18**	**13.57**	**-16.82**
85	38145-17A	**-8.03**	**15.55**	**-14.90**
86	38145-18A	**-8.24**	**15.12**	**-15.31**
87	38145-19A	**-8.14**	**13.49**	**-16.89**
88	38145-20A	**-7.95**	**13.32**	**-17.05**
89	38145-21A	**-8.24**	**14.19**	**-16.21**
90	38145-22A	**-7.84**	**14.38**	**-16.03**
91	38145-23A	**-7.82**	**14.53**	**-15.89**
92	38145-24A	**-7.52**	**15.75**	**-14.70**
93	38145-25A	**-7.61**	**16.22**	**-14.25**
94	38145-25A dup	**-7.58**	**16.39**	**-14.09**
95	38145-26A	**-7.84**	**13.01**	**-17.36**
96	38145-27A	**-7.85**	**13.34**	**-17.04**
97	38145-28A	**-7.70**	**14.46**	**-15.96**
98	38145-1B	**-8.77**	**13.90**	**-16.49**
99	38145-1M	**-9.04**	**16.04**	**-14.43**
100	38145-2M	**-8.71**	**16.17**	**-14.29**
101	38145-3M	**-8.30**	**16.53**	**-13.95**
102	38145-1TA	**-7.78**	**15.78**	**-14.67**
103	38145-2TA rpt	**-8.13**	**16.86**	**-13.62**
104	38145-3TA	**-7.75**	**15.95**	**-14.51**
105	38145-4TA	**-7.78**	**13.80**	**-16.59**
106	38145-4TA dup	**-7.71**	**15.75**	**-14.70**
107	38145-4TArpt	**-8.56**	**17.20**	**-13.30**
108	38145-4TA rpt	**-8.19**	**21.11**	**-9.50**

Num	**Sample**	**VPDB**	**VSMOW**	**VPDB**
109	38148-1A	**-8.68**	**18.34**	**-12.19**
110	38148-1A dup	**-8.53**	**19.68**	**-10.89**
111	38148-1A rpt	**-9.26**	**16.72**	**-13.76**
112	38148 2A	**-8.82**	**19.41**	**-11.15**
113	38148 2A dup	**-8.29**	**15.83**	**-14.63**

114	38148 2A rpt	**-8.23**	**13.38**	**-17.00**
115	38148 2A rpt dup (in 70°c oven ovenight)	**-8.16**	**13.00**	**-17.37**
116	38148 2A rpt dup (in 70°c oven overnight)	**-8.15**	**13.15**	**-17.22**
117	38148-3A	**-8.84**	**17.25**	**-13.24**
118	38148-4A	**-8.66**	**18.04**	**-12.48**
119	38148-5A	**-8.57**	**17.22**	**-13.28**
120	38148-6A	**-8.45**	**17.43**	**-13.07**
121	38148-6A dup	**-8.44**	**17.53**	**-12.97**
122	38148-7A	**-8.41**	**17.88**	**-12.63**
123	38148-8A	**-8.61**	**17.21**	**-13.28**
124	38148-9A	**-8.21**	**17.48**	**-13.03**
125	38148-10A	**-8.04**	**18.22**	**-12.31**
126	38148-11A	**-8.23**	**18.18**	**-12.34**
127	38148-11A dup	**-8.15**	**18.11**	**-12.41**
128	38148-12A	**-8.38**	**17.75**	**-12.76**
129	38148-13A	**-7.49**	**14.12**	**-16.28**
130	38148-14A	**-7.46**	**14.87**	**-15.55**
131	38148-15A	**-7.31**	**14.52**	**-15.90**
132	38148-16A	**-7.45**	**15.30**	**-15.14**
133	38148-16A dup	**-7.51**	**16.18**	**-14.29**
134	38148-16A rpt	**-8.23**	**18.07**	**-12.46**
135	38148-17A	**-8.37**	**17.22**	**-13.27**
136	38148-17A dup	**-8.32**	**17.67**	**-12.84**
137	38148-18A	**-8.14**	**18.34**	**-12.19**
138	38148-19A	**-8.24**	**17.41**	**-13.09**
139	38148-20A	**-8.07**	**17.17**	**-13.33**
140	38148-20A dup	**-8.12**	**17.42**	**-13.09**
141	38148-21A	**-8.42**	**17.57**	**-12.94**
142	38148-22A	**-8.23**	**17.51**	**-12.99**
143	38148-22A dup	**-8.16**	**17.62**	**-12.88**
144	38148-23A	**-8.24**	**18.38**	**-12.16**
145	38148-24A	**-7.92**	**17.95**	**-12.57**
146	38148-25A	**-7.79**	**17.51**	**-13.00**
147	38148-1M	**-9.19**	**17.26**	**-13.23**
148	38148-1M dup	**-9.03**	**17.17**	**-13.33**
149	38148-2M	**-8.86**	**16.70**	**-13.78**
150	38148-3M	**-8.61**	**15.95**	**-14.51**
151	38148-1B	**-9.27**	**16.84**	**-13.65**
152	38148-2TA	**-8.02**	**17.76**	**-12.75**

