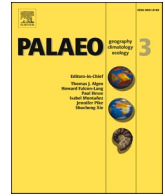


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Invited Research Article

Dietary reconstruction and evidence of prey shifting in Pleistocene and recent gray wolves (*Canis lupus*) from Yukon TerritoryZoe Landry^{a,*}, Sora Kim^b, Robin B. Traylor^b, Marisa Gilbert^c, Grant Zazula^{c,d}, John Southon^e, Danielle Fraser^{a,c,f,g}^a Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada^b Department of Life and Environmental Sciences, University of California, Merced, 5200 N. Lake Road, Merced, CA 95343, United States of America^c Palaeobiology Department, Canadian Museum of Nature, PO Box 3443 Stn "D", Ottawa, ON K1P 6P4, Canada^d Palaeontology Program, Department of Tourism and Culture, Government of Yukon, 133 Industrial Road, Whitehorse Y1A 2V2, Canada^e Department of Earth System Science, University of California, Irvine, 3200 Crout Hall St, Irvine, CA 92697, United States of America^f Department of Earth Sciences, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada^g Department of Paleobiology, Smithsonian National Museum of Natural History, Washington, DC 20560, United States of America

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ABSTRACT

We investigate if and how diets of gray wolves from the Yukon Territory, Canada, have changed from the Pleistocene (>52.8 ka BP to 26.5 ka BP [± 170 y BP]) to the recent Holocene (1960s) using dental microwear analysis of carnassial teeth and stable isotope analyses of carbonates ($\delta^{13}\text{C}_{\text{CO}_3}$ and $\delta^{18}\text{O}_{\text{CO}_3}$) and collagen ($\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$) from bone. We find that dental microwear patterns are similar between the Pleistocene and Holocene specimens, indicating that there has been no change in carcass utilization behaviours, where flesh, not bone, is primarily consumed. Based on minimal changes in $\delta^{13}\text{C}_{\text{CO}_3}$ and $\delta^{13}\text{C}_{\text{col}}$ values, we find that, over thousands of years, Yukon gray wolves have remained generalist predators feeding upon several large ungulate species. Interestingly, $\delta^{15}\text{N}_{\text{col}}$ values suggest that the extinction of megafaunal species at ~ 11.7 Ka induced a shift from a diet comprised primarily of horse (*Equus* sp.) to one based on cervids (i.e. moose and caribou). Survival of large-bodied cervids, such as caribou (*Rangifer tarandus*), was likely key to wolf survival. Although gray wolves survived the end Pleistocene megafauna extinction and demonstrate a degree of ecological flexibility, we suggest that failure to preserve major elements of their current niche (e.g. *caribou*) may result in continued population declines, especially in the face of increasing anthropogenic influences.

1. Introduction

The Canadian Arctic and northern boreal forest ecosystems are among those most severely affected by anthropogenically-driven climate change (Screen and Simmonds, 2010; Simmonds, 2015; Rowland et al., 2016). These ecosystems are experiencing increases in mean annual temperature far exceeding that of the global average (Glig et al., 2012; Rowland et al., 2016) and landscape-scale ecosystem changes such as shrubification of tundra environments (Danby et al., 2011; Hope et al., 2015). The long-term impacts of anthropogenic climate change on northern biota are, however, as of yet uncertain; whether northern species can and will adapt to changing environmental conditions in the long-term remains unknown. What is certain, however, is that the effects

will reach far beyond our lifetimes (i.e., centuries to millennia; Karl and Trenberth, 2003). The fossil record is our only resource for understanding the past responses of organisms to global change, offering insights into the vulnerability and resilience of ecosystems and species on timescales over which the effects of global change are expected to play out (Dietl et al., 2015; Lanier et al., 2015). In particular, the fossil records of the Pleistocene (>52.8 ka BP to 26.5 ka BP [± 170 y BP]) and Holocene (1960s) provide unparalleled opportunities for understanding the ecological responses of extant species to global change.

Gray wolves (*Canis lupus* Linnaeus, 1758) are among the best studied extant carnivores and their present-day ecology is well-understood (Gauthier and Theberge, 1986; Hayes, 1995; Hayes et al., 2000; Paquet and Carbyn, 2003; Merkle et al., 2017; Dalerum et al., 2018;

* Corresponding author.

E-mail addresses: zoelandry@cmail.carleton.ca (Z. Landry), skim380@ucmerced.edu (S. Kim), rtraylor@ucmerced.edu (R.B. Traylor), mgilbert@nature.ca (M. Gilbert), Grant.Zazula@gov.yk.ca (G. Zazula), jsouthon@uci.edu (J. Southon), dfraser@nature.ca (D. Fraser).<https://doi.org/10.1016/j.palaeo.2021.110368>

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Gable et al., 2018; Zrzavy et al., 2018). Records from North America suggest that wolves crossed the Bering Land Bridge from Eurasia during the mid-Pleistocene (800 ka BP) (Kurtén, 1968; Tedford et al., 2009), but were restricted to northern parts of the continent until the late Pleistocene (~28 ka BP), potentially due to competitive exclusion by the now-extinct dire wolves (*Canis dirus* Leidy, 1858) (Meachen et al., 2016; Meachen et al., 2021; Perri et al., 2021). During much of the Holocene, gray wolves ranged from the Canadian Arctic to northern Mexico (Paquet and Carbyn, 2003; Wang and Tedford, 2010). However, they have been recently extirpated from large portions of their range by intense human hunting and habitat loss (Paquet and Carbyn, 2003; Vilà et al., 2003). Still, gray wolves are well-represented as both fossils (Tedford et al., 2009; Harington, 2011) and living animals (Carmichael et al., 2001; Hayes et al., 2003) in the Yukon Territory of Canada. Genomic evidence suggests that Pleistocene gray wolves in Yukon had affinity with other Pleistocene wolves of Eurasia and Beringia (Koblmüller et al., 2016; Meachen et al., 2021; Wang et al., 2020), and these Beringian populations gave rise to modern lower latitude populations following an expansion at the end of the Last Glacial Maximum, ~21 ka (Clark et al., 2012; Loog et al., 2020). Though the gray wolves that inhabit the Yukon today may be morphologically distinct (Leonard et al., 2007; Meachen et al., 2016; Meachen et al., 2021), it is unclear whether they were ecologically distinct. The records from the Yukon bracket major climate change and extinction events during the latest Pleistocene and early Holocene (Guthrie, 2006), through which gray wolves persisted, providing the opportunity for comparing their ecologies.

The climate of the Yukon during the late Pleistocene was generally cooler and drier than today (Zazula et al., 2006b; Zimov et al., 2012), which allowed for the development of open, largely treeless habitats dominated by steppe-like grasslands (Zimov et al., 1995; Zazula et al., 2003). The northern Yukon Territory was part of the mammoth steppe, a megacontinental ecosystem characterized by the presence of herbivorous megafauna (>44 kg) (Harington, 2011; Jürgensen et al., 2017; Schwartz-Narbonne et al., 2019). During this time, the interiors of Alaska and the Yukon were too dry to permit the establishment of continental glaciers and served as glacial refugia (Zazula et al., 2006b; Froese et al., 2009; Schwartz-Narbonne et al., 2019). Fossils of gray wolves are commonly recovered at Yukon localities associated with the mammoth steppe fauna, including those in the Old Crow Basin and the Klondike goldfields (Harington, 2011). During the Pleistocene to Holocene transition (~11.7 ka), the global climate became progressively warmer and wetter (Nogués-Bravo et al., 2008; Rabanus-Wallace et al., 2017). This climate shift led to a transition from steppe-tundra towards boreal forest ecosystems, though some tundra ecosystems still remain in the northern Yukon and in alpine regions (Rowland et al., 2016).

