May 2013

Origins of stable isotopic variations in Late Pleistocene horse enamel and bone from Alberta

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

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ORIGINS OF STABLE ISOTOPIC VARIATIONS IN LATE PLEISTOCENE HORSE ENAMEL AND BONE FROM ALBERTA

(Thesis format: Monograph)

by

Nicolle Sara Bellissimo

Graduate Program in Geology

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

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Abstract

Oxygen and carbon isotopic measurements of coevally formed bone and tooth enamel bioapatite from a modern equid show that these tissues record drinking water and diet isotopic signals in an identical fashion. Hence, data for both tissues can be combined to track movement, dietary changes, and seasonal variability over the animal’s lifetime, and climatic variability over longer time periods. This tool was tested for horses using ten paired tooth and bone samples to reconstruct conditions in Alberta during the Late Pleistocene. While post-mortem isotopic alteration confounded interpretation of the results, two key findings emerged: (i) pre- and post-Last Glacial Maximum (LGM) time periods based on radiocarbon dates correspond with high and low δ¹⁵N collagen values, respectively; and (ii) pre- and post-LGM horses have similar drinking water δ¹⁸O and diet δ¹³C values, suggesting that environmental conditions, including seasonality, were similar across the time periods represented by these samples.

Keywords: stable isotopes, phosphate, carbonate, Pleistocene, Alberta, palaeoenvironment, horses, enamel, bone, tissue spacing, incremental enamel sampling
Acknowledgments

I would like to thank my supervisor, Fred Longstaffe, for allowing me to choose a research project that I was interested in and for providing financial, professional, and personal support to help me complete this project. Thank you for dedicating your time to guide, help, and advise me during my Masters. Thank you Emily Webb for being my peer mentor and taking time out of your schedule to teach me the foundations needed to build my masters. Thank you for your help in Edmonton, and for going with me to get my modern horse sample (which I wouldn't have gotten if you weren't there, you know why).

This research was supported through funding provided by: the University of Western Ontario, Ontario Graduate Scholarship in Science and Technology (OGSST), Queen Elizabeth II Scholarship, Graduate Thesis Research Fund (GTRF), and by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant and Canada Foundation for Innovation (CFI) and Ontario Research Fund (ORF) infrastructure grants to F.J. Longstaffe, and in part, through release time provided to F.J. Longstaffe by the Canada Research Chairs Program.

To Kim Law, Li Huang, Miss Grace Yau, Lisa Munro, Zoe Morris, Rachel Schwartz-Narbonne, Alicia Donis, and Andrea Prentice for helping me complete my lab work in the Laboratory for Stable Isotope Science. I would not have been able to finish without your knowledge and help. You, as well as the other LSIS members (i.e. the other graduate students) made my time in the lab fun and very enjoyable and I will cherish those memories forever.

To Chris Jass, Peter Milot, and the Royal Alberta Museum for providing access to Pleistocene horse samples. To Ed Eastaugh for providing access to and help in the Zooarchaeology lab to deflesh the modern horse.

I would like to thank Keith, my family, and Toby for their love, support, and encouragement throughout my Masters. Thank you to my friends in the department, for being supportive and providing me with sanity via the many social, sport, and grad club activities (i.e. beer drinking), you made the department feel like an extended family.
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Chapter 1

Introduction

1.1 General background and objectives

The oxygen isotopic composition of vertebrate bone or enamel bioapatite can been used to reconstruct climatic conditions recorded during bioapatite formation (e.g. Longinelli, 1984; Sharp & Cerling, 1998; Fricke et al., 1998, etc.). Similarly, the carbon isotopic composition of bioapatite has been used to reconstruct dietary components consumed during an animal’s life (e.g. Sullivan & Krueger, 1981; Lee-Thorp & van der Merwe, 1987; Wang et al., 1994, etc.). The bioapatite phosphate and structural carbonate oxygen and carbon isotope fractionation between enamel and bone in modern and Pleistocene horses is investigated in this thesis. An understanding of these fractionations is important for accurate reconstruction of climate and migratory histories, and dietary preferences. This is especially true in light of the proposition of Warinner and Tuross (2009), that bone and enamel in pigs may not be isotopically equivalent tissues, possibly the result of different biological factors occurring during bioapatite formation (see section 1.2.2). If this applies to different taxa, it will have many influences on palaeoecological and palaeoclimatological reconstructions, such as: what tissues (enamel or bone) are sampled for analysis, the relevance of tissue comparisons, and whether or not a fractionation factor has to be applied to bone or enamel, etc. Therefore, it is necessary to determine what isotopic signals are incorporated during enamel and bone formation for reliable environmental reconstructions. To investigate this question, the oxygen isotopic composition of phosphate and the oxygen and carbon isotopic compositions of structural carbonate have been analyzed from modern (Equus caballus) and Pleistocene horse bone and enamel samples.

In addition, the organic component of bone, mainly collagen, from modern and Pleistocene horses has been analyzed for its carbon and nitrogen isotopic compositions. These data have been utilized to examine environmental conditions during the Late Pleistocene (126–12 ka BP) in central Alberta (Head et al., 2008). This is an area for which current understanding of Pleistocene ecosystems is quite limited because there is
minimal isotopic research, particularly on large herbivores, for that region. It is important to fill this gap of knowledge for a key area affected by continental ecosystem and climatic changes at the end of the Pleistocene. Choosing a large herbivore for this study also simplifies interpretation of the carbon and nitrogen isotopic results for bone collagen, as these compositions are a direct measure (± metabolic effects) of the flora consumed by these animals.

The Pleistocene Epoch (2.588 Ma–12 ka BP) was characterized by extensive ice sheet cover over most of the Northern Hemisphere (Head et al., 2008; Miller et al., 2010). For the past 700 ka BP, glacial cycles in the Northern Hemisphere have lasted ~100 ka and were separated by short warm interglacial periods (Miller et al., 2010). During glacial periods, ocean levels decreased enough to periodically expose the land between the Bering Strait, creating the landmass known as Beringia. Beringia had an area of ~34 million km$^2$, which included eastern Russia, northwestern North America and the ‘Bering land bridge’ (Harrington, 2005). Beringia was a significant part of the mammoth steppe, a very large continental biome that extended from western Europe to North America (Guthrie, 1968; Szpak et al., 2010). Beringia was ice-free during glacial intervals, thus permitting the exchange of flora and fauna among various areas of Beringia (i.e. between Alaska and Siberia) via the Bering Strait (Miller et al., 2010; Shapiro et al., 2004 and references therein). The array of flora (e.g. sage and grass-like plants, etc.) and megafauna (animals >44kg, e.g. woolly mammoth, bison, horse, etc.) that lived in Beringia and the mammoth steppe during the Late Pleistocene created an ecosystem for which there is no modern analogue (Zazula et al., 2003; Harrington, 2005; Koch & Barnosky, 2006).

Throughout the Late Pleistocene, especially during periods of reduced ice cover, the plants and megafauna that occupied Beringia were able to extend their ranges into much of North America, including Alberta and the continental United States (Shapiro et al., 2004 and references therein; Fox-Dobbs et al., 2008; Burns, 2010 and references therein). After the Last Glacial Maximum (LGM) 22–18 $^{14}$C ka BP (26.2–21.4 ka BP) the animals returned to these areas, including Alberta (e.g. Burns, 1996). There is some debate as to when the ice sheets retreated in Alberta after the LGM and whether or not an 'ice-free' passage existed (e.g. Burns, 1996; Munyikwa et al., 2011, etc.). For example,
Dyke and Prest (1987) and Dyke et al. (2003) (based on radiocarbon chronologies) portray Alberta as ice-free between 12–11 $^{14}$C ka BP (13.8–12.8 ka BP, converted from INTCAL09), which was thought to be too late to account for humans already being south of the ice sheets in North America (Munyikwa et al., 2011 and references therein). But recent estimates using optically stimulated luminescence dating suggest it was ice-free a thousand years earlier, which would be early enough for humans to have used Alberta as an 'ice-free' passage from Beringia to south of the ice sheets (Munyikwa et al., 2011 and references therein). Regardless, Alberta played an important role in megafauna migrations and was central to large-scale Late Pleistocene glacial events. [Dates reported in this thesis are given as uncalibrated radiocarbon years before AD 1950, for example 10 $^{14}$C ka BP, where ka is thousands of years. Conversion to calibrated radiocarbon years (ka BP) was performed using INTCAL09 (Reimer et al., 2009), and is shown in brackets beside the radiocarbon date. The only exceptions made are for research papers that report only calibrated radiocarbon years.]

Horses were chosen for this study because they were a common herbivore throughout Beringia, the mammoth steppe, and Alberta and are abundantly represented in the fossil record (Burns, 1996; Guthrie, 2001; Hoppe et al., 2004a). The isotopic analysis undertaken on these samples is important for several reasons. First, understanding more about the phosphate oxygen and carbonate oxygen isotope fractionation between horse bone and teeth is necessary because these patterns have not been clearly defined for horses and large-bodied herbivores. This information will add to the overall physiological knowledge of large-bodied mammals. Second, this research will improve our understanding of the terrestrial record of environmental change in central Alberta during the Pleistocene for a significant group of animals. This is especially important considering Alberta's possible role as an 'ice-free' passage, facilitating human migration from Beringia to south of the ice sheets. In addition, a better understanding of the environment in which extinct wild horses lived will help characterize the changing nature of their ecological niche within North America near the Pleistocene-Holocene transition when 70% of megafaunal genera became extinct, including Pleistocene horses (Guthrie, 2003; Koch & Barnosky, 2006; Gill et al., 2009). Radiocarbon dates for 220 Alaska and Yukon Territory horses suggest they went extinct ~12.5 $^{14}$C ka BP (~14.9–14.8 ka BP),
with a rapid decline in body size occurring beforehand (Guthrie, 2003 and references therein). The causes of the extinction are still debated but commonly attributed to either human impact or climate change, or both (Guthrie, 2003; Shapiro et al., 2004; Koch & Barnosky, 2006).

1.2 Background - Stable isotopes

Stable isotopes are atoms that contain the same number of protons and electrons, but a different number of neutrons, resulting in different atomic masses. This difference in mass (e.g. $^{12}$C vs. $^{13}$C) causes isotopes of the same element to have slightly different thermodynamic and kinetic properties (Urey, 1947; Koch, 1998; Hoefs, 2009). These differences can cause separation of isotopes during physical and chemical processes, making stable isotopes useful tracers. For example, light isotopes (those with fewer neutrons) of a given element take part in chemical reactions at a faster rate than heavier isotopes (Ambrose, 1993; Koch, 1998; Hoefs, 2009). Fractionations of stable isotopes are relatively small and are therefore reported using delta ($\delta$) notation expressed in per mil (‰):

$$\delta(‰) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000,$$  

where $R_{\text{sample}}$ and $R_{\text{standard}}$ are the high-mass to low-mass isotope ratios of the same element (e.g. $^{18}$O/$^{16}$O, $^{13}$C/$^{12}$C, $^{15}$N/$^{14}$N) in the sample and standard, respectively (Hoefs, 2009).

1.2.1 Carbon and nitrogen

The stable carbon and nitrogen isotopic compositions of food consumed by an animal are transferred to the animal's tissues during the incorporation and digestion of food (Koch, 1994; Stevens, 2009). As such, the isotopic composition of bones and teeth can act as a tracer of different dietary inputs (i.e. different plants in the case of herbivores) with specific isotopic compositions (Koch et al., 1994). The carbon isotope composition of animal tissues (e.g. bone and teeth) is mostly related to $C_3$ versus $C_4$ plant
intake (Kohn & Cerling, 2002). Ingestion of C₃ (trees, most shrubs and herbs, and grasses adapted to cool or wet climates) or C₄ (grasses adapted to warm or arid habitats) plants can be identified because their different photosynthetic pathways produce distinct isotopic compositions (average δ¹³C: C₃ −26.5 ‰; C₄ −12.5 ‰, respectively) (Smith, 1972; O'Leary, 1988; Ambrose, 1993; Koch et al., 1994). The δ¹³C values of plants can also be influenced by canopy cover, light intensity, moisture level, atmospheric CO₂, temperature changes, and salinity (Kohn & Cerling, 2002; Clementz et al., 2009; Heaton, 1999; Stevens et al., 2009).

The carbon isotopic composition of the diet is transferred to animal tissues with some associated fractionations. The carbon isotope composition of the structural carbonate that substitutes for PO₄³⁻ and OH⁻ sites in bioapatite (part of bone and teeth; see below) reflects bulk carbon from the whole diet (i.e. carbohydrates, lipids, and proteins) in most situations (Ambrose & Norr, 1993; Tieszen & Fagre, 1993; Kohn & Cerling, 2002; Clementz et al., 2009). Cerling and Harris (1999) determined that structural carbonate is enriched in ¹³C by +14.1 ± 0.5 ‰ compared to the diet of large ungulate mammals.

The carbon and nitrogen isotopic compositions of bone collagen mainly reflect dietary protein (Ambrose & Norr, 1993; Stevens & Hedges, 2004; Jim et al., 2004). Because collagen takes a long time to turnover (>10 years), the carbon and nitrogen isotope compositions of bone collagen will represent the average composition of dietary protein consumed over a significant period of time (Ambrose, 1993; Stevens & Hedges, 2004; Ayliffe et al., 2004; Hedges et al., 2007). For large herbivores, there is a carbon isotope fractionation between collagen and diet of about +5.5 ‰ (Vogel et al., 1978; Sullivan & Krueger, 1981; van der Merwe, 1989; Ambrose & Norr, 1993). As to whether there are systematic differences in collagen δ¹³C values among different bones in an animal, Warinner and Tuross (2009) reported no difference for a humerus vs. a mandible bone (both formed by different ossification processes) from a pig. This suggests that collagen from different bones within such larger animals record very similar δ¹³C values.

The δ¹⁵N values of collagen are mainly determined by the nitrogen isotopic composition of the diet and there is little difference (typically ≤1‰) between the δ¹⁵N values of various tissues within a body (e.g. nail vs. hair vs. liver vs. muscle; DeNiro &
Epstein, 1981; Ambrose, 1993; Nardoto et al., 2006; Crowley et al., 2010). With an increase in δ\(^{15}\)N values of ~3 ‰ each step up the food chain under most circumstances, nitrogen isotopic compositions can be used to determine the trophic level status of an animal (DeNiro & Epstein, 1981; Schoeninger & DeNiro, 1984; Koch et al., 1994; Bocherens & Drucker, 2003; Sponheimer et al., 2003a). However, horses are herbivores and therefore any variation in the nitrogen isotopic compositions of their tissues would not normally reflect a change in trophic level (Stevens & Hedges, 2004).

The δ\(^{15}\)N and δ\(^{13}\)C values of soils, plants, and herbivores can be influenced by stress, climate, and physiological factors (Ambrose, 1993; Sponheimer et al., 2003a; Fuller et al., 2005). For example, canopy cover, light availability, moisture level, atmospheric CO\(_2\), temperature, and salinity changes can affect the δ\(^{13}\)C values of plants because these factors influence the amount of isotopic fractionation that occurs during photosynthesis (see Heaton, 1999; Stevens et al., 2009; Kohn & Cerling, 2002; Clementz et al., 2009). Environmental factors also play a role. The canopy effect is one such factor; it causes \(^{13}\)C depletion in plants growing under a closed forest canopy compared to plants growing in more open environments. This effect is influenced by the incorporation of \(^{13}\)C-depleted CO\(_2\) from respiration and/or soil decomposition (e.g. van Der Merwe & Medina, 1989; see review in Heaton, 1999). Likewise, plant δ\(^{15}\)N values tend to decrease with decreasing average annual temperature and with increasing average annual precipitation (Amundson et al., 2003).

As mentioned earlier, different tissues (e.g. bone collagen vs. tooth dentin) have different turnover rates for both carbon and nitrogen. Thus their isotopic compositions can be used to identify certain 'trophic level effects', for example, those associated with weaning. Tooth dentin laid down during nursing would have an offset (up to about +3 ‰ relative to the mother's nitrogen isotope composition) compared to bone/tooth dentin remodelled/deposited after weaning (which has nitrogen isotopic compositions closer to adult values) (Fogel et al., 1989; Richards et al., 2002; Metcalfe et al., 2011).

1.2.2 Oxygen

The oxygen isotope compositions of biogenic materials, such as bones and teeth and the bioapatite that they contain, record a range of environmental parameters and
biological processes, and hence can be used to reveal palaeoclimate information (Kohn & Cerling, 2002). The oxygen isotope composition of bioapatite is determined by the temperature and composition of body water when it formed (Koch et al., 1994). Bioapatite is thought to precipitate in oxygen isotopic equilibrium with body water. Body water composition is determined by drinking water and water in the diet. The oxygen isotope composition of large herbivores, in particular, may be affected by the water in their food, especially if it has a high water content (Koch et al., 1994; Daux et al., 2008). The oxygen isotope composition of ingested water is affected by several interrelated factors including the \( \delta^{18}O \) value of: (i) precipitation, which is related to average annual temperature, humidity, and air mass source, and (ii) plant water, which varies with environmental stressors such as temperature, and humidity, as well as soil-water composition, rooting depth, photosynthetic pathway, and plant morphology (Dansgaard, 1964; Koch et al., 1994).

Palaeoclimate information (e.g. during the Pleistocene) can be obtained from vertebrate fossils by analyzing the oxygen isotope composition of bioapatite phosphate and/or structural carbonate. These isotopic signatures are related to the isotopic composition of ingested water, which is related to local rainfall \( \delta^{18}O \) values, which in turn are related to climate (Dansgaard, 1964; Longinelli, 1984; Luz et al., 1984; Bryant & Froelich, 1995; Zazzo et al., 2004a). Since mammalian body temperature is constant at ~37 °C, the oxygen isotope composition of bioapatite is not directly influenced by external temperature. Bioapatite mineralizes in equilibrium with body water and reflects only the composition of oxygen inputs. Because horses are large, water-dependent herbivores, their body-water \( \delta^{18}O \) values are mainly controlled by drinking water (Longinelli, 1984; Luz et al., 1984; Bryant & Froelich, 1995); there is usually an offset of about +18 ‰ between the \( \delta^{18}O \) of bioapatite PO\(_4\) and body water in mammals (Kohn & Cerling, 2002). \( \delta^{18}O \) values of precipitation in mid- to high-latitudes usually follow a sinusoidal pattern on a seasonal basis, with one cycle representing one year (Rozanski et al., 1993). Therefore, an animal such as a horse whose teeth grow for a period of many months to a few years, will display a sinusoidal curve in \( \delta^{18}O \) of enamel bioapatite if it is drinking water from a somewhat constant source with an input dominated by
precipitation (e.g. Sharp & Cerling, 1998; Balasse et al., 2003a; Zazzo et al., 2005; Feranec et al., 2009; Metcalfe et al., 2011).