	13C	**18O**	**18O**

Num	Sample	VPDB	VSMOW	VPDB
153	39149-1A	**-8.55**	**21.33**	**-9.29**
154	39149-1A dup	**-8.65**	**18.93**	**-11.62**
155	39149-6A	**-8.24**	**19.77**	**-10.81**
156	39149-6A dup	**-8.31**	**18.87**	**-11.67**
157	39149-7A	**-6.73**	**18.76**	**-11.78**
158	39149-7A dup	**-8.33**	**17.22**	**-13.27**

		13C	18O	18O
Num	Sample	VPDB	VSMOW	VPDB
159	39151-1A	**-8.83**	**18.60**	**-11.93**
160	39151-1A dup	**-8.79**	**18.33**	**-12.20**
161	39151-2A	**-8.56**	**19.46**	**-11.10**
162	39151-3A	**-8.64**	**18.86**	**-11.68**
163	39151-4A	**-8.81**	**18.98**	**-11.57**
164	39151-5A	**-8.58**	**19.03**	**-11.52**
165	39151-6A	**-8.88**	**19.75**	**-10.83**
166	39151-6A dup	**-8.77**	**20.71**	**-9.89**
167	39151-7A	**-8.57**	**20.15**	**-10.43**
168	39151-8A	**-8.65**	**19.67**	**-10.90**
169	39151-9A	**-8.58**	**19.32**	**-11.24**
170	39151-11A	**-8.77**	**21.04**	**-9.57**
171	39151-10A	**-8.48**	**19.11**	**-11.44**
172	39151-10A dup	**-8.70**	**18.22**	**-12.30**
173	39151-12A	**-8.82**	**18.81**	**-11.74**
174	39151-13A	**-8.65**	**19.97**	**-10.61**
175	39151-14A	**-8.78**	**18.98**	**-11.57**
176	39151-16A	**-8.72**	**18.76**	**-11.78**
177	39151-15A	**-8.49**	**19.67**	**-10.90**
178	39151-15A dup	**-8.55**	**19.60**	**-10.96**
179	39151-17A	**-8.56**	**20.66**	**-9.94**
180	39151-18A	**-8.64**	**19.69**	**-10.88**
181	39151-19A	**-8.64**	**20.06**	**-10.52**
182	39151-20A	**-8.61**	**19.03**	**-11.52**
183	39151-21A	**-8.75**	**18.93**	**-11.61**
184	39151-22A	**-8.56**	**20.20**	**-10.39**
185	39151-23A	**-8.29**	**19.37**	**-11.19**
186	39151-24A	**-8.27**	**20.37**	**-10.22**
187	39151-25A	**-8.37**	**19.48**	**-11.09**
188	39151-26A	**-8.43**	**19.72**	**-10.85**

Appendix 2: Collagen Stable Isotope Data

*Individual samples represented by their CMN number (i.e. 39***). Samples with A indicate powder collected along the length of the antler, B indicate samples collected along the mandible, M indicate samples taken long the third molar, and finally TA indicate samples collected on the tine of the antler.*

Num	Sample	δ^{13}C (VPDB)	C%	δ^{15}N (AIR)	N%
189	39079-1A	**-18.90**	30.23	**4.90**	10.61
190	39079-2A	**-18.97**	43.45	**5.52**	15.73
191	39079-3A	**-18.91**	40.22	**5.53**	14.43
192	39079-4A	**-18.98**	44.46	**5.68**	16.06
193	39079-5A	**-19.05**	39.56	**5.30**	14.27
194	39079-6A	**-18.93**	43.64	**5.67**	15.79
195	39079-7A	**-18.82**	37.40	**5.57**	13.32
196	39079-7A dup	**-18.84**	37.55	**5.65**	13.42
197	39079-8A	**-18.83**	35.67	**5.57**	12.65
198	39079-9A	**-18.83**	41.90	**5.88**	14.99
199	39079-10A	**-18.49**	32.19	**5.72**	11.12
200	39079-11A	**-18.75**	36.36	**5.79**	12.76
201	39079-12A	**-18.90**	36.06	**5.71**	12.70
202	39079-13A	**-18.73**	26.60	**5.37**	8.99
203	39079-14A	**-18.97**	36.53	**5.91**	12.74
204	39079-15A	**-19.01**	41.15	**5.97**	14.55
205	39079-15A dup	**-18.91**	33.45	**5.74**	11.52
206	39079-16A	**-18.83**	37.65	**5.84**	13.18
207	39079-17A	**-19.02**	44.58	**6.01**	15.55
208	39079-18A	**-18.94**	38.93	**5.86**	13.26
209	39079-19A	**-18.86**	35.02	**5.65**	11.63
210	39079-1TA	**-19.19**	41.40	**5.62**	14.63
211	39079-2TA	**-18.93**	37.58	**5.82**	13.20
212	39079-1B	**-18.52**	22.88	**3.30**	7.57

