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Spatial and temporal variations in the isotopic composition of bison tooth enamel from the Early Holocene Hudson–Meng Bone Bed, Nebraska

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Abstract

The Hudson–Meng bone bed in northwestern Nebraska is an early Holocene deposit of hundreds of bison of differing ages that were killed in a catastrophic event of unknown origin. The M_1, M_2, and M_3 molars of individuals, ranging from ca. 1 to 7 years of age at the time of their death, were examined to determine inter- and intra-tooth isotopic variability, and to link this variability to time in order to better understand the environments that existed prior to the mass death event. The $\delta^{13}C$ value of molar structural carbonate increases by 2–3‰ from the M_1 to M_3 molars, reflecting increasing direct forage signals, and decreasing maternal influences. The $\delta^{18}O$ value of molar structural carbonate in a given individual shows no consistent trend with time from birth, indicating a fairly direct linkage to 'dietary water' regardless of tooth ontogeny. Detailed 'down-tooth' isotopic measurements indicate a small seasonal signal in the $\delta^{13}C$ value of forage/maternal milk, which appeared to have been largely dominated by C_4 flora. In contrast, there was almost a 10‰ range in the isotopic composition of dietary water, a range that is within present-day summer versus winter precipitation in the region. The $\delta^{18}O$ value of bulk tooth enamel, arranged by approximate time prior to the death event, indicate a consistent increase in the $\delta^{18}O$ value of body water (2–3‰), suggesting a long-term decrease in winter/spring precipitation and/or drought. The $\delta^{18}O$ trend with time, coupled with the high C_4 grass abundance, suggest environmental stresses on the herd preceding the catastrophic death event. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The isotopic composition of carbonate in mammalian tooth enamel has been the focus of numerous publications on paleodiet and paleoecology of large grazing herbivores (e.g. MacFadden et al., 1994; Tieszen, 1994; Wang et al., 1994; Quade et al., 1995; Kohn et al., 1996; MacFadden and Cerling, 1996). In general, the $\delta^{13}C$ values of structural carbonate in enamel apatite indicate the relative abundance of C_4 grass in a herbivore's diet (Wang et al., 1993; Quade et al., 1994) and by inference, the plant ecology of paleoenvironments (Cerling et al., 1993). Similarly, the oxygen isotope ratio of enamel carbonate or phosphate has potential to provide information on isotopic composition of surface water and surface temperatures in the paleoenvironments (Longinelli, 1984;

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Luz et al., 1984; Luz and Kolodny, 1985). There is a growing recognition of complexity in this isotopic record, and this paper addresses aspects of developmental complexity and what it can additionally tell us about past environmental settings.

There is a growing appreciation of significant spatial enamel isotopic variation within a given individual:

1. between teeth: the $\delta^{18}O$ value of single teeth from different positions in the jaw of single individuals shows ranges in phosphate $\delta^{18}O$ values because of seasonal water intake, type of diet, differences in water conservation capabilities of different animals, body size and other factors (e.g. Koch et al., 1989, Bryant et al., 1996a,b; Fricke and O'Neil, 1996; Stuart-Williams and Schwarcz, 1997);
2. within teeth: Pioneering studies by Sharp and Cerling (1996), Cerling and Sharp (1996) and Fricke and O'Neil (1996) revealed significant 'down-tooth' isotopic variability linked to the long time span (and changing diet) encompassed in the molars of many large herbivores.

These aggregate studies show that proper interpretation of enamel isotopic ratios in fossil teeth requires knowledge of both the tooth type and its ontogeny.

The Hudson–Meng bison bone bed in northwestern Nebraska is an early Holocene deposit containing the skeletal remains of up to 1000 bison individuals, all of whom died at the same time (Todd and Rapson, 1993). In this paper we use this deposit to:

1. amplify our understanding of between- and in-tooth isotopic variability (focusing here on bison); and
2. use this isotopic data to illuminate aspects of the events that led to the sudden, and unexplained, deaths of such a large number of individuals.

2. Background

2.1. Carbon isotope systematics of diet and tooth enamel

The present-day Great Plains are characterized by a mixture of C_3 and C_4 grasses (Sims et al.,

1978; French, 1979; Boutton et al., 1980; Tieszen, 1994) which bison consume indiscriminately (Peden et al., 1974; Schwartz and Ellis, 1981). Growing season temperature and moisture are predominant factors affecting the proportion of C_3 to C_4 flora in a given region (Boutton et al., 1980). Seasonal studies show early spring and late fall predominance of C_3 grasses while the C_4 grasses are most dominant in mid summer (Tieszen, 1994). A strong correlation has been found between relative abundance of C_4 grass species and minimum temperatures during the growing season (Terri and Stowe, 1976), which allows paleoclimate information to be determined from the C_4 abundance in dietary proxies such as the $\delta^{13}C$ value of tooth enamel.

The difference in the $\delta^{13}C$ values of C_3 (a $\delta^{13}C$ value of $-26.5\pm2.5‰$) and C_4 grasses (a $\delta^{13}C$ value of $-12.5\pm1.2‰$) (Tieszen et al., 1983) is the basis for using bison diet, as estimated from the $\delta^{13}C$ values of enamel, as a means of determining paleoenvironmental information. It has been observed that the $\delta^{13}C$ value of tooth enamel is ca. 12‰ more positive than the animal's dietary intake (e.g.Wang et al., 1994; Morgan et al., 1994).

2.2. Oxygen isotope systematics of surface water and enamel

The weighted annual $\delta^{18}O$ value of global precipitation is generally correlated with mean annual surface temperature (e.g. Dansgaard, 1964; Rozanski et al., 1993), although on a more local scale, other factors such as the source of the precipitation may have an major influence on precipitation values (e.g. Amundson et al., 1996). The $\delta^{18}O$ value of body water of animals is determined by the isotopic composition, and fluxes, of oxygen into and out of the body, the most important of which is oxygen in ingested water (liquid and plant water) (Luz et al., 1984; Luz and Kolodny, 1985; Bryant and Froelich,1995; Bryant et al., 1996a, b; Kohn, 1996). It is likely that water in grass leaves is an important contributor to bison body water because bison on the Great Plains ingest a substantial amount of plant material, and leaf water has higher $\delta^{18}O$ values than that of other surface water reservoirs (Dongmann et al.,

1974; Epstein et al., 1977; Burke and Stuiver, 1981; Sternberg, 1989; Yakir, 1992). However, we know of no studies performed on the body water oxygen budget of freely ranging bison, so the quantitative linkage between body water and precipitation remains unknown.

2.3. Bison tooth development

Mammalian tooth enamel has been shown to form continuously over several years (Hillson, 1986; Dean, 1987; Aiello and Dean, 1990; Bromage, 1990; Huda and Bowman, 1995). This results in a high-resolution 'down-tooth' record of changing body $\delta^{13}C$ and $\delta^{18}O$ values driven by seasonal changes in water and carbon intake. In order to accurately interpret seasonal trends via isotope analysis, it is imperative to understand enamel formation patterns for different specific teeth of bison.