In bioapatite, oxygen contained in both its structural carbonate (CO$_3$) and phosphate (PO$_4$) components has exchanged isotopes with body water, resulting in linearly correlated oxygen isotope compositions (Bryant et al., 1996a; Iacumin et al., 1996). The offset between CO$_3$ and PO$_4$ is slightly variable: Lecuyer et al. (2010) estimated 1000 Inα(CO$_3$-PO$_4$) values, based on O'Neil et al. (1969) and Kim and O'Neil (1997)'s equations, of +7 to +8 ‰ for temperatures ranging from 10–37 °C. This matches the calculations of Bryant et al. (1996a), who combined the fractionation equations of Shemesh et al. (1988) and Zheng (1996) to achieve the same result. The measured oxygen isotope fractionation between hydroxyapatite-bound carbonate and phosphate has a value of +7.5 ‰ at 37 °C (Lecuyer et al., 2010; Kolodny et al., 1983). Lecuyer et al. (2010) compiled oxygen isotope data for structural carbonate and phosphate from modern mammals to obtain a value of +8.1 ‰ for the bioapatite oxygen isotope CO$_3$-PO$_4$ fractionation. They suggest that this slightly higher value might result from non-equilibrium oxygen isotope fractionation or changes in pH of the extracellular fluid.

It has been proposed that differences can exist in the oxygen (and carbon) isotope compositions of structural carbonate between different tissues of the same animal, e.g. enamel bioapatite vs. bone bioapatite. Warinner and Tuross (2009) examined pig bone and enamel bioapatite formed at the same time and found them to have different isotopic compositions. They reported oxygen and carbon isotope fractionations between enamel bioapatite and bone bioapatite of +1.7 ‰ and 2.3 ‰, respectively. They suggest the offset could be because of "the differential crystallinity indices of the two mineral types or poorly understood local fractionation factors in different body water and micronutrient pools" but that more work is needed to identify the cause. It is presently unknown whether or not a similar offset exists for the oxygen isotope composition of enamel vs. bone bioapatite PO$_4$, particularly for larger mammals.
1.3 Background - Skeletal tissues

1.3.1 Bones and teeth

Bones and teeth in animals are composed of an inorganic component (mineral: bioapatite) and an organic component (protein: collagen). Bones are made of ~70 wt% mineral and ~20–30 wt% collagen and a small portion of other proteins and water. This mineral/collagen ratio varies among animals, among bones in an animal, and with age (Ambrose, 1993; Lee-Thorpe, 2008; Pasteris et al., 2008). Unlike bones, mammalian teeth have three types of mineralized tissue: enamel, dentin, and cementum. Dentin and cementum have a similar amount of mineral and collagen as bone, while enamel has a much higher mineral component of 96 wt% (Driessens & Verbeeck, 1990; Skinner, 2005; Hillson, 2005). The mineral component in both bones and teeth consists of carbonated hydroxylapatite (~Ca\textsubscript{9}[(PO\textsubscript{4})\textsubscript{4.5}(CO\textsubscript{3})\textsubscript{1.5}](OH)\textsubscript{1.5}), which has a range of compositional variability arising from the ease of elemental substitution (Driessens & Verbeeck, 1990; Hoppe et al., 2004a). An example of this range is provided by Skinner (2005): (Ca, Na, Mg, K, Sr, Pb...\textsubscript{10}(PO\textsubscript{4}, CO\textsubscript{3}, SO\textsubscript{4}...)\textsubscript{6}(OH, F, Cl, CO\textsubscript{3})\textsubscript{2}.

During bone formation, collagen is first laid down and plate-like bioapatite crystallites then nucleate on and grow within the collagen framework. Bone is remodelled throughout an animal’s life, and depending on the size and health of the animal, it can take many years; the original collagen-mineral network laid down dissolves allowing osteoblasts to deposit new bone material (Pasteris et al., 2008; Clementz et al., 2009).

The cells that form the components of teeth are very different from those that form bones. The most common tooth-forming cells are odontoblasts, which produce dentin, and ameloblasts, which produce enamel. Dentin is formed in a similar way to bone, but it does not undergo remodelling (exceptions apply to secondary and tertiary dentin, see section 1.3.2) (Pasteris et al., 2008). Mammalian enamel grows from the occlusal surface (at the enamel-dentin junction, EDJ) to the root (moving outwards towards the outer enamel surface as it progresses downwards) (Hillson, 2005). Enamel formation takes place in a two-stage process. The matrix production stage is first: ameloblast cells secrete a lightly mineralized organic matrix; at this point it is only one third mineral. A successive series of incremental lines form during matrix production,
providing a record of the direction and rate of tooth crown formation. These lines, which under an optical microscope appear as regularly spaced light and dark lines, referred to as "Retzius' striae", represent material that started mineralizing at the same time; however, they may have finished mineralizing at different times during enamel maturation (Fig. 1.1) (Hillson, 2005; Hoppe et al., 2004a). The second stage is maturation: the ameloblasts then remove the proteins and water as crystallites increase in size, producing heavily mineralized enamel (Hillson, 2005; Pasteris et al., 2008). Zazzo et al. (2005) showed two aspects of the mineralization front in bovid teeth: "a vertical component from the tip of the tooth crown to the neck, and a horizontal component from the enamel-dentine junction to the outer enamel". Enamel bioapatite crystallites are larger and have higher crystallinity than bone bioapatite crystals (LeGeros, 1991; Lee-Thorp, 2008).

Figure 1.1 An illustration of Retzius stria (identified by white arrows) in horse enamel. Note: this is a magnified view of a thin section under crossed polarized light. C=cementum; d=dentin (from Hoppe et al., 2004a).
1.3.2 Horse dentition

As is the case for all mammalian teeth, horse teeth contain three calcified dental tissues: enamel, cementum, and dentin. Horse teeth contain two types of enamel: equine type-1 and type-2 (Dixon, 2002 and references therein). Equine type-1 enamel consists of parallel layers of bioapatite prisms and flat, interprismatic, bioapatite plates, which makes this enamel hard and brittle. Cheek teeth, particularly the upper cheek teeth are composed of this type of enamel. Equine type-2 enamel is composed of groups of enamel prisms that are intricately interwoven in three dimensions, and is the main component of incisor teeth. The whole tooth is covered by cementum before tooth wear, which involves surrounding the deep infoldings in the lower cheek teeth (Dixon, 2002; Hillson, 2005).

There are three types of horse dentin tissues: primary, secondary, and tertiary. Primary dentin is laid down during tooth eruption. Secondary dentin is laid down over the life of the tooth by the odontoblasts that line the outer pulp (the soft tissue in dental pulp cavities containing connective tissues, blood, lymphatic vessels, and nerves). Secondary dentin slowly replaces the pulp over the life of the tooth. Tertiary dentin is laid down where insults (i.e. infection or trauma) have occurred (Dixon, 2002 and references therein).

1.3.3 Sequence of tooth eruption and mineralization in horses

The Triadan system of dental nomenclature, used in veterinary medicine, is used here for describing equine teeth (Fig. 1.2). A horse's dentition is classified as diphyodont because they have two sets of teeth: temporary/deciduous teeth and permanent (Dixon, 2002). The deciduous cheek teeth (premolars 06s–08s) are replaced by three permanent teeth in the adult. The first premolar (05s) and three molars (09s–11s) have no deciduous teeth. In modern equids, there are 24 cheek teeth in the maxillary (upper jaw is composed of the maxillae and premaxillae) and mandibular (lower jaw) bones (Dixon, 2002; Hillson, 2005). They are rectangular in shape (except the 06s and 07s, which are triangular). The upper cheek teeth are wider than the lower cheek teeth, which have a taller erupted crown. The first cheek teeth (06s) are the shortest, with the remaining cheek teeth reaching 7–8 cm in height before wear. All permanent cheek teeth are in wear by the time the horse reaches 5 years of age. These teeth wear at a rate of ~2–3 mm/yr; therefore a 7.5 cm tall tooth will be fully worn at about 30 years of age (Dixon, 2002).
domestic horses, the 09s are the first permanent teeth to erupt at ~8–12 months of age. The eruption of the 10s follows at ~20–26 months. Permanent premolars, 06s and 07s, replace their deciduous precursors at 2.5 and 3 years of age, respectively. The last teeth to erupt are the 11s at 3.5 years and the 08s at 4 years (Hoppe et al., 2004a and references therein).

Figure 1.2 The Triadan system of dental nomenclature, showing adult dentition. The quadrants are numbered from 1 to 4 in a clockwise direction, beginning at the 'right' (as labelled in figure) maxillary quadrant (from Dixon, 2002).

Teeth grow while being completely encased in the jaw. Understanding the sequence and timing of tooth mineralization in horses is very important when considering which tooth and what part of that tooth to sample for isotopic investigation. Hoppe et al. (2004a) showed that the relative order of mandibular tooth mineralization in domestic horses is in close agreement with the sequence of tooth eruption; mandibular teeth can either form at the same time as maxillary teeth or several months before. The first adult teeth to mineralize are the 09s, which start at 2 weeks of age. The 10s are the second teeth to mineralize, followed by the 06s, 07s, and 08s. The last to mineralize are the 11s. It is important to consider that horses typically nurse for 9 months; therefore the isotopic compositions of tooth enamel that mineralized during this time (i.e. parts of 09s and 10s) could be offset relative to adult compositions (Hoppe et al., 2004a and references therein).
All teeth continue to mineralize after eruption. The duration of mineralization varies among the different teeth and is independent of the order in which they erupt and begin mineralization. The 06s have the shortest mineralization time of 18 months. The 09s and 07s finish mineralizing at 2 years of age, the 10s finish by 2.5 years of age. The 08s and 11s are the last to appear and finish mineralizing at 2.6 and 2.8 years, respectively (Hoppe et al., 2004a).

The following is a list of important considerations for interpreting isotopic compositions of horse enamel: (1) Enamel is lost as teeth wear; therefore the worn teeth of older individuals preserve isotopic signatures only from the last stages of tooth mineralization (Hoppe et al., 2004a). (2) Our understanding of tooth eruption and mineralization sequences are based on the modern horse; therefore only inferences can be made for extinct species. The similarities in tooth pattern and eruption observed amongst different species of extant equids implies they have similar patterns of mineralization and that this could also be true for extinct species (Bryant et al., 1996c; Hoppe et al., 2004a). The rate of tooth growth varies amongst modern equid species, again suggesting that such variation could have existed for extinct species (Hoppe et al., 2004a). (3) Mandibular tooth eruption/mineralization patterns are assumed to be similar to maxillary patterns.

1.4 Background - Horses

The modern domesticated horse (*Equus caballus*) is quite different from the first members of its family (Equidae), who lived 58 million years ago. In terms of diet, the evolution of horses involved a change from a predominantly C\textsubscript{3}-based diet (e.g. trees and shrubs) to a diet consisting mainly of C\textsubscript{4} and C\textsubscript{3} grasses for several taxa during the Late Miocene to Early Pliocene (Simpson, 1951; MacFadden, 1988; Wang et al., 1994; MacFadden et al., 1999 and references therein). That said – Pleistocene and modern C\textsubscript{3} grasses in North America dominated/dominate cooler environments at higher elevations or above 45°N latitude. Ancestor horses were much smaller (25–50 kg) and had brachyodont (low-crowned) teeth (Bryant et al., 1996b). They lived in wooded biomes and fed on woody plants (browsers). With evolution, modern horses became much larger.
(75–400 kg), developed hypsodont (high-crowned) teeth and fed on grasses (grazers) (MacFadden, 1986; Bryant et al., 1996b).

1.4.1 Pleistocene horses

Horses are abundantly represented in the fossil record over the course of their 58 Ma history. Fossil horses, as well as modern horses, are widespread geographically (Bryant et al., 1994). As such, they serve as ideal proxies for palaeodiet and palaeoclimate isotopic reconstructions, because the different physiological fractionation factors associated with interspecies comparisons can be eliminated. Their abundance also allows for multi-continental examination of a climatic event (e.g. glacial-interglacial cycles). In addition, access to modern equids provides the opportunity to perform controlled studies on close analogues.

MacFadden et al. (1999) for example, used the carbon isotope compositions of horse tooth enamel to learn the Pleistocene distribution of C_3 vs. C_4 grasses over the range of latitudes spanning Alaska to Argentina. They reported a δ^{13}C gradient of 10 ‰, reflecting the changing proportion of C_3 to C_4 grasses between high and equatorial latitudes.

Stevens and Hedges (2004) analyzed the carbon and nitrogen isotope compositions of bone and tooth dentin collagen of horses that occupied northwest Europe between 40^{14}C ka BP (~44.1 ka BP) and now. While glacial conditions existed 40^{14}C ka BP (~44.1 ka BP) years ago in northwest Europe (British Isles, Belgium, and Germany), steppe and tundra environments were present in areas not occupied by glacial ice (Stevens & Hedges, 2004 and references therein). Stevens and Hedges (2004) observed a decrease in the δ^{13}C values of horse collagen towards the present time, which they attributed mainly to an increase in atmospheric CO_2 concentrations over the same period. This is supported by the observed relationship between decreasing plant δ^{13}C values and increasing CO_2 concentrations (e.g. Polley et al., 1993). Stevens and Hedges (2004) also observed a post-glacial lowering in horse δ^{15}N compared to pre-glacial values, followed by an increase around the onset of the Holocene. They suggested that the lower δ^{15}N values in the British Isles and Germany relative to Italy (and France to a lesser extent) could be the result of changing temperatures and the resulting interrelated changes in
other factors (e.g. water availability, soil nitrogen cycling, etc.). They also suggested that
there could be a delay of possibly a few thousand years in the response of plant (and
therefore bone collagen) $\delta^{15}$N values to retreating glacial conditions (e.g. post-glacial soil
development, increased water availability because of increased permafrost melting rates,
etc.).

Feranec et al. (2009) analyzed the carbon and oxygen isotope compositions of
bioapatite from Late Pleistocene horse and bison tooth enamel from Rancho La Brea in
southern California to learn how herbivores can partition and compete for resources
within an ecosystem. They predicted, based on data from modern horse and bison, that
the isotopic compositions for fossil horse and bison would be characteristic of grazers
with some browse in their diets. Their oxygen isotope data revealed a sinusoidal seasonal
growth pattern (see section 1.2.2) for enamel, while their carbon isotope data suggested a
weaker seasonal pattern of dietary preferences. Both herbivores ate mainly C$_3$ plants, but
the bison ate more C$_4$ plants than the horses. Relative to the horses, the bison exhibited
more variation in their carbon isotope compositions, suggesting that they migrated away
from Rancho La Brea for part of the year. In fact, the bison ate more C$_4$ plants in the
winter and only ate from the C$_3$ dominated ecosystem of Rancho La Brea in the late
spring.

Stevens et al. (2009) analyzed the carbon and nitrogen isotope compositions of
horse bone collagen to reconstruct the past environment at two Belgian cave sites, Trou
de Chaleux and Goyet, located about 30 km apart. Very limited radiocarbon dating (e.g.
Hedges et al., 1993; Germonpré, 1997; see Table 1 in Stevens et al., 2009, for complete
list) had suggested that these sites were occupied at about the same time during the Late-
Glacial interstadial (~14.6 ka BP) (Svensson et al., 2006; Weaver et al., 2003). Stevens et
al. (2009) predicted that if the sites were occupied at the same time then the nitrogen and
carbon isotope compositions of the horse collagen from those two sites should be similar.
However, the carbon and nitrogen isotopic results presented by Stevens et al. (2009) were
different, suggesting that the two populations had different diets acquired in different
environments. Additional radiocarbon dates verified that horse bones from the Trou de
Chaleux cave represented the Late-Glacial interstadial, whereas those from Goyet Cave
were 32–27 $^{14}$C ka BP (~36.4–31.4 ka BP) in age, mixed together with Late-glacial (between the Last-Glacial interstadial and the Holocene) horse remains.

Numerous other stable isotope studies have also been performed on Pleistocene horse fossils (e.g. Wang, 1994; Chillón et al., 1994; Higgins & MacFadden, 2004). There are also as many studies on 'non-Pleistocene' horse fossils (e.g. Hoppe et al., 2004a; Hoppe et al., 2004b; Bryant et al., 1996c; Huertas et al., 1995; Bryant et al., 1994; Chillón et al., 1994); these investigate a suite of topics ranging from the relationship between the isotopic composition of modern vs. Miocene horses and their environments to the timing of enamel mineralization in modern equid cheek teeth.

1.5 Background - Pleistocene Alberta

Alberta, Canada, was one of the areas where horses roamed in North America during the Pleistocene. Alberta is located along the eastern slopes of the Cordillera mountain range, adjacent to the northern Great Plains. Alberta was central to large-scale Late Pleistocene glacial events that affected many North American megafauna (Burns, 2010, Fig. 1.3). Burns (1996) conducted a study on the megafauna population dynamics during the Late Pleistocene. They recovered thousands of Pleistocene megafauna bone and tooth specimens, including twenty mammal species, mainly grazers, from a number of gravel sites in the Edmonton, Alberta, region. Radiocarbon dates from bone collagen samples indicated that many of the animals (e.g. horse, mammoth, etc.) were of Mid-Wisconsinan age (non-glacial, ~30–25 $^{14}$C ka BP (~34.7–29.6 ka BP)), just before the LGM. This suggests that this was a productive time period in Alberta, during which it contained vast grasslands (Burns, 1996). During this time horses had the highest abundance among the three most common megafauna in the region (bison, horse, mammoths) (Jass et al., 2011).

These megafauna were also present in the region after the LGM, indicating that 'mammoth steppe' like communities, which had existed in Alberta prior to the LGM, had returned (Burns, 1996). Therefore, the megafauna occupied Alberta throughout the Wisconsinan, but during the LGM when Alberta was glaciated, they were present only in unglaciated areas like eastern Beringia and south of the ice sheets (Burns, 1996). After
the LGM, several megafauna species migrated into southern and central Alberta from the unglaciated United States as the Cordilleran and Laurentide ice sheets slowly separated (depending on when exactly they separated, Alberta may have also provided an 'ice-free' passage for humans to use to move from Beringia to south of the ice sheets, see section 1.1). These megafaunal species included bison, muskox, camel, horse, mammoth, and lion. At the same time, populations of bison, moose, and wapiti were moving south to the United States from Beringia (Shapiro et al., 2004; Burns, 2010). Bison became the most abundant megafauna in Alberta after the LGM. The 'ice-free' passage in western Alberta contained mainly herb and shrub vegetation, which supported grazing megafauna like the bison. But towards the Holocene, this vegetation was replaced by boreal forests that spread northward from the eastern plains and westward mountains. This likely impeded the northward migration of some megafauna by $10^{14}$C ka BP (~11.6–11.4 ka BP), for example *Bison* (MacDonald & McLeod, 1996; Burns, 2010).