During the late Pleistocene, gray wolves had access to a variety of large-bodied herbivores as potential prey or carrion, including both now-extinct and extant species, such as horses (*Equus* sp. Linnaeus, 1758), steppe bison (*Bison priscus* Bojanus, 1827), woolly mammoths (*Mammuthus primigenius* Blumenbach, 1799), Dall sheep (*Ovis dalli* Nelson, 1884), muskox (*Ovibos moschatus* Zimmermann, 1780), helmeted muskox (*Bootherium bombifrons* Harlan, 1825), saiga antelope (*Saiga tatarica* Linnaeus, 1766), and caribou (*Rangifer tarandus* Linnaeus, 1758) (Harington, 2011; de Manuel et al., 2020). Wolves also coexisted with several large-bodied carnivorous species, including the cave lion (*Panthera leo spelaeus* Goldfuss, 1810) and giant short-faced bear (*Arctodus simus* Cope, 1879) (Harington, 2011), with which they may have competed for prey resources (Ripple and Van Valkenburgh, 2010; Pardi and Smith, 2016). After the arrival of humans in North America at ~14 ka (Lanoë and Holmes, 2016), populations of several large-bodied mammals began to decline (Lorenzen et al., 2011), culminating in the Quaternary Megafaunal Extinction (Koch and Barnosky, 2006; Barnosky, 2008; Fox-Dobbs et al., 2008; Ripple and Van Valkenburgh, 2010; Mann et al., 2015). By ~11.7 ka, most of the Arctic megafauna had become extinct in the Yukon (Harington, 2011), with North America as a whole having lost ~72% of large-bodied mammal genera

(Barnosky, 2008). There were widespread, landscape-scale ecological consequences of the megafauna extinction, including changes to plant biomes, fire regimes, nutrient and seed transport, and how species were ecologically associated (Piers et al., 2015; Doughty et al., 2016; Lyons et al., 2016; Piers et al., 2018; Tóth et al., 2019; Pineda-Munoz et al., 2021). Gray wolves are among the few large predators that survived this extinction (Harington, 2011; Pardi and Smith, 2016), alongside a 'skeleton crew' of prey species including caribou, moose (*Alces alces* Linnaeus, 1758), and elk (*Cervus canadensis* Erxleben, 1777) (Harington, 2011; Yeakel et al., 2013). Modern Yukon gray wolves are, however, increasingly threatened due to climate change (Callaghan et al., 2011; Glig et al., 2012), habitat loss (Paquet and Carbyn, 2003), and decreases in large ungulate abundances (Boulanger et al., 2011; Callaghan et al., 2011; Klaczek et al., 2016).

To our knowledge, there has been no long-term study that explicitly focused on the dietary ecology of gray wolves from the Yukon, and that encompass the timescales over which the effects of anthropogenic perturbation are expected to operate (i.e. tens of thousands of years); further, no study has attempted to compare the dietary ecology of ancient and present-day Yukon wolves. The objective of the present study is to characterize and compare the dietary ecology of Pleistocene and recent gray wolves from the Yukon as a means of understanding their response to the late Pleistocene through Holocene climatic and ecological disturbance. We characterize the dietary ecology of gray wolves using dental microwear analysis and stable isotope analysis of oxygen ($\delta^{18}\text{O}$), carbon ($\delta^{13}\text{C}$), and nitrogen ($\delta^{15}\text{N}$) from bone. We use Bayesian stable isotope mixing models to estimate the primary prey species consumed by wolves during the Pleistocene and compare the results to recent and present-day observational studies (Gauthier and Theberge, 1986; Hayes et al., 2016; Merkle et al., 2017). We offer new insight into the feeding ecology of wolves over a period characterized by significant climate change and shifts in prey availability, providing novel information on the ecological flexibility of gray wolves over thousands of years.

2. Background

2.1. Dental microwear analysis

Dental microwear analysis is the quantification of the patterns of microscopic wear in teeth generated by chewing (Walker et al., 1978). Animal diets are inferred based on reliable relationships between the relative proportions of scratches and pits and preferred food type (Walker et al., 1978; Grine, 1986; Van Valkenburgh et al., 1990; Solounais and Sempredon, 2002; Merceron et al., 2005; Fraser et al., 2009; Schubert et al., 2010; Ungar et al., 2010). Wear patterns on the teeth provide a record of the food items that the individual has consumed over the last few weeks of its life and offers insight into its general feeding ecology (Grine and Kay, 1988; Winkler et al., 2020). Microwear features are divided into two main categories: pits and scratches. Pits are characterized by their circular or sub-circular outline and are similar in length and width (Grine, 1986; Sempredon et al., 2004). Scratches are straight, elongated features with parallel sides that are longer than they are wide (Sempredon et al., 2004).

Carnassial teeth tend to have a relatively large wear facet that is generated by the upper carnassial sliding over the lower during chewing, which offers great insight into dietary preference using microwear analysis (Van Valkenburgh et al., 1990; Schubert et al., 2010). Furthermore, gray wolves (and other caniformes) have retained the sub-equal bicuspid talonid morphology (Van Valkenburgh, 1991), allowing the carnassial teeth, the upper fourth premolar (hereafter P4) and the lower first molar (hereafter m1), to serve a dual purpose; in combination with the retention of post-carnassial molars (Flower and Schreve, 2014), they enable wolves to both crush bones and slice through tough connective tissues (Van Valkenburgh, 1991; Severtsov et al., 2016). Since the carnassials of canids are capable of both bone crushing and flesh

slicing (Tanis et al., 2018), they are ideal for this study as they record the two types of eating behaviours wolves exhibit.

2.2. Stable isotope analysis

Stable isotope analysis (SIA) is commonly used to reconstruct diets and trophic interactions (Clementz, 2012). SIA is based on the principle that ratios of certain stable isotopes within digestible food and water are recorded with predictable fractionation in the tissue of consumers (Bearhop et al., 2004; Schwartz-Narbonne et al., 2019). The axiom of SIA is ‘you are what you eat and drink, given the effects of physiology.’ Herein, we analyze variation in three stable isotopes systems: oxygen ($\delta^{18}\text{O}_{\text{CO}_3}$), carbon ($\delta^{13}\text{C}_{\text{CO}_3}$ and $\delta^{13}\text{C}_{\text{col}}$), and nitrogen ($\delta^{15}\text{N}_{\text{col}}$) from bone.

2.2.1. Oxygen

Stable oxygen isotope compositions ($\delta^{18}\text{O}_{\text{CO}_3}$) from the carbonate component of hydroxyapatite (i.e., bone, tooth enamel) can be used as environmental indicators, particularly of temperature and the hydrological cycle (Luz et al., 1984; Bryant et al., 1996). Oxygen isotopes undergo constant fractionation at $\sim 37^\circ\text{C}$ (the internal body temperature of most mammals), as they are assimilated into the animal’s tissues from the water, and therefore strongly reflect consumed water (Bryant et al., 1996; Kohn, 1996). Therefore, $\delta^{18}\text{O}$ values reflect a complex interaction between local temperature, precipitation sourcing, altitude, and relative humidity (Dansgaard, 1964; Luz et al., 1984; Kohn, 1996). Below 20°C , there is a positive correlation between the $\delta^{18}\text{O}$ value of precipitation and the mean annual temperature ($\sim 0.7\text{‰}/^\circ\text{C}$; Dansgaard, 1954; Dansgaard, 1964; Kohn and Welker, 2005). The $\delta^{18}\text{O}_{\text{CO}_3}$ value in biological apatite is also reflective of habitat; higher $\delta^{18}\text{O}_{\text{CO}_3}$ values reflect more open, arid habitats (e.g. tundra) where there is high evaporative stress, while lower $\delta^{18}\text{O}_{\text{CO}_3}$ values reflect closed, moist habitats (e.g. boreal forest), where the stress of evaporation is lower (Van der Merwe and Medina, 1991; Bocherens, 2003). The combination of $\delta^{13}\text{C}_{\text{CO}_3}$ and $\delta^{18}\text{O}_{\text{CO}_3}$ values is used to provide environmental context because together, they reflect both the local composition of vegetation and climatic conditions.

2.2.2. Carbon

The stable carbon isotope composition of bone carbonate ($\delta^{13}\text{C}_{\text{CO}_3}$) and collagen ($\delta^{13}\text{C}_{\text{col}}$) reflect an animal’s diet and are useful in determining trophic interactions in ancient and modern ecosystems (Bocherens and Drucker, 2003; Clementz, 2012). In animals, $\delta^{13}\text{C}_{\text{CO}_3}$ values reflect whole diet, while $\delta^{13}\text{C}_{\text{col}}$ values are thought to primarily reflect ingested proteins (Ambrose and Norr, 1993; Jim et al., 2004). In general, there is a $\sim 1\text{‰}$ increase in $\delta^{13}\text{C}$ values per trophic level increase (Bearhop et al., 2004), with the lowest values in primary producers and highest in apex predators (Pilot et al., 2012). Since carnivores occupy a high trophic level and ingest both a range of prey body tissues as well as a variety of herbivorous species, isotopic values derived from their tissues are an averaged representative of multiple dietary carbon sources (Bump et al., 2007). In ecosystems with both C_3 and C_4 plants $\delta^{13}\text{C}$ values of herbivores are commonly used to distinguish which of these two plant types are primarily consumed by the animal (Jim et al., 2004; Codron et al., 2013); however, as the Yukon is occupied only by C_3 plants (Zazula et al., 2006a; Wooller et al., 2007; Tahmasebi et al., 2017), the $\delta^{13}\text{C}$ values of this study are solely reflective of C_3 plants as the ecosystem’s primary producers. Variation in $\delta^{13}\text{C}$ values among C_3 plants can be due to differences between species (i.e., forest species vs. tundra species) or changes in climate (Zimov et al., 1995; Clark et al., 2012).