Bison exhibit reproductive synchronicity: their birthing season occurs in spring and is a tightly constrained and predictable event (Berger and Cunningham, 1994). Thus, isotopic data from the same type of tooth in different individuals of the same biological age are very likely to reflect the same seasonal periods. Seasonal eruption and wear patterns for cattle, and bison in particular, have been studied (Brown et al., 1960; Frison and Reher, 1970; Haynes 1984; Wegrzyn and Serwatka, 1984), and the time frame for enamel development in bison molars was further clarified in preparation for this study. The growth patterns of enamel crowns for bison mandibular molars (Fig. 1) were determined from two sources. First, an initial timeline of enamel crown development was outlined using bison tooth eruption and wear patterns as documented by Great Plains archaeologists. The researchers have aged animals and determined season of death using measurements of different individual teeth from bison bone beds, as well as teeth from large numbers of animals butchered in modern bison herds (Frison and Reher, 1970; Frison and Stanford, 1982; Frison and Todd, 1987; Todd et al., 1990). Additionally, 61 radiographs were taken from a University of Wyoming collection of 35 individual modern bison mandibles of known age at death. The crown enamel on these
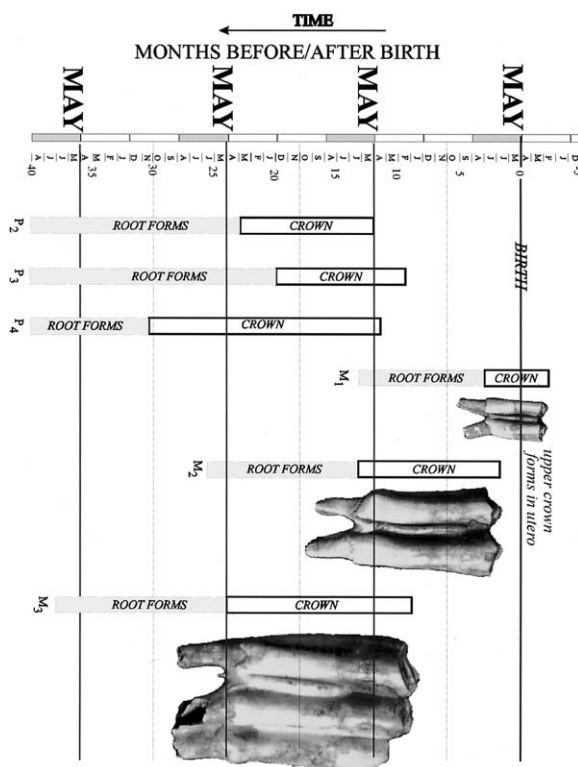

Fig. 1. Modern bison enamel formation timeline (see text for discussion). Enamel growth begins at the top of the crown and proceeds to the root in all teeth. Months begin with December (D) at 5 months prior to birth. The M_1 molar beings formation en utero beginning in February (F). Along with the timeline for mandible molars, premolars (P_2, P_3, P_4) are also presented.

specimens was identifiable via radiographs which established enamel growth patterns from fetal specimens to 2 year olds. In this work, we made observation based on knowledge that teeth grow sequentially from the top of the crown downwards to the cementum–enamel junction (CEJ). Enamel is biomineralized daily and is not remetabolized like other components such as bone collagen. Microscopic cross-striations in the enamel crown mark the daily and weekly variation in this enamel matrix excretion.

The results of these observations (Fig. 1) suggest that the formation of the M_1 molar enamel crown occurs partially en utero and is completed several months after birth. The M_2 molar enamel crown forms between the time of birth and ca. 13 months of age. The M_3 molar forms between

Table 1
Isotopic composition of various bulk samples and subsamples from all individuals[a]

Individual	Side	Tooth	Cusp	Sample	$\delta^{13}C$	$\delta^{18}O$	Time (months)
Modern	L	M_2	Meta	1	−11.5	22.1	
				2	−11.6	24	
				3	−11.7	23.6	
				4	−10.9	23	
				5	−10.4	22.1	
				6	−10.1	21.1	
				7	−9.8	19.4	
				8	−9.5	19.4	
730-44							
Cohort 2	L	M^1	Meta	Bulk	−7	23.5	13
				CEJ1/3	−6.9	20.2	
		M_2	Meta	Bulk	−5.9	23.8	2
				CEJ1/3	−11.4	20.1	
				MID1/3	−5.9	23.3	
				OCC 1/3	−7.7	20	
				1	−6.4	22.2	
				2	−6.7	19	
				3	−6.7	19.5	
				4	−6.4	21.3	
				5	−6.1	22.2	
				6	−5.3	25.3	
				7	−3.7	28.6	
				8	−5	27.5	
226-14a							
Cohort 3	L	M_1	Meta	Bulk	−8	21.9	25
				CEJ 1/3	−7.6	19	
		M_2	Meta	Bulk	−6.2	21.5	14
				CEJ 1/3	−4.5	24.5	
		M_3	Meta	Bulk	−5.3	23.5	4
				CEJ 1/3	−5.7	25.5	
212-31							
Cohort 3	L	M_1	Meta	Bulk	−7.5	23.3	25
		M_2	Meta	Bulk	−6	22.6	14
				CEJ 1/3	−3.5	27.2	
		M_3	Meta	Bulk	−4.7	23.9	4
				CEJ 1/3	−4.8	24.3	
I84-23-160							
Cohort 4	L	M_1	Meta	Bulk	−8	22.5	37
				CEJ 1/3	7.6	18.8	
		M_2	Meta	Bulk	−5.9	22.6	26
				CEJ 1/3	−5.7	23.5	
		M_3	Meta	Bulk	−5.2	22.1	16
224-172							
Cohort 5	L	M_1	Meta	Bulk	−6.8	23.6	49
				CEJ 1/3	−6.9	19.4	
		M_2		CEJ 1/3	−5.6	21.8	
		M_3	Meta	Bulk	−5.5	21.5	28
				CEJ 1/3	−5.2	24.5	

Table 1 (*continued*)

Individual	Side	Tooth	Cusp	Sample	$\delta^{13}C$	$\delta^{18}O$	Time (months)
145.8							
Cohort 7	L	M_1	Meta	Bulk	−6.6	19.8	73
			Ento	Bulk	−6.5	19.4	
		M_2	Meta	Bulk	−5.2	22.8	
				CEJ 1/3	−5.9	18.1	
			Ento	Bulk	−5.6	23.1	
		M_3	Meta	Bulk	−5.1	21.4	
				CEJ 1/3	−4.7	23	
			Entob	Bulk	−5	21.1	
			Hypo	Bulk	−5.2	21.3	
	R	M_1	Meta	Bulk	−7.3	17.9	
				CEJ 1/3	−6.9	19	
			Ento	Bulk	−6.8	19.3	
		M_2	Meta	Bulk	−5.7	22.5	
				CEJ 1/3	−5.6	21.4	
			Ento	Bulk	−5.6	23	
		M_3	Meta	Bulk	−4.7	22.4	
			Ento	Bulk	−5.1	21.4	
			Hypo	Bulk	−5.4	21	

[a] Location of samples are listed by tooth cusps which are: meta, metaconid; ento, entoconid; hypo, hypoconulid (see Fig. 1) (CEJ, cementum–enamel junction third subsample; MID, middle third subsample; OCC, occlusal third subsample; and samples listed as 1–8 are the eighth subsamples). Time is in months from completion of enamel crown until time of herd death.

nine months and ca. 2 years of age. Therefore, the data available suggest that the enamel of the M_1, M_2, and M_3 of a bison represent a continuous record of bison carbon and oxygen in diet from en utero to 2 years of age.

3. Materials and methods

3.1. Fossil samples

The fossil bison teeth analyzed in this study were excavated from the Hudson–Meng (25SX115) bison bonebed located in the southern half of the Oglala National Grasslands in north-eastern Sioux county, northwest Nebraska (Miller, 1994). The bonebed contains the remains of 500–1000 bison and has yielded ^{14}C dates of 9500 B.P. (Todd and Rapson, 1993). All individuals died at the same time although the cause of death remains enigmatic (Todd and Rapson, 1993). Evidence from patterns of wear on teeth and from tooth eruption patterns on calf mandibles (Frison and

Reher, 1970; Frison and Stanford, 1982; Frison and Todd, 1987; Frison, 1991; Todd et al., 1990) indicate that the herd died in late summer (Todd and Rapson, 1993).