![Figure 1.3 Location of Alberta, Canada under glacial ice during the LGM. The red circle identifies the Edmonton region, from where the Pleistocene horse samples were excavated (modified from Munyikwa et al., 2011).](image-url)
Despite its central location during past glacial episodes, and its importance as a possible migration route for animals via the 'ice-free' passage, there is a paucity of literature on the isotopic compositions of Alberta's Pleistocene-Holocene megafauna (with exception of Bocherens et al., 1994) and the changing environment during this time. Isotopic analysis of the Pleistocene horses in this study will help address some of these topics, including if and how ecosystem changes affected the horses, especially in terms of habitat quality and plant availability.
Chapter 2

Materials and Methods

2.1 Samples

Ten Late Pleistocene horse teeth and bone (from the mandible, below the incisors) samples were obtained from the Royal Alberta Museum (RAM). The samples had been excavated from gravel pits in the Edmonton region, near the North Saskatchewan River (Fig. 1.3, Table 2.1). In addition, a mandible from a modern domesticated horse was obtained from Atwood Pet Food Supplies LTD, a deadstock removal company in Atwood Ontario. The modern horse sample was used as a control for comparing teeth and bone isotopic compositions of the Pleistocene horse samples. Teeth that mineralized after weaning (see section 1.3.3) were selected for isotopic analysis and were labelled according to the Triadan system (Fig. 1.2): 308 (LP4), 408 (RP4), 306 (LP2), 406 (RP2).

Table 2.1 Sample information

<table>
<thead>
<tr>
<th>LSIS Label</th>
<th>RAM Label</th>
<th>Pit Location</th>
<th>Tooth</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAM 1 B, T</td>
<td>P97.11.2A</td>
<td>Riverview Pit</td>
<td>306 (LP2)</td>
</tr>
<tr>
<td>RAM 2 B, T</td>
<td>P96.3.13</td>
<td>Cloverbar Sand &amp; Gravel</td>
<td>306 (LP2)</td>
</tr>
<tr>
<td>RAM 3 B, T</td>
<td>P96.17.12A</td>
<td>Island Bluff</td>
<td>306 (LP2)</td>
</tr>
<tr>
<td>RAM 4 B, T</td>
<td>P02.10.4</td>
<td>Pit #48</td>
<td>108 (URP4)</td>
</tr>
<tr>
<td>RAM 5 B, T</td>
<td>P99.3.163</td>
<td>Pit #48</td>
<td>406 (RP2)</td>
</tr>
<tr>
<td>RAM 6 B, T</td>
<td>P05.10.103</td>
<td>Pit #48</td>
<td>406 (RP2)</td>
</tr>
<tr>
<td>RAM 7 B, T</td>
<td>P94.8.79</td>
<td>Riverview Pit</td>
<td>406 (RP2)</td>
</tr>
<tr>
<td>RAM 8 B, T</td>
<td>P94.1.380</td>
<td>Pit #48</td>
<td>306 (LP2)</td>
</tr>
<tr>
<td>RAM 9 B, T</td>
<td>P04.3.33B</td>
<td>Pit #48</td>
<td>406 (RP2)</td>
</tr>
<tr>
<td>RAM 10 B, T</td>
<td>P94.2.33</td>
<td>Pit #80</td>
<td>406 (RP2)</td>
</tr>
</tbody>
</table>

Samples: Laboratory for Stable Isotope Science (LSIS) labels; RAM labels are the Royal Alberta Museum catalogue numbers
Materials: B= bone, T= tooth
Tooth designations: L= left, R= right, U=upper (all other teeth are from the lower mandible)
2.2 Sampling techniques

The first sampling problem faced was to determine whether or not the teeth obtained from the modern horse were deciduous or adult. This question arose because, upon their removal from the mandible, it was evident that they had not fully mineralized (the enamel did not fully extend to the root, Fig. 2.1). Confirmation that the teeth were adult (and hence not influenced by weaning) is based on several observations. First, the permanent 06s and 08s erupt at 2.5 and 4 years, respectively. The 11s (M3s), which had erupted in the jaw, erupt at 3.5 years (with no deciduous precursor). Therefore, since the 11s were present in the jaw the permanent 06s and 08s should have erupted as they have coinciding eruption times. Second, radiographs of the mandible, made using a Faxitron 43855D X-ray, showed that there were not any teeth below the 06s and 08s, implying that they were permanent premolars and not deciduous (Fig.2.2). Third, the overlapping mineralization times of 06s, 08s, and 11s support the labelling of 06s and 08s as permanent teeth; the 06s and 08s mineralize from 13–31 months and 19–51 months respectively, and the 11s mineralize from 21–55 months.

Figure 2.1 Modern horse tooth 308 (LP4). Dashed line marks the outline of the enamel layer, illustrating that it does not extend all the way to the root. Sample lines are shown within the inner enamel (IE) layer.
Bulk (enamel pieces representing the length of the tooth, i.e. more than one growing season) samples were collected and ground using a mortar, pestle, and a Dremel tool. The next sampling problem faced was how best to obtain serial samples from the Pleistocene and modern horse teeth in order to examine seasonal variation. There have been several studies on the use of serial sampled enamel $\delta^{18}O$ values as a tool to track seasonal variations during enamel mineralization (e.g. Sharp & Cerling, 1998; Fricke et al., 1998; Balasse, 2003a; Frémondeau et al., 2012). Most sampling techniques for studies of seasonality using stable isotope compositions of enamel involve drilling a sequence of horizontal lines on the outer enamel surface perpendicular to the height of the tooth, starting from the crown to the root (Balasse, 2003b). An alternate sampling strategy that samples the innermost enamel (IE) layer was used in this thesis. This strategy is preferred because the innermost enamel layer is heavily mineralized during matrix secretion, therefore samples obtained represent a more specific time period of growth, whereas the other method collects samples across a longer period of growth. For this reason, sampling the IE layer limits isotopic signal damping of the compositions obtained by body water
and therefore ingested water (Suga, 1982; Balasse, 2003b; Zazzo et al., 2005). In
addition, Hoppe et al. (2004a), using radiographic analyses, observed that the
mineralization front in modern equine cheek teeth is more or less parallel to the Retizius
striae (which progress outward from the enamel-dentin junction, EDJ, at an angle of 5–
10° and runs almost parallel to the height of the tooth, Fig. 1.1). Therefore, inner enamel
(IE) samples with a lengthwise preference were taken along the height of the tooth.

Prior to analysis, the occlusal surface of all Pleistocene teeth was removed using a
Buehler saw and preserved for future dental morphology or microwear studies. The
Pleistocene and modern teeth were then cut along the lingual-buccal axis (Fig. 2.3 A),
perpendicular to the long axis of the mandible. The teeth were cut in this way to expose
the mesial and buccal aspects of the modern teeth as they contained the most enamel
mineralization. In order to maximize the amount of enamel for serial sampling in the
modern teeth and to be consistent, all teeth were cut so that the sampling surface was
orientated in the mesial direction (Fig. 2.3 B), on the buccal side.

Figure 2.3  A) Location of 06 and 08 mandibular cheek teeth. Teeth were cut along
buccal-lingual axis (dental orientations are shown) before isotopic analysis. B) Position
of cut tooth on drill stage; mesial side of tooth is orientated towards the stage for serially
sampling. Enamel layer is marked in red (modified from Hillson, 2005 and Metcalfe,
2012).
The mesial half of the tooth was mounted on a microdrill plate, IE orientated upwards. Lines were drilled on the IE surface using a Merchantek MicroMill. The inner two-thirds of the enamel was sampled (except where sample limitations required sampling most of the enamel layer) along lines with the following general dimensions: 400–600 µm wide (lingual-buccal direction), ~2500 µm long (occlusal-basal direction), and 720–900 µm deep (mesial-distal direction), with sample-specific adjustments made depending on enamel variations in teeth (Fig. 2.4 A). The drill lines were separated from each other using a spacing needed to collect material representing a few months of mineralization. Based on observations made by Hoappe et al. (2004a), 06-teeth mineralize for ~18 months, and 11-teeth mineralize for ~32 months. Hence, a monthly growth rate could be estimated by dividing the length of the tooth (after occlusal surface was removed) by the mineralization rate of the tooth. Accordingly, drill lines were separated from each other by 1090 to 3000 µm, depending on growth rate (Fig. 2.4 B).

Drilled enamel powder was transferred to glass vials by dabbing a fine-tipped paintbrush into the sample; the paintbrush was cleaned between samples with pressurized air, distilled water, and acetone. Micromilled samples were not pretreated in any fashion prior to isotopic analysis; this allowed analysis of smaller sample sizes (and therefore less averaging of the growth period represented by the sample). Enamel is much more resistant to isotopic alteration than bone (see below), making pretreatment less important for enamel than bone. There was also less difference between pretreated and untreated isotopic compositions for enamel samples compared to bone (see section 3.4.1).
2.3 Diagenesis

It is important to verify that bone and teeth samples of palaeontological material have retained their original isotopic compositions, both for bioapatite and collagen components. There are several post-mortem mechanisms that can alter the isotopic composition of bioapatite. These processes include chemical weathering, precipitation of secondary minerals, adsorption of ions at the surface of bioapatite crystals, and isotopic exchange during dissolution and re-precipitation (Zazzo et al., 2004a). This alteration (referred to as diagenesis) of bioapatite can reduce its usefulness as a tool for
reconstructing palaeodiet and palaeoenvironmental conditions from its isotopic compositions (Munro et al., 2007). Zazzo et al. (2004a), for example, showed that bone and enamel crystallites are susceptible to varying degrees of carbon and/or oxygen isotopic exchange during early diagenesis, depending on the presence or absence of microbial activity to facilitate the process. They also showed that enamel was more resistant to isotopic alteration than bone or dentin. This difference occurs because bones and dentin consist of smaller crystallites (with more surface area exposed for reaction), are more porous/permeable, and have a higher organic content than enamel.

Consequently, bone and dentin can be much more susceptible to recrystallization and isotopic alteration than enamel (Kohn & Cerling, 2002; Zazzo et al., 2004a). The degree of isotopic alteration affecting different chemical constituents, specifically the structural carbonate (CO$_3^{2-}$) and phosphate (PO$_4^{3-}$) groups within bioapatite crystals, can also vary. In the absence of microbial effects, PO$_4^{3-}$ oxygen is less affected by isotopic exchange than CO$_3^{2-}$ oxygen because of the greater strength of the P-O bonds relative to C-O bonds (Kohn & Cerling, 2002; Zazzo et al., 2004a).

There are several methods that can be used to estimate the degree of alteration affecting bioapatite. One of these is the Crystallinity Index (CI), which provides a measure of the degree of order in the bioapatite structure (Surovell & Stiner, 2001). Because bioapatite crystals are small in bone and dentin, any fossil samples of these materials containing large crystals and a more ordered structure have almost certainly undergone post-mortem recrystallization. The CI values can be used as a semi-quantitative measure of such recrystallization (Shemesh, 1990; Wright & Schwarcz, 1996; Munro et al., 2007 and references therein). Fourier Transform infra-red (FTIR) spectroscopy is typically used to measure CI.

To obtain FTIR analyses in this study, 2 mg of bone or enamel powder (sieved to a particle size of 45–63 µm) were mixed with 200 mg of KBr, and then placed under 10 tons of pressure for 10 minutes. The resulting pellet was then scanned using a Bruker Vector 22 FTIR spectrometer and the resulting spectrum interrogated using the spectral analysis software provided with the instrument. In this method, crystallinity is determined from the degree of PO$_4^{3-}$ band splitting and calculated as follows:
FTIR CI= \frac{(A_{605}+A_{565})}{A_{595}}, \quad [\text{Equation 2.1}]

where $A_x$ is the absorbance at wave number 'x', assuming a straight baseline between 750 and 450 cm\(^{-1}\) (Weiner & Bar-Yosef, 1990; Shemesh, 1990; Munro et al., 2007 and references therein). FTIR CI values typically range from \(~2.50–3.25\) for modern bone, and from \(~3.40–4.50\) for archaeological bone (Munro et al., 2007 and references therein). Bioapatite samples with FTIR CI values less than 3.8 are considered unaltered (Shemesh, 1990).

A second test for bioapatite post-mortem alteration is provided by powder X-ray diffraction (pXRD), which was used in this study to confirm the presence of carbonated hydroxylapatite (bioapatite) in the bone and tooth samples, and to check for the presence of inorganic contaminants, such as secondary calcite, that can precipitate in void spaces of bone or teeth. The pXRD data were collected using a Rigaku RU-200BVH rotating anode diffractometer, operated in step-scan mode and employing Co K\(\alpha\) radiation ($\lambda=0.1790210$ nm) at 45 kV and 160 mA. The measurements were collected from 28 to 44° 2\(\theta\), using a step size of 0.010° and a scan speed of 1.000°min\(^{-1}\).

The degree of correlation between the $\delta^{18}$O values of co-existing phosphate and structural carbonate from bone or enamel can also be used to test the isotopic integrity of bioapatite samples. Iacumin et al. (1996) analyzed 31 bone and tooth samples of modern mammals for their structural carbonate and phosphate oxygen isotope compositions and found them to be positively correlated along a so-called ‘equilibrium’ line. They suggested that the $\delta^{18}$O values of samples plotting on or close to this line represent unaltered material. Since then, however, Martin et al. (2008) and Pellegrini et al. (2011) have demonstrated that the primary oxygen isotope compositions of structural carbonate and phosphate do not always conform to such a strict relationship at the microscopic scale.

Information obtained from collagen analysis can also test the quality of bone. Well-preserved collagen has a yield >1\%, a carbon content of 30–43\% and a nitrogen content of 11–16\% (Ambrose, 1990; van Klinken, 1999). In addition, the atomic C/N ratio of collagen can be used to assess preservation; the C/N ratio of well-preserved collagen falls between 2.9–3.6 (DeNiro, 1985).
2.4 Stable isotopes

Stable isotope compositions are presented in standard delta (δ) notation in per mil (‰) relative to VPDB for carbon, VSMOW for oxygen and AIR for nitrogen (Coplen, 1996, 2011; Hoefs, 2009).

2.4.1 Phosphate preparation

In order to analyze the oxygen isotope composition of the phosphate component of bioapatite (from bulk enamel and bone samples), the bioapatite samples had to be purified and precipitated as Ag₃PO₄. This was done following a procedure adapted from Firsching (1961), Crowson et al. (1991), and Stuart-Williams and Schwarcz (1995).

Approximately 15 ml of 3 M acetic acid was added to 45<63 µm powdered bone (34 mg) or enamel (29–32 mg) for a minimum of 24 hours. To remove calcium from bioapatite, 0.55 M potassium oxalate was added to each sample and the pH was adjusted to 3.5–4.0 using 8 M potassium hydroxide (KOH) to precipitate calcium in the form of calcium oxalate. Samples were then centrifuged and the supernatant solution retained and reacted with 5 mL of 0.5 M lead acetate. The pH was then adjusted to 5.3–5.5 with 8 M KOH and the samples were centrifuged to isolate the lead phosphate precipitate. This white precipitate (in each sample tube) was placed in a 95 °C water bath and 2 treatments of 1 mL of concentrated nitric acid and 1.5 mL of peroxide were added to remove any traces of K-oxalate and organics. After the peroxide had finished reacting, the samples were washed with 1 mL of distilled water and evaporated to low liquid levels. This wash was repeated 3 times to ensure that all peroxide had been removed. At this point, 8 M KOH was then added to neutralize the samples until the precipitate dissolved, and once it dissolved 3 mL of 0.5 M lead acetate was added. The pH was adjusted to 5.5–5.7 using 8 M and 4 M KOH. After reaction, the samples were centrifuged, the precipitate retained, and 2 mL of 0.25 M nitric acid added. The remaining lead-phosphate precipitate was dissolved to completion by dropwise addition of 3 M nitric acid. Once dissolved, 2 mL of 0.25 M ammonium sulphate was added to remove any remaining lead. The samples were then reacted, centrifuged, and the supernatant retained. Two or three drops of
bromothymol blue solution were added with 5 mL of distilled water, and the pH was adjusted to 5.5–6.5 with 4 M KOH.

The next day, 0.6 g of silver nitrate was dissolved in 10 mL of distilled water for each sample and ammonium hydroxide was added until the (ammoniacal) solution turned clear. Then, 1 mL of ammonium hydroxide, 1.5 mL of ammonium nitrate, and 10 mL of the ammoniacal silver solution (silver nitrate, water, NH₄OH) were added to each sample. Sample volumes were each topped up (and maintained) at a 60 mL volume with millipore water, and were left to precipitate on a hotplate for 6–6.5 hours at 55 °C. The precipitate was then cooled to room temperature and the resultant Ag₃PO₄ crystals were filtered and washed 3 times with millipore water. Samples were dried overnight at 65 °C and then were ready to be weighed for isotopic analysis.

2.4.1.1 Phosphate analysis: BrF₅ method

About 20–25 mg of the precipitated silver phosphate samples was loaded into Ni reaction vessels. Samples were loaded into the reaction vessels within a drybox (under flow of dry N₂ gas). A dry filter and fresh phosphorous pentoxide (P₂O₅) powder was put into the drybox during loading to maintain a relative humidity of < 13 %. Once loaded, samples were heated under vacuum for 2 hours at 300 °C, following which 80 mmHg of bromine pentafluoride (BrF₅) was added to each reaction vessel, which was then closed. Furnaces were also added to each reaction vessel, which were then heated at 600 °C overnight. Using an extraction line and procedures modified after Clayton and Mayeda (1963), Crowson et al. (1991), and Stuart-Williams and Schwarcz (1995), the O₂ released from the silver phosphate by high temperature reaction with BrF₅ was converted quantitatively to CO₂ by reaction with incandescent carbon. The sample gas was isolated cryogenically in order to calculate its yield (µmol/mg). The pressure of the gas (in a known volume) was measured with an electronic gauge and then the displacement of the gas was measured after it was transferred to evacuated sample tubes for oxygen isotopic analysis using a VG Prism II, dual-inlet, triple-collecting, isotope-ratio mass spectrometer (IRMS).

The theoretical yield of CO₂ produced from Ag₃PO₄ is 4.78 µm/mg. During the course of these experiments, standard yields averaged 4.87 µm/mg (n=7), and sample
yields averaged 4.96 µm/mg (n=22). The oxygen isotopic results were calibrated to VSMOW following Coplen (1996), using a 2-point curve based on the following standards and accepted values: TU-1 (+21.11 ‰) and TU-2 (+5.45 ‰). Using this curve, a δ¹⁸O value of +11.25 ± 0.22 ‰ (n=5, SD) was obtained for the internal laboratory phosphate standard (Aldrich 2), which compares well with its accepted value of +11.2 ‰. The one set of duplicate samples were reproducible to ± 0.13 ‰ of the average.