Num	Sample	δ^{15}N (AIR)	N%	δ^{13}C (VPDB)	C%
214	39090-1A	**5.28**	16.05	**-18.40**	44.16
215	39090-2A	**5.43**	16.08	**-18.41**	44.39
216	39090-3A	**5.85**	14.76	**-18.37**	40.90
217	39090-4A	**6.05**	15.35	**-18.37**	42.15
218	39090-5A	**5.80**	15.69	**-18.34**	43.00
219	39090-6A	**5.53**	15.78	**-18.37**	42.87
220	39090-7A	**5.60**	15.84	**-18.41**	43.19
221	39090-8A	**6.26**	14.81	**-18.37**	40.79
222	39090-9A	**6.56**	15.82	**-18.41**	43.32
223	39090-10A	**6.31**	15.59	**-18.38**	42.83
224	39090-10A dup	**6.31**	15.50	**-18.37**	42.55

225	39090-11A	**6.11**	13.50	**-18.38**	37.58
226	39090-12A	**6.04**	13.44	**-18.42**	37.21
227	39090-13A	**6.06**	15.46	**-18.50**	42.80
228	39090-14A	**5.93**	12.76	**-18.37**	35.25
229	39090-15A	**6.25**	15.56	**-18.53**	42.82
230	39090-16A	**6.18**	15.82	**-18.53**	43.60
231	39090-17A	**6.29**	16.08	**-18.51**	44.15
232	39090-18A	**6.51**	15.11	**-18.54**	42.10
233	39090-18A dup	**6.47**	15.04	**-18.60**	42.07
234	39090-19A	**6.43**	15.97	**-18.53**	44.16
235	39090-20A	**6.36**	15.75	**-18.50**	43.38
236	39090-21A	**6.71**	15.58	**-18.57**	42.98
237	39090-22A	**6.62**	15.46	**-18.57**	42.57
238	39090-22A dup	**6.61**	15.48	**-18.60**	42.79
239	39090-23A	**6.82**	16.20	**-18.69**	45.13
240	39090-24A	**6.86**	15.83	**-18.61**	44.00
241	39090-25A	**6.92**	15.83	**-18.60**	44.32
242	39090-26A	**6.91**	16.08	**-18.71**	45.19
243	39090-1TA	**5.56**	15.82	**-18.41**	43.24
244	39090-2TA	**5.70**	15.75	**-18.36**	43.23
245	39090-3TA	**6.78**	15.73	**-18.53**	43.44
246	39090-1B	**4.27**	15.23	**-18.82**	43.52

Num	**Sample**	**δ^{13}C (VPDB)**	**C%**	**δ^{15}N (AIR)**	**N%**
247	39102-1A	**-19.12**	31.89	**4.19**	10.99
248	39102-1A dup	**-18.97**	24.33	**3.79**	8.20
249	39102-2A	**-18.98**	44.72	**4.36**	16.29
250	39102-3A	**-19.05**	44.81	**4.73**	16.29
251	39102-4A	**-18.97**	44.15	**4.83**	16.10
252	39102-5A	**-18.90**	44.32	**4.82**	16.23
253	39102-6A	**-18.49**	20.26	**4.13**	6.99
254	39102-7A	**-18.82**	44.33	**5.20**	16.22
255	39102-8A	**-18.67**	37.13	**5.29**	13.29
256	39102-9A	**-18.81**	44.55	**5.27**	16.17
257	39102-10A	**-18.88**	45.24	**5.19**	16.43
258	39102-11A	**-18.87**	42.48	**5.01**	17.50
259	39102-12A	**-18.87**	39.43	**5.16**	16.08
260	39102-13A	**-18.87**	43.94	**5.23**	18.15
261	39102-13A dup	**-18.94**	43.82	**5.03**	18.04
262	39102-14A	**-18.91**	43.84	**5.26**	18.14
263	39102-15A	**-18.94**	44.18	**5.25**	18.26
264	39102-16A	**-19.00**	40.48	**5.21**	16.49
265	39102-17A	**-18.96**	39.68	**5.29**	16.06
266	39102-18A	**-19.04**	44.01	**5.35**	18.05

267	39102-19A	**-18.84**	33.48	**5.06**	13.30
268	39102-20A	**-18.91**	32.08	**4.88**	12.62
269	39102-20A dup	**-18.89**	31.57	**5.01**	12.47
270	39102-21A	**-18.96**	42.49	**5.39**	17.02
271	39102-22A	**-18.66**	39.73	**5.29**	15.74
272	39102-1TA	**-19.03**	31.95	**4.44**	12.55
273	39102-2TA	**-18.91**	34.55	**4.75**	13.81
274	39102-3TA	**-18.70**	42.96	**5.46**	17.27
275	39102-1B	**-19.21**	16.12	**2.29**	5.31