Left mandibular molars from five individuals as well as right and left mandibular molars from a sixth individual were collected and sampled. By convention, animals in a herd are classified into cohort groups with animals born in the most recent spring defined as cohort group 1. The animals in this study comprise cohort groups 2 (youngest) through 7 (oldest) (Table 1). Cohort group 1 individuals were present in the bison bed, but none were available for this study. Two of the animals were the same age at death (cohort group 3). The animals ranged in age from 1.3 to ca. 7.3 years of age at the time of their death. Age of death was determined by tooth wear/eruption patterns. As expected from a normal aging process, wear increased from the 1st to the 3rd molar. Similarly, the teeth of older animals are more worn than for younger ones. The height/length of each tooth is given in Table 2.

Table 2
Measurements (in mm) of molar crown attributes of teeth used for isotopic analyses (BK indicates broken teeth)

Individual	Molar No.	Metaconid height	Entoconid height	Saddle height	Occlusal width mesial	Occlusal width distal	Occlusal surface length	Ectostylid to wear
730-44	M1	46.5	49.0	42.0	12.0	13.0	31.5	9.0
Cohort group 2	M2	66.5	67.5	60.5	12.0	12.0	37.5	22.0
(1.3 years)	M3	NA	NA	NA	NA	NA	NA	NA
226-14a	M1	BK	BK	BK	BK	BK	BK	10.0
Cohort group 3	M2	63.9	66.0	58.0	15.0	14.0	38.5	16.0
(2.3 years)	M3	66.0	62.0	59.5	13.5	14.5	44.5	19.0
212-31	M1	39.4	41.6	35.9	13.1	13.8	28.6	1.5
Cohort group3	M2	56.7	58.1	51.0	14.4	13.4	37.4	11.5
(2.3 years)	M3	63.8	61.5	52.3	11.7	11.4	44.6	15.1
I84-23-160	M1	35.0	38.0	29.0	15.0	14.5	33.0	00.0
Cohort group 4	M2	53.0	56.0	47.0	14.5	15.0	37.0	4.6
(3.3 years)	M3	62.0	62.5	55.0	14.0	13.0	44.5	15.0
224-172	M1	27.5	31.5	23.5	15.0	BK	27.5	00.0
Cohort group 5	M2	43.0	46.0	42.0	15.5	16.0	36.0	00.0
(4.3 years)	M3	59.0	60.5	52.0	15.0	14.5	47.5	9.5
145-8 right side	M1	BK	26.0	15.5	BK	BK	BK	00.0
Cohort group 7	M2	42.2	45.5	37.5	15.0	16.0	33.5	00.0
(6.3 years?)	M3	48.0	53.5	46.5	15.5	14.0	44.0	6.0
Left side	M1	21.5	21.6	15.7	17.0	15.5	BK	00.0
	M2	38.0	40.0	32.5	17.0	15.5	33.5	00.0
	M3	50.6	53.0	48.0	15.0	15.0	44.5	4.0

3.2. Modern samples

In order to compare to fossil samples, one left mandibular molar from a modern bison was collected and sampled. This animal, part of the Fort Robinson herd located ca. 20 miles SE of Hudson–Meng, was a year old when it died. Therefore, it was similar in age to the cohort group 2 individual from Hudson–Meng fossil bed. Despite this age similarity, the Fort Robinson individual is not an ideal isotopic proxy for modern ecological conditions because its supply of food and water has been altered by human activity. For example, pasture forage at Fort Robinson is regularly supplemented in winter months by a feed mix of hay (alfalfa, millet, oats, sudex) and corn while drinking water comes from both surface reservoirs and from a windmill that supplies ground water year round.

3.3. Sampling strategy

From the large number of fossil specimens, the following isotopic comparisons/observations were made:

1. isotopic variation, in bulk enamel, between M_1, M_2, and M_3 molars;
2. isotopic variation, in enamel just above the CEJ (i.e. just above gum line), between M_1, M_2, M_3 molars and between the bulk samples from each molar;
3. isotopic variation between left and right molars in a given individual;
4. 'down-tooth' isotopic variability in the M_2 molar from the fossil and modern cohort 2 individuals; and
5. isotopic variability in bulk enamel with time preceding an individual's death.

Bison molars exhibit cusps (i.e. metaconid, entoconid, hypoconulid) as discrete sections of a tooth that were used for sampling purposes. Fig. 2 illustrates the types of samples taken from the bison molars used in this study. All 20 fossil teeth were sampled lengthwise on the metaconid cusp for a 'bulk sample'. The bulk sample consists of a 2–3 mm wide vertical strip of enamel that spanned the entire height of the tooth (down to the gum line). Bulk samples were also taken lengthwise for
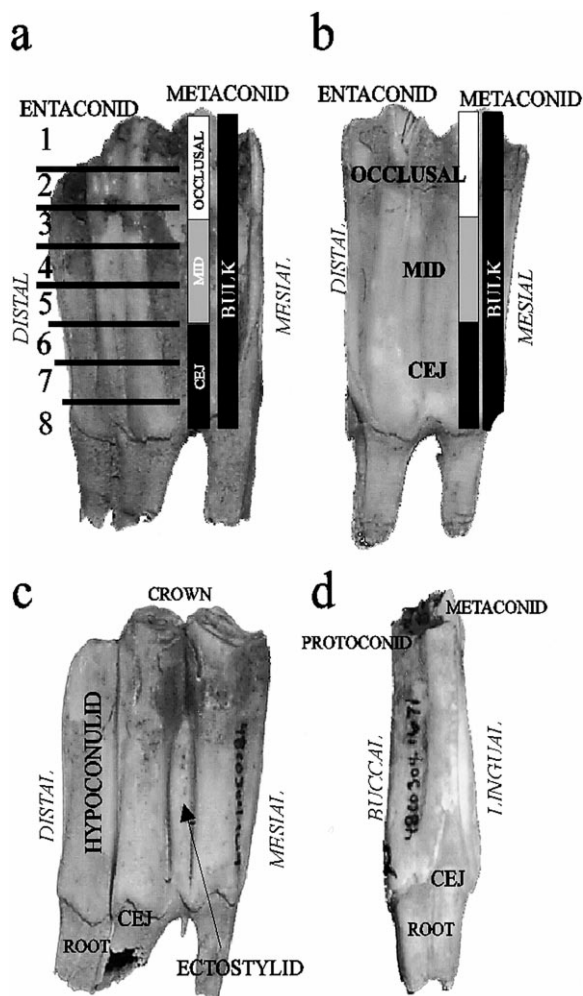
Fig. 2. Tooth terminology and illustration of different enamel sub-samples examined in this study: (a) lingual side of a left M_2 with enamel sub-samples identified; the entoconid and metaconid cusps are also identified, (b) lingual side of a left M_1 with identification of additional enamel sub-samples, (c) buccal side of a right M_2 with root and CEJ identified; the hypoconulid cusp is also identified, (d) left M_2, distal view.

several additional cusps on both right and left mandibular molars of the oldest animal (entoconid and hypoconulid; see Fig. 2) in order to compare cusp-to-cusp variability and to assess right-to-left side of mouth variability. On 16 of the fossil molars, subsamples were taken from the CEJ to about one-third of the total upward direction in order to compare late forming enamel with the bulk enamel from the same tooth. Detailed 'down-

tooth' isotopic variability was examined in two ways:

1. M_2 from the fossil cohort 2 was sampled into thirds (uppermost: 'OCC' for occlusal chewing surface, middle: 'MID' for the middle third, and lower-most: 'CEJ'); and
2. a vertical strip from the M_2 fossil and modern molars were divided into eighths for our most detailed time-resolution.