2.4.1.2 Phosphate analysis: TC-EA method

The δ¹⁸O values of phosphate samples were also determined using a ThermoFinnigan TC-EA coupled to a ThermoFinnigan Delta Plus XL IRMS. Powdered samples in silver capsules (~0.4 mg) were introduced into the TC-EA using a Zero Blank auto sampler and reacted rapidly for a few seconds via pyrolysis at 1350 °C. The carbon monoxide gas produced was passed through a homemade GC column, packed with a 5 Å molecular sieve heated to 90 °C, to eliminate any impurities present (e.g. water vapour, etc.), and then transferred by helium flow to the IRMS for analysis. The δ¹⁸O values were calibrated to VSMOW following Coplen (1996), using a 2-point curve based on the following standards and accepted values: Aldrich 2 (+11.2 ‰) and IAEA-CH-6 (+36.4 ‰). An average value of +21.8±0.4 ‰ (n=15, SD) was obtained for NBS 120c during these experiments, which compares well with its accepted value of +21.7 ‰.

2.4.2 Structural carbonate analysis

Bone and teeth samples were gently scrubbed with a wire brush to remove adhering dirt, rinsed with distilled water, and dried at room temperature. The bulk enamel and bone samples were then ground with a mortar and pestle to fine powder and sieved to less than 180 µm. In order to remove organic material, a portion of the bulk samples was treated with 0.04 ml of 2 % reagent-grade bleach per mg of sample at room temperature for 24 and 72 hours, for enamel and bone, respectively, and rinsed five times with deionized (Millipore) water. (Another sample portion was untreated to allow for comparisons with unbleached incremental samples; see below). To remove any secondary carbonates acquired during post-mortem processes, 0.1 M acetic acid was added in a ratio of 0.04 ml per mg of sample for 4 hours at room temperature. The
samples were then rinsed five times with Millipore water and freeze-dried (Garvie-Lok et al., 2004; Koch et al., 1997). A Micromass MultiPrep auto sampler was then used to react ~0.5–1 mg of bone or enamel with H$_3$PO$_4$ at 90 °C for 25 minutes and the gas evolved analyzed using a VG Optima dual-inlet IRMS, following the method of Metcalfe et al. (2009).

The structural carbonate $\delta^{13}$C values were calibrated to VPDB following Coplen (1994), using a 2-point curve based on the following standards and accepted values: NBS-19 (+1.95 ‰) and Suprapur (−35.28 ‰). The average $\delta^{13}$C values obtained for NBS-18 and the internal laboratory standard WS-1 were −4.95 ± 0.19 ‰ (n= 22, SD) and +0.75 ± 0.17 ‰ (n=14, SD), in close agreement with their accepted values of −5.0 and +0.76 ‰, respectively. The $\delta^{18}$O values were calibrated relative to VSMOW following Coplen (1996), using a 2-point calibration curve based on the following standards and accepted values: NBS-19 (+28.60 ‰) and NBS-18 (+7.20 ‰). The average $\delta^{18}$O values of internal standards Suprapur and WS-1 were +13.22 ± 0.25 ‰ (n=20, SD) and +26.32 ± 0.25 ‰ (n=14, SD), in close agreement with their accepted values of +13.20 and +26.23 ‰, respectively.

2.4.3 Collagen analysis

A variation of the procedure described by Longin (1971) was used to extract collagen from the bone samples. The bone samples were mechanically cleaned, ground, and sieved, retaining the fraction between 0.18 and 0.85 mm. Lipids were removed from the bone sample using 8 mL of 2:1 chloroform:methanol solution. To dissolve the inorganic (mineral) phase of bone, 0.25–0.5 M HCl was added to each sample at room temperature. Humic and fulvic acids were removed using 0.1 M NaOH at room temperature and then rinsed ~8 times with distilled water. The collagen was gelatinized in 0.25 M HCl at 90 °C and then dried at 90 °C. The collagen samples were lightly ground and weighed into tin capsules (0.400 ± 0.02 mg). The $\delta^{13}$C and $\delta^{15}$N values and C/N ratios were analyzed using a Costech elemental combustion system (ECS 4010) coupled to a ThermoFinnigan Delta Plus XL IRMS (operated in continuous flow mode).

The $\delta^{13}$C values of collagen were calibrated to VPDB following Coplen (1994) using a 2-point curve based on the following standards and accepted values: USGS-40 (−
The δ¹⁵N values were calibrated to AIR using a 2-point curve based on the following standards and accepted values: USGS-40 (−4.52 ‰) and USGS-41 (+37.63 ‰). Nitrogen and carbon contents, which were then used to calculate atomic C/N ratios, were also calibrated using the accepted abundances in USGS-40 and USGS-41 (n= 9.5 and C= 40.7 %). An internal standard (Keratin), and international standards IAEA-CH-6 and IAEA-N2 were included in each run to monitor the accuracy and precision of measurements. The results for Keratin were: δ¹³C = −24.04 ± 0.15 ‰ (n=11, SD; accepted = −24.04 ‰); δ¹⁵N = +6.25 ± 0.23 ‰ (n=11, SD; accepted = +6.36 ‰; C/N measured= 3.78 ± 0.06 (n=12, SD; theoretical: 3.4, O’Connell & Hedges, 1999). The results for IAEA-CH-6 were: δ¹³C = −10.47 ± 0.13 ‰ (n=5, SD; accepted = −10.45 ‰). The results for IAEA-N2 were: δ¹⁵N = +20.40 ± 0.17 ‰ (n=4, SD; accepted = +20.30 ‰). Collagen C/N ratios of samples were reproducible to better than ± 0.01 (n=9, SD).

2.5 Drinking water oxygen isotope composition

The δ¹⁸O values of drinking water were calculated for the Pleistocene and modern horses using the following approach. Phosphate δ¹⁸O values were converted to drinking water composition using the relationship of Huertas et al. (1995), who combined their data with the δ¹⁸O results of Bryant et al. (1994) and Chillón et al. (1994):

\[ \delta^{18}O (PO_4^{3-}) = 0.71[\delta^{18}O (H_2O)] + 22.60, \]  

[Equation 2.2]

To obtain drinking water oxygen isotopic compositions from structural carbonate δ¹⁸O values, they first had to be converted to phosphate δ¹⁸O values (assuming that both components formed in isotopic equilibrium with each other; see section 1.2.2) using the fractionation factor between structural carbonate (SC) and phosphate (P) for mammalian systems (Bryant et al., 1996a, Iacumin et al., 1996):

\[ \alpha_{SC:P}(1.0086) = \frac{1000 + \delta_{SC}}{1000 + \delta_{P}}, \]  

[Equation 2.3]
2.6 Radiocarbon analysis

Four Pleistocene horses were radiocarbon dated at the University of Arizona Accelerator Mass Spectrometry (AMS) Laboratory. Bone collagen was extracted using an automated flow-cell apparatus, following a modification of the technique used by Longin (1971), and combusted with copper oxide at >800 °C. Radiocarbon dates were calculated using the Libby half-life (Libbey et al., 1949). The dates were corrected for natural and ion source fractionation using measured $^{13}$C/$^{12}$C ratios for bone collagen (Stuiver & Polach, 1977). Conversion to calibrated years (ka BP) from uncalibrated radiocarbon years was performed using INTCAL09 (Reimer et al., 2009).

2.7 Defleshing considerations

The modern horse sample needed to be defleshed before isotopic analysis could be completed. The mandible was cut in half (between the incisors), the left mandible was put into water at 20 °C, and the right mandible was put into water at 29 °C for a total of 17 days. After the first week, most of the tissue was scraped off, and the jaws were put back into clean water at their respective temperatures for the remainder of defleshing time. Two different water temperatures were used to determine which approach removed adhering tissues better (i.e. 'cold' or 'warm' water). Tests were conducted on the modern horse mandible to ensure that isotopic changes did not occur during defleshing. The mandibular 'hinges' were used for testing three defleshing techniques: cold water (~20 °C), warm water (~29 °C), and warm water with an enzyme (30 mL Protease at 29 °C). It was learned later, however, that the enzyme was past its expiration date and therefore unlikely to have much effect on tissue breakdown.

Bone samples from the modern mandible used for defleshing tests were analysed using the BrF$_5$ method for phosphate (Table 2.2 and Fig. 2.5). The piece of mandible put in cold water (CW) had a phosphate oxygen isotope composition of $+11.5 \pm 0.4 \%_o$ (n=2). The samples put in warm water (WW) and water with the enzyme (E) both had a $\delta^{18}$O phosphate value of $+12.1 \%_o$. (Table 2.2). No difference was expected between WW and E bones because they were both from the hinge part of the right mandible (Fig. 2.5) and
the enzyme was past its expiration date and expected to behave similar to the WW experiment. Given that the Pleistocene bone samples were taken from the mandible below the incisors, a sample of the modern horse bone was also taken from this position from the right mandible (Fig. 2.5) that had been defleshed in warm water (sample MH). It had a $\delta^{18}O$ phosphate value of $+11.3\%$, as determined using the TC-EA method. The $\delta^{18}O$ phosphate values of all defleshed bone samples were within $\pm 0.4 \%$ (SD). This range is acceptable given the inherent isotopic variability that can exist within the same bone sample. For example, collagen isotopic compositions of bone samples from the same human rib can differ on average by 0.1 and 0.3 \% for $\delta^{13}C$ and $\delta^{15}N$ values, respectively (K. Olsen, personal communication).

Table 2.2  Phosphate and structural carbonate (pretreated and untreated) bone isotopic compositions of the defleshing tests.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Phosphate $\delta^{18}O$ (%)</th>
<th>Untreated $\delta^{18}O$ (%)</th>
<th>Untreated $\delta^{13}C$ (%)</th>
<th>Pretreated $\delta^{18}O$ (%)</th>
<th>Pretreated $\delta^{13}C$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>+12.1</td>
<td>+19.3</td>
<td>-15.0</td>
<td>+19.8</td>
<td>-14.9</td>
</tr>
<tr>
<td>E</td>
<td>+12.1</td>
<td>+19.4</td>
<td>-14.9</td>
<td>+19.8</td>
<td>-15.0</td>
</tr>
<tr>
<td>CW</td>
<td><strong>+11.5</strong></td>
<td>+19.9</td>
<td>-14.8</td>
<td>+20.0</td>
<td>-15.3</td>
</tr>
</tbody>
</table>

Values in **bold** are the average of duplicate analyses
Figure 2.5  Diagram of a horse mandible showing modern bone and teeth samples. CW, WW, and E were defleshing tests and represent cold water, warm water, and enzyme experiments, respectively (see section 2.7). The right mandible was defleshed in warm water and the left mandible was defleshed in cold water. MH is the bone sample for the modern horse (modified from Bendrey, 2007).

The carbon and oxygen isotope compositions of structural carbonate were also analyzed from the WW, E, and CW defleshed samples (Fig. 2.5). Untreated WW, E, and CW had an average δ^{13}C value of $-14.9 \pm 0.08\, \%$ (SD) and an average δ^{18}O value of $+19.5 \pm 0.34\, \%$ (SD) (Table 2.2). Pretreated WW, E, and CW had an average δ^{13}C value of $-15.1 \pm 0.21\, \%$ (SD) and an average δ^{18}O value of $+19.9 \pm 0.11\, \%$ (SD) (Table 2.2). Given the limited range of carbonate and phosphate isotopic compositions (Table 2.2) and 'background variability', all results from the defleshing tests were incorporated into the isotopic baseline used to evaluate tissue isotopic spacings, which are discussed later in the thesis.
Chapter 3

Results

3.1 Assessment of diagenesis of bioapatite and associated collagen

FTIR CI bone and enamel results for the modern and Pleistocene horses are listed in Table 3.1. The modern horse bone has a CI of 2.7 and the modern teeth have an average CI of 3.3. All modern and Pleistocene horse FTIR CI bone and teeth values (except for RAM 3B) fall within the range of accepted values for modern and archaeological bone samples, 2.50–3.25 and 3.40–4.50, respectively (see section 2.3).

The pretreated RAM 3B had a CI of 2.6, which is higher than its untreated CI of 1.7. The increase in CI (within the acceptable range) for RAM 3B was likely because of the removal of secondary carbonate during pretreatment. The horse bone and teeth CI values are similar to those reported by Trueman et al. (2004) for other mammals of similar size (2.7–3.4; cow, elephant, zebra, rhinoceros, wildebeest, and buffalo bone samples). All of the peaks in the horse bone and teeth FTIR and pXRD spectra are characteristic of carbonated hydroxylapatite; no calcite or unusual phases (e.g. siderite, brushite, etc.) were detected. The modern horse has a carbon content of 40.1 %, a nitrogen content of 14.7 %, and a C/N ratio of 3.19. The Pleistocene bone collagen has a carbon content of 36.6–39.4 % (n=9), a nitrogen content of 12.9–14.0 % (n=9), and a C/N ratio of 3.29–3.34 (n=9) (Table 3.1). All values are typical of well-preserved collagen (see section 2.3). The collagen yield for the modern horse is 23.5 %, whereas for the Pleistocene samples it ranges from 5.6 to 15.0 % (7.8 ± 3.0 %, SD; n=9), well above the limit (≥1 %) for well-preserved collagen. RAM 3B did not contain extractable collagen, again indicating poor bone preservation. As such, results for RAM 3B are excluded from the environmental interpretations that follow.
Table 3.1  Horse bone and tooth diagenetic tests.

<table>
<thead>
<tr>
<th>Sample</th>
<th>FTIR</th>
<th>CI</th>
<th>N (%)</th>
<th>C (%)</th>
<th>Yield (%)</th>
<th>C/N</th>
<th>FTIR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
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<td>Modern</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>aMH (bone)</td>
<td>2.7</td>
<td>14.7</td>
<td>40.1</td>
<td>23.5</td>
<td>3.2</td>
<td></td>
<td>3.4</td>
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<tr>
<td>308(P4L)</td>
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<tr>
<td>408(P4R)</td>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.1</td>
<td></td>
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<tr>
<td>306(P2L)</td>
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<tr>
<td>406(P2R)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>3.2</td>
<td></td>
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<tr>
<td>Pleistocene</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>RAM 1</td>
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<td>5.6</td>
<td>3.3</td>
<td>3.4</td>
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</tr>
<tr>
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<td>2.7</td>
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<td>6.2</td>
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<td>14.0</td>
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<td>37.5</td>
<td>6.8</td>
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<tr>
<td>RAM 6</td>
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<td>39.1</td>
<td>7.8</td>
<td>3.3</td>
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<td>RAM 7</td>
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<td>38.9</td>
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<td>37.5</td>
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<td>8.3</td>
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<td></td>
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</tr>
</tbody>
</table>

aMH= modern horse bone
Dental arcade location of teeth is shown in brackets beside the Triadan sample label
Results indicated in **bold** are the average of duplicate analyses

3.2 Oxygen isotopic analysis of bioapatite phosphate

3.2.1 BrF₅ method versus TC-EA

The phosphate oxygen isotopic compositions (δ¹⁸O_p) of bone and enamel from the modern and Pleistocene horses are listed in Table 3.2, as produced using both the TC-EA and BrF₅ methods. Both methods produced comparable results for standard materials (see sections 2.4.1.1 and 2.4.1.2). For samples, replicate analyses are reproducible ± 0.2 ‰ (n=15; SD) using the TC-EA method, and ±0.1 ‰ (n=2, SD) using the BrF₅ method. For samples, there is no statistical difference between the oxygen isotopic results produced using the TC-EA and BrF₅ methods (bone phosphate; Independent-samples t-test, p=0.131; tooth phosphate; Independent-samples t-test, p=0.570). Accordingly, the results are pooled in the environmental and physiological interpretations that follow (averages from both methods are plotted in Fig. 3.1).
For future work, the TC-EA method is the preferred method for phosphate oxygen isotope analysis for several reasons: (1) A greater number of phosphate standards can be analyzed as unknowns within each analytical session, thus improving the ability to assess precision and accuracy; (2) More samples can be analyzed in a shorter period of time (e.g. up to 18 samples in 1 day using the TC-EA versus no more than 5 samples in three days using the BrF₅ method); (3) greater potential for analytical error exists in the BrF₅ method, particularly from adsorption of water by samples and reaction vessels during sample loading; and (4) the BrF₅ method requires use of this highly hazardous (toxic, explosive) reagent.

Table 3.2  Horse bone and tooth δ¹⁸Oᵣ and associated drinking water oxygen isotope compositions (δ¹⁸O₅ₒ).  

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bone TC-EA δ¹⁸O</th>
<th>Bone BrF₅ δ¹⁸O</th>
<th>Teeth TC-EA δ¹⁸O</th>
<th>Teeth BrF₅ δ¹⁸O</th>
<th>Bone Teeth *Average δ¹⁸O</th>
<th>Radiocarbon dates (¹⁴C BP)</th>
<th>Radiocarbon lab #</th>
<th>Bone δ¹⁸O</th>
<th>Teeth δ¹⁸O</th>
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</tr>
<tr>
<td>308(P4L)</td>
<td>+11.3</td>
<td>+11.8</td>
<td>+11.3</td>
<td>+11.3</td>
<td>+11.5</td>
<td>11 240 ± 110</td>
<td>AA97948</td>
<td>−15.6</td>
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<tr>
<td>408(P4R)</td>
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<td>+11.4</td>
<td>+11.6</td>
<td>+11.4</td>
<td>+11.5</td>
<td>11 260 ± 110</td>
<td>AA97949</td>
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<td>306(P2L)</td>
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<td>+11.5</td>
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<td>RAM 1</td>
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<td>+7.7</td>
<td>+6.2</td>
<td>+6.7</td>
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<td>11 204 ± 110</td>
<td>AA97948</td>
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<td>−22.8</td>
</tr>
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<td>+8.4</td>
<td>+9.0</td>
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<td>+9.2</td>
<td>11 260 ± 110</td>
<td>AA97949</td>
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<tr>
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<td>+9.2</td>
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<td>−19.8</td>
</tr>
<tr>
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<td>+10.5</td>
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<td>+9.7</td>
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<td></td>
<td></td>
<td>−17.6</td>
<td>−18.3</td>
</tr>
</tbody>
</table>

*Calculated using the average δ¹⁸Oᵣ value obtained for all analyses, regardless of method
*MH= modern horse bone
Dental arcade location of teeth is given in brackets beside the Triadan sample label
Values in **bold** are the average of duplicate analyses
**Water δ¹⁸O values are calculated using Equation 2.2; see section 2.5
3.3 Radiocarbon dating

Two Pleistocene bone samples, RAM 1B and 2B, have radiocarbon dates (11 240 ± 110 and 11 260 ± 110 14C BP) that postdate the LGM (22–18 14C ka BP; 26.2–21.4 ka BP). These samples will be referred to as post-LGM. The two other radiocarbon-dated samples, RAM 5B and 8B, have dates (>38 900 and >46 700 14C BP) that precede the LGM (Table 3.2). These samples will be referred to as pre-LGM.