Num	**Sample**	**δ^{15}N (AIR)**	**N%**	**δ^{13}C (VPDB)**	**C%**
276	39107-1A	**2.99**	12.94	**-19.47**	35.07
277	39107-2A	**2.79**	11.89	**-19.29**	32.33
278	39107-3A	**2.94**	13.18	**-19.44**	34.99
279	39107-3A dup	**2.76**	11.08	**-19.38**	32.24
280	39107-4A	**3.33**	16.80	**-19.35**	43.05
281	39107-5A	**3.48**	16.99	**-19.43**	44.01
282	39107-6A	**3.28**	15.88	**-19.43**	41.06
283	39107-7A	**2.59**	11.34	**-19.19**	33.14
284	39107-8A	**2.94**	15.14	**-19.43**	42.61
285	39107-9A	**2.37**	9.84	**-19.28**	28.96
286	39107-10A	**2.94**	15.97	**-19.46**	44.89
287	39107-11A	**2.81**	14.47	**-19.38**	40.98
288	39107-12A	**2.76**	9.08	**-19.11**	26.79
289	39107-13A	**2.94**	16.10	**-19.43**	45.30
290	39107-14A	**3.06**	16.02	**-19.43**	45.15
291	39107-15A	**3.10**	16.11	**-19.41**	45.15
292	39107-16A	**3.02**	16.12	**-19.51**	44.93
293	39107-17A	**3.21**	16.16	**-19.44**	45.02
294	39107-18A	**2.94**	13.64	**-19.61**	39.99
295	39107-18A dup	**3.13**	13.78	**-19.43**	39.13
296	39107-19A	**2.97**	13.61	**-19.61**	39.38
297	39107-20A	**3.13**	16.07	**-19.52**	44.54
298	39107-21A	**3.36**	16.27	**-19.55**	45.01
299	39107-22A	**3.34**	16.12	**-19.53**	44.68
300	39107-23A	**3.14**	13.42	**-19.54**	38.05
301	39107-24A	**3.25**	13.71	**-19.60**	39.06
302	39107-25A	**3.40**	10.79	**-19.45**	31.43
303	39107-26A	**3.86**	15.77	**-19.52**	43.98
304	39107-27A	**4.11**	16.18	**-19.45**	45.36
305	39107-27A dup	**3.93**	15.95	**-19.44**	44.36
306	39107-28A	**4.08**	15.64	**-19.42**	43.96
307	39107-29A	**3.57**	11.16	**-19.25**	33.06
308	39107-30A	**4.44**	15.84	**-19.41**	44.18

309	39107-31A	**4.55**	14.02	**-19.13**	40.02
310	39107-1TA	**2.84**	13.23	**-19.73**	37.82
311	39107-2TA	**2.77**	11.50	**-19.31**	32.78
312	39107-3TA	**3.97**	16.03	**-19.34**	43.92
313	39107-4TA	**4.20**	16.03	**-19.02**	44.07
314	39107-5TA	**3.65**	10.67	**-19.02**	31.08
315	39107-5TA dup	**4.01**	11.99	**-19.07**	33.45
316	39107-6TA	**4.23**	12.80	**-18.96**	36.44
317	39107-7TA	**4.17**	12.63	**-18.99**	35.69
318	39107-1B	**2.67**	14.72	**-19.28**	42.75

Num	**Sample**	**δ13C (VPDB)**	**C%**	**δ15N (AIR)**	**N%**
319	39108-1A	**-18.87**	34.02	**5.59**	11.83
320	39108-1A dup	**-18.79**	28.90	**5.41**	10.11
321	39108-2A	**-18.49**	20.45	**5.35**	6.72
322	39108--2A RPT	**-18.90**	38.21	**6.11**	13.54
323	39108-3A	**-18.61**	25.11	**5.24**	8.64
324	39108-4A	**-18.53**	25.59	**5.79**	8.90
325	39108-5A	**-18.75**	44.38	**6.65**	16.01
326	39108-6A	**-18.71**	39.14	**6.35**	14.01
327	39108-7A	**-18.64**	30.87	**5.99**	10.77
328	39108-8A	**-18.77**	40.64	**6.28**	14.60
329	39108-9A	**-18.85**	39.88	**6.08**	14.20
330	39108-10A	**-18.63**	25.87	**5.14**	8.64
331	39108-11A	**-18.50**	20.59	**5.72**	6.84
332	39108--11A RPT	**-18.81**	33.95	**6.00**	11.96
333	39108-12A	**-18.85**	33.36	**5.89**	11.54
334	39108-12A dup	**-18.63**	26.24	**5.72**	8.99
335	39108-13A	**-18.50**	25.57	**5.69**	8.73
336	39108-14A	**-18.28**	16.72	**5.42**	5.22
337	39108-14A RPT dup	**-18.73**	32.03	**6.20**	11.09
338	39108--14A RPT	**-18.61**	27.56	**6.15**	9.49
339	39108-15A	**-18.66**	44.66	**6.52**	16.11
340	39108-16A	**-18.86**	44.24	**6.49**	16.07
341	39108-17A	**-18.58**	31.65	**6.13**	10.98
342	39108-18A	**-18.88**	33.68	**6.20**	11.85
343	39108-19A	**-18.86**	34.36	**6.46**	12.07
344	39108-20A	**-18.87**	39.64	**6.50**	14.03
345	39108-21A	**-18.79**	34.63	**6.39**	12.26
346	39108-21A dup	**-18.52**	22.98	**5.58**	7.84
347	39108--21A RPT	**-18.72**	28.10	**6.17**	9.62
348	39108-22A	**-18.90**	41.06	**6.64**	14.73
349	39108-23A	**-18.58**	29.66	**6.09**	10.16