2.2.3. Nitrogen

Stable nitrogen isotope composition ($\delta^{15}\text{N}_{\text{col}}$) can be used as a means of reconstructing trophic webs (Bocherens and Drucker, 2003). The tissues of consumers are enriched in ^{15}N and produce a $\delta^{15}\text{N}$ increase by

~ 3 to $\sim 5\text{‰}$ per trophic level increase (Bocherens and Drucker, 2003; Fox-Dobbs et al., 2008). Interpretation of $\delta^{15}\text{N}_{\text{col}}$ values poses some difficulty, however, as there are a variety of factors that influence the $\delta^{15}\text{N}_{\text{col}}$ values of animal tissues (McMahon and McCarthy, 2016). For example, animals that live in more arid environments tend to have greater $\delta^{15}\text{N}_{\text{col}}$ values compared to animals that live in environments with greater precipitation (Heaton et al., 1986; Crowley et al., 2011; Hixon et al., 2018), as a result of differences in ^{15}N uptake among plant species (Codron et al., 2013). Furthermore, intense and prolonged nutritional stress results in tissues becoming enriched in ^{15}N as the animal breaks down (catabolizes) its own tissues for energy (Keenan and DeBruyn, 2019). The combination of $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ values is often used to infer dietary inputs using stable isotope mixing models (Semmens et al., 2009; Derbridge et al., 2012; Hopkins and Ferguson, 2012).

3. Materials and Methods

We obtained specimens of Pleistocene gray wolves ($n = 31$) from the Yukon Territory from the Canadian Museum of Nature (CMN) Palaeobiology Collection and the Yukon Government (YG) Palaeontology Program Collection (Table S1). Samples from recent Yukon specimens ($n = 17$) were obtained from the CMN Zoology Collection (Table S2). Specimens were selected based on the following criteria: 1) at least one well-preserved P4 or m1; and 2) no large cracks visible along the shearing facet to be molded. Due to previously poor storage facilities, some teeth were split along the sagittal plane, although no teeth used had cracks that compromised the integrity of the shearing facet to be molded. To enable differentiation between baseline (i.e. environmental change) and dietary shifts, we also collected specimens of Pleistocene caribou ($n = 8$) from the Yukon from the CMN Palaeontology Collection (Table S3) and recent caribou ($n = 8$) from the Yukon obtained from the CMN Zoology Collection (Table S4). We do not possess fossilized plant remains and, therefore, herbivores represent the best approximation of the isotopic baseline.

3.1. Radiocarbon dating

Wolves were divided between two temporal bins, late Pleistocene (~ 40 ka BP) and recent (1960’s). Bulk radiocarbon dates were taken from Harington (2003) and new dates were acquired using AMS radiocarbon dating performed at the University of California Irvine. Sample preparation at UC Irvine was based on Shammas et al. (2019): briefly, following mechanical cleaning, samples of ~ 200 mg of wolf bone plus known-age bone standards and ^{14}C -free blanks were crushed to 1–2 mm chips and decalcified overnight in a measured volume of 1 N HCl just sufficient to dissolve a mass of bone mineral (hydroxyapatite) corresponding to the entire sample weight. After washing with ultra-pure Milli-Q water, the decalcified samples were gelatinized in 0.01 N HCl at 60°C overnight, ultrafiltered to select a high molecular weight fraction (>30 kDa), and freeze-dried overnight. For details of sample combustion and graphitization and the actual AMS radiocarbon measurements, see Santos et al. (2007) and Southon et al. (2004). Radiocarbon dates were not calibrated as the majority of the dates were beyond the scope of IntCal13 (Reimer et al., 2013).

3.2. Dental microwear methods

The carnassial teeth of gray wolf specimens were used in the microwear analysis. Although we recognize that canids (including wolves) primarily use their post-carnassial molars for bone processing (Van Valkenburgh, 1989; Ungar et al., 2010; Tanis et al., 2018; Prassack et al., 2020), Tanis et al. (2018) noted that the m1 can be used to provide some insight regarding bone consumption in the absence of a suitable post-carnassial molar (m2). Because certain fossil specimens only possessed suitable m1 teeth (i.e., m2 were either missing or poorly preserved), we selected the m1 for this analysis. Additionally, by

choosing to analyze the m1, we are able to directly compare the wear patterns to those of the P4 (i.e., when m1 teeth were unavailable in fossil specimens), as these teeth act in conjunction to process food (Van Valkenburgh et al., 1990).

Molds of the carnassial teeth (lingual P4, labial m1) of suitable specimens were made with polyvinylsiloxane dental impression material (President's microSystem regular body, Coltène-Whaledent Inc., Cuyahoga Falls, OH) after cleaning the teeth with ethanol and/or acetone (Solounais and Semprebon, 2002; Fraser et al., 2009). Retaining walls were built around the tooth molds using molding putty (President the Original: Putty Fast Soft Base and Catalyst Coltène-Whaledent Inc., Cuyahoga Falls, OH). Casts were then made by filling the molds with EpoKwick epoxy resin and hardener, and were left to harden for seven days. The epoxy casts were then removed and gold-coated to prepare for analysis using a scanning electron microscope (FEI Apreo FE-SEM; referred to as SEM hereafter) at the Natural Heritage Campus of the CMN. Casts were mounted on pegs and secured with copper tape to avoid charging the sample, and mounted in the specimen holder. The high-vacuum mode was used, set to 5.00 kV. Magnification was set to 100× and 2–3 images per tooth cast were captured using the ETD detector. Each specimen was photographed at various locations on the wear facets of the cast.

The SEM images obtained were then analyzed in ImageJ Software (Abramoff et al., 2004). The scale on the image was set to 500 μm (0.5 mm) and two square boxes set to 300 μm × 300 μm (0.3 mm × 0.3 mm) were drawn on locations where microwear patterns were the clearest on each image. Within each box, the number of scratches and the number of pits were counted separately (Solounais and Semprebon, 2002) using the multiple selection tool (Fraser et al., 2009). Pits were defined by the qualification of the wear features and the scoring followed the protocol outlined in Van Valkenburgh (1991), Solounais and Semprebon (2002), Merceron et al. (2005), and Fraser et al. (2009). Two images were analyzed for each specimen, yielding four counts each of scratches and pits, which were then averaged to obtain the mean value for that specimen. The presence of gouges and cracks were noted for each specimen, but were not counted.

Previous studies have successfully used SEM imaging and feature counting to infer the diets of animals (e.g. Grine, 1986; Solounais and Semprebon, 2002; Fraser et al., 2009; Fraser and Theodor, 2013; Henton et al., 2017). As with all methods that involve visual counting, there is potential for observer error (DeSantis et al., 2013). However, studies suggest that the observer error associated with SEM-based microwear quantification is no more problematic than the intra- and interobserver errors associated with other morphometric parameters (Grine et al., 2002). Furthermore, dental microwear texture analysis is not without error, as it may suffer significantly from inter-microscope variability (Kubo et al., 2017).

3.3. Stable isotope analysis

The mandibles of the Pleistocene and recent gray wolves were sampled using a Dremel tool with a diamond tapered point bit (part #7144) and the powdered bone was collected in safe lock micro test tubes. We pretreated bone samples for carbonate analysis following the methods of Koch et al. (1997). Briefly, 3–4 mg of powdered bone was reacted with 1 mL of 2–3% NaOCl for 48 h to remove organic matter and then rinsed 5 times with deionized water. The samples were then reacted with 0.5 mL 1 M CH₃COOH (acetic acid) buffered with calcium acetate (pH ~5) for 24 h to remove labile carbonates. Samples were agitated and left to react in a refrigerator for 24 h. The samples were again rinsed 5 times with deionized water and dried at 60 °C overnight. For collagen analysis, we demineralized ~4 mg of bone powder with ~1.5 mL 0.1 M HCl for 30 min at 4 °C, rinsed 5 times using deionized water, froze purified collagen overnight, and lyophilized overnight. Samples were not subjected to defatting as C:N ratios did not indicate substantial lipid content.