3.4 Oxygen and carbon isotopic analysis of bioapatite structural carbonate

3.4.1 Bulk structural carbonate

The bulk carbon (δ13Csc) and oxygen (δ18Osc) values of untreated and pretreated bone and enamel samples are listed in Table 3.3. Replicate analyses for untreated and pretreated bone δ18Osc values vary by ± 0.4 ‰ (n=4, SD) and ± 0.4 ‰ (n=6, SD), respectively. RAM 3B exhibits the poorest reproducibility both for treated (± 0.7 ‰) and untreated (± 1.5 ‰) samples, which is likely indicative of post-mortem alteration. These data for this sample are therefore excluded from subsequent discussions. The reproducibility of replicate δ13Csc analyses of bone is much better than for oxygen.
(pretreated, ± 0.2 ‰; n=6, SD; untreated, ± 0.2 ‰; n=4, SD). Duplicate tooth analyses for pretreated samples are within ± 0.2 ‰ for δ¹³C-sc and ± 0.1 ‰ for δ¹⁸O-sc (n=2, SD), while untreated duplicates are within ± 0.1 ‰ and ± 0.1 ‰ (n=3, SD) for δ¹³C-sc and δ¹⁸O-sc, respectively.

There is no statistical difference between untreated and pretreated Pleistocene enamel δ¹⁸O-sc values (excluding RAM 3, Independent-samples t-test). The average difference between untreated and pretreated bone samples is +1.2 ± 0.6 ‰ for δ¹³C-sc and +1.4 ± 1.5 ‰ for δ¹⁸O-sc (excluding RAM 3B). The average differences between untreated and pretreated enamel δ¹³C-sc values is +0.9 ± 1.0 ‰. There is a larger difference between untreated and pretreated bone δ¹³C-sc and δ¹⁸O-sc values than for teeth. Bone is more susceptible to isotopic alteration than enamel (see section 2.3), and thus it is likely pretreatment had more effect on restoring original bone isotopic signals because their crystallites had absorbed more secondary carbonates. Accordingly, pretreated bone and enamel isotopic compositions (δ¹⁸O-sc values are shown in Fig. 3.2) have been used to determine the diet and drinking water compositions listed in Table 3.3. Diet δ¹³C values were calculated assuming a Δ¹³C-sc-diet value of +14.1 ‰ (Table 3.3, see section 1.2.1)

Figure 3.2  Pretreated bone and tooth δ¹⁸O-sc values of the Pleistocene horses.
Table 3.3  Stable isotopic compositions for structural carbonate (untreated and pretreated) and collagen, and corresponding diet and drinking water δ-values.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Radiocarbon dates (¹⁴C BP)</th>
<th>Untreated Bone</th>
<th>Teeth</th>
<th>Pretreated Bone</th>
<th>Teeth</th>
<th>Collagen</th>
<th>Diet δ¹³C, from:</th>
<th>Water δ¹⁸O</th>
<th>Water δ¹³O</th>
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<tr>
<td>aMH (bone)</td>
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<td>¹³C</td>
<td>¹⁸O</td>
<td>¹³C</td>
<td>¹⁸O</td>
<td>¹³C</td>
<td>¹⁸O</td>
<td>¹³C</td>
<td>¹⁸O</td>
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<td>308(P4L)</td>
<td>11 240 ± 110</td>
<td>-10.0</td>
<td>16.3</td>
<td>-11.0</td>
<td>16.6</td>
<td>-11.3</td>
<td>12.5</td>
<td>-11.0</td>
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<tr>
<td>408(P4R)</td>
<td>11 260 ± 110</td>
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<td>16.4</td>
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<td>-11.6</td>
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<tr>
<td>306(P2L)</td>
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<tr>
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<td>-9.4</td>
<td>17.1</td>
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<td>15.2</td>
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<tr>
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<td>15.6</td>
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<td>15.3</td>
</tr>
</tbody>
</table>

aMH= modern horse bone
Dental arcade location of teeth is given in brackets beside the Triadan sample label.

Values in **bold** are the average of duplicate analyses.

Radiocarbon ages reported as ¹⁴C age BP

* δ¹³C<sub>sc</sub> diet values are calculated assuming a Δ¹³C<sub>sc-diet</sub> value of +14.1 ‰ (Cerling & Harris, 1999)

** δ¹³C<sub>col</sub> diet values are calculated assuming a Δ¹³C<sub>col-diet</sub> value of +5.5 ‰ (Vogel et al., 1978; Sullivan & Krueger, 1981; van der Merwe, 1989; Ambrose & Norr, 1993)

^ δ¹⁸O<sub>sc</sub> water values are calculated using Equations 2.2 and 2.3, see section 2.5
3.4.2 Incremental structural carbonate

The $\delta^{13}C_{sc}$ and $\delta^{18}O_{sc}$ values for untreated samples of serially sampled modern and Pleistocene horse enamel are listed in Table 3.4. Secondary carbonate precipitation, which the pretreatment method is used to remove, is generally minimal for enamel, and the use of untreated samples makes it possible to analyze narrower increments in the direction of tooth growth. The isotopic results for the Pleistocene horses are plotted against tooth sampling position in Figure 3.3 (incremental distance starting near the occlusal surface and ending near the root). The carbon isotope variation within individual teeth ranges from +0.6 to +2.0 ‰ (excluding RAM 8T for which there was only two samples). The variation in oxygen isotopic composition within individual teeth ranges from +2.0 to +5.1 ‰ (excluding RAM 8T). Cyclicity is apparent in the oxygen isotopic compositions of RAM 2 and RAM 10. RAM 1, RAM 3, and RAM 4 display irregular fluctuations in their oxygen and carbon isotopic compositions with growth direction in the tooth. RAM 5, RAM 6, RAM 7, and RAM 9 have carbon isotope patterns that track those of the oxygen isotopic variation for most of the occlusal-root length, but at certain times during tooth growth, covariation between the $\delta^{13}C_{sc}$ and $\delta^{18}O_{sc}$ values is not synchronized. The RAM 8 tooth is worn down substantially, indicating it was an older horse when it died (see section 1.3.3, Table 4.2); only two serial samples could be obtained.
Table 3.4 Stable isotopic compositions of serially sampled enamel and corresponding diet and drinking water δ-values.

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<th>Increment (mm)</th>
<th>Structural Carbonate</th>
<th>*Diet</th>
<th>**Water</th>
<th>Structural Carbonate</th>
<th>*Diet</th>
<th>**Water</th>
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<td>δ¹⁸O (%)</td>
<td>δ¹³C (%)</td>
<td>δ¹⁸O (%)</td>
<td>δ¹³C (%)</td>
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<tr>
<td>14.4</td>
<td>14.0</td>
</tr>
<tr>
<td>–25.9</td>
<td>–25.0</td>
</tr>
<tr>
<td>–23.8</td>
<td>–24.2</td>
</tr>
</tbody>
</table>

### Table Notes

- **Values in bold** are the average of duplicate analyses.
- ^Sampling sequence reset to zero on cut occlusal surface because of minimal amount of buccal enamel.
- *δ¹³C<sub>sc</sub> diet values are calculated assuming a Δ¹³C<sub>sc-diet</sub> value of +14.1 ‰ (Cerling & Harris, 1999).
- **δ¹⁸O<sub>sc</sub> water values are calculated using Equations 2.2 and 2.3, see section 2.5.**
Figure 3.3  $\delta^{13}\text{C}_{\text{sc}}$ and $\delta^{18}\text{O}_{\text{sc}}$ values for serially sampled Pleistocene horse teeth. Bulk $\delta^{13}\text{C}_{\text{sc}}$ and $\delta^{18}\text{O}_{\text{sc}}$ values are also plotted for each tooth. Isotopic results are plotted against tooth sampling position (incremental distance starting near the occlusal surface and ending near the root).
3.5 Collagen

The modern horse has a collagen carbon isotopic composition ($\delta^{13}C_{\text{col}}$) of $-22.7\%$ (n=1). Replicate Pleistocene $\delta^{13}C_{\text{col}}$ values have a standard deviation of $\pm 0.3\%$ (n=2; SD). Pleistocene horse $\delta^{13}C_{\text{col}}$ values average $-20.7 \pm 0.28\%$ (SD, n=9) (Table 3.3). The $\delta^{13}C_{\text{col}}$ variation is much narrower than obtained for the pretreated bulk bone $\delta^{13}C_{\text{bc}}$ ($\pm 1.0\%$; SD, n=9). Diet carbon isotopic compositions ($\delta^{13}C_{\text{diet}}$) derived from bone $\delta^{13}C_{\text{col}}$ values are also shown in Table 3.3, and determined assuming a $\Delta^{13}C_{\text{col-diet}}$ separation of $+5.5\%$. The modern horse has a $\delta^{15}N_{\text{col}}$ value of $+4.7\%$ (n=1). The $\delta^{15}N_{\text{col}}$ analyses of Pleistocene duplicates are reproducible to $\pm 0.03\%$ (n=5, SD). There are two distinct groups of bone collagen nitrogen isotopic compositions in the Pleistocene horse bone samples (Table 3.3). RAM 1, RAM 2, and RAM 4 have low $\delta^{15}N_{\text{col}}$ values, averaging $+0.2\%$, whereas the remaining samples average $+6.4\%$ (n=6). Samples with radiocarbon dates that followed the LGM belong to the grouping with low $\delta^{15}N$ values, while samples predating the LGM have high $\delta^{15}N$ values (Fig. 3.4). There is no statistical difference in $\delta^{13}C_{\text{col}}$ values between these two groupings (Independent-samples t-tests).

![Figure 3.4](image)

Figure 3.4 Collagen stable carbon and nitrogen isotopic compositions for Pleistocene horses. Radiocarbon ages are listed adjacent to the dated samples. Samples with high $\delta^{15}N$ values predate the LGM, while samples with low $\delta^{15}N$ values postdate the LGM.
3.6 Enamel-bone tissue spacing

3.6.1 Phosphate spacing

The phosphate $\Delta^{18}O_{\text{bone-tooth}}$ separations for all horses are listed in Table 3.5. There is no statistical difference between modern tooth and bone $\delta^{18}O_p$ values (Mann-Whitney U Test, $p > 0.650$). The average difference between modern bone and teeth $\delta^{18}O$ phosphate values is $-0.1 \permil$, which is less than the standard deviation from the mean obtained for duplicate samples. The average $\Delta^{18}O_{\text{bone-tooth}}$ separation for the Pleistocene horse samples is $+1.2 \pm 1.0 \permil$ (SD).

3.6.2 Structural carbonate spacing

The modern and Pleistocene horse structural carbonate $\Delta^{13}C_{\text{bone-tooth}}$ and $\Delta^{18}O_{\text{bone-tooth}}$ spacing are listed in Table 3.5. The average structural carbonate $\Delta^{18}O_{\text{bone-tooth}}$ and $\Delta^{13}C_{\text{bone-tooth}}$ separations for the modern horse are $0.0 \permil$ and $-0.4 \permil$, respectively. Within analytical error, there is no difference between modern horse bone and tooth isotopic compositions. The structural carbonate $\Delta^{13}C_{\text{bone-teeth}}$ and $\Delta^{18}O_{\text{bone-teeth}}$ separations for the Pleistocene horses range from $-0.1$ to $+2.8 \permil$, and $-1.0$ to $+3.2 \permil$, respectively.
Table 3.5  Structural carbonate and phosphate bone and tooth isotopic compositions (pre-treated, bulk samples) and corresponding bone-tooth isotopic tissue spacing.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Radiocarbon dates (14C BP)</th>
<th>Carbonate</th>
<th>*Phosphate</th>
<th>Δbone-tooth tissue spacing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bone</td>
<td>Tooth</td>
<td>Bone</td>
</tr>
<tr>
<td>Modern</td>
<td></td>
<td>δ13C</td>
<td>(%)</td>
<td>δ18O</td>
</tr>
<tr>
<td>aMH (bone)</td>
<td>–14.9 +20.1</td>
<td>+11.5</td>
<td>–0.4 +0.0</td>
<td>–0.1 +8.6</td>
</tr>
<tr>
<td>308(P4L)</td>
<td>–14.6 +19.9</td>
<td>+11.3</td>
<td>2.4 –0.6</td>
<td>+0.6</td>
</tr>
<tr>
<td>408(P4R)</td>
<td>–14.4 +20.3</td>
<td>+11.5</td>
<td>–0.8 +3.8</td>
<td>+2.4</td>
</tr>
<tr>
<td>306(P2L)</td>
<td>–14.6 +20.0</td>
<td>+11.7</td>
<td>1.9 1.2</td>
<td>+1.7</td>
</tr>
<tr>
<td>406(P2R)</td>
<td>–14.3 +20.2</td>
<td>+11.8</td>
<td>1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Pleistocene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAM 1</td>
<td>11 240 ± 110</td>
<td>–11.3 +12.5</td>
<td>–11.0 +14.9</td>
<td>+7.7</td>
</tr>
<tr>
<td>RAM 2</td>
<td>11 260 ± 110</td>
<td>–9.2 +14.6</td>
<td>–11.6 +15.1</td>
<td>+9.0</td>
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<tr>
<td>RAM 3</td>
<td>–13.6 +4.2</td>
<td>–13.3 +15.7</td>
<td>+9.3</td>
<td>+8.6</td>
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<tr>
<td>RAM 4</td>
<td>–11.2 +16.0</td>
<td>–12.1 +16.8</td>
<td>+10.5</td>
<td>+9.1</td>
</tr>
<tr>
<td>RAM 5</td>
<td>&gt;38,900</td>
<td>–10.5 +16.1</td>
<td>–12.4 +14.9</td>
<td>+10.0</td>
</tr>
<tr>
<td>RAM 6</td>
<td>–11.5 +13.8</td>
<td>–11.7 +15.7</td>
<td>+11.3</td>
<td>+7.6</td>
</tr>
<tr>
<td>RAM 7</td>
<td>–10.1 +17.5</td>
<td>–12.9 +14.2</td>
<td>+10.5</td>
<td>+9.3</td>
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<tr>
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<td>&gt;46,700</td>
<td>–11.9 +16.1</td>
<td>–13.2 +15.7</td>
<td>+9.3</td>
</tr>
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<td>–12.3 +13.5</td>
<td>–11.5 +13.7</td>
<td>+8.0</td>
<td>+7.8</td>
</tr>
<tr>
<td>RAM10</td>
<td>–11.3 +15.9</td>
<td>–11.1 +15.3</td>
<td>+10.1</td>
<td>+9.6</td>
</tr>
</tbody>
</table>

aMH= modern horse bone
*Calculated using the average δ18O p results for both methods
Chapter 4

Discussion

4.1 Possible limitations on the life history record for the modern horse

The life history of the modern horse, including its residence and migratory record, was unavailable. This can be a limitation when comparing isotopic differences between teeth (which record isotopic signals for a limited part of an animal's life) and bone (which can record adult isotopic signals because of tissue remodelling). If the modern horse had ranged widely during its life, then comparisons of isotopic compositions between bone and teeth would include isotopic signals recorded during migration in addition to physiological processes. However, there is some evidence to rule out this possible limitation, particularly for δ¹⁸O values (δ¹³C values might be less sensitive to location changes depending on the staple food of the horse, see section 4.3.1). First, the supplier of the horse sample, Atwood Pet Food Supplies Ltd., only collects animals from southwestern Ontario, and in particular from the Chatham to Kincardine area (Fig. 4.1). If the horse moved within this region, its δ¹⁸Odw values would show only limited variation as the average precipitation δ¹⁸O value in this area is around −9 ‰ (F.J. Longstaffe, personal communication), assuming it drank predominantly from local surface waters (Fig. 4.1 B), allowing tissue offsets to be identified. Second, it is unlikely that the horse travelled extensively given it was relatively young when it died; its 06 and 08 teeth hadn't finished mineralizing (see section 2.2), which mineralize from ~13–19 months and ~31–51 months, respectively (Hoppe et al., 2004a). Based on this evidence, the horse was probably less than 4 years old when it died. For these reasons, it is very likely that the modern horse bone and teeth were recording the same diet and drinking water isotopic signals.
Figure 4.1  A) Map of amount-weighted monthly precipitation δ¹⁸O values for North America. The black box identifies the general area in which the modern horse lived. B) Monthly precipitation δ¹⁸O range for southwestern Ontario, where the modern horse lived. This area has an average precipitation δ¹⁸O value of −9 ‰ (F.J. Longstaffe, personal communication). The dashed line marks the north easternmost extent of the possible range for the modern horse (modified from IAEA, 2001; The Atlas of Canada, 2012). Legend as in Figure 4.1 A.
4.2 Oxygen isotope tissue-spacing

4.2.1 Modern horse

Warinner and Tuross (2009) proposed that bone and enamel in pigs may not be isotopically equivalent tissues in terms of their bioapatite structural carbonate compositions (see section 1.2.2). This was not found to be the case for the modern horse analysed in this study; the average structural carbonate $\Delta^{18}O_{\text{bone-tooth}}$ spacing of the modern horse was ~0 ‰ (see section 3.6.2, Table 3.5). The small average structural carbonate $\Delta^{13}C_{\text{bone-tooth}}$ spacing of ~0.4 ‰ can be attributed to normally encountered variation. For example, Brookman and Ambrose (2012) sampled different lophs (ridges) of kangaroo teeth in southern Australia and found that the difference between lophs from the same tooth could vary from 0.1 to 0.7 ‰ for $\delta^{13}C_{sc}$ and 0.1 to 0.8 ‰ for $\delta^{18}O_{sc}$. They also found that differences between left- and right-paired molars ranged from 0.0 to 1.0 ‰ for $\delta^{13}C_{sc}$ and 0.0 to 1.7 ‰ for $\delta^{18}O_{sc}$. Brookman and Ambrose (2012) suggested that the differences correspond to the 'inherent isotopic variability' caused by different timing of enamel mineralization or because of slight variations in sampling techniques (e.g. depth of sample interval, etc.). This example illustrates the isotopic variability that is present in a tissue like enamel. Similar isotopic variability may be present in the teeth and bone of the modern horse in this study.

There is a chance that because the horse teeth sampled here were not fully mineralized their isotopic compositions may be seasonally biased, reflecting maximum or minimum isotopic compositions (corresponding to summer highs or winter lows, respectively). However, if this was the case more of an offset would be predicted between teeth and bone isotopic compositions, the latter of which represents more of a seasonal average. Horses grow very quickly in the first years after birth. At birth, a foal has only ~17 % of their mature bone mineral content (Lawrence, 2005). They reach 90 % of their adult weight (~500 kg) by the time they are 22 months old (Thompson, 1995; Lawrence, 2005). However, maximum bone mineral content is not reached until ~6 years of age (Lawrence, 2005). Therefore, if the horse sampled here died after the 08 teeth began mineralizing (which starts at ~19 months of age), then its bones would still have been
forming. Hence, it is highly likely that both tissues were incorporating the same body water at the same time.