350	39108-24A	**-18.65**	35.53	**6.01**	12.32
351	39108-25A	**-18.45**	39.72	**6.27**	13.98
352	39108-1TA	**-19.07**	30.26	**5.48**	10.45
353	39108-2TA	**-18.91**	34.75	**5.68**	12.53
354	39108-3TA	**-18.92**	39.03	**6.70**	14.16
355	39108-4TA	**-18.16**	34.86	**6.27**	12.60
356	39108-1B	**-20.47**	17.99	**3.62**	4.93
357	39108-1B dup	**-19.01**	17.66	**3.70**	5.48
358	39108--1B RPT	**-19.02**	16.63	**3.18**	4.96

Num	**Sample**	**δ^{15}N (AIR)**	**N%**	**δ^{13}C (VPDB)**	**C%**
359	39110-1A	**3.44**	12.05	**-19.47**	35.53
360	39110-2A	**4.73**	15.12	**-19.23**	43.75
361	39110-3A	**4.61**	11.30	**-18.93**	32.96
362	39110-3A dup	**3.67**	5.29	**-18.49**	16.55
363	39110-4A	**4.84**	12.15	**-18.98**	35.41
364	39110-5A	**5.32**	13.55	**-18.87**	39.13
365	39110-6A	**5.63**	15.25	**-18.88**	43.84
366	39110-7A	**4.50**	11.51	**-19.00**	33.99
367	39110-8A	**4.80**	15.19	**-19.19**	43.79
368	39110-9A	**5.01**	15.35	**-19.21**	43.97
369	39110-10A	**4.94**	15.40	**-19.14**	44.18
370	39110-11A	**5.89**	15.16	**-18.91**	43.74
371	39110-12A	**5.56**	9.97	**-18.63**	29.52
372	39110-13A	**6.17**	15.19	**-18.74**	43.69
373	39110-14A	**5.95**	15.95	**-18.83**	44.22
374	39110-15A	**5.80**	15.88	**-18.95**	44.00
375	39110-16A	**5.13**	12.01	**-19.08**	33.96
376	39110-17A	**2.53**	14.01	**-18.78**	39.61
377	39110-18A	**5.36**	15.74	**-19.23**	43.93
378	39110-19A	**5.02**	14.66	**-19.34**	41.32
379	39110-20A	**5.31**	16.30	**-19.12**	45.15
380	39110-20A dup	**5.28**	14.40	**-19.19**	40.35
381	39110-21A	**5.19**	15.97	**-19.19**	44.31
382	39110-22A	**5.12**	12.50	**-18.95**	35.32
383	39110-23A	**5.93**	15.85	**-18.87**	43.94
384	39110-24A	**6.14**	14.20	**-18.69**	39.75
385	39110-25A	**6.13**	15.99	**-18.78**	44.48
386	39110-26A	**6.29**	15.92	**-18.73**	44.35
387	39110-27A	**6.12**	10.22	**-18.37**	29.48
388	39110-27A dup	**6.09**	9.90	**-18.34**	28.69
389	39110-28A	**6.27**	11.16	**-18.40**	31.84
390	39110-29A	6.77	14.54	**-18.33**	41.16
391	39110-1TA	**3.85**	13.14	**-19.33**	36.52
392	39110-2TA	**6.27**	11.62	**-18.43**	33.09
393	39110-1B	**3.53**	8.09	**-18.96**	23.95