All stable isotope analyses took place at the University of California, Merced Stable Isotope Lab. To analyze carbonate isotope composition, ~1 mg of pre-treated sample was weighed into borosilicate glass extender tubes and the headspace was flushed with He. The samples were reacted with >100% H₃PO₄ at 70 °C for 4 h, then CO₂ gas within the headspace was sampled for isotopic composition measurement using a ThermoFisher GasBench II coupled with a ThermoFisher Delta V Plus continuous flow isotope ratio mass spectrometer. Carbon and oxygen isotope compositions were corrected for instrument drift, mass linearity, and normalized to the VPDB scale using the NBS-18 ($n = 9$), USGS 44 ($n = 9$), and a Carrara Marble (CM; Elemental Microanalysis; $n = 8$) calcite reference materials. The average reproducibility was $\pm 0.2\text{‰}$ and $\pm 0.1\text{‰}$ (1σ) for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively. We also analyzed several replicates of NIST 120c ($n = 7$), a phosphorite with chemistry similar to enamel and bone. Average isotope compositions for NIST 120c were $-6.6 \pm 0.2\text{‰}$ ($\delta^{13}\text{C}$) and $-2.5 \pm 0.2\text{‰}$ ($\delta^{18}\text{O}$).

To analyze collagen isotope composition, 0.7 mg of demineralized bone were weighed into 3x5mm tin boats (EA Consumables) for combustion on a Costech Elemental Analyzer 4010 coupled via Conflo IV to a ThermoFisher Delta V Plus continuous flow isotope ratio mass spectrometer. Carbon and nitrogen isotope compositions were corrected for instrument drift, mass linearity and standardized to the international VPDB ($\delta^{13}\text{C}$) and AIR ($\delta^{15}\text{N}$) scales using the USGS 40 ($n = 4$) and USGS 41a ($n = 5$) reference materials. We also analyzed several replicates of a homogenized squid tissue ($n = 6$) as an internal check. The average isotope compositions were $-18.7 \pm 0.04\text{‰}$ ($\delta^{13}\text{C}$) and $12.1 \pm 0.2\text{‰}$ ($\delta^{15}\text{N}$) which is indistinguishable from the long term average ($\delta^{13}\text{C} = -18.7 \pm 0.1\text{‰}$, $\delta^{15}\text{N} = 11.8 \pm 0.3\text{‰}$, $n = 112$). Isotope ratios are expressed using δ notation, where:

$$\delta^h X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values are reported relative to the standard Vienna Pee Dee Belemnite (VPDB) and $\delta^{15}\text{N}$ values are reported relative to AIR.

3.4. Statistical analysis

The mean microwear values of the gray wolf specimens were translated into the R software environment (R Core Team, 2019). A paired t -test assuming equal variance was then performed to test for statistically significant differences between the mean wear values of pits and scratches, respectively, of the Pleistocene and recent wolf specimens.

Prior to statistical analysis, the $\delta^{13}\text{C}$ values obtained for the Pleistocene carbonate and collagen samples of the wolves were corrected for recent anthropogenic CO₂ levels using the equation and parameter values detailed in Long et al. (2005):

$$\delta^{13}\text{C} = k - e^{at^2}$$

where k and a are derived parameters equal to -5.5656 and 6.0932×10^{-5} , respectively, and t is an index to year where 1 represents 1880 (Long et al., 2005). We corrected all Pleistocene $\delta^{13}\text{C}$ values to the year 1965 to correct for differences in the ^{13}C composition of atmospheric CO₂ of the Pleistocene, so that values may be directly compared to recent wolves. Thus, the equation that we used to determine our correction factor for these values is as follows:

$$\delta^{13}\text{C} = -5.5656 - e^{(6.0932 \times 10^{-5})(1965 - 1880)^2}$$

Paired t -tests assuming equal variance were performed on the $\delta^{18}\text{O}_{\text{CO}_3}$, $\delta^{13}\text{C}_{\text{CO}_3}$, $\delta^{13}\text{C}_{\text{col}}$, and $\delta^{15}\text{N}_{\text{col}}$ values of the Pleistocene and recent Yukon wolf specimens in R (R Core Team) to test for statistically significant differences among the wolves from different time periods.

Bayesian stable isotope mixing models were run in MixSIAR (v.3.1.10) (Stock et al., 2018) using JAGS software (v.4.3.0) in R (R Core Team, 2019), with the $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ values from the Pleistocene

Table 1
Source data and assigned priors for the Bayesian stable isotope mixing models.

Species	No. of specimens	Mean $\delta^{13}\text{C}_{\text{col}}$ (‰)	Mean $\delta^{15}\text{N}_{\text{col}}$ (‰)	Assigned prior (uninformed model)	Assigned prior (informed model)	Reference
<i>Equus</i> sp.	6	-20.9	7.1	1	0.5307345728	Schwartz-Narbonne et al. (2019)
<i>Mammuthus primigenius</i>	22	-21.1	8.2	1	0.0537711142	Metcalf et al. (2016)
<i>Ovibos moschatus</i>	10	-19.5	5.5	1	0.573733304	Raghavan et al. (2014)
<i>Ovis dalli</i>	8	-19.1	6.0	1	2.230114383	Schwartz-Narbonne et al. (2019)
<i>Rangifer tarandus</i>	20	-19.3	4.1	1	1.6098907693	Schwartz-Narbonne et al. (2019); this study

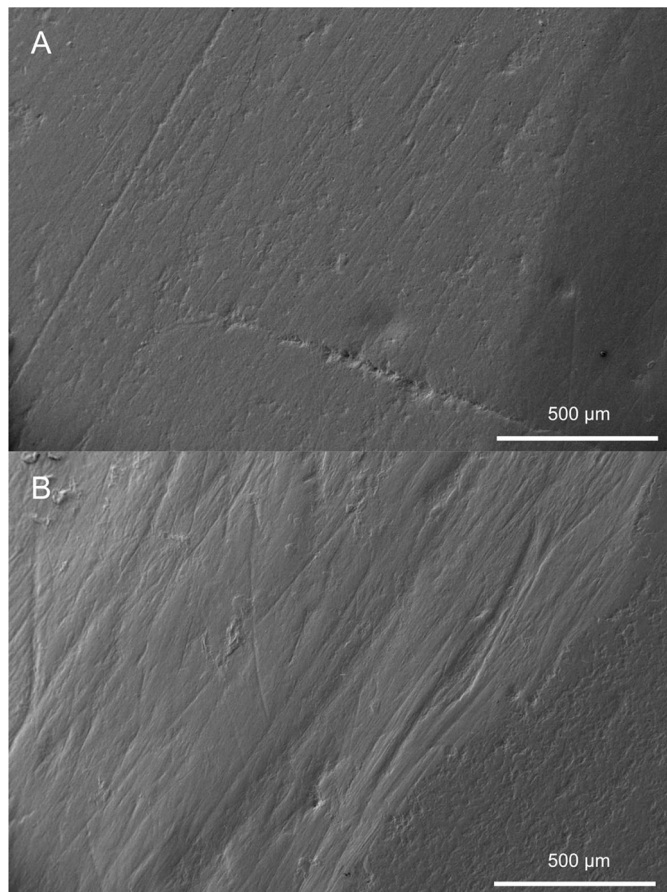


Fig. 1. A) SEM image of typical carnassial dental microwear from a Pleistocene gray wolf (*Canis lupus*) specimen (CMNFV 28862) taken with the EDT detector at 100 \times magnification; B) SEM image of typical carnassial dental microwear from a recent gray wolf (*Canis lupus*) specimen (CMNMA 30924) taken with the EDT detector at 100 \times magnification.

wolves (the mix; $n = 29$). We did not run a Bayesian stable isotope mixing model for the recent wolves because there are an abundance of published, observational studies conducted on modern gray wolves (Gauthier and Theberge, 1986; Hayes, 1995; Hayes et al., 2000; Paquet and Carbyn, 2003; Hayes et al., 2016; Dalerum et al., 2018), thus their diets have been well-documented. Five prey species (the sources) were included in the Pleistocene wolf model. All prey fossils present are from Eastern Beringia and are of similar radiocarbon age to the wolf specimens ~ 50 – 30 ka BP (Raghavan et al., 2014; Metcalfe et al., 2016; Schwartz-Narbonne et al., 2019). Prey isotope compositions ($\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$) are from bone collagen and were either borrowed from published literature or obtained de novo for this study (Table 1, S3, S4).