The enamel strips (of all types) were removed from teeth using a Dremel tool and a diamond-blade attachment. Dentine adhering to the enamel was removed using stainless steel dental instruments. Bulk and subsamples of enamel were then ground into powder using an aluminum oxide mortar and pestle. Enamel-occluded carbonate was prepared as in Wang et al. (1993). Enamel powder was reacted overnight in a 5% solution of sodium hypochlorite to remove organic material. It was then washed with distilled water over a micro-glass filter paper. Samples were reacted with 1M acetic acid overnight under weak vacuum to remove surficial carbonates, and were again washed over micro-glass filter paper and dried. Approximately 100 mg of this enamel powder was converted to CO_2 with 100% phosphoric acid at $25°$ for ca. 3 days (McCrea, 1950). The evolved CO_2 was purified cryogenically and then analyzed on a VG prism stable isotope ratio mass spectrometer at the Center for Isotope Geochemistry, Lawrence Berkeley National Laboratory. Values are reported in permil (‰) units relative to SMOW (^{18}O) and PDB (^{13}C) standards. Standard deviation in δ^{18}O and δ^{13}C values associated with triplicate measurements on a single sample are $< 0.3‰$.

4. Results and discussion

4.1. Inter-molar isotopic variability

Although considerable isotopic variability exists between the fossil individuals, the δ^{13}C values of enamel consistently increased from the M_1 to M_3 molars [Fig. 3(a)]. The overall shift was on the order of 2–3‰. This δ^{13}C trend suggests that en utero enamel formation and the ingestion of moth-
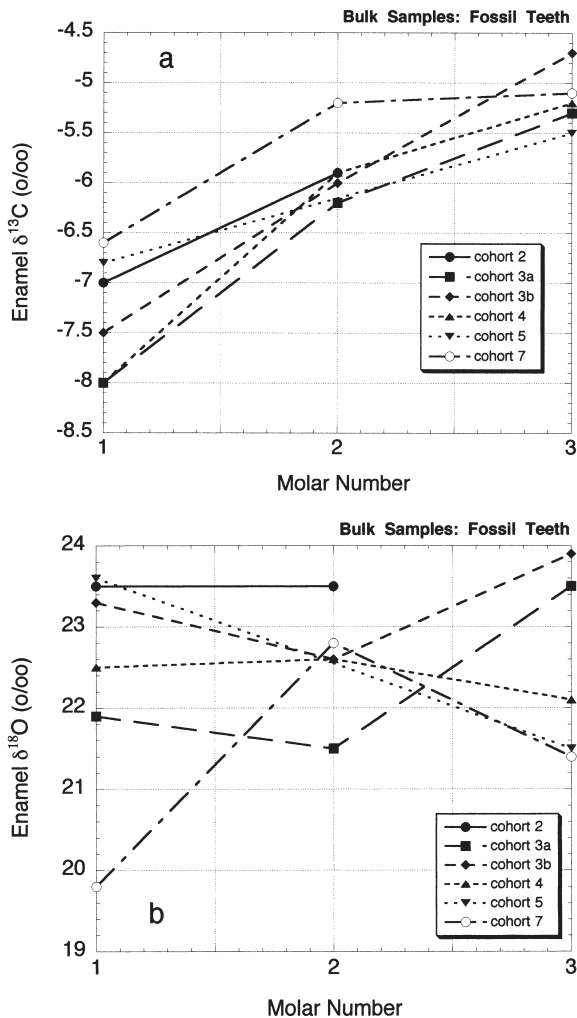
Fig. 3. (a) Variation in the $\delta^{13}C$ value of bulk tooth enamel and (b) variation in the $\delta^{18}O$ value of bulk tooth enamel as a function of tooth type (M_1, M_2, M_3) for six fossil individuals.

provide evidence which indicates that carnivore $\delta^{13}C$ apatite values are $+8‰$ greater than the diet of prey (compared to the $+12‰$ between the herbivore enamel and its diet). The actual mechanisms behind our observations remain to be determined by experimentation. The M_2 $\delta^{13}C$ values were also lower than those of the M_3, but greater than those of the M_1. The M_2 reflects diet consumed during both nursing and post-weaning. The M_3 $\delta^{13}C$ values primarily reflect forage intake. These data show that the C isotope composition of molars in the mouth of one animal can vary by $>2‰$, re-emphasizing the need to recognize the type and ontogeny of teeth in C isotopic studies for paleodiet. For future investigations, we would recommend the M_3 molar as the 'molar of choice', as that would yield the most direct evidence of forage consumed. This molar was also utilized by Bryant et al. (1996a) for an isotopic study of fossil equids. However, the other molars may yield exciting biological data, as well as fractionation factors en utero. Clearly a better understanding of the biology of modern, living, organisms are needed to better understand these enamel isotopic signals.

There is no consistent trend in enamel $\delta^{18}O$ values from M_1 to M_3 molars [Fig. 3(b)]. There are, however, enormous ranges in the $\delta^{18}O$ values for a given molar position among the individuals studied (up to 3.5‰). Although speculative, this suggests that there is no significant change in the $\delta^{18}O$ value of body water obtained from mother blood/milk and that of general dietary intake of weaned individuals. Bryant et al. (1996a) also did not find a consistent pattern from the M_1 to M_3 molars in bulk enamel phosphate in Oligocene to Miocene-aged Equids.

4.2. Cementum-enamel junction enamel versus bulk enamel

The CEJ material should represent enamel formed over a more restrictive period of time, and thus should not reflect the integrated signal that the bulk enamel does. For samples where both bulk and CEJ samples were available, the $\delta^{13}C$ values are roughly correlated, although there are several outliers [Fig. 4(a)]. As we will discuss later (Section 4.4), and from what is apparent from the
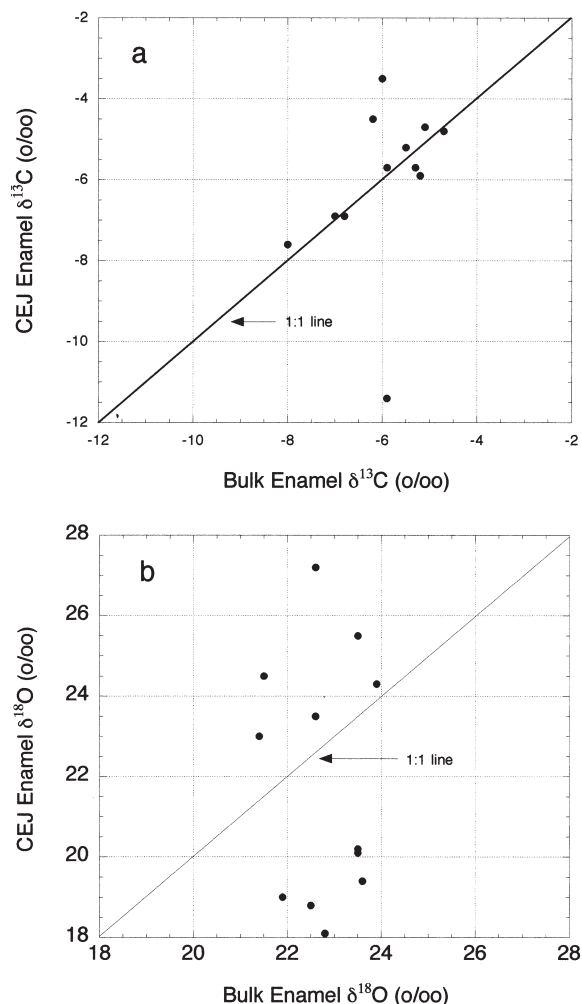
er's milk influence the $\delta^{13}C$ values of the enamel of molars formed prior to weaning. The M_1, formed partially en utero, has the lowest $\delta^{13}C$ values of all the molars. The fetus, which is nourished by the blood of the mother, might be consuming a C compound with a different $\delta^{13}C$ value than the mother's bulk diet. Alternatively, the physiology of the developing fetus may have an unusual fractionation factor of biosynthesis. For example, if unborn calves are viewed as 'predators' of their mother, Krueger and Sullivan (1984)