Num	Sample	δ^{15}N (AIR)	N%	δ^{13}C (VPDB)	C%
394	39120-1A	**4.54**	5.14	**-18.33**	17.40
395	39120-2A	**5.65**	15.26	**-18.71**	44.26
396	39120-3A	**5.34**	10.63	**-18.66**	32.07
397	39120-4A	**5.54**	12.92	**-18.67**	38.43
398	39120-5A	**5.55**	15.37	**-18.78**	44.75
399	39120-6A	**5.81**	14.52	**-18.63**	42.83
400	39120-7A	**5.63**	11.82	**-18.54**	35.36
401	39120-7A dup	**5.13**	7.42	**-18.29**	23.11
402	39120-8A	**5.90**	15.32	**-18.59**	45.16
403	39120-9A	**5.67**	11.82	**-18.61**	35.61
404	39120-10A	**5.14**	7.46	**-18.35**	23.30
405	39120-11A	**5.84**	12.41	**-18.75**	37.46
406	39120-12A	**5.68**	11.23	**-18.71**	33.78
407	39120-13A	**5.71**	12.44	**-18.93**	37.29
408	39120-14A	**5.89**	13.39	**-18.88**	40.12
409	39120-15A	**5.73**	11.77	**-18.76**	35.40
410	39120-16A	**5.85**	14.50	**-18.70**	43.00
411	39120-17A	**5.54**	11.82	**-18.92**	37.04
412	39120-1TA	**5.89**	14.37	**-18.94**	42.60
413	39120-2TA	**5.93**	15.15	**-18.84**	44.56
414	39120-1B	**7.00**	14.34	**-18.99**	43.79

Num	Sample	δ^{15}N (AIR)	N%	δ^{13}C (VPDB)	C%
415	39132-1A	**-19.39**	28.33	**4.60**	10.80
416	39132-2A	**-19.29**	44.00	**4.65**	18.19
417	39132-3A	**-19.28**	42.92	**5.15**	17.48
418	39132-4A	**-19.30**	44.11	**5.01**	18.20
419	39132-5A	**-19.25**	44.35	**5.08**	18.28
420	39132-6A	**-19.29**	44.13	**4.72**	18.34
421	39132-6A dup	**-19.28**	44.12	**5.05**	18.24
422	39132-7A	**-19.28**	44.01	**4.96**	18.24
423	39132-8A	**-19.03**	44.04	**5.54**	18.00
424	39132-9A	**-18.94**	44.53	**5.52**	18.53
425	39132-10A	**-19.19**	44.05	**5.17**	17.94
426	39132-11A	**-19.01**	44.33	**5.31**	18.41
427	39132-12A	**-18.93**	44.02	**5.66**	18.30
428	39132-13A	**-18.84**	44.32	**5.35**	18.45
429	39132-14A	**-18.88**	39.78	**5.29**	16.47
430	39132-14A dup	**-18.81**	36.02	**5.37**	14.72
431	39132-15A	**-19.68**	41.18	**5.50**	15.90
432	39132-16A	**-19.19**	42.12	**5.53**	16.95
433	39132-17A	**-19.07**	35.03	**5.25**	13.93
434	39132-18A	**-19.05**	29.70	**5.33**	11.62

435	39132-19A	**-19.03**	35.81	**5.51**	14.10
436	39132-20A	**-18.91**	43.04	**5.75**	17.27
437	39132-21A	**-18.60**	23.27	**5.10**	8.76
438	39132-22A	**-18.56**	23.92	**4.97**	8.91
439	39132-23A	**-18.75**	39.29	**5.47**	12.64
440	39132-1TA	**-19.28**	44.57	**5.34**	15.04
441	39132-2TA	**-19.34**	44.22	**4.88**	14.99
442	39132-3TA	**-18.81**	44.21	**5.73**	15.12
443	39132-3TA dup	**-18.74**	43.90	**5.83**	14.99
444	39132-1B	**-19.54**	22.19	**3.18**	6.78