We generated two models for the Pleistocene wolves, each with different prior distributions of prey fraction in diet (Table 1). All priors were scaled to a total weight of five, since there were five sources

included in the model, and were applied in alphabetical order to the sources. The first model was run with uninformative priors, which automatically weighed all prey species as being equally likely to contribute to the diets of the wolves. The second model was run with informative priors, these being based on models of prey abundance derived from prey body mass (Damuth, 1981; Alroy, 1998; Smits, 2015). The trophic enrichment factors ($\Delta X = \delta X_{\text{consumer}} - \delta X_{\text{prey}}$, where X is the element) we used for the wolves are $\Delta^{13}\text{C}_{\text{col}} = +1.3\text{‰} \pm 0.6\text{‰}$ $\Delta^{15}\text{N}_{\text{col}} = +4.6\text{‰} \pm 0.7\text{‰}$, based on wolf-specific trophic enrichment factors for bone collagen calculated by Fox-Dobbs et al. (2007). Models were run for 100,000 iterations.

4. Results

A subset of Pleistocene wolves (selected based on shared localities) were ^{14}C dated and all fell within the Middle Wisconsinan interstadial of Marine Isotope Stage 3 (Harington, 2011), ranging from >52.8 ka BP to 26.5 ka BP (± 170 y BP) (Table S1). These ages place the Pleistocene wolves in a warmer interstadial period that occurred between two glacial maxima (Lemieux et al., 2008; Harington, 2011). One wolf that was initially assigned to the Pleistocene bin (YG 498.1) yielded a ^{14}C date of 160 ± 25 y BP, and was grouped for further analyses alongside the recent wolves.

The tooth wear surfaces of the Pleistocene specimens were characterized by a mix of coarse and fine scratches, as well as small and large pits (Fig. 1A). Approximately half of the specimens had gouges in the wear surfaces. Several specimens also exhibited large cracks throughout the wear surface extending through the tooth itself that may not be the result of mastication behaviours and are likely caused by taphonomic processes. The tooth wear surfaces of the recent specimens were generally characterized by fine or mixed scratches, with several specimens having few coarse scratches (Fig. 1B). The recent specimens also generally exhibited mixed pit sizes, although there was a wider range of pit sizes among the recent specimens compared to the Pleistocene specimens. There were few specimens with gouges in the wear surface, and several specimens had cracks in the teeth, likely caused by suboptimal storage conditions of the specimens in the past. The mean numbers of pits and scratches for Pleistocene and recent wolves do not differ significantly (Pits: Two-tailed t -test, $t = -0.810$, $df = 66.138$, $p = 0.421$, Scratches: Two-tailed t -test, $t = -1.747$, $df = 70.350$, $p = 0.085$), indicating similar feeding strategies (Fig. 2).

The stable isotope composition of recent and Pleistocene wolves had varying patterns between carbonate and collagen as well as isotope systems. For carbonates, there is extensive overlap, yet still differences, among both isotope systems of recent and Pleistocene wolves. The $\delta^{18}\text{O}_{\text{CO}_3}$ values differed significantly between the recent and Pleistocene wolves, with higher values in recent (mean: -15.5‰ , SD: $\pm 0.9\text{‰}$) compared to Pleistocene (mean: -16.7‰ , SD: $\pm 1.0\text{‰}$) wolves with statistical significance (Two-tailed t -test, $t = 3.228$, $df = 35.393$, $p = 0.003$) (Fig. 3A). The $\delta^{13}\text{C}_{\text{CO}_3}$ values also overlapped between recent (mean: -14.8‰ ; SD: $\pm 1.3\text{‰}$) and Pleistocene (mean: -13.9‰ , SD: $\pm 1.0\text{‰}$), but the two time periods were significantly different in means (Two-tailed t -test, $t = -2.189$, $df = 26.333$, $p = 0.039$) even after the Pleistocene values had been corrected for 1965 atmospheric CO_2 ^{13}C composition

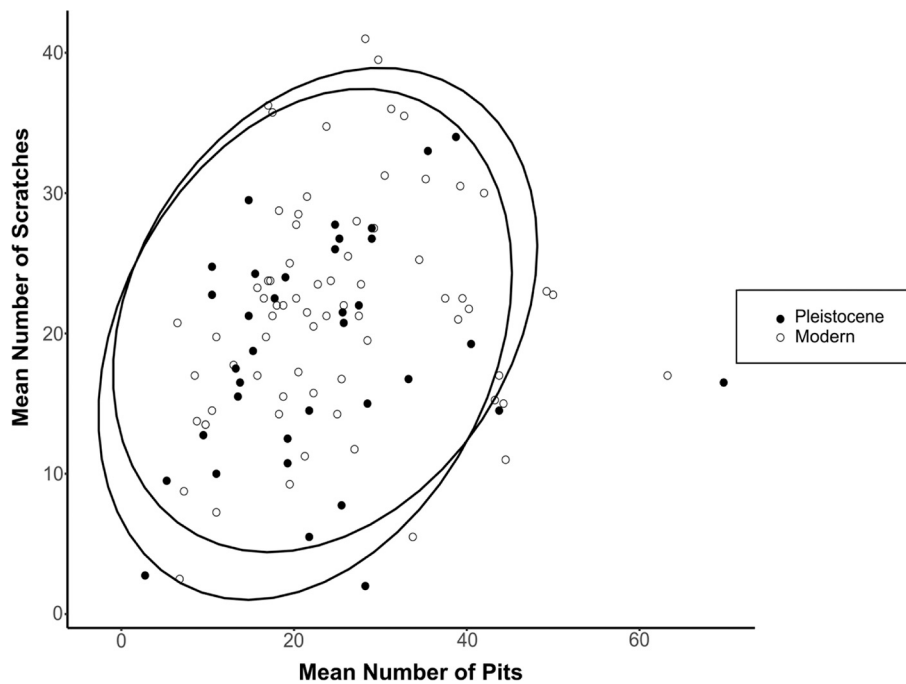


Fig. 2. Mean number of pits and average number of scratches exhibited by Pleistocene and recent gray wolves (*Canis lupus*) from the Yukon Territory, with 95% confidence ellipses.

(Fig. 3A). However, due to the extensive overlap, we believe that the difference is largely driven by few outliers, as the majority of both recent and Pleistocene wolves $\delta^{13}\text{C}_{\text{CO}_3}$ values range from -16‰ to -12‰ .

For collagen, there is more variation between the two isotope systems, and greater differences in the isotope trends of recent and Pleistocene wolves. There is still a large overlap between the $\delta^{13}\text{C}_{\text{col}}$ values of recent and Pleistocene wolves, with a wider range of values exhibited by the recent wolves compared to the Pleistocene wolves. The $\delta^{13}\text{C}_{\text{col}}$ values obtained from recent (mean: -21.5‰ , SD: $\pm 1.6\text{‰}$) and Pleistocene (mean: -20.9‰ , SD: $\pm 1.4\text{‰}$) wolves are not significantly different (Two-tailed t-test, $t = 1.179$, $df = 29.639$, $p = 0.248$), and fall within a general range of -22‰ to -19‰ , though we note that one recent wolf specimen has substantially lower $\delta^{13}\text{C}_{\text{col}}$ values than other recent specimens (Fig. 3B). There is a clear difference in the $\delta^{15}\text{N}_{\text{col}}$ values between Pleistocene and recent wolves, indicating that there has been a change in these values over time (Fig. 3B). The recent wolves had significantly lower $\delta^{15}\text{N}$ values and variation (mean: 6.7‰ , SD: $\pm 1.3\text{‰}$) than Pleistocene wolves (mean: 9.4‰ , SD: $\pm 2.0\text{‰}$); while significantly different (Two-tailed t-test, $t = 5.468$, $df = 42.600$, $p = 2.213 \times 10^{-6}$), which is clearly visible in Fig. 3B.

The two iterations of MixSIAR with uninformed and informed priors, produced different results. The posterior probabilities from the uninformed priors indicated that horses constituted the majority of the diet ($\sim 52.2\% \pm 19.0\%$), followed by woolly mammoths ($\sim 15.5\% \pm 10.6\%$), and caribou ($\sim 15.0\% \pm 12.3\%$) (Table 2; Fig. 6A). The informative priors based on models of prey abundance derived from body mass indicated that, again, horse was the primary contributor to diet ($\sim 54.2\% \pm 19.5\%$), followed by caribou ($\sim 21.5\% \pm 13.9\%$), and Dall sheep ($\sim 16.2\% \pm 0.1\%$) (Table 2; Fig. 6B). We do not repeat this analysis for the recent wolves due to the abundance of studies that directly observe the dietary inputs of Yukon gray wolves.