Fig. 4. Relationship between the (a) $\delta^{13}C$ value of bulk enamel and the CEJ sections and (b) $\delta^{18}O$ value of bulk tooth enamel and CEJ sections for six fossil individuals.

total isotopic range in Fig 4(a), this appears to be due to an apparent narrow seasonal range in the $\delta^{13}C$ value of the early Holocene diet of these animals (ca. 4‰). In contrast, there is no correlation between the $\delta^{18}O$ value of bulk and CEJ enamel [Fig. 4(b)]. This is likely due to an apparent large seasonal to annual trend in the $\delta^{18}O$ values of dietary water of these early Holocene animals [up to 9‰ range in Fig. 4(b)]. The simple conclusion that can be reached from this comparison is that fossil tooth fragments will give a far different isotopic signal, in highly sea-

sonal environments, than bulk tooth enamel. The environmental interpretation of tooth fragments therefore required sound knowledge of enamel development timelines.

4.3. Isotopic variability between left and right molars in same individual

Fig. 5 illustrates the variation in isotopic composition between mandible molars of a single individual. The individual selected is from fossil cohort group 7 — the oldest individual examined in the study. Significant wear had occurred on the teeth of the individual, with nearly half of the M_1 molars missing due to wear (Table 2). In this particular comparison, there was up to a 1.3 and 1.9‰ variation, in $\delta^{13}C$ and $\delta^{18}O$ values, respectively, from left to right in a single individual. Obviously, the observed variation was relatively small in most of the comparisons, indicating that left or right mandible makes little difference in a molar's isotopic composition.

4.4. Inter-tooth isotopic variability

In the fossil cohort 2 individual, a strip of bulk enamel was divided into 3 segments (CEJ, MID, OCC) which were isotopically analyzed (Table 1). Each segment has different $\delta^{13}C$ and $\delta^{18}O$ values. Below, we will discuss in more detail the possible seasons corresponding to each segment. However, we note that the measured $\delta^{13}C$ value for the CEJ sample ($-11.4‰$) is anomalously negative, far more negative than any other sample from the fossil collection. We therefore suspect an analytical error in this sample.

The detailed 'down-tooth' isotopic variability for both the fossil and modern samples are illustrated in Fig. 6. Clearly, as has been recognized by previous authors (Cerling and Sharp, 1996; Sharp and Cerling, 1998; Fricke and O'Neil, 1996), there is a seasonal dietary record in this variation. The tooth formation data, summarized in Fig. 1, suggest that the crown of M_2 should form in mid-summer and the base should form in the following spring to early summer. Given what is well-known about Great Plains ecology and meteorology, we expect that the most positive $\delta^{13}C$ values (prepon-

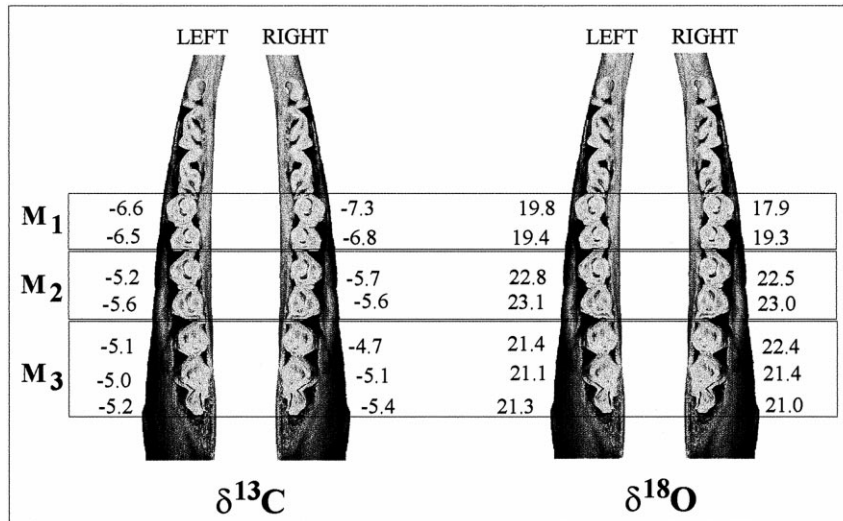Fig. 5. The $\delta^{13}C$ and $\delta^{18}O$ values of bulk enamel carbonate from molars on the left and right mandibles of fossil 145-8, the animal of oldest age at time of death (fossil cohort 7).

derance of C_4 grasses) and $\delta^{18}O$ values (summer precipitation from Gulf of Mexico) should occur in mid summer. The minimum $\delta^{13}C$ values may occur in spring during C_3 grass production, while the most negative $\delta^{18}O$ values may occur in the winter, if animals consume significant quantities of snow. These hypothesized dietary signals do not match well with the estimated tooth formation timeline and the isotope data (Figs. 1 and 6): that is, the O isotope data suggest that the enamel began forming in the fall and ended in the following late summer. It is plausible that the environmental water isotope signals that are ultimately reflected in the M_2 molars are obscured via physiological changes that the growing animal is subjected to over this period (weaning, strong seasonal climate and water stress oscillations, changes in body and water pool size, etc.). The importance of these physiological processes is deserving of more research. Another way of reconciling this apparent isotopic discrepancy is that enamel timeline we have developed may vary a few months among individuals (assuming physiological processes are not responsible). Such an offset or variation would then reconcile 'time of enamel formation' with the C and O isotope data in the fossil individual we examined.