In these modern samples, there is no statistical difference in $\delta^{18}O_p$ values between tooth enamel and bone that formed at the same time (see section 3.6.1), implying that the phosphate component of bioapatite fractionates in the same way in both tissues in horses. Similarly, the lack of difference in structural carbonate isotopic compositions between horse bone and tooth enamel formed at the same time suggests that oxygen and carbon isotopes, respectively, fractionate very similarly in these two tissues, at least in horses. Hence any significant measured differences are not of metabolic origin, and therefore can be used to study external factors such as migration, dietary, and climatic change. The difference between the findings of this study and those of Warinner and Tuross (2009) may indicate that isotopic offsets arising from metabolic processes are species-specific (e.g. pigs vs. horses). That said, it should be noted that only one horse has been studied here, whereas Warinner and Tuross (2009) had a larger sample set (9 pigs). Certainly, more horse samples fed a controlled diet need to be analyzed to confirm the results of the one set of observations reported here.

4.2.2 Phosphate-structural carbonate

The use of bone-enamel isotopic offsets for both oxygen and carbon to detect migration and/or dietary changes is only possible if both tissues have retained their original isotopic compositions. The relationship between $\delta^{18}O_{sc}$ and $\delta^{18}O_p$ values in mammalian bioapatite has been used as a tool for identifying post-mortem alteration (see section 2.3). The use of this tool is based on the assumption that during enamel and bone mineralization, structural carbonate and phosphate components form at the same time from the same body water (Iacumin et al., 1996; Pellegrini et al., 2011). However, it has been shown previously that the $\Delta^{18}O_{sc-p}$ relationship can vary depending on the mammalian species analyzed, and that $\delta^{18}O_{sc}$ and $\delta^{18}O_p$ do not adhere to a strictly defined relationship with each other (e.g. Bryant et al., 1996a; Zazzo et al., 2004b; Martin et al., 2008; Pellegrini et al., 2011). Possible causes of such variation are that the phosphate and carbonate components of bioapatite do not precipitate at exactly the same time and/or from the same body water (Pellegrini et al., 2011).
Bryant et al. (1996a) and Pellegrini et al. (2011) calculated fractionation factors for equids between $\delta^{18}O_{\text{sc}}$ and $\delta^{18}O_{\text{p}}$ ($\alpha_{\text{sc-p}}$) of 1.0086 ± 0.0007 (for bone and enamel) and 1.0086 ± 0.0005 (for enamel), respectively. The average $\alpha_{\text{sc-p}}$ for the modern horse analyzed in this study (using both bone and enamel compositions) is 1.0085 ± 0.0001, which is identical within error of the values reported by Bryant et al. (1996a) and Pellegrini et al. (2011). The average $\alpha_{\text{sc-p}}$ for the bone and enamel of Pleistocene horses sampled here is 1.0061 ± 0.0012, which is substantially lower than expected. The average $\Delta^{18}O_{\text{sc-p}}$ for Pleistocene horses is $+6.2 \pm 1.4$ ‰ (SD, for both bone and teeth, n=19), which is lower than the average value for the modern horse of $+8.6 \pm 0.2$ ‰ (SD, for bone and teeth, n=5, Table 3.5) and the $+8.7$ ‰ offset determined by Bryant et al. (1996a).

As mentioned earlier, $\Delta^{18}O_{\text{sc-p}}$ can vary depending on the species analysed; the extent of deviation from the established norm for a species before post-mortem alteration is suspected has not been established conclusively in most cases. Pellegrini et al. (2011) compiled the available $\alpha_{\text{sc-p}}$ values for modern animals, noting the average value to be $+1.00867 \pm 0.00077$. In other words, the average $\Delta^{18}O_{\text{sc-p}}$ offset was $+8.8 \pm 0.8$ ‰ with a range of $+6.6$ to $+10.6$ ‰. While a better understanding of the relationship between $\delta^{18}O_{\text{sc}}$ and $\delta^{18}O_{\text{p}}$ is needed to use the relationship as a test for post-mortem alteration with high confidence, Pellegrini et al. (2011) suggested that $\Delta^{18}O_{\text{sc-p}}$ offsets $<+7$ ‰ or $>+11$ ‰ should be approached with caution as they likely indicate diagenetic alteration of one tissue or the other, or perhaps both.

Coexisting $\delta^{18}O_{\text{p}}$ and $\delta^{18}O_{\text{sc}}$ values for Pleistocene bone and enamel are plotted in Figure 4.2. If the recommended 'envelope' of accepted values is applied to the bulk isotopic analyses, then only RAM 7B and RAM 1T, 2T, 3T, 4T, and 6T samples lie within the ‘acceptable’ range for unaltered material. If the other Pleistocene bone and teeth samples have undergone post-mortem alteration that affected their bulk $\delta^{18}O$ (and $\delta^{13}C$) values, it is important to try to identify which component (i.e. carbonate and/or phosphate) has been affected and by what mechanism. Under inorganic conditions and at low temperatures the phosphate moiety is very resistant to isotopic exchange (e.g. Blake et al., 1997; Lecuyer et al., 1999), while the structural carbonate moiety is much more susceptible to alteration because of faster rates of exchange (Zazzo et al., 2004a).
Significant oxygen isotope exchange, however, can occur in the phosphate moiety under certain conditions because microbial enzymes are able to break inorganic P-O bonds (Blake et al., 1997; Zazzo et al., 2004a). Zazzo et al. (2004b) plotted intratooth sequential δ\(^{18}\)O values in a δ\(^{18}\)O\(_p\)-δ\(^{18}\)O\(_{sc}\) space and proposed a model to determine whether microbial mediated or inorganic diagenesis affected tooth δ\(^{18}\)O values. This model is based on the different kinetics of *post-mortem* alteration reactions involving phosphate and carbonate moieties under inorganic and microbial conditions. They suggested that a slope of phosphate versus structural carbonate δ\(^{18}\)O < 1 indicates bioapatite-water alteration dominated by microbial conditions, which mainly affects the phosphate moiety, whereas a slope > 1 indicates alteration dominated by inorganic processes, which mainly affects the structural carbonate moiety. The oxygen isotope phosphate/structural carbonate slopes obtained for both the Pleistocene teeth and bone samples are < 1 (Fig. 4.2), which suggests a role for microbial alteration of phosphate oxygen isotopic compositions according to the Zazzo et al. (2004b) model. In this scenario, it appears that bone was affected more than teeth as the Δ\(^{18}\)O\(_{sc-p}\) spacings of the former deviate most from the 'envelope' of accepted values described by Pellegrini et al. (2011).

All bone δ\(^{18}\)O\(_p\) values obtained for the Pleistocene samples are higher than their corresponding enamel δ\(^{18}\)O\(_p\) values (Fig. 3.1). This offset is in contrast to the lack of a significant difference between modern horse bone and teeth δ\(^{18}\)O\(_p\) (or δ\(^{18}\)O\(_{sc}\)) values (see section 3.6.1). In addition, this consistent bone>tooth offset was not observed in Pleistocene δ\(^{18}\)O\(_{sc}\) values (Fig. 3.2). The same pattern of higher bone δ\(^{18}\)O\(_p\) values relative to teeth δ\(^{18}\)O\(_p\) has been reported elsewhere for large animals (e.g. elephants, bison, horses, etc.; Ayliffe et al., 1992; Tütken et al., 2008), and was attributed to *post-mortem* isotopic exchange of δ\(^{18}\)O\(_p\) with surrounding fluids. While Ayliffe et al. (1992) did not rule out microbial activity as its cause, Tütken et al. (2008) found no evidence for a *post-mortem* microbial influence on their samples. Ayliffe et al. (1992) suggested that the observed δ\(^{18}\)O\(_p\) alteration was caused by re-equilibration with soil waters through replacement of the original bioapatite or because of isotopic exchange. Tütken et al. (2008) speculated that changes in the δ\(^{18}\)O\(_p\) values of bone could result from *post-mortem* inorganic processes on the microscopic scale, during which isotopic exchange with surrounding fluids occurs despite the presence of collagen.
Figure 4.2 $\delta^{18}O_p$ versus $\delta^{18}O_{sc}$ for individual samples of Pleistocene teeth A) and bone B). The line of best fit for modern equids is shown by the solid black line (Bryant et al., 1996a). The dashed line represents the lower limit (+7 ‰) of the acceptable 'envelope' for unaltered samples, according to Pellegrini et al. (2011). The regression line (and its equation) for the isotopic data for the Pleistocene horses are shown in blue.
For the Pleistocene horses studied here, both the bone and teeth $\delta^{18}O_p$ values suggest some degree of microbially mediated *post-mortem* alteration (if the Zazzo et al. 2004b model is accepted). The $\delta^{18}O_p$ values of bone are more severely affected than those of teeth, which might explain the observed offset in $\delta^{18}O_p$ values between these two tissues (Table 3.2 and Fig. 3.1). The Pleistocene horse samples were obtained from gravel deposits that were frequently saturated by meteoric water. Modern Edmonton has an average meteoric water $\delta^{18}O$ value of $-17.1$ ‰ (IAEA/WMO, 2010). Some of the Pleistocene horse bones could have undergone microbially mediated isotopic exchange with such meteoric water, causing a shift in $\delta^{18}O$ values towards such compositions. If this was the case, it would explain why a bone-enamel oxygen isotope offset wasn't observed in structural carbonate compositions for these samples; structural carbonate has been shown to be more resistant to microbial alteration (Zazzo et al., 2004a). This does not rule out the possibility that the structural carbonate component also experienced *post-mortem* alteration under inorganic conditions. For example, based on the calcite-water oxygen isotopic geothermometer of Friedman and O’Neil (1977), modern secondary calcite formed at 5 ° and 10 °C from Edmonton meteoric water (avg. $-17.1$ ‰) has $\delta^{18}O$ values (+16.0 and +14.7 ‰, respectively). Such compositions lie within the range of $\delta^{18}O_{sc}$ values measured for the Pleistocene teeth and bone (Fig. 3.2). Therefore, it is not impossible that some of the Pleistocene horse bones and teeth underwent isotopic exchange with meteoric water (under inorganic conditions), causing a shift in $\delta^{18}O_{sc}$ values towards meteoric compositions. However, it is very likely that the teeth samples would have been less affected by such alteration because enamel is more resistant to alteration because of its smaller crystallite surface area and lower porosity (Zazzo et al., 2004a). Also, it seems that not all Pleistocene samples were affected equally by this putative *post-mortem* alteration; for example, the $\delta^{18}O_p$ value of RAM 7B, and the $\delta^{18}O_{sc}$ and $\delta^{18}O_p$ values of RAM 1T, 2T, 3T, 4T, and 6T likely reflect their original composition, given their $\Delta^{18}O_{sc-p}$ spacings (Table 3.5). Preservation of original isotopic compositions is particularly probable for RAM 2T, given its sinusoidal $\delta^{18}O_{sc}$ curve that follows expected seasonal patterns of growth (see section 4.4.3).
Figure 4.3  Calculated $\delta^{18}O_{dw}$ for Pleistocene and modern bone and teeth based on $\delta^{18}O_p$ values (Equation 2.2). The average annual meteoric water $\delta^{18}O$ value for Edmonton is represented by the black line (IAEA/WMO, 2010).

The possibility also exists that the pattern of phosphate $\Delta^{18}O_{\text{bone-tooth}}$ spacings observed for the Pleistocene samples reflects the age of individual horses. Some teeth had been worn down substantially; hence the isotopic compositions of the remaining bulk material could be seasonally biased. If the teeth reflect minimum $\delta^{18}O_{dw}$ values, representing the winter season, this would be reflected in lower teeth $\delta^{18}O_p$ values, and comparatively higher $\delta^{18}O_p$ values in the associated bone (and vice versa). Table 4.1 lists the Pleistocene horses in descending order from oldest to youngest and their corresponding phosphate $\Delta^{18}O_{\text{bone-tooth}}$ spacing. The ordering of horses from oldest to youngest is based on the height of the teeth, and the fact that young horses have relatively unworn teeth. For example, before wear, the 06s (the shortest cheek teeth) can be ~5 cm long, while the rest of the cheek teeth (e.g. 08s) can be up to ~7–8 cm long in young Thoroughbreds. Cheek teeth wear at a rate of ~2–3 mm/yr, and therefore – the shorter the tooth, the older the horse (Dixon, 2002). All of the Pleistocene teeth analysed in this study were 06s except RAM 4T, which was an 08 (Table 2.1). Therefore RAM 4T is
considered to be the oldest given that it was likely a taller tooth at the outset and because its tooth is most worn. The results of this examination, however, found no statistical relationships between the age of the horse (‘youngest’ five and ‘oldest’ five) and phosphate $\Delta^{18}O_{\text{bone-tooth}}$ spacing (Independent-samples $t$-test, $p= 0.294$) or structural carbonate $\Delta^{18}O_{\text{bone-tooth}}$ spacing (Independent-samples $t$-test, $p= 0.339$).

Table 4.1 The height of Pleistocene horse teeth (distal end, mm) and corresponding phosphate and structural carbonate $\Delta^{18}O_{\text{bone-tooth}}$ spacing, listed from oldest to youngest horse.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tooth height, distal end (mm)</th>
<th>$\text{PO}_4$ bone-enamel $\Delta^{18}O$ (%)</th>
<th>$\text{CO}_3$ bone-enamel $\Delta^{18}O$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAM 4</td>
<td>35.5</td>
<td>+1.4</td>
<td>-0.8</td>
</tr>
<tr>
<td>RAM 8</td>
<td>34.5</td>
<td>+0.2</td>
<td>+0.4</td>
</tr>
<tr>
<td>RAM 1</td>
<td>51.5</td>
<td>+1.3</td>
<td>-2.4</td>
</tr>
<tr>
<td>RAM 10</td>
<td>53.5</td>
<td>+0.5</td>
<td>+0.7</td>
</tr>
<tr>
<td>RAM 2</td>
<td>58.0</td>
<td>+0.6</td>
<td>-0.6</td>
</tr>
<tr>
<td>RAM 3</td>
<td>60.5</td>
<td>+0.8</td>
<td></td>
</tr>
<tr>
<td>RAM 7</td>
<td>61.5</td>
<td>+1.2</td>
<td>+3.2</td>
</tr>
<tr>
<td>RAM 5</td>
<td>67.5</td>
<td>+1.7</td>
<td>+1.2</td>
</tr>
<tr>
<td>RAM 9</td>
<td>73.0</td>
<td>+0.2</td>
<td>-0.1</td>
</tr>
<tr>
<td>RAM 6</td>
<td>75.5</td>
<td>+3.8</td>
<td>-1.9</td>
</tr>
</tbody>
</table>

If the Pleistocene horses (particularly the bone) underwent post-mortem isotopic alteration, why was this not detected by the other diagenetic tests, for example C/N ratio and CI index? While all but one of the Pleistocene bone samples have C/N ratios within the acceptable range, there was still significant post-mortem loss of collagen. Collagen can retain its amino acid composition, its C/N ratio, and $\delta^{13}C_{\text{coll}}$ and $\delta^{15}N_{\text{coll}}$ values until 99% of the collagen by weight has been lost (e.g. Dobberstein et al., 2009; Tütken & Vennemann, 2011). Collagen loss increases bone porosity and the exposure of bone bioapatite, which facilitates its dissolution and/or recrystallization – typically into larger crystallites (Tütken & Vennemann, 2011 and references therein). That said, a significant increase in bioapatite crystallite size was not detected in the Pleistocene samples; all bone and enamel CI values remained within the accepted range (see section 3.1). Tütken et al.
(2008) also report alteration of bone $\delta^{18}$O$_p$ values without an associated increase in crystallinity, which they could not explain. Tütken and Vennemann (2011) noted that the best-preserved bone typically has a collagen yield of ~14 % or higher, similar to that of modern bone. All but one of the Pleistocene bone samples have collagen yields lower than 14 % (Table 3.1), with an average yield of 6.9 % (excluding RAM 4B which has a yield of 15 %). Breakdown of collagen by microbial attack and/or hydrolysis are common and important processes in bone degradation (Child, 1995; Collins et al., 2002; Tütken & Vennemann, 2011). Therefore, while original Pleistocene bone collagen isotopic signals may have been preserved, the low amount of collagen remaining in these samples may have increased microbial access to the bioapatite crystallites, thus facilitating post-mortem alteration via this pathway.

4.3 Bulk and serially sampled structural carbonate

4.3.1 Modern horse structural carbonate drinking water and diet isotopic signatures

The $\delta^{18}$O$_{dw}$ value calculated for the modern horse from its bulk $\delta^{18}$O$_{sc}$ value is $-15.7 \%$ for both bone and teeth (similar to the average $\delta^{18}$O$_{dw}$ value of $-15.5 \%$ calculated using $\delta^{18}$O$_p$). This is lower than the average annual precipitation $\delta^{18}$O value known for the region where the horse most likely lived: $-9 \%$ (F.J. Longstaffe, personal communication, Fig. 4.1). This area has a seasonal range in meteoric water $\delta^{18}$O values of $-14.2$ to $-7.0 \%$ (F.J. Longstaffe, personal communication). The serially sampled $\delta^{18}$O$_{dw}$ values, by comparison, display a smaller range: 308 (P4L), $-20.7$ to $-16.4 \%$, avg. $-17.9 \%$; 408 (P4R), $-21.1$ to $-15.9 \%$, avg. $-18.8 \%$ (06 results are not included because of the limited amount of enamel present, Table 3.4). The smaller range and lower bulk average $\delta^{18}$O$_{dw}$ values could arise in several ways. First, it is very likely that the horse did not drink directly from precipitation, but rather drank from well water. Shallow groundwater in southwestern Ontario typically has $\delta^{18}$O values that are $~2 \%$ lower than average precipitation, because of preferential recharge during winter months (F.J. Longstaffe, personal communication). Average groundwater $\delta^{18}$O values range from around $-11.5 \%$ near the southern shores of Lake Huron to around $-12.5 \%$ in the vicinity of Barrie, Ontario, on the shores of Lake Simcoe (North et al., 2013). Second,
some groundwater in the region has still lower $\delta^{18}O$ values because it still contains some components of Late Pleistocene glacial meltwater (Husain et al., 2004). Third, there are errors associated with the two-step calculation of $\delta^{18}O_{dw}$ values from $\delta^{18}O_{sc}$ values, as discussed in section 4.4.2. Without further details on the exact drinking water composition of the modern horse analyzed here, it is not possible to speculate further.

The modern horse diet compositions, as calculated from their pretreated bulk $\delta^{13}C_{sc}$ values, are $-29.0$ and $-28.6$ ‰ for bone and teeth, respectively (serially sampled diet $\delta^{13}C_{sc}$ values are similar; Table 3.3, 3.4). These compositions are indicative of an entirely C$_3$ diet (see section 1.2.1). Modern domesticated horses in Ontario mainly eat hay, pasture, grains, or concentrates (OMFRA, 2012). Pieces of hay were found imbedded between the teeth and jawbone of the modern horse. Sponheimer et al. (2003) reported $\delta^{13}C$ values of modern alfalfa hay feed at $-27.0 \pm 0.4$ ‰, similar to what is suggested here. It is likely that the modern horse’s diet largely consisted of hay, with little to no supplement from C4 plants, such as corn. The diet composition determined from the $\delta^{13}C_{col}$ values ($-28.2$ ‰) for this horse lead to the same conclusion (Table 3.3).