5. Discussion

During the late Pleistocene (15–11.7 ka), invasion of North America by humans (Guthrie, 2006; Lanoë and Holmes, 2016), climate changes (Allen et al., 2012; Clark et al., 2009) and the loss of 72% of large bodied

mammal genera (Barnosky, 2008) had profound ecological consequence, which included changes to plant biomes, fire regimes, nutrient and seed transport, among others (Piers et al., 2015; Doughty et al., 2016; Lyons et al., 2016; Piers et al., 2018; Tóth et al., 2019; Pineda-Munoz et al., 2021). Gray wolves are one of the few large predators that survived this extinction (Harington, 2011; Pardi and Smith, 2016). Herein, we tested whether the ecological upheaval of the Pleistocene-Holocene transition had significant ecological consequences for gray wolves, specifically, whether these changes induced dietary shifts. To do so, we used dental microwear and stable isotope analysis of oxygen ($\delta^{18}\text{O}$), carbon ($\delta^{13}\text{C}$), and nitrogen ($\delta^{15}\text{N}$) from bone.

During the megafaunal extinction in North America (~ 11.7 ka), the majority of large-bodied mammals became extinct or extirpated (Barnosky, 2008), including many species that may have been prey to Yukon gray wolves (Harington, 2011). When prey are either scarce, more difficult to catch, or there is high competitive stress among congeneric species, more of a prey carcass may be consumed, which leads to increased numbers of large pits and gouges as well as tooth breakage due to the mastication of bone (Van Valkenburgh et al., 2019). During the Pleistocene, there were many potential prey species available to gray wolves compared to the present-day (Slough and Jung, 2007; Harington, 2011; de Manuel et al., 2020). But there were also more species of carnivores (e.g., short-faced bears, scimitar cats) that were possible competitors of wolves (Harington, 2011; Tseng et al., 2019). In contrast, gray wolves in the present-day Yukon have relatively few potential competitors. Wolves are one of the largest modern terrestrial northern predators, alongside brown bears (*Ursus arctos*) and black bears (*Ursus americanus*; Merkle et al., 2017), and the only pursuit hunter (Flower and Schreve, 2014). These species co-exist through partitioning prey resources, with the ursids mainly feeding on vegetation and scavenged ungulate carcasses, and wolves feeding almost exclusively on large ungulates (Merkle et al., 2017). Due to the relatively low competitive pressure (Merkle et al., 2017), wolves are able to feed on all species of ungulates in the present-day Arctic, with diets comprised mainly of moose and caribou (Gauthier and Theberge, 1986; Hayes et al., 2016; Merkle et al., 2017).

Despite potentially large differences in the competitive landscapes,

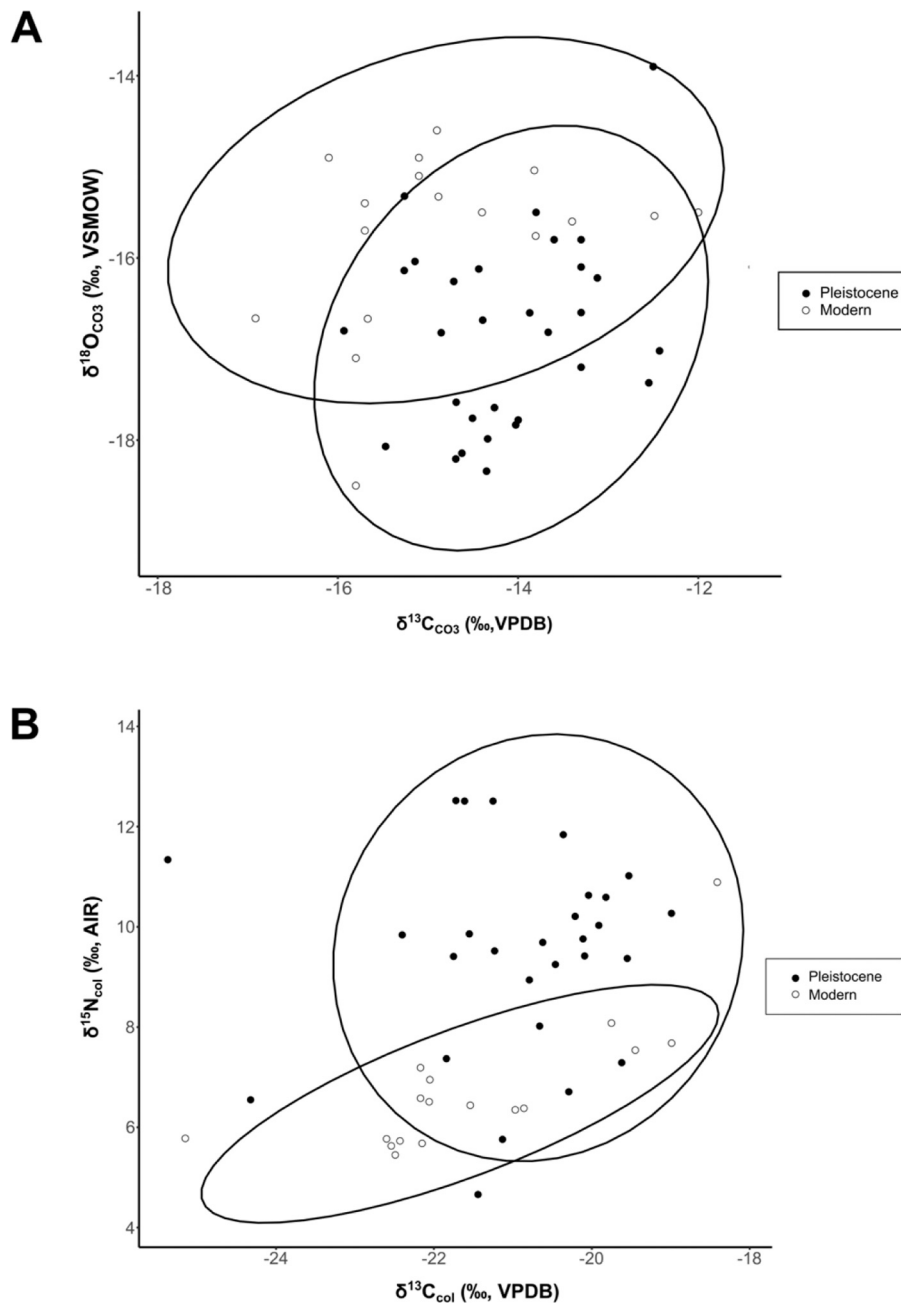


Fig. 3. A) $\delta^{13}\text{C}_{\text{CO}_3}$ and $\delta^{18}\text{O}_{\text{CO}_3}$ values of Pleistocene and recent gray wolves (*Canis lupus*) from the Yukon Territory, with 95% confidence ellipses; B) $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ values of Pleistocene and recent gray wolves (*Canis lupus*) from the Yukon Territory, with 95% confidence ellipses.

Table 2
Results of the uninformed and informed Bayesian stable isotope mixing models.

Species	Mean dietary contribution (%) - uninformed model	Standard deviation (%) - uninformed model	Mean dietary contribution (%) -informed model	Standard deviation (%) - informed model
<i>Equus</i> sp.	52.2	19.0	54.2	19.5
<i>Mammuthus primigenius</i>	15.5	10.6	1.7	5.3
<i>Ovibos moschatus</i>	10.6	9.8	6.4	7.9
<i>Ovis dalli</i>	6.8	5.9	16.2	10.1
<i>Rangifer tarandus</i>	15.0	12.3	21.5	13.9

the dental microwear patterns of both the Pleistocene and recent specimens are dominated by scratches (Fig. 1A, B), indicating that flesh-slicing was the primary mastication behavior (Schubert et al., 2010). Though there are small pits present on the occlusal surfaces of both sets of specimens, they do not exhibit the large pits and gouges that are characteristic of species that exhibit extensive osteophagy (Van Valkenburgh et al., 1990; Schubert et al., 2010). We also do not observe many teeth that appear to have been broken during life. Thus, it is unlikely that wolves from either temporal interval relied significantly upon extensive carcass consumption to fulfil their dietary requirements. Though wolves may have consumed bones to access the nutritious marrow (Flower and Schreve, 2014; Van Valkenburgh et al., 2019), dental microwear analysis suggests there was little change in carcass utilization behavior.