It is clear that there is a moderate (4‰) C and large (9.6‰) O isotope range within the fossil tooth — and in all the teeth examined in this study. This suggests, at face value without accounting for possible effects of nursing versus grass intake, a C_4 grass intake ranging from 56 to 77% of total diet. Kelly et al. (1998) measured the C isotope composition of soil organic matter in paleosols underlying and encasing the bonebed, and reported $\delta^{13}C$ values of -14.9 and $-16.6‰$ (89 and 71% C_4 grass, respectively) for underlying soil organic matter and -12.9 and $-22.1‰$ (100 and 31% C_4 grass, respectively) for encasing organic matter. These (with one exception) indicate C_4 grass percentages consistent with the fossil tooth data (which integrates a much shorter time span than the soil organic matter). The present abundance of C_4 grasses in this portion of the Great Plains is considerably lower than this value. First, in this general region of the Plains, C_4 grasses comprise ca. 35% of the flora (Tieszen, 1994). Second, Kelly et al. (1998) measured the $\delta^{13}C$ values of soil organic matter in modern soils at Hudson–Meng, and reported values between -19.2 and $-21‰$. These C isotope values indicate that C_4 grasses make up between 39 and 52% of the present flora, far less than that indicated by
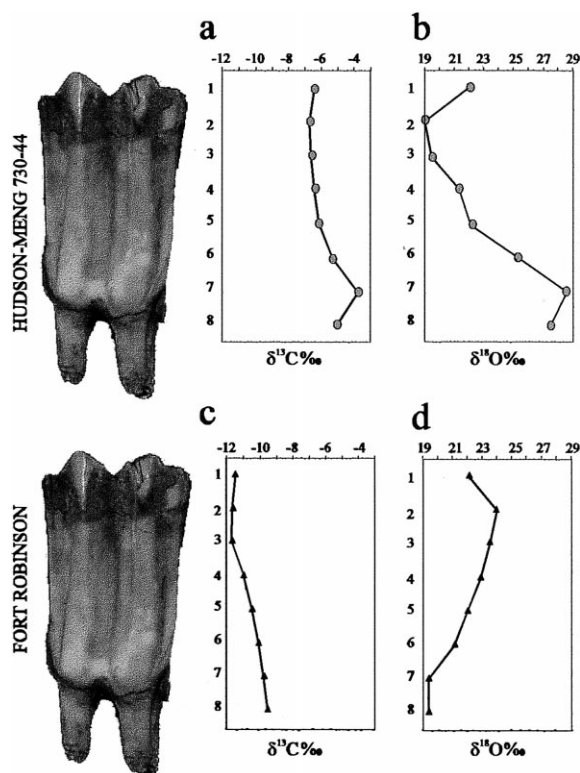
Fig. 6. Isotopic composition of eight subsections (crown to CEJ) of a fossil (730-44) and modern (Ft. Robinson) M_2 molar: (a) $\delta^{13}C$ values of fossil, (b) $\delta^{18}O$ values of fossil, (c) $\delta^{13}C$ values of modern, and (d) $\delta^{18}O$ values of modern.

the C isotope composition of the early Holocene fossil tooth enamel. Therefore, it seems that the fossil animals foraged a more C_4-rich prairie than that which presently exists [see also Kelly et al. (1998), Fig. 4(b)].

In contrast, the modern individual consumed between 20 and 36% C_4 grasses. While the modern individual was given a supplemental diet, obscuring the significance of the C isotope data, the fraction of C_4 grasses on the present range near Ft. Robinson is about 32% (Lora O'Rourke, Pine Ridge Ranger Station, personal communication; Tieszen, 1994).

The large O isotope variation in the fossil molar suggests a seasonal range in the $\delta^{18}O$ of 'dietary waters' from ca. -9.4 to $0.2‰$ [Fig. 6(b) — using a fractionation factor of 28.4 at 25°C, calculated from O'Neil et al. (1969)], values that are consis-

tent with the winter to summer isotopic transition, and with the present average mean precipitation values which are likely between ca. -7 and $-10‰$ (see Amundson et al., 1996 for summary of published data in region; E.F. Kelly, personal communication of O isotopes in soil carbonates). In contrast, the O isotopic variation in the modern animal is smaller, suggesting dietary water extremes of -4.8 and $-9‰$ [Fig. 6(d)]. Clearly, supplemental water has tended to dampen the natural seasonal water trends that are reflected in free-ranging animals.

In summary, the C and O dietary ranges exhibited in the fossil molar are expected given the known seasonality in dietary sources in the Great Plains. It may be that the proportion of C_4 grass available to the fossil animal is greater than the abundance of C_4 grasses on the modern range, a hypothesis also proposed by Jahren et al. (1998) based on isotopic work on bone and tooth enamel from the Hudson–Meng site. However, further work is needed to verify this hypothesis.

4.5. Isotopic variability in bulk enamel with respect to time prior to death

Here we take advantage of our tentative understanding of tooth/enamel formation patterns for bison, and our assessment of animal age at time of death, to arrange the isotopic data from the bulk fossil teeth as a function of time (*months from estimated enamel crown completion to the death of the herd*). Roughly, this provides an isotopic perspective of diet from $6+$ years to a few months before the mass death of the herd.

The plot of the $\delta^{13}C$ value of the bulk enamel versus time [Fig. 7(a)] suggests, without critical analysis, that the proportion of C_4 grass in the diet decreased as the time of the mass death approached. However, this is an artifact induced by molar type. All $\delta^{13}C$ values less than $-6.5‰$ are present in M_1 molars, teeth that obviously are greatly influenced by processes other than the abundance of forage. In summary, the M_3 (and secondarily M_2) molars indicate no significant dietary shift over the course of time. However, as mentioned above, there was already a very high
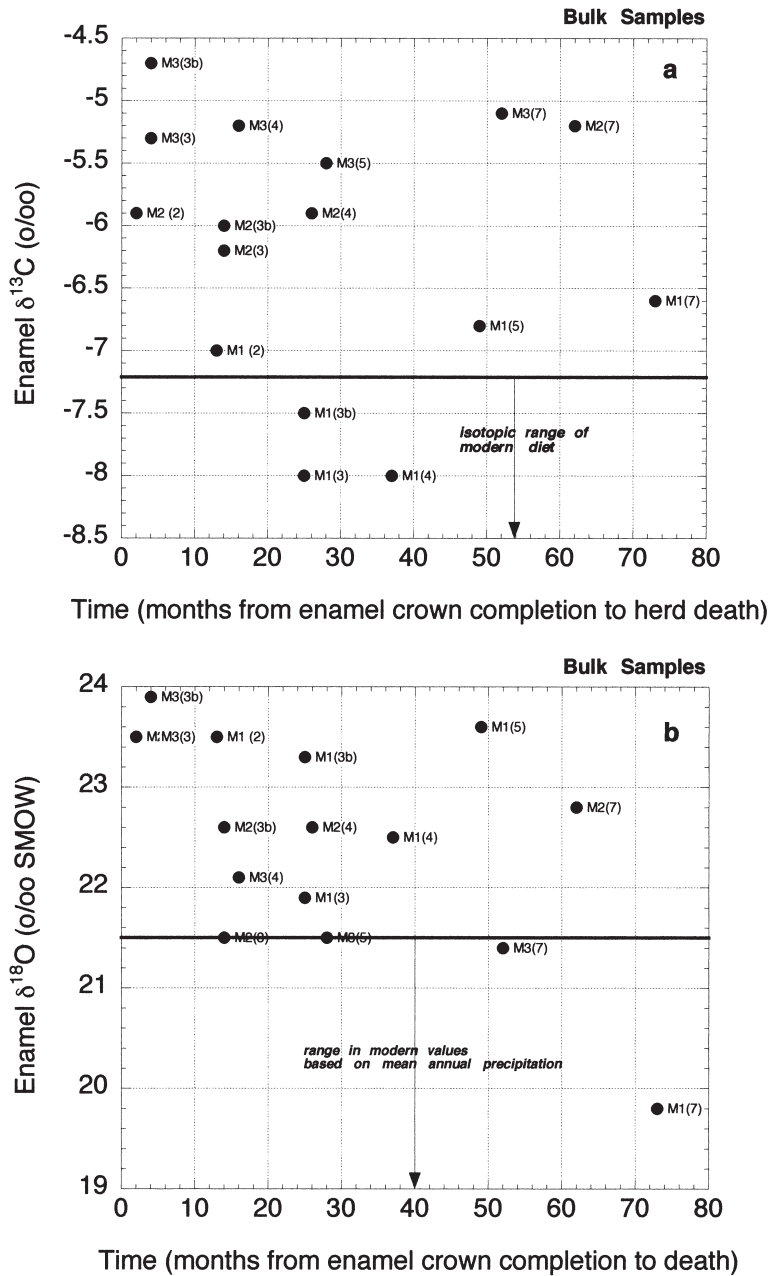
Fig. 7. The variation in (a) $\delta^{13}C$ and (b) $\delta^{18}O$ values of bulk tooth enamel in fossil teeth as a function of time from the completion of the enamel crown to the time of the herd death. The symbols next to data points indicate molar type (e.g. M_3) and, in parentheses, the cohort group (e.g. 2–7). The probable range in isotopic values of modern grazers is calculated from isotopic data on soil organic matter and rainfall discussed in the text.

percentage of C_4 grass in the animal's diet relative to modern values [Fig. 7(a)].