4.3.2 Differences between bulk and serially sampled structural carbonate

The majority of the average $\delta^{13}C_{sc}$ and $\delta^{18}O_{sc}$ values obtained for the serially sampled teeth are consistently lower than their corresponding bulk isotopic compositions (Table 3.3, 3.4). Serially sampled average $\delta^{13}C_{sc}$ and $\delta^{18}O_{sc}$ values are on average 0.6 ‰ and 1.4 ‰ (n=14) lower than bulk values, respectively (for both modern and Pleistocene horses). If this difference was caused by a seasonal bias in the material sampled, the compositions obtained for serially sampled materials wouldn’t be expected to be consistently lower than bulk compositions. This is also unlikely to be a species-specific effect. The same pattern of lower average incremental $\delta^{18}O_{sc}$ values has been observed for mammoths and mastodons (Metcalfe, 2011). Metcalfe (2011) attributed the difference to faster mineralization of the innermost enamel (used for serially sampling) compared to the rest of the enamel (used for bulk samples) and associated compositional differences in the fluid from which the enamel precipitates.

4.3.3 Pleistocene bulk structural carbonate drinking water and diet isotopic signatures
The average bone and tooth $\delta^{18}O_{dw}$ values ($\pm$ SD) for the Pleistocene horse samples were $-22.7 \pm 2.2$ $\%$ and $-22.6 \pm 1.2$ $\%$, respectively (Table 3.3). The similarity between bone and tooth $\delta^{18}O_{dw}$ values suggests that the horses drank from comparable drinking water sources during bone and tooth mineralization.

To interpret the carbon isotopic data for the Pleistocene horses, we must first consider the Suess effect. The $\delta^{13}C$ value of atmospheric CO$_2$ has been decreasing over the past 200 years as a result of the increase in CO$_2$ added to the atmosphere by fossil fuel burning and deforestation (Verburg, 2007). This decrease in atmospheric CO$_2$ $\delta^{13}C$ values from $-6.5$ $\%$ (around 1744 AD) to $-8.0$ $\%$ (modern day) is known as the Suess effect (Friedli et al., 1986; Dotsika et al., 2011 and references therein). Modern C$_3$ plant isotopic compositions must therefore be adjusted upwards by 1.5 $\%$ for comparison with Pleistocene bone or tooth carbon isotopic compositions (Friedli et al., 1986; Marino & McElroy, 1991).

The average bone and tooth diet $\delta^{13}C$ values ($\pm$ SD) obtained for the Pleistocene horse samples were $-25.2 \pm 1.0$ $\%$ and $-26.2 \pm 0.9$ $\%$, respectively (excluding RAM 3B; Table 3.3). This suggests that the Pleistocene horses had a diet consisting almost entirely of C$_3$ plants (C$_3$ plants have an average value of $-25.0$ $\%$; after correcting for the Suess effect). This was expected as Pleistocene Alberta was overwhelmingly dominated by C$_3$ grasses and Pleistocene horses consumed predominantly grasses (see section 1.4). The narrow range among bulk bone and teeth $\delta^{13}C_{sc}$ and $\delta^{18}O_{sc}$ values, respectively, suggests that the Pleistocene horses sampled here consumed similar food and water during enamel (which mineralizes early on in an animal's life) and bone (which remodels itself throughout an animal's life) formation.

4.3.4 Growth rate estimates for serially sampled Pleistocene horse teeth.

The collective amount of time (in months) represented by the serial samples of the Pleistocene horse teeth (Table 4.2) has been estimated as:

$$\text{Total growth time (in months)} = \frac{(DL*n_{DL} + S*n_S)}{(GR)}.$$  
[Equation 4.1]
where DL = drill line length (in µm), usually ~2500 µm parallel to tooth growth direction; \( n_{DL} \) = number of growth lines; \( S \) = space (in µm) between drill lines parallel to tooth growth direction; \( n_S \) = number of spaces, and GR = growth rate in µm/month (see section 2.2).

This provides an estimate (in months) of the time represented by serially sampled enamel collected along the length of the tooth. Important caveats are that this calculation assumes a constant growth rate. Enamel growth rates are not completely constant and are known to vary among modern equid species (and likely ancient equids, e.g. Hoppe et al., 2004a).

Table 4.2 Growth time estimates (in months) for serially sampled Pleistocene horse teeth.

<table>
<thead>
<tr>
<th>Sample</th>
<th>RAM 1</th>
<th>RAM 2</th>
<th>RAM 3</th>
<th>RAM 4</th>
<th>RAM 5</th>
<th>RAM 6</th>
<th>RAM 7</th>
<th>RAM 8</th>
<th>RAM 9</th>
<th>RAM 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time represented (months)</td>
<td>13.2</td>
<td>15</td>
<td>14.1</td>
<td>21</td>
<td>16.6</td>
<td>13.5</td>
<td>12.8</td>
<td>3.8</td>
<td>13.6</td>
<td>16.7</td>
</tr>
</tbody>
</table>

4.4 Palaeoecological implications

4.4.1 Late Pleistocene climate

As mentioned in sections 3.3 and 3.5, there is an association between \( \delta^{15}N_{col} \) values and pre- versus post-LGM radiocarbon dates. Horses with pre-LGM dates have significantly higher \( \delta^{15}N \) values (averaging +6.0 ‰, \( n=2 \)) than post-LGM horses (averaging +0.1 ‰, \( n=2 \)) (Fig. 4.4). Low post-LGM \( \delta^{15}N_{col} \) values have also been reported elsewhere. For example, reindeer from Siberia have a range of \( \delta^{15}N_{col} \) values from +1.3 to +1.5 ‰ (Iacumin et al., 2000), horses and deer from northwest Europe have \( \delta^{15}N_{col} \) values ranging between +0 to ~+1.2 ‰ (Richards & Hedges, 2003), and horses from northwest Europe have \( \delta^{15}N_{col} \) values as low as –0.9 to +2.2 ‰ (Stevens & Hedges, 2004). This relationship of low \( \delta^{15}N_{col} \) values in post-LGM time can be used to tentatively classify the other Pleistocene horse samples as either pre- (RAM 6, 7, 9, 10) or post-LGM (RAM 4) (RAM 3 did not contain extractable collagen).
In light of the different horse $\delta^{15}N_{col}$ values between pre- and post-LGM time periods, it is important to review the North American (and Eurasian) environments in which the horses (and other megafauna) lived during these times. During pre-LGM conditions in the Mid-Wisconsinan (concurrent with Marine Isotope Stage 3, MIS 3, ~70–30 ka BP), the ice sheets were smaller compared to ice volumes before MIS 3 and during the LGM (Anderson & Lozhkin, 2001 and references therein; Miller et al., 2010). Northern areas (e.g. Alaska, Beringia, and Yukon Territory) supported open herb or steppe tundra as well as shrubland, and conifers were present in interior regions (Anderson & Lozhkin, 2001; MacDonald et al., 2012). Grasses and willows were abundant during the Mid-Wisconsinan, but birch was less common than during the Pleistocene-Holocene transition (Anderson & Lozhkin, 2001). In Alaska, which was cool and dry during the Mid-Wisconsinan, megafauna were dominated by horse, bison, and mammoths (Guthrie, 2001). These three megafauna were also dominant in Alberta prior to the LGM (Burns, 1996; Jass et al., 2011). This observation suggests that this period in Alberta was also cool and dry (but warmer than during the LGM) with open grasslands; for example, 40% of mammals found in an Early-Wisconsinan Alberta floodplain deposit are known to have occupied open grassland habitats (Anderson & Lozhkin, 2001; Harington, 1990 and references therein; Dredge & Thorleifson, 1987).
The LGM was particularly cold globally and arid in the north (Miller et al., 2010; Guthrie, 2001). Most of southern Alberta did not experience a full glaciation during the Pleistocene until this time (Young et al., 1994; Burns, 1996; Burns, 2010). An estimate for the appearance of ice cover in the Edmonton area is $\sim$21.3 $^{14}$C ka BP ($\sim$25.5 ka BP), based on radiocarbon dated fossils (Young et al., 1994). At the LGM, forests were absent north of 55° N (including Edmonton), there was a mixture of steppe and tundra in the northern Eurasian interior, and a mixture of graminoid and forb in Beringia (Bigelow et al., 2003).

After the LGM, the ice sheets retreated as temperatures rose in North America. Deglaciation occurred slowly between 18–13 $^{14}$C ka BP ($\sim$21.4–15.5 ka BP) (Dyke & Prest, 1987; Dyke et al., 2003). There is some debate as to when central Alberta became ice-free (see section 1.1). Dyke and Prest (1987) and Dyke et al. (2003) (based on radiocarbon chronologies) portrayed it as ice-free between 12–11 $^{14}$C ka BP ($\sim$13.8–12.8, conversion from INCAL, 2009) (Fig. 4.5), while Munyikwa et al. (2011) suggested it was ice-free one thousand years earlier (using optically stimulated luminescence dating).
After the ice retreat, northern populations of boreal taxa such as *Picea*, *Pinus*, and *Betula* expanded into areas where ice had been, while southern taxa declined (based on fossil pollen records, Williams et al., 2004). From ~15–10 ka BP, there was development of large peatlands, dense *Betula* cover, and conifer forest (e.g. *Pinus*, *Picea*, etc.) in Beringia (including Alaska and Yukon territory) (MacDonald et al., 2012 and references therein). Pollen records from 12–10 $^{14}$C ka BP (~13.8–11.4 ka BP) indicate that central Alberta was dominated by herbs, grasses, and deciduous shrubs, with few trees (MacDonald & McLeod, 1996). *Picea* and *Pinus* also increased in abundance in central Canada, connecting with western species that were expanding north into the Canadian Rockies and *Picea* populations from Alaska (Williams et al., 2004 and references therein). After the onset of the Holocene at ~10 $^{14}$C ka BP (~11.6–11.4 ka BP), dense spruce forests were established across Alberta (MacDonald & McLeod, 1996; MacDonald et al., 2012). There was also an increase in the abundance of temperate trees moving north from the southeastern United States (Williams et al., 2004). The increase in temperature and moisture availability was quite notable in Alaska with the formation of lakes, bogs, shrub tundra, forests, and soil paludification (Guthrie, 2003; Mann et al., 2002 and references therein). In summary, the environment in Alberta changed from a relatively cool and dry climate through the Mid-Wisconsinan, to a drastically colder and more arid climate during the LGM. It then became warmer and moister after the LGM than it had been during the Mid-Wisconsinan.
4.4.2 Bulk patterns in δ¹⁸O<sub>sc</sub> and δ¹³C<sub>sc</sub> values

Enamel is assumed to be more resistant to post-mortem alteration than bone, and therefore of most value for palaeoenvironmental reconstruction. Accordingly, only the isotopic results for enamel are considered in this section. For the pre- and post-LGM horses the δ¹⁸O<sub>dw</sub> values (calculated from enamel δ¹⁸O<sub>p</sub> values) are similar, avg. −19.7 ‰ (n=6) and −20.6 ‰ (n=3), respectively, as are pre- and post-LGM δ¹⁸O<sub>dw</sub> values (avg. −23.0 ‰, n=6, and −22.1 ‰, n=3, respectively) calculated using bulk (pretreated) δ¹⁸O<sub>sc</sub> values. The relatively small discrepancy between these two sets of results could arise in a number of ways. First, and most likely, either or both of the δ¹⁸O<sub>sc</sub> and δ¹⁸O<sub>p</sub> values were affected to some extent by post-mortem alteration. This is likely the case as there was no significant difference between the δ¹⁸O<sub>dw</sub> values calculated using structural carbonate versus phosphate oxygen for the modern horse (see section 4.3.1). Another factor is that two equations were required (Equations 2.2 and 2.3) to calculate δ¹⁸O<sub>dw</sub> from δ¹⁸O<sub>sc</sub> values, whereas only one equation was needed to convert δ¹⁸O<sub>p</sub> values (Equation 2.2).
Third, both of these equations were determined for different equid species from different time periods (see Bryant et al., 1996a and Huertas et al., 1995).

The similarity between $\delta^{18}O_{dw}$ values for pre- and post-LGM times suggest that the horses drank from comparable water sources and/or seasonal climatic patterns were similar during both time periods. Average pre-LGM $\delta^{18}O_{dw}$ values calculated using $\delta^{18}O_{sc}$ for mammoths from Alberta are higher, $-19.1$‰ ($n=4$) (Metcalfe, 2011). However, the pre-LGM mammoth and horse $\delta^{18}O_{dw}$ values are not directly comparable for several reasons: (1) the mammoths were sampled from different localities in Alberta, not only the Edmonton region; (2) there may be $\delta^{18}O$ variations arising from species-specific metabolic effects, given that mammoth physiology, and diet and drinking behaviour was likely different from horses, and (3) the mammoths and horses are likely not temporally comparable; some of the mammoths had ages beyond the limits of radiocarbon dating, and hence likely represent much earlier pre-LGM time than some of the horses sampled here. That said, some of the horses labelled as pre-LGM were not radiocarbon dated and therefore could also be well beyond the radiocarbon dating limit. Therefore, a potentially large span of time could be represented by the pre-LGM horses, and hence only general environmental reconstructions can be made for such a potentially long interval.

The pre-LGM horses have slightly lower $\delta^{13}C_{\text{diet}}$ values (calculated from bulk pretreated $\delta^{13}C_{sc}$ values) compared to post-LGM horses: $-26.2$‰ ($n=6$) and $-25.7$‰ ($n=3$), respectively. As mentioned in section 1.2.1, $\delta^{13}C_{sc}$ values represent bulk carbon from the whole diet. The pre-and post-LGM structural carbonate $\delta^{13}C_{\text{diet}}$ values suggest that the horses were consuming relatively similar plants, with little change in horse diet between these time periods. The average $\delta^{13}C_{\text{diet}}$ values (calculated from bulk $\delta^{13}C_{\text{col}}$ values) for the pre- and post-LGM horses are identical: $-26.2$‰ ($n=6$) and $-26.2$‰ ($n=3$), respectively. The $\delta^{13}C_{\text{col}}$ values are mainly influenced by dietary protein, and because horses are herbivores, their protein input would have been from plant material. The similar pre- and post-LGM collagen $\delta^{13}C_{\text{diet}}$ values indicate that the horses were eating similar plant types with similar protein components. It is important to mention the climatic factors that can affect the carbon isotope composition of plants (and therefore structural carbonate and collagen), which include light, nutrient and water availability, temperature, CO$_2$ atmospheric concentrations, etc. (as reviewed in Heaton, 1999). These
environmental parameters can influence stomatal conductance and carboxylation rates of plants, therefore affecting the amount of isotopic fractionation during photosynthesis (Stevens & Hedges, 2004; as reviewed in Heaton, 1999). The similar structural carbonate (bulk diet) and identical pre- and post-LGM collagen (protein source) $\delta^{13}C_{\text{diet}}$ values suggests that the climatic differences between these two time periods were too small to cause large changes in plant and corresponding bulk diet and protein $\delta^{13}C$ values.

The pre-LGM horse structural carbonate $\delta^{13}C_{\text{diet}}$ values are slightly lower than dated pre-LGM ($\sim 40$ $^{14}C$ ka BP, 44.1 ka BP) mammoth structural carbonate $\delta^{13}C_{\text{diet}}$ values from Alberta ($-24.6$‰, n=3) (Metcalfe, 2011 and references therein). Overall similarities and only minor differences in diet selection (both animals are grazers) likely explain this small variation (Metcalfe, 2011 and references therein). The horse collagen $\delta^{13}C_{\text{diet}}$ values are similar to pre- ($\sim 40$ $^{14}C$ ka BP, 44.1 ka BP) and post- ($\sim 11$ $^{14}C$ ka BP, 12.9–12.8 ka BP) LGM collagen $\delta^{13}C_{\text{diet}}$ values of proboscideans from Alberta ($-25.5$‰, n=4; $-25.4$‰, n=1, respectively; Metcalfe, 2011 and references therein). Iacumin et al. (2000) also reported little variation in $\delta^{13}C_{\text{coll}}$ variation between Late Pleistocene mammoths and reindeer from Eurasia (i.e. Russia and Siberia), and suggest that pre- and post-LGM environmental conditions were similar, but more arid before the LGM. Based on the collagen isotopic compositions of mammoths, Szpak et al. (2010) likewise suggested that Siberia was cold and arid before the LGM, on average more so than central Alaska and Yukon (eastern Beringia) at this time, with eastern Beringia becoming colder and more arid after the onset of the LGM.

4.4.3 Serial sampling patterns in $\delta^{18}O_{\text{sc}}$ and $\delta^{13}C_{\text{sc}}$

Home ranges are undefended areas in which a horse restricts its activities and seeks shelter, food, and mates (McCort, 1984; Linklater et al., 2000). The extent of a home range can vary seasonally, depending on water and food availability (McCort, 1984; Smuts, 1975). Larger home ranges are typically associated with poorer habitat quality, and the size of a home range usually increases during the winter (Smuts, 1975; Linklater et al., 2000). The size of a horse’s home range can influence the seasonal isotopic signal recorded in enamel. The average maximum variation in pre-LGM serially sampled $\delta^{18}O_{\text{sc}}$ values is $3.7$‰ (n=5) ($3.4$‰ including RAM 8T), with the maximum
variation ranging from 2.0 to 5.1 ‰. The average maximum variation in post-LGM δ¹⁸O_sc values is 3.0 ‰ (range, 2.2 to 4.9 ‰; n=3). Variability in the δ¹³C_sc values of serially sampled pre-LGM horses ranges from 0.2 to 2.0 ‰ (n=5) and in post-LGM horses, from 0.6 to 1.5 ‰ (n=3). This intra tooth variation in δ¹³C_sc lies within the range of annual variation typical for a C₃ environment (Heaton, 1999; Sharp & Cerling, 1998 and references therein). The general similarity of these oxygen and carbon isotopic ranges between time periods suggest that seasonal environmental conditions varied in more or less the same fashion during both time periods.

The δ¹⁸O_sc pattern of the pre-LGM RAM 5T contains an abrupt increase, suggestive of a change in water source to one enriched in ¹⁸O, perhaps because of evaporative enrichment or domination by summer precipitation. This sample’s δ¹⁸O_sc pattern of variation is tracked moderately well by its variation in δ¹³C_sc of 2.0 ‰. The somewhat larger variability of RAM 5T δ¹³C_sc values (compared to other pre-LGM horses, Table 3.4) may indicate that it occupied a larger home range or consumed a more varied diet. However, such a range in enamel δ¹³C_sc values may also reflect annual variations in C₃ plant δ¹³C values. For example, variations of ±1.5 ‰ can be produced over the growing season because of changes in weather conditions and/or changes in plant physiology (Heaton, 1999; Sharp & Cerling, 1998 and references therein).