Differences in the stable isotope compositions of animal tissues

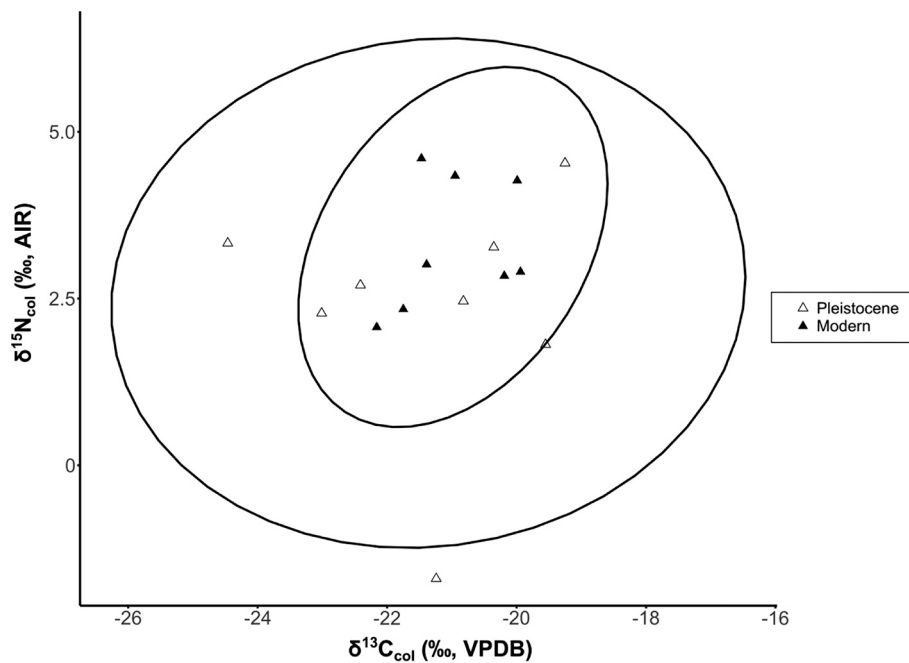


Fig. 4. $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ values of Pleistocene and recent caribou (*Rangifer tarandus*) from the Yukon Territory, with 95% confidence ellipses.

reflect differences in ecology over space and time, including prey selection, habitat use, and behavior (Ben-David and Flaherty, 2012). $\delta^{18}\text{O}$ values reflect consumed waters and, thus, environmental and climate differences among populations or time periods (Luz et al., 1984; Bryant et al., 1996). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are dietary indicators (Bocherens and Drucker, 2003) and also vary, to a lesser degree, with habitat and environmental conditions (Heaton et al., 1986; Crowley et al., 2011; Hixon et al., 2018). We observed an increase in wolf $\delta^{18}\text{O}_{\text{CO}_3}$ values over time, consistent with documented climate shifts from the Pleistocene to present-day (Fitzgerald, 1991; Zazula et al., 2006b; Glig et al., 2012; Irvine et al., 2012; Rowland et al., 2016). Caribou show a small yet significant increase in $\delta^{13}\text{C}_{\text{col}}$ values over time, although these results are largely driven by a single specimen (Fig. 4). Though the vegetation structure of the Yukon has remained C_3 plant-dominated from the Pleistocene to the recent Holocene (Zazula et al., 2006a; Wooller et al., 2007; Tahmasebi et al., 2017), the minor change in caribou $\delta^{13}\text{C}_{\text{col}}$ could reflect a shift in the isotopic baseline resulting from changes in the species composition of the plant community (the shift from arid steppe-tundra to moist boreal forest) and climate (Zimov et al., 1995; Clark et al., 2012). The $\delta^{13}\text{C}_{\text{CO}_3}$ and $\delta^{13}\text{C}_{\text{col}}$ values of both Pleistocene and recent gray wolves fall within the expected range for predators feeding on prey from C_3 -based ecosystems (Ben-David and Flaherty, 2012). The marginal increase in $\delta^{13}\text{C}_{\text{CO}_3}$ values between Pleistocene and recent wolves could reflect a switch in prey species (Hedges and van Klinken, 2000; Hedges, 2003) or a change in the isotopic baseline (Post, 2002; Long et al., 2005). An increase is, however, not observed in the wolf $\delta^{13}\text{C}_{\text{col}}$ values, which reflect consumed protein rather than whole diet, as is the case for $\delta^{13}\text{C}_{\text{CO}_3}$ (Ambrose and Norr, 1993; Jim et al., 2004), suggesting dietary rather than baseline changes. Similarly, Yukon wolves show a significant increase in $\delta^{15}\text{N}_{\text{col}}$ from the Pleistocene to recent (Fig. 3A, B), which could be explained by either environmental changes (relating to the shift from arid steppe-tundra to moist boreal forest; Heaton et al., 1986; Zimov et al., 1995; Crowley et al., 2011; Harington, 2011; Rowland et al., 2016; Rabanus-Wallace et al., 2017; Hixon et al., 2018) or changes in primary prey species (Hedges and van Klinken, 2000; Hedges, 2003). However, caribou do not show a similar increase in $\delta^{15}\text{N}_{\text{col}}$ (Fig. 4A, B), further suggesting a dietary shift among Yukon wolves.

Modern Yukon gray wolves are relatively inflexible in their feeding

behavior, feeding primarily on large-bodied ungulates (e.g. moose, caribou) (Gauthier and Theberge, 1986; Paquet and Carbyn, 2003; Hayes et al., 2016; Merkle et al., 2017; Dalerum et al., 2018), except when their abundances are low (Gauthier and Theberge, 1986; Hayes, 1995; Hayes et al., 2000). The primary species consumed by recent/modern Yukon wolves are caribou (18%–35%; Gauthier and Theberge, 1986; $50\% \pm 0.10\%$; Merkle et al., 2017), moose (30–41%; Gauthier and Theberge, 1986; $29\% \pm 0.09\%$), and caribou/moose (37%; cases where stomach contents and scat could not be further identified) (Hayes et al., 2016). The offset between the $\delta^{15}\text{N}_{\text{col}}$ of recent caribou and gray wolves is consistent with the expected trophic enrichment from prey to predator (Fig. 4B), lending further support to caribou being one of the primary prey species of present-day gray wolves. Given the transient presence of moose and elk in the Yukon during the Pleistocene (Meiri et al., 2014; Meiri et al., 2020), however, it is unlikely that wolves retained the same proportions of prey species consumption over thousands of years.

When the abundances of ungulates are low, modern wolves have been documented to switch to feeding mainly on smaller, non-ungulate prey (Merkle et al., 2017; Dalerum et al., 2018). This behavior typically occurs over very short timescales, often in response to seasonal variation (Gable et al., 2018). In these instances, modern wolves will occasionally feed on various berries, American beaver (*Castor canadensis* Kuhl, 1820), snowshoe hare (*Lepus americanus* Erxleben, 1777), muskrat (*Ondatra zibethicus* Linnaeus 1766) (Gable et al., 2018), and salmon (*Oncorhynchus* sp. Walbaum, 1792) (Adams et al., 2010), all of which were also present in the Yukon during the Pleistocene (Harington, 2011; Meachen et al., 2021). However, recent/modern wolves exhibit relatively infrequent prey-switching, and will only feed upon small prey opportunistically or when large prey abundances are very low (Gauthier and Theberge, 1986; Hayes, 1995; Hayes et al., 2000). At present, however, we cannot distinguish seasonal patterns of isotopic variation.