Num	**Sample**	**δ^{15}N (AIR)**	**N%**	**δ^{13}C (VPDB)**	**C%**
445	39145-1A	**3.98**	14.41	**-18.75**	39.68
446	39145-2A	**3.86**	16.32	**-18.78**	44.44
447	39145-3A	**4.17**	16.38	**-18.72**	43.87
448	39145-4A	**4.07**	16.24	**-18.77**	44.21
449	39145-5A	**3.58**	9.80	**-18.65**	28.18
450	39145-5A dup	**2.49**	6.39	**-18.45**	19.48
451	39145-6A	**4.27**	16.27	**-18.87**	44.61
452	39145-7A	**3.46**	9.80	**-18.70**	28.47
453	39145-8A	**3.72**	10.81	**-18.71**	31.19
454	39145-9A	**4.44**	12.00	**-18.39**	34.09
455	39145-10A	**4.62**	14.38	**-18.77**	40.00
456	39145-11A	**4.46**	9.80	**-18.35**	28.68
457	39145-12A	**4.25**	9.73	**-18.71**	28.20
458	39145-13A	**4.39**	14.43	**-18.78**	40.18
459	39145-14A	**4.66**	16.04	**-18.66**	44.55
460	39145-15A	**4.39**	11.67	**-18.74**	33.21
461	39145-16A	**4.67**	15.34	**-18.65**	42.19
462	39145-16A dup	**4.62**	14.70	**-18.81**	40.32
463	39145-17A	**4.33**	10.28	**-18.70**	29.75
464	39145-18A	**4.84**	14.44	**-18.85**	40.54
465	39145-19A	**4.79**	11.83	**-18.82**	33.33
466	39145-20A	**4.38**	9.62	**-18.78**	28.51
467	39145-21A	**5.20**	13.33	**-18.90**	37.09
468	39145-22A	**4.90**	9.40	**-18.55**	26.95
469	39145-23A	**5.27**	11.85	**-18.76**	32.95
470	39145-24A dup	**5.42**	13.72	**-18.76**	38.18
471	39145-25A	**5.48**	13.92	**-18.68**	41.87
472	39145-26A	**5.32**	11.52	**-18.59**	32.88
473	39145-27A	**5.85**	15.47	**-18.89**	42.72
474	39145-28A	**5.79**	13.07	**-18.84**	37.28
475	39145-1TA	**4.11**	10.86	**-18.58**	30.61
476	39145-2TA	**4.28**	10.58	**-18.70**	29.92

477	39145-3TA	**5.76**	11.25	**-18.68**	31.96
478	39145-4TA	**4.71**	7.07	**-18.40**	21.59
479	39145-4TA dup	**5.04**	9.32	**-18.52**	26.73
480	39145-1B	**3.22**	6.29	**-18.64**	18.99
481	39145-1B RPT	**3.79**	6.43	**-18.78**	19.74
482	39145-5A RPT	**3.47**	7.32	**-18.62**	21.66

Num	Sample	δ^{15}N (AIR)	N%	δ^{13}C (VPDB)	C%
483	39148-1A	**4.04**	16.49	**-19.35**	44.43
484	39148-2A	**3.99**	16.60	**-19.24**	43.72
485	39148-3A	**3.91**	16.36	**-19.24**	42.94
486	39148-4A	**3.91**	16.57	**-19.29**	43.48
487	39148-5A	**4.31**	16.59	**-19.22**	43.91
488	39148-6A	**4.40**	16.32	**-19.22**	43.09
489	39148-7A	**3.98**	16.17	**-19.07**	44.08
490	39148-8A	**4.25**	16.30	**-19.13**	44.19
491	39148-8A dup	**4.27**	15.94	**-19.51**	44.78
492	39148-9A	**4.37**	16.18	**-19.20**	44.22
493	39148-10A	**4.40**	16.30	**-19.24**	45.33
494	39148-11A	**4.58**	16.45	**-19.14**	45.28
495	39148-12A	**4.68**	15.99	**-19.24**	44.17
496	39148-13A	**4.39**	16.08	**-19.22**	44.36
497	39148-14A	**4.46**	16.26	**-19.16**	44.84
498	39148-15A	**4.32**	16.37	**-19.13**	44.74
499	39148-16A	**4.48**	16.40	**-19.11**	44.70
500	39148-17A	**4.62**	16.56	**-19.02**	44.79
501	39148-18A	**4.64**	15.96	**-19.25**	44.80
502	39148-19A	**4.55**	16.45	**-19.11**	44.79
503	39148-20A	**4.53**	16.48	**-19.04**	44.88
504	39148-20A dup	**4.52**	16.57	**-19.01**	45.02
505	39148-21A	**4.60**	16.23	**-19.06**	44.38
506	39148-22A	**4.76**	16.47	**-18.98**	45.05
507	39148-23A	**4.68**	16.02	**-19.02**	44.56
508	39148-24A	**4.73**	16.10	**-18.80**	44.54
509	39148-25A	**4.96**	15.96	**-18.89**	44.88
510	39148-1TA	**4.67**	16.50	**-19.12**	44.84
511	39148-1TA dup	**4.43**	16.43	**-19.13**	45.20
512	39148-2TA	**4.93**	16.57	**-18.96**	44.90
513	39148-1B	**3.23**	15.75	**-19.07**	44.85

Num	Sample	δ^{15}N (AIR)	N%	δ^{15}N (AIR)	N%
514	39149-1A	**4.60**	15.48	**-19.05**	43.29
515	39149-2A	**4.61**	15.75	**-18.94**	42.86
516	39149-3A	**4.58**	16.75	**-18.95**	45.37

