Micro-sampling of human bones for mobility studies: diagenetic impacts and potentials for elemental and isotopic research

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The nature of long bone formation and the pathways of interaction between bone samples and the burial environment suggest that portions of the bones disconnected from the arterial system are resistant to diagenetic alteration. Preliminary work on femurs from Early Bronze Age hunter-gatherers in Cis-Baikal, Siberia shows that the nature and progression of chemical changes in the bone matrix due to microbial attack can be analyzed using laser ablation inductively coupled plasma mass spectrometry. Intra-osteon variability in elemental concentrations and strontium isotope ratios \(^{87}\text{Sr}/^{86}\text{Sr}\) indicate the presence of unaltered portions of bone within diagenetically modified bone and suggest that useful data remain accessible. These biogenic signals can potentially be useful for mobility research in broad terms and the smaller timescales within an individual's lifetime (months, years), accessible therein. Laser ablation micro-sampling of femur specimens showed that intra-osteon elemental composition of Ba, Re, and Cs varied within and was correlated between multiple osteons of a single bone. Portions of chemically unaffected bone were identified within, and effectively discriminated from diagenetically altered bone tissue. Areas showing visual alterations and erratic or uncorrelated Ca and Sr elemental results also had anomalous Sr isotope ratios, suggesting diagenetic alteration in those places. Compositional and isotopic analysis of intact portions of bone supports the hypothesis that hunter-gatherer groups in Cis-Baikal made numerous major movements during their lives. Microscopic analysis of long bones clarifies aspects of biodeterioration and correlations between trace elemental results and diagenetic alteration. Micro-sampling of intact portions of bone expands the scope of available materials for research on mobility and other aspects of human past behavior.

1. Introduction

The goals of this study are: 1) to examine the applicability of laser ablation as a sample introduction method for trace elemental and strontium isotope analysis using ICP-MS on human skeletal materials; 2) to assess its potential to access unaltered portions of archaeological bone; and 3) to provide useful insights on human behavior in general and mobility specifically. For mobility studies, tooth enamel is the preferred material due to its resistance to diagenetic alterations, leaving bone samples either neglected or regarded with suspicions of providing spurious data. In the current study we used femur samples from individuals from Khuzhir-Nuge XIV (KN XIV), an Early Bronze Age hunter-gatherer cemetery on the west coast of Lake Baikal, Siberia (Fig. 1). Both TIMS and ICP-MS techniques have been previously applied to human teeth and bones from KN XIV (e.g., Haverkort, et al., 2010, Haverkort et al., 2008, Weber et al., 2003, Weber et al., 2007) and these data are useful in assessing the efficacy of laser-ablation trace element and Sr isotopic analyses of bones. More precisely, the study addresses the following two main questions: 1) Are there intact portions of bones, specifically intact osteons, which can be effectively analyzed for trace element concentrations and strontium isotope ratios by laser-ablation? and 2) How effective is micro-sampling of individual osteons for tracking mobility within the context of Cis-Baikal geology and human interactions with different plant and animal resources?

Sampling and analytical protocols are always integral part of geochemical tests performed on archaeological materials and involve a series of deliberate decisions. For geological specimens, these choices often reflect the specific research question(s) being pursued. The situation is rather more involved with complex and organic mineral structures as there are frequently difficulties in accessing the desired target data. Geochemical analysis of archaeological human skeletal materials includes such considerations as
the analytical technique, the kind and extent of diagenetic alteration of the samples, and the formation of the skeletal material itself. For skeletal materials, the relationship between the formation event of the physical sample and the chemical interactions occurring during life being reconstructed based on the obtained results can be complicated and open to interpretation. Strontium isotope analysis has traditionally relied on thermal ionization mass spectrometry (TIMS) due to its reliability and analytical precision. The advent of multi-collector inductively-coupled-plasma mass spectrometry (MC-ICP-MS) as an alternative to TIMS, coupled with either a laser micro-drill or a laser ablation (LA) unit for micro-sampling, greatly expanded the possibilities for application of Sr isotope tracers in archaeological research. Both laser micro-drills and laser ablation are far less destructive and enable higher spatial resolution for analysis than the TIMS and MC-ICP-MS techniques. However, micro-sampling for solution preparation still requires significant laboratory processing whereas laser ablation requires virtually no such special handling (Copeland et al., 2008; Nowell and Horstwood, 2009; Prohaska et al., 2002; Scharlotta, 2010). In spite of several studies using LA on human teeth, bone and high-Sr apatites (Prohaska et al., 2002, Weber et al., 2007), analysis of phosphate minerals by LA has not been tested extensively for strontium work until quite recently (Copeland et al., 2010; Horstwood et al., 2006; Pye, 2004; Scharlotta, 2010, 2012; Simonetti et al., 2008; Vroon et al., 2008; Weber et al., 2008, Weber et al., 1998; Woodhead et al., 2005).