Unlike in modern ecosystems, we cannot directly observe predator-prey interactions in the Pleistocene (Domingo et al., 2016). Therefore, to investigate the composition of Pleistocene wolf diets, we used Bayesian Stable Isotope Mixing Models, running versions with and without ecologically informed priors (see Materials and Methods). The first Bayesian mixing model (uninformative priors, no accounting for abundance based on body size) indicated that horses contributed the most to Pleistocene wolf diets, followed by woolly mammoths, and

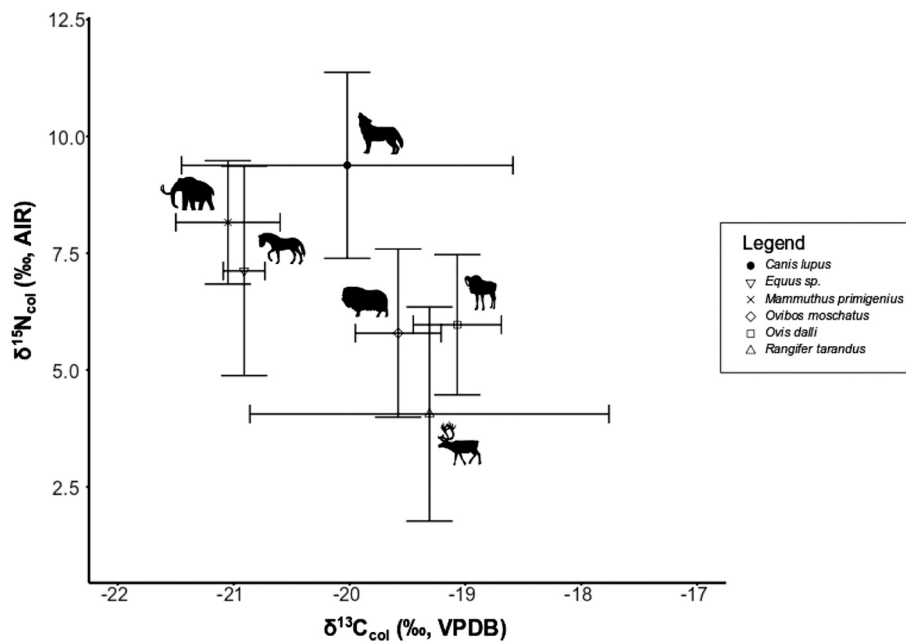


Fig. 5. Isospace plot of mean $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ values (\pm standard deviation) of potential Pleistocene wolf prey species and Pleistocene gray wolves from Beringia. *M. primigenius* values from Metcalfe et al. (2016); *Ovis moschatus* values from Raghavan et al. (2014); *E. sp.*, *Ovis dalli*, and some *R. tarandus* values from Schwartz-Narbonne et al. (2019).

caribou (Table 2). There is a great deal of uncertainty associated with the results of the uninformed Bayesian mixing model (Fig. 6A). Therefore, we believe that the results do not fully reflect the most likely predator-prey interactions for late Pleistocene. The second Bayesian mixing model (informative priors based on models of prey abundance derived from their body mass; Damuth, 1981), however, also indicated that horses were the primary prey of Pleistocene wolves, contributing to over half of their overall diets, followed by caribou, and Dall sheep (Table 2). We believe these results to be more ecologically relevant, due to our use of ecologically-informed priors based on prey size (Damuth, 1981) and horses having been the most abundant prey in the steppe-tundra ecosystems of Eastern Beringia during the Pleistocene (Guthrie, 2003; Harington, 2011).

One potential drawback of our Bayesian mixing models is that there is extensive overlap in the $\delta^{13}\text{C}_{\text{col}}$ values of all of Pleistocene prey species (Fig. 5). The ability of Bayesian stable isotope mixing models to discriminate among isotopic sources is strongly influenced by the isotopic separation (distinctiveness) of the sources and can produce biased results when sources are not differentiable along multiple isotope axes (Lerner et al., 2018). Due in part to the number of sources included in the model (Lerner et al., 2018) and the overlapping isotopic ranges of the sources, our models primarily differentiated sources by their $\delta^{15}\text{N}_{\text{col}}$ values (Fig. 5). This resulted in the model assigning similar contributions for many sources to the overall diet and producing relatively large uncertainties (standard deviations; Franco-Trecu et al., 2013). However, we consider the overall contribution of horse to be the most ecologically probable, based on their abundance during the Pleistocene and previous work (Fox-Dobbs et al., 2008). We therefore conclude that, during the Pleistocene, Yukon gray wolves relied primarily upon horse to fulfil their dietary requirements.

6. Conclusion

During the Quaternary Megafaunal Extinction (~ 11.7 ka), North America saw widespread, landscape-scale ecological changes that ultimately contributed to the loss of many species, including horses (Guthrie, 2006) and mammoths (Fisher, 1996; Koch and Barnosky, 2006). We show that extinction of much of the mammal megafauna

during the Pleistocene to Holocene transition induced a dietary change among wolves. Recent Yukon wolves rely primarily on the extant large ungulates, caribou and moose (Gauthier and Theberge, 1986; Hayes et al., 2016; Merkle et al., 2017), while Pleistocene wolves primarily consumed horses. Wolves, however, did not change their carcass utilization behaviours, consuming primarily flesh and little bone. Though wolves exhibit some degree of ecological flexibility, they have remained large ungulate specialists. Future studies should include modern (early 2000s to present) wolf specimens from the Yukon to observe whether wolf diets have continued to change throughout time. It will be crucial to conserve populations of caribou and moose as well as the ecosystems with which they are associated, particularly as climate continues to alter the Canadian Arctic at rates far exceeding the global average (Glig et al., 2012; Rowland et al., 2016).

Credit author statement

Zoe Landry: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Visualization, Project administration.; **Sora Kim:** Validation, Investigation, Resources, Writing – Review & Editing.; **Robin B. Traylor:** Methodology, Validation, Investigation, Resources, Writing – Review & Editing.; **Marisa Gilbert:** Resources, Writing – Review & Editing.; **Grant Zazula:** Resources, Writing – Review & Editing, Funding acquisition.; **John Southon:** Methodology, Investigation, Resources, Writing – Review & Editing.; **Danielle Fraser:** Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

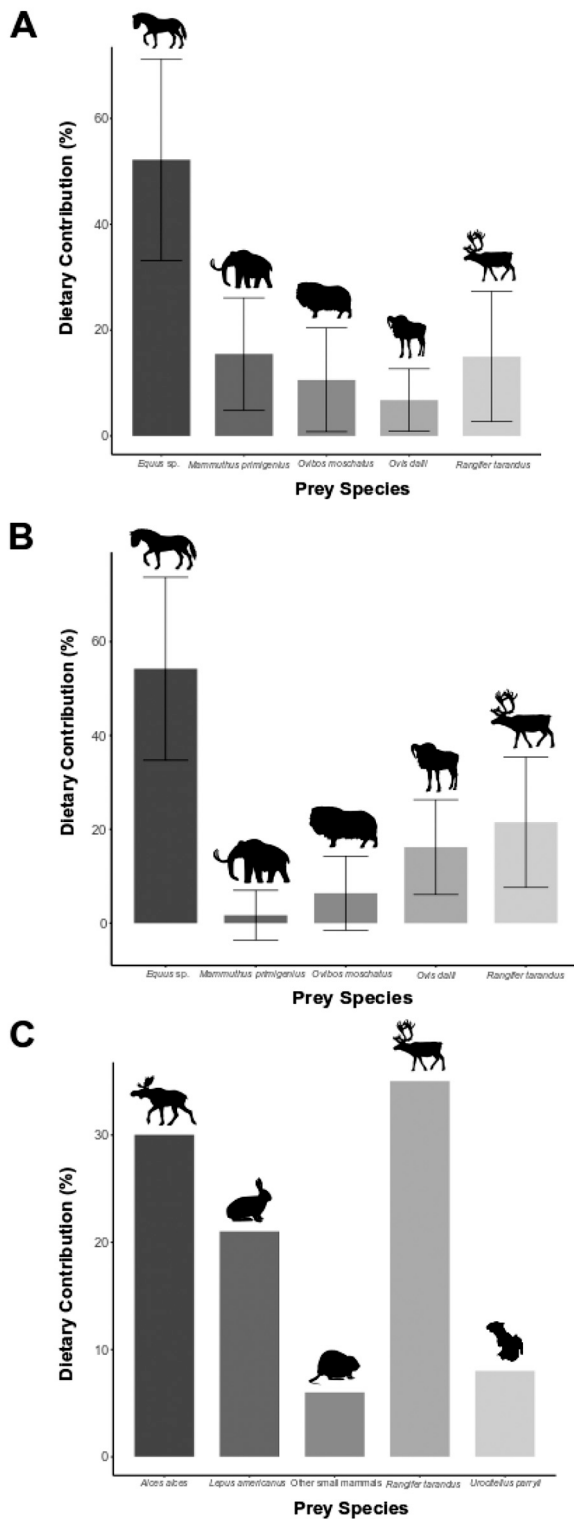


Fig. 6. Percent contributions of prey species sources to the diets of: A) Pleistocene-age Yukon gray wolves generated by the uninformed mixing model (\pm standard deviation); B) Pleistocene-age Yukon gray wolves generated by the informed mixing model (\pm standard deviation); C) recent Yukon gray wolves, from Gauthier and Theberge (1986).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.palaeo.2021.110368>.

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