Pre-LGM serial samples from RAM 6T, 7T, and 9T have δ¹⁸O_sc patterns similar to RAM 5T, in that they are irregular and exhibit abrupt changes. This may reflect short-term seasonal climate changes (e.g. Brookman & Ambrose, 2012), consumption of different drinking water sources at different times because of a larger home range (and poorer habitat quality), and/or consumption of drinking water that did not mirror the oxygen isotopic compositions of local precipitation. RAM 6T, 7T, and 9T exhibit ranges in carbon isotopic composition that were unaccompanied by recognizable patterns in δ¹⁸O_sc. The δ¹³C_sc values of these horses fall within the range of annual variations in C₃ plant δ¹³C values. Only two datapoints are available for pre-LGM RAM 8T, with a range of 2.1 ‰ for δ¹⁸O_sc and 0.2 ‰ for δ¹³C_sc.

Post-LGM sample RAM 2T is the only horse to display a well-developed sinusoidal variation in its δ¹⁸O_sc and hence its inferred δ¹⁸O_dw values (Table 3.4, Fig. 3.3). Such patterns are typical of mid- to high-latitude precipitation. High δ¹⁸O_dw values
correspond to summer precipitation and low values correspond to winter precipitation (Rozanski et al., 1993). The seasonal differences at mid- to high-latitudes are caused by several factors, including seasonally changing temperature, humidity, and air masses (Rozanski et al., 1993). If this horse's drinking water was mainly controlled by precipitation input, then this should be reflected in the enamel’s oxygen isotopic composition, which appears to be the case. RAM 2T's δ^{18}O_{sc} curve spans a period of ~15 months (Table 4.2), with two summer highs and one winter low. In addition, RAM 2T has a δ^{13}C_{sc} range of 1.5 ‰, which is within the range of annual variations in C_{3} plant δ^{13}C values. For these reasons, RAM 2 likely had a small home range with a water source dominated by precipitation input. Had RAM 2 occupied a large home range, it would have imbibed a variety of water sources with isotopic compositions not necessarily dominated by seasonal signals.

Pre-LGM RAM 10T has a somewhat sinusoidal pattern of δ^{18}O_{sc} values, with one possible summer high and a winter low. These data are interpreted to indicate that this horse drank from a more 'constant' water source dominated by precipitation input during enamel mineralization, as was the case for RAM 2T. This pattern of δ^{18}O_{sc} values, combined with the relatively low range of measured δ^{13}C_{sc} values (1.4 ‰; similar to RAM 2T), suggests that RAM 10 had a relatively small home range.

The pattern of δ^{18}O_{sc} variation in post-LGM sample RAM 1T shows a weak inverse relationship with its δ^{13}C_{sc} values. This horse likely drank from a water source (i.e. river, lake, etc.), which was not highly responsive to seasonal changes in isotopic composition (Metcalfe, 2011 and references therein). Post-LGM RAM 4T has an irregular δ^{18}O_{sc} pattern similar to RAM 5T. RAM 1T and 4T have a low range of δ^{13}C_{sc} values (0.6 ‰ for both), suggesting that these individuals occupied a relatively small home range or consumed a restricted diet during enamel formation. Low δ^{13}C_{sc} enamel variability was also observed in Late Pleistocene horses from California by Feranec et al. (2009), who concluded that these horses had a more restricted range in habitat or plant choice compared to bison from the same general area. The extent of variation in δ^{13}C_{sc} values, however, doesn't necessarily correlate to home range size. As discussed earlier, RAM 2T (which had a sinusoidal δ^{18}O_{sc} pattern, interpreted to indicate a 'constant' water source) displays a larger range in δ^{13}C_{sc} values compared to RAM 1T and 4T but likely
also occupied a small home range (see above). Furthermore, the serially sampled $\delta^{13}C_{sc}$ values for all horses fall within the range of annual variations expected for a $C_3$ environment, and the similarity in $\delta^{13}C_{sc}$ and $\delta^{13}C_{col}$ values before and after the LGM suggest that the horses were eating similar plants that grew under very similar conditions. RAM 3T (which does not have an associated date) has an irregular $\delta^{18}O_{sc}$ pattern, like RAM 5T, and exhibits a low range in $\delta^{13}C_{sc}$ values (0.7 ‰) similar to RAM 1T and RAM 4T.

Modern Edmonton has a seasonal range in meteoric water $\delta^{18}O$ values of $-28.0$ to $-12.6$ ‰ with an average value of $-17.1 \pm 0.9$ ‰ (IAEA/WMO, 2010). The calculated serially sampled $\delta^{18}O_{dw}$ values, by comparison, display a much smaller range (see above). The smaller ranges and/or poor sinusoidal $\delta^{18}O_{dw}$ patterns determined for these Pleistocene horses (and for serially sampled teeth more generally) could have several origins. One possibility is consumption of water from lakes or groundwater springs, which average precipitation inputs and outputs over the residence time of these reservoirs. Another is that the horses drank from multiple water sources with different oxygen isotopic compositions. Perhaps of most importance is the averaging of isotopic compositions that occurs during enamel mineralization. The sampling approach is critical in this regard (Metcalfe et al., 2011; Metcalfe & Longstaffe, 2012). While innermost enamel was sampled here in an attempt to limit damping of the isotopic signal, this approach required long drill lines along the length of the enamel in order to obtain enough material for analysis, resulting in fewer serial samples overall. Finally, the absence of higher $\delta^{18}O_{dw}$ seasonal values closer to those present in the modern precipitation record could indicate overall cooler climatic conditions (compared to modern conditions) for the Pleistocene horses, with less pronounced seasonality. This would yield lower average precipitation $\delta^{18}O$ values and less variation among them over the seasonal cycle compared to the modern Edmonton region.

4.4.4 Structural carbonate-collagen carbon isotope relationship

The nature of the $\Delta^{13}C_{sc-coll}$ relationship in bone is complex (Loftus & Sealy, 2012). Lee-Thorp et al. (1989) analyzed the carbon isotopic composition of bone collagen and structural carbonate from southern African fauna. They interpreted the patterns in
bone $\Delta^{13}C_{\text{sc-col}}$ spacings to be related to trophic level differences, with herbivores having a value of $+6.8 \pm 1.5 \%$. According to Ambrose et al. (1997), $\Delta^{13}C_{\text{sc-col}}$ spacings $>+4.4 \%$ indicate that the $\delta^{13}C$ value of dietary protein is more negative than whole diet, whereas spacings $<+4.4 \%$ indicate that dietary protein is less negative than whole diet. It has also been shown (e.g. Tieszen & Fagre, 1993; Clementz et al., 2009) that $\Delta^{13}C_{\text{sc-col}}$ spacings are useful for understanding diet quality (i.e. lipid and protein content). In addition, Clementz et al. (2009) found that herbivore $\Delta^{13}C_{\text{sc-col}}$ spacings in bone were consistent spatially and temporally, having a value of $+7.6 \pm 0.5 \%$. The $\Delta^{13}C_{\text{sc-col}}$ spacing of the modern horse analyzed here ($+7.8 \%$) is consistent with this observation (Table 3.3). The average bone $\Delta^{13}C_{\text{sc-col}}$ spacing for the Pleistocene horses is $+9.6 \pm 1.0 \%$ (SD) (Table 3.3), similar to that obtained for mammoths from Alberta ($+9.6 \pm 0.2 \%$, n=7) (Metcalfe, 2011). Pre- and post-LGM horses have average $\Delta^{13}C_{\text{sc-col}}$ values of $+9.4$ and $+10.1 \%$, respectively. The post-LGM horses have a higher $\delta^{13}C_{\text{sc}}$ value (the $\delta^{13}C_{\text{col}}$ values from both time periods were identical, see above), perhaps suggesting that their bulk diet contained less lipids and protein and was of somewhat lower quality (Ambrose & Norr, 1993; Tieszen & Fagre, 1993; Iacumin et al., 2000; Clementz et al., 2009). That said, both pre- and post-LGM $\Delta^{13}C_{\text{sc-col}}$ spacings are typical of large herbivores in the wild (~$+7$ to $+10 \%$) (Sullivan & Krueger, 1981; Lee-Thorp et al., 1989; Bocherens et al., 1994; Iacumin et al., 2000).

### 4.4.5 Collagen $\delta^{15}N$ values

As previously described, higher $\delta^{15}N_{\text{col}}$ values are associated with the pre-LGM horses and lower $\delta^{15}N_{\text{col}}$ values are associated with the post-LGM horses (Fig. 4.4), and low post-LGM $\delta^{15}N_{\text{col}}$ values have also been reported from elsewhere (see section 4.4.1). Decreases in soil and plant $\delta^{15}N$ values are known to accompany increased precipitation and soil moisture availability (e.g. Austin & Vitousek, 1998; Amundson et al., 2003; Szpak et al., 2013). Accordingly, Iacumin et al. (2000) suggested that low $\delta^{15}N_{\text{col}}$ values of reindeer in Siberia reflected increased precipitation after the LGM and that melting ice sheets and permafrost increased the amount of soil moisture in areas adjacent to the glaciers. Richard and Hedges (2003) proposed that low $\delta^{15}N_{\text{col}}$ values of horses and deer from northwest Europe arose from a combination of changing soil organic $\delta^{15}N$ values
during cold periods (because of increased retention of $^{15}$N-depleted volatile compounds), and low $\delta^{15}$N values in soils that were undergoing regeneration after deglaciation. The $\delta^{15}$N$_{col}$ values of a consumer are mainly determined by their diet, which in the case of horses refers to the type of plants eaten (additional factors such as stress can also influence $\delta^{15}$N values, e.g. Fuller et al., 2005; see section 1.2.1). Stevens and Hedges (2004) reviewed the many factors that can lead to low plant $\delta^{15}$N values (and therefore $\delta^{15}$N$_{col}$ values), and concluded that many might be at play, including a delay in the response of plants and bone collagen to deglaciation, post-LGM soil development, and increased water availability.

Because of the possible influence of plant $\delta^{15}$N values on $\delta^{15}$N$_{col}$ values, it is helpful to review processes that cause changes in plant $\delta^{15}$N values. Most plants (with the exception of legumes, which live in symbiosis with bacteria attached to their roots) do not utilize atmospheric nitrogen directly; rather, they rely on soil inorganic nitrogen (Stevens & Hedges, 2004 and references therein; Ambrose, 1993). Most of this nitrogen comes from soil nitrates, ammonia, ammonium, and animal urea (Ambrose, 1993). Some of these products are produced by soil microbial processes (as part of the nitrogen cycle), which fractionate $^{15}$N. The main soil transformations produced by microbes include mineralization, nitrification, and denitrification (Dawson, 2002 and references therein). These processes lead to soil products that have low $\delta^{15}$N values relative to the soil in which they were produced (Dawson, 2002 and references therein).

Is it possible that an increase in nitrification and denitrification processes (which produce N$_2$O) following the LGM caused lower soil and consequently lower plant $\delta^{15}$N values? Atmospheric concentrations of N$_2$O tend to be higher in warm intervals (e.g. interglacials) than in colder periods (e.g. LGM) (Wolff & Spahni, 2007 and references therein; Flückiger et al., 2004). The increase in soil disturbance from LGM ice sheet advance and retreat (freeze/thaw) could have led to increased N$_2$O production (which would lower soil and plant $\delta^{15}$N values). For example, Christensen and Christensen (1991) observed that freeze/thaw cycles increased denitrification in soils. However, identifying these processes as the cause for low $\delta^{15}$N plant (and therefore collagen) values might oversimplify the different nitrogen transformations involved in the nitrogen cycle, which vary geographically and with different environmental conditions (Dawson et al.,
2002 and references therein). For example, modern terrestrial sources of N2O in high latitudes are relatively small, and can be assumed to have been even less significant during glacial times; temperature and precipitation changes can also strongly influence the source strength in these regions (Flückiger et al., 2004 and references therein).

In the Arctic, δ¹⁵N values can vary with plant type. For example, spruce trees, evergreen shrubs, and deciduous grass in Alaska can have a δ¹⁵N value of −7.7, −4.3, and +0.9 ‰, respectively (Schulze et al., 1994; Bocherens, 2003; Ben-David, 2001). This pattern of δ¹⁵N values among vegetation types suggests the possibility that the horses incorporated more browse into their diets as a result of changing vegetation in the region or as a means to compensate for poorer food availability/quality proximal to the ice sheets. However, such an explanation is not well supported by the very similar structural carbonate and collagen δ¹³C values measured for the pre- and post-LGM horses (see section 4.4.2). Also, as Stevens and Hedges (2004) pointed out, deer, reindeer, and horses in Eurasia exhibited similar ¹⁵N-depletion; if diet was the cause they all would have had to choose similar diets and foods with low δ¹⁵N values, which seems unlikely. In summary, the low post-LGM horse δ¹⁵N values may reflect low plant δ¹⁵N values, which can be attributed to a combination of the factors listed above, as well as other factors that affect the nitrogen cycle, including permafrost activity, nutrient availability in the soil, and microbial activity. A comprehensive study of Pleistocene δ¹⁵N plant values is needed to evaluate their role in determining the δ¹⁵N values reported here for the pre- and post-LGM horses from Alberta.
Chapter 5
Conclusions and Future Work

5.1 Major Conclusions
1. Coeval bone and tooth enamel in the modern horse sample show no significant differences between these tissues for each of $\delta^{13}C_{sc}$, $\delta^{18}O_{sc}$, and $\delta^{18}O_p$ values. In other words, bioapatite carbon and oxygen isotopic signatures are recorded in almost the same way in bone and tooth enamel from this animal. This observation implies that tissue-dependent physiological and/or metabolic processes do not cause differences between bone and tooth oxygen or carbon isotopic compositions in horses. Since bone and tooth enamel record the diet and drinking water isotopic signals in virtually identical fashion, differences in $\delta^{13}C_{sc}$, $\delta^{18}O_{sc}$, and/or $\delta^{18}O_p$ values between these tissues can be used to infer migration behaviour and diet changes in horses.

2. Previously determined relationships between $\delta^{18}O_{sc}$ and $\delta^{18}O_p$ values in mammalian bioapatite have been used to identify post-mortem alteration in the Pleistocene horse samples from Alberta. Together, $\Delta^{18}O_{sc-p}$ spacings of $<+7$‰ (following Pellegrini et al., 2011) and an array of $\delta^{18}O_p$ versus $\delta^{18}O_{sc}$ values with a slope of $<1$ (following Zazzo et al., 2004b) suggest that microbially mediated post-mortem alteration may have affected the bioapatite phosphate component of most bone and some tooth samples. Most tooth samples have likely retained primary $\delta^{18}O_{sc}$ values, as indicated by their CI values, and for a few samples, by variations in the $\delta^{18}O_{sc}$ values of serially sampled enamel that match patterns expected for seasonal variation.

3. The $\delta^{18}O_{dw}$ water values calculated for Alberta using bulk enamel phosphate and structural carbonate oxygen isotopic compositions are similar for the Pleistocene horses from both before and immediately after the Last Glacial Maximum (LGM). This observation suggests that the horses drank from sources with very similar oxygen isotopic compositions. The broader implication is that climatic conditions controlling the oxygen isotopic composition of meteoric water were similar during the lifetimes of the pre- and post-LGM horses.
4. The bulk $\delta^{13}C_{\text{diet}}$ values calculated from enamel structural carbonate compositions of all Pleistocene horse samples examined here are very similar between bone and tooth tissues, as is also the case for the $\delta^{13}C_{\text{diet}}$ values calculated from bulk collagen compositions. All of these measures indicate consumption exclusively of $C_3$ plants and also suggest that horse diets did not vary significantly between infancy and adulthood. There is also very little difference in these carbon isotopic compositions between pre- and post-LGM horse samples. This indicates ecological conditions yielding very similar carbon isotopic compositions for grazed vegetation during both time periods.

5. The $\delta^{18}O_{\text{sc}}$ values of most serial samplings of the Pleistocene tooth enamel exhibited irregular variation, interpreted to indicate larger home ranges and/or consumption of varied drinking water sources by these horses. The $\delta^{18}O_{\text{sc}}$ values for a few serially sampled teeth are consistent with seasonal variations in drinking water expected within a smaller home range. Serially sampled $\delta^{13}C_{\text{sc}}$ values display the range of variation typical for a $C_3$ environment.

6. The post-LGM horses have much lower $\delta^{15}N_{\text{col}}$ values than the pre-LGM horses. This difference may indicate lower plant $\delta^{15}N$ values following the LGM, perhaps arising from a significant change in the parameters controlling the nitrogen cycle following the LGM. Regardless of its origin, this shift in $\delta^{15}N_{\text{col}}$ values may provide a coarse screening test for distinguishing between horses (and perhaps other megafauna) that lived before and after the LGM.

5.2 Future Work

To use equid enamel and bone tissue $\delta^{18}O_{\text{sc}}$, $\delta^{18}O_{p}$, and $\delta^{13}C_{\text{sc}}$ values in reconstruction of Pleistocene (and other) environments, it is important to verify first whether or not these isotopic signals are recorded free of differences arising from metabolic or physiological processes during formation of each tissue. This has been shown to be the case for the one modern horse specimen here. However, to extend this finding more broadly with real confidence requires a much larger sample population. Ideally, the isotopic composition of drinking water and diet for domestic horses would be
monitored in a controlled setting from birth to death. Such controlled studies are also needed for other large animals that can serve as models for isotopic systematics in other Pleistocene megafauna (>44 kg).

Preservation of primary isotopic compositions in bone and enamel is necessary for these tissue signatures to be useful in palaeoenvironmental reconstruction. Given the possibility of post-mortem alteration of bioapatite phosphate and/or structural carbonate components in some of the horse bone and tooth samples analyzed here, it would be helpful to identify more directly the extent and mechanism(s) (i.e. inorganic or microbial) of alteration. A good starting point is the use of scanning electron microscopy to identify physical traces of microbial activity such as tunneling or tissue reworking (Tütken et al., 2008, and references therein). Such observations could then be followed by in situ isotopic analysis of affected versus unaffected areas at the μm level, such as described by Brady et al. (2008).

Given the very large shift in nitrogen isotopic compositions observed between the pre- and post-LGM horses, it would be of great interest to study the influence of environmental parameters on plant (and faunal) δ¹⁵N values. In particular, detailed investigations are recommended to determine: (i) how changing temperature and moisture conditions directly affect plant and herbivore δ¹⁵N values, and (ii) how nitrogen isotopic fractionations in the water-soil-plant nitrogen cycle may have changed from pre-to post-LGM times.

To further understand the Late Pleistocene environment of Alberta, more studies are warranted of the stable isotopic composition of temporally and geographically related megafauna. The stable isotopic compositions of a suite of well-dated megafauna – spanning time from before the LGM into the Holocene – would provide more detailed description of terrestrial environmental change. Stable carbon isotopic analysis of a variety of megafauna, in particular, would capture the range of dietary items available to contemporary animals and hence the ecological variability of the landscape. Oxygen and carbon isotopic analysis of serially sampled enamel from a large population of herbivores during this time span would also provide a more robust characterization of seasonal climatic patterns and vegetation availability.


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