A significant amount of research has been devoted to the subject of diagenesis of archaeological bone materials, highlighting both

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**Fig. 1.** Lake Baikal, Siberia showing the location of the KN XIV cemetery, cultural micro regions, and the age of the dominant bedrock formations.
the importance and the complexity of the matter. The majority of archaeological bone is likely to be biologically altered to some extent (Jans et al., 2004). Microbial attack is a significant factor of diagenetic alteration with a growing body of work focused on the specific causes, processes and effects of this alteration (e.g., Jans, 2008; Jans et al., 2004; Smith et al., 2007). This work has shed light on changes in the porosity, bone mineral (i.e., crystallinity changes), the decreases and ultimate loss of bone collagen and the relationship between many agents involved in biodeterioration, although the question regarding the potential for utilizing unaltered portions of bone for geochemical research has been largely left unaddressed (Jans, 2008; Smith et al., 2007; Trueeman et al., 2008).

Previous work (Copeland et al., 2008; Scharlotta, 2010; Simonetti et al., 2008; Woodhead et al., 2005) has indicated a number of potential problems in gathering accurate strontium isotopic data from calcium phosphate matrices using LA-ICP-MS. In addition to interference from rubidium (\(^{87}\text{Rb}\)), doubly charged rare earth elements (Paton et al., 2007), and calcium dimers (Woodhead et al., 2005), there is the production of a polyatomic species of CaPO that interferes with the \(^{87}\text{Sr}\) (Scharlotta, 2010, 2012, Scharlotta et al., 2011, Simonetti et al., 2008, Vroon et al., 2008). This polyatomic species appears to be unique to the laser ablation technique as it is not apparent with solution mode (SM) MC-ICP-MS.

The problem of CaPO polyatomic interference is limited to certain matrix types. For archaeological teeth, method to correct for this interference is necessary in order to obtain accurate data from samples with low concentrations of Sr (Scharlotta et al., 2011). However, for bone samples, there does not appear to be any significant offset in isotopic values resulting from this polyatomic species as the ratios measured previously by (SM) MC-ICP-MS (Haverkort et al., 2008) and those measured by LA-ICP-MS and reported here are quite compatible (Figs. 26–28, Table 1). Since, similar results were noted by Radloff et al. (2010), confirmation that this problem is not applicable to bone tissue would ease the pathways of analysis and interpretation.

Bones themselves provide enough ambiguity regarding structural formation, reformation, and subsequent alteration to cause concerns about sampling methodology without additional concerns regarding the use of micro-sampling. A long bone can reflect well over 20 years of an individual’s life. Individual osteons generally have a lifespan of no more than 20–25 years and form themselves by cutting through and replacing old bone material (Frost, 1963). However, this does not necessarily translate to a simple age of the bone as equivalent to that of the current osteons for remnants of the old bone are frequently visible in histological analysis. These old portions are also represented in the homogenized powders and solutions of traditional macro- or bulk-sampling methods. The advantage of direct micro-sampling is that it can alleviate this aspect of uncertainty by providing a clear link between what is being sampled and the target analytical data.