The plot of bulk enamel $\delta^{18}O$ values shows a pattern of increasing values with time (ca. 2–3‰). Unlike the C isotope data, this is not caused by the effect of any one molar type, since as discussed above, the $\delta^{18}O$ value of the molars appears to reflect 'environmental waters' regardless of the ontogeny of the tooth. The trend toward increasingly positive integrated $\delta^{18}O$ values in the enamel suggests a significant, and long-term, increase in isotopically positive precipitation. Additionally, the values all appear (based on sparse data) to be several per mil greater than teeth that would reflect modern average precipitation [Fig. 7(b)]. In the Plains, this could be accomplished by some combination of changing winter/summer precipitation ratios. One hypothesis is that winter/early spring and or late fall/winter precipitation decreased with time (which also agrees with the low C_3 grass component of the diet). In general, the data may indicate continual drying over the roughly 6 year period.

The cause of the mass bison death in the Hudson–Meng bed remains unknown. The steadily increasing $\delta^{18}O$ values of the enamel may suggest a long-term drought (or absence of spring/winter precipitation). Therefore, a very tentative hypothesis for the herd death is that of prolonged drought, starvation, poor reproductive success, and ultimately mass death. Obviously, more research is needed to determine the validity of this admittedly speculative hypothesis. However, it would appear that stable isotopes in tooth enamel will greatly inform any hypotheses advanced for the formation of this unusual geological deposit.

5. Conclusions

We have examined the spatial and temporal patterns of C and O isotope variation in fossil tooth enamel from the Hudson–Meng bone bed. Some general conclusions are that:

- C isotope composition of bulk enamel is highly dependent on tooth type, with M_1 (and M_2 to a lesser degree) influenced by diet obtained from the individual's mother.
- O isotope composition is apparently unrelated to tooth type, reflecting environmental water directly (M_3) or in a relatively unaltered state through the mother (M_1 and partially M_2).
- There is only a small isotopic difference in a given molar from the left or right mandible of a given individual.
- Seasonal isotopic patterns are clearly expressed 'down-tooth' in a given molar, although our understanding of the possible range in the timing of enamel formation among individuals makes the assignment of isotopes to season a somewhat circular process at the present time.
- The environment about 9500 years ago, just prior to the bison mass death, was possibly dominated by far more C_4 grass than the present local environment and by a trend of increasing $\delta^{18}O$ values in the years leading up to the bison deaths. This suggests severe environmental stress.

The environmental interpretations are preliminary, but point in a direction of future research to learn more about the factors contributing to this enigmatic geological deposit.

What is sorely lacking is a detailed study of the relationship between diet and tooth enamel in modern bison. Such a study should be a long-term, time series isotopic study from an individual's inception to an age of 3 or 4 years. Coupled with this should be improved models of the timeline of enamel development. However, in the absence of this critical work, the rudiments of isotope systematics and bison biology as they are presently known provide intriguing, though less quantitative, perspectives into many paleoenvironments.

References

Aiello, L., Dean, C., 1990. An Introduction to Human Evolutionary Anatomy. Academic Press, New York.

Amundson, R., Chadwick, O., Kendall, C., Wang, Y., DeNiro, M., 1996. Isotopic evidence for shifts in atmospheric circulation patterns during the late Quaternary in mid-North America. Geology 24 (1), 23–26.

Berger, J., Cunningham, C., 1994. Bison: Mating and Conservation in Small Populations. Columbia University Press, New York.

Boutton, T.W., Harrison, A.T., Smith, B.N., 1980. Distribution of biomass of species differing in photosynthetic pathway along an altitudinal transect in southeastern Wyoming grassland. Oecologia 45, 287–298.

Bromage, T.G., 1990. Early hominid development and life history. In: Rousseau, C.J. (Ed.), Primate Life History and Evolution. Wiley–Liss, New York, pp. 105–114.

Brown, W.A.B., Christofferson, P.V., Massler, M., Weiss, M.B., 1960. Postnatal tooth development in cattle. American Journal of Veterinary Research 21 (80), 7–34.

Bryant, J.D., Froelich, P.H., 1995. A model of oxygen isotope fractionation in body water of large mammals. Geochimica et Cosmochimica Acta 59 (21), 4523–4537.

Bryant, J.D., Froelich, P.H., Showers, W.J., Genna, B.J., 1996a. Biologic and climatic signals in the oxygen isotopic composition of Eocene–Oligocene equid enamel phosphate. Palaeogeography, Palaeoclimatology, Palaeoecology 126, 75–89.

Bryant, J.D., Froelich, P.H., Showers, W.J., Genna, B.J., 1996b. A tale of two quarries: biologic and taphonomic sygnatures in the oxygen isotope composition of tooth enamel phosphate from modern and Miocene equids. Palaios 11 (4), 397–408.

Burke, R.L., Stuiver, M., 1981. Oxygen isotope ratios in trees reflect mean annual temperature and humidity. Science 211, 1417–1419.

Cerling, T.E., Sharp, Z.D., 1996. Stable carbon and oxygen isotope analysis of fossil tooth enamel using laser ablation. Palaeogeogr., Palaeoclimat., Palaeoecol. 126, 173–186.

Cerling, T.E., Wang, Y., Quade, J., 1993. Expansion of C4 ecosystems as an indicator of global ecological change in the late Miocene. Nature 361, 344–345.

Dansgaard, W., 1964. Stable isotopes in precipitation. Tellus 16, 436–468.

Dean, M.C., 1987. Growth layers and incremental markings in hard tissues, a review of the literature and some preliminary observations about enamel structure in *Paranthropus boisei*. Journal of Human Evolution 16, 157–172.

Dongmann, G., Nurnberg, H.W., Forstel, H., Wagener, K., 1974. On the enrichment of H_2 ^{18}O in the leaves of transpiring plants. Radiation and Environmental Biophysics 11, 41–52.

Epstein, S., Thompson, P., Yapp, C.J., 1977. Oxygen and hydrogen isotopic ratios in plant cellulose. Science 198, 1209–1215.

French, N.R. (Ed.), Perspectives in Grassland Ecology 1979. Springer-Verlag, New York.

Fricke, H.C., O'Neil, J.R., 1996. Inter- and intro-tooth variation in the oxygen isotope composition of mammalian tooth enamel phosphate: implication for paleoclimatological and paleobiological research. Palaeogeography, Palaeoclimatology, Palaeoecology 126, 91–99.

Frison, G.C., 1991. Prehistoric Hunters of the Great Plains. Academic Press, New York.

Frison, G.C., Reher, C., 1970. Appendix 1: age determination of buffalo by teeth eruption and wear In: The Glenrock Buffalo Jump 48CO304: late Prehistoric Period buffalo procurement and butchering in the Northwest Plains. Plains Anthropologist Memoir 7, 46–50.

Frison, G.C., Stanford, D.J. (Eds.), The Agate Basin Site: A Record of Paleoindian Occupation of the Northwestern High Plains 1982. Academic Press, San Diego.