517	39149-4A	**4.47**	13.82	**-18.82**	38.17
518	39149-5A	**2.26**	5.01	**-18.36**	17.12
519	39149-5A dup	**3.49**	6.61	**-18.42**	19.99
520	39149-6A	**4.91**	15.11	**-18.98**	41.94
521	39149-7A	**4.66**	13.68	**-18.83**	37.71
522	39149-8A	**4.80**	16.59	**-18.87**	44.98
523	39149-9A	**3.95**	9.94	**-18.66**	28.54
524	39149-9A dup	**3.90**	9.35	**-18.62**	27.18
525	39149-10A	**4.68**	12.03	**-18.62**	33.75
526	39149-11A	**5.01**	16.71	**-18.87**	45.75
527	39149-12A	**4.27**	9.95	**-18.60**	29.08
528	39149-13A	**5.04**	15.10	**-18.64**	41.41
529	39149-14A	**3.63**	7.61	**-18.35**	23.05
530	39149-14A dup	**4.69**	11.53	**-18.60**	32.83
531	39149-15A	**4.79**	12.56	**-18.65**	35.08
532	39149-16A	**5.10**	16.11	**-19.05**	45.23
533	39149-17A	**5.19**	16.62	**-18.64**	45.08
534	39149-18A	**5.02**	16.69	**-18.69**	45.24
535	39149-19A	**5.37**	16.58	**-18.73**	45.26
536	39149-20A	**4.67**	12.75	**-18.86**	35.95
537	39149-21A	**5.06**	15.99	**-18.87**	43.88
538	39149-22A	**4.58**	11.47	**-18.85**	32.97
539	39149-23A	**4.50**	12.58	**-18.89**	36.02
540	39149-24A	**4.86**	13.86	**-18.73**	38.73
541	39149-24A dup	**4.86**	13.26	**-18.72**	37.02
542	39149-25A	**4.48**	12.52	**-18.37**	35.40
543	39149-26A	**4.25**	11.92	**-18.32**	34.10
544	39149-27A	**4.05**	10.60	**-18.50**	32.13
545	39149-1TA	**4.84**	13.36	**-18.87**	37.33
546	39149-2TA	**4.35**	9.89	**-18.94**	29.45
547	39149-3TA	**4.34**	10.74	**-18.22**	30.90
548	39149-4TA	**4.27**	12.65	**-18.17**	36.35
549	39149-1B	**4.31**	13.96	**-19.52**	43.71

Num	**Sample**	**δ^{13}C (VPDB)**	**C%**	**δ^{15}N (AIR)**	**N%**
550	39151-1A	**-19.05**	45.32	**4.40**	16.76
551	39151-2A	**-19.14**	45.55	**4.57**	16.96
552	39151-3A	**-19.10**	45.59	**4.65**	16.93
553	39151-4A	**-19.07**	45.08	**4.70**	16.78
554	39151-5A	**-19.06**	44.91	**4.77**	16.78
555	39151-6A	**-19.02**	45.94	**4.89**	17.18
556	39151-7A	**-19.06**	45.26	**5.21**	16.92
557	39151-7A dup	**-19.04**	45.03	**4.79**	16.89
558	39151-8A	**-19.08**	45.15	**5.13**	16.81

559	39151-9A	**-19.01**	45.36	**5.32**	16.98
560	39151-10A	**-19.04**	45.07	**5.11**	16.67
561	39151-11A	**-19.10**	45.59	**5.09**	16.97
562	39151-11A dup	**-19.17**	45.26	**5.07**	16.92
563	39151-12A	**-19.00**	45.51	**5.58**	16.86
564	39151-13A	**-19.04**	45.26	**5.35**	16.91
565	39151-14A	**-19.15**	44.41	**5.13**	16.59
566	39151-15A	**-19.08**	44.97	**5.40**	16.86
567	39151-16A	**-19.01**	45.06	**5.51**	16.95
568	39151-17A	**-19.05**	44.77	**5.67**	16.93
569	39151-18A	**-18.98**	44.59	**5.63**	16.09
570	39151-19A	**-19.00**	44.11	**5.64**	15.84
571	39151-20A	**-18.96**	44.63	**5.72**	16.06
572	39151-20A dup	**-19.02**	44.59	**5.79**	16.01
573	39151-21A	**-19.09**	44.70	**5.60**	16.02
574	39151-22A	**-19.10**	44.43	**5.72**	16.00
575	39151-23A	**-19.03**	44.71	**5.80**	16.09
576	39151-24A	**-18.96**	44.94	**6.02**	16.06
577	39151-25A	**-18.93**	44.23	**6.06**	15.75
578	39151-26A	**-18.76**	46.00	**6.18**	16.30
579	39151-1TA	**-19.14**	35.23	**4.52**	12.44
580	39151-2TA	**-18.96**	37.02	**4.63**	13.35
581	39151-3TA	**-19.11**	41.47	**4.47**	14.98
582	39151-4TA	**-18.84**	44.73	**6.15**	15.96
583	39151-4TA dup	**-18.87**	44.77	**6.06**	15.87
584	39151-1B	**-18.79**	45.14	**4.99**	15.60