Frison, G.C., Todd, L.C. (Eds.), The Horner Site: The Type Site of the Cody Cultural Complex 1987. Academic Press, New York.

Haynes, G., 1984. Tooth wear rate in northern bison. Journal of Mammology 85 (3), 487–491.

Hillson, S., 1986. Teeth. Cambridge University Press, London.

Huda, T.F., Bowman, J.E., 1995. Age determination from dental microstructure in juveniles. American Journal of Physical Anthropology 97, 135–150.

Jahren, A.H., Todd, L.C., Amundson, R.G., 1998. Stable isotopic dietary analysis of bison bone bed samples from the Hudson–Meng bonebed: effects of paleotopography. Journal of Archaeological Science 25, 465–475.

Kelly, E.F., Blecker, S.W., Yonker, C.M., Olson, C.G., Wohl, E.E., Todd, L.C., 1998. Stable isotope composition of soil organic matter and phytoliths as paleoenvironmental indicators. Geoderma 82, 59–81.

Koch, P.L., Fisher, D.C., Dettman, D., 1989. Oxygen isotope variation in the tusks of extinct proboscideans: a measure of season of death and seasonality. Geology 17, 515–519.

Kohn, M.J., Schoeninger, M.J., Valley, J.W., 1996. Herbivore tooth oxygen isotope compositions: effects of diet and physiology. Geochimica et Cosmochimica Acta 60 (20), 3889–3896.

Krueger, H.W., Sullivan, C.H., 1984. Models for carbon isotope fractionation between diet and bone. In: Turland, J.R., Johnson, P.E. (Eds.), Stable Isotopes in Nutrition. American Chemical Society, Washington, DC, pp. 205–220.

Longinelli, A., 1984. Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research? Geochimica et Cosmochimica Acta 48, 1689–1693.

Luz, B., Kolodny, Y., 1985. Oxygen isotope variations in phosphate of biogenic apatites, IV: mammal teeth and bones. Earth and Planetary Science Letters 75, 29–36.

Luz, B., Kolodny, Y., Horowitz, M., 1984. Fractionation of oxygen isotopes between mammalian bone phosphates and environmental drinking water. Geochimica et Cosmochimica Acta 48, 1689–1693.

MacFadden, B.J., Cerling, T.E., 1996. Mammalian herbivore communities, ancient feeding ecology, and carbon isotopes: a 10 million-year sequence from the Neogene of Florida. Journal of Vertebrate Paleontology 16 (1), 103–115.

MacFadden, B.J., Wang, Y., Cerling, T.E., Anaya, F., 1994. South American fossil mammals and carbon isotopes: a 25

million-year sequence from the Bolivian Andes. Palaeogeography, Palaeoclimatology, Palaeoecology 107, 257–268.

McCrea, J.M., 1950. On the isotope chemistry of carbonates and paleotemperature scale. Journal of Chemical Physics 18, 849–857.

Miller, J.C., 1994. Geology of the Hudson–Meng site (24SX115), Sioux County, Nebraska. In: Todd, L.C., Rapson, D.J., Wandsrider, L. (Eds.), Hudson–Meng Bison Bonebed and Oglala National Grasslands 1993. Archaeological Research Design and Field Work Summary. Unpublished report on file with Nebraska National Forest, Chadron, Nebraska, pp. 84–129.

Morgan, M.E., Kingston, J.D., Marino, B.D., 1994. Carbon isotopic evidence for the emergence of C_4 plants in the Neogene from Pakistan and Kenya. Nature 367, 162–165.

O'Neil, J.R., Clayton, R.N., Mayeda, T.K., 1969. Oxygen isotope fractionation in divalent metal carbonates. Journal of Chemical Physics 51, 5547–5558.

Peden, D.G., Van Dyne, G.M., Rice, R.W., Hanson, R.M., 1974. The trophic ecology of *Bison bison* L. on shortgrass plains. Journal of Applied Ecology 11 (2), 489–497.

Quade, J., Solounias, N., Cerling, T.E., 1994. Stable isotopic evidence from paleosol carbonates and fossil teeth in Greece for forest or woodlands over the past 11 ma. Palaeogeography, Palaeoclimatology, Palaeoecology 108, 41–53.

Quade, J., Cerling, T.E., Andrews, P., Alpagut, B., 1995. Paleodietary reconstruction of Miocene faunas from Pasalar Turkey using stable carbon isotopes of fossil tooth enamel. Journal of Human Evolution 28, 373–384.

Rozanski, K., Araguas-Araguas, L., Gonfiantini, R., 1993. Isotopic patterns in modern precipitation. In: Swart, P.K. (Ed.), Climate Change in Continental Isotopic Records. American Geophysical Union, Washington, pp. 1–36.

Schwartz, C.C., Ellis, J.E., 1981. Feeding ecology and niche separation in some native and domestic ungulates on the shortgrass prairie. Journal of Applied Ecology 18, 343–353.

Sharp, Z., Cerling, T.E., 1996. A laser GC-IRMS technique for in situ stable isotope analyses of carbonates and phosphates. Geochimica et Cosmochimica Acta 60, 2909–2916.

Sharp, Z.D., Cerling, T.E., 1998. Fossil isotope records of seasonal climate and ecology: straight from the horse's mouth. Geology 26, 219–222.

Sims, P.L., Singh, J.S., Lauenroth, W.K., 1978. The structure and function of ten western North American grasslands. Journal of Ecology 66, 251–285.

Sternberg, L.S.L., 1989. Oxygen and hydrogen isotope ratios in plant cellulose: mechanisms and applications. In: Rundel, R.W. (Ed.), Stable Isotopes in Ecological Research. Ecological Studies Board, Los Angeles, pp. 124–141.

Stuart-Williams, H., Schwarcz, H.P., 1997. Oxygen isotopic determination of climatic variation using phosphate from beaver bone, tooth enamel and dentine. Geochimica et Cosmochimica Acta 61 (12), 2539–2550.

Teeri, J.A., Stowe, L.G., 1976. Climatic patterns and the distribution of C_4 grasses in North America. Oecologia 23, 1–12.

Tieszen, L.L., 1994. Stable isotopes on the plains: vegetation analyses and diet determinations. In: Owsley, D.W., Jantz, R.G. (Eds.), Skeletal Biology in the Great Plains. Smithsonian Institution Press, Washington, pp. 261–282.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., Slade, N.A., 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57, 32–37.

Todd, L.C., Rapson, D.J.,1993. Excavations at the Hudson–Meng Bison bonebed. Unpublished report on file with Nebraska National Forest, Chadron, Nebraska.

Todd, L.C., Hofman, J.L., Schultz, C.B., 1990. Seasonality of the Scottsbluff and Lipscomb bison bonebeds: implications for modeling paleoindian subsistence. American Antiquity 55 (4), 813–827.

Wang, Y., Cerling, T.E., Quade, J., Bowman, J.R., Smith, G.A., Lindsay, E.H., 1993. Stable Isotopes of Paleosols and Fossil Teeth as Paleoecology and Paleoclimate Indicators: An Example from the St. David Formation Arizona. Climate Change in Continental Records, Geophysical Monograph 78. AGU.

Wang, Y., Cerling, T.E., MacFadden, B.J., 1994. Fossil horses and carbon isotopes:new evidence for Cenozoic dietary, habitat, and ecosystem changes in North America. Palaeogeography, Palaeoclimatology, Palaeoecology 107, 269–279.

Wegrzyn, M., Serwatka, S., 1984. Teeth eruption in the European bison. Acta Theriologica 29 (9), 111–121.

Yakir, D., 1992. Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. Plant, Cell and Environment 15, 1005–1020.