Previous Sr isotopic research has focused primarily on sedentary agrarian groups (e.g., Bentley and Knipper, 2005; Grue et al., 1997; Price et al., 2002, Price et al., 2008; Price et al., 2007, Price et al., 1994). With such groups, the goal of research is to identify the local signature so that organisms of nonlocal origin (people and animals) can be recognized. Such an approach is of only limited utility for the study of hunter-gatherers, as many groups utilized large ranges of territory and would thus have less localized (i.e., averaged over larger areas) isotopic signatures reflecting their lifetime mobility. It is this circumstance that drives interest in micro-sampling of skeletal materials to access insights into mobile individuals with greater chronological resolution. However, further research is needed to understand better the dynamic interaction between direct chemical contact of living organisms with the biologically available strontium, the formation of skeletal tissues, and data recovery from these tissues. This study is focused on the data recovery side of this problem, examining the range of variability in strontium isotope ratios and trace element composition found within human femur samples.

2. Cis-Baikal resources and geology

The Cis-Baikal region of Siberia refers to the area including the western coast of Lake Baikal, the upper sections of the Angara and Lena river drainages, and the Tunka region adjacent to the southwestern tip of Lake Baikal (approximately between 52° and 58° N and 101° and 110° E; Fig. 1). The topographic complexity of the rift valley that formed Lake Baikal led to the formation of a large number of micro-habitats, with a variety of seasonally available resources (Galazii, 1993; Weber, 2003; Weber et al., 2002). The thermal capacity of Lake Baikal itself moderates the local climate immediately along its coastline, resulting in generally milder temperatures during the winter and cooler temperatures during the summer. As a result, the Angara River Valley remains relatively free of snow during the long winter which attracts various species of ungulates looking for forage and less restricted mobility (Formozov, 1964). There is a variety of large and medium-size game found in the region including moose (Alces alces), red deer (Cervus elaphus), roe deer (Capreolus capreolus pygargus), reinddeer (Rangifer tarandus), mountain goat (Capra sibirica), and wild boar (Sus scrofa). Smaller species such as hare (Lepus sp.), marmot (Marmota sibirica), suslik (Spermophilus citellus), and waterfowl such as geese are also abundant in many areas around the lake. During the summer, large runs of black grayling (Thymallus arcticus baikalensis) are found in the uppermost section of the Angara River near its source at Lake Baikal, and several fish species enter the tributaries of the Angara in large numbers to spawn. The shallow coves and bays in the Little Sea region of Lake Baikal, between O’llkhon Island and the west coast of the lake, also provide excellent opportunities for fishing and during the late winter and early spring when the lake is frozen, nerpas, the Lake Baikal seal (Phoca sibirica) can be hunted (Levin and Potapov, 1964; Losey et al., 2008; Marchiafava et al., 1974; Weber, 1995). Ethnographic studies of boreal forest populations highlight

Table 1

<table>
<thead>
<tr>
<th>Burial</th>
<th>Master ID</th>
<th>Sample ID</th>
<th>Age</th>
<th>Sex</th>
<th>Avg. laser (^{87}\text{Sr}/^{86}\text{Sr})</th>
<th>Zn error (^{87}\text{Sr}/^{86}\text{Sr})</th>
<th>Soln. (^{87}\text{Sr}/^{86}\text{Sr})</th>
<th>Zn error (^{87}\text{Sr}/^{86}\text{Sr})</th>
<th>Birth</th>
<th>C/N</th>
<th>(^{13}\text{C})</th>
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<th>(^{15}\text{C}) age BP</th>
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<td>11.8</td>
<td>GF</td>
<td>4060 ± 120</td>
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<td>0.71012</td>
<td>0.00002</td>
<td>Non-local</td>
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<td>11.7</td>
<td>GF</td>
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</tr>
<tr>
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<td>0.00175</td>
<td>0.71036</td>
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<td>Non-local</td>
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<td>GF</td>
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<td>GFS</td>
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<td>GFS</td>
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the use of mushrooms, berries, and pine nuts as other food resources (Haverkort et al., 2010; Katzenberg and Weber, 1999; Lam, 1994; Marles, 2000). While archaeological evidence for plant use during the Baikal's middle Holocene is very limited, there is sufficient ethnographic information about the use of plants in boreal forager subsistence systems around the world to speculate upon their usage as diverse but limited in overall energetic contribution (e.g., Kelly, 1983; Marles, 2000; Winterhalder, 1981, 1982).

Within the Cis-Baikal region there are four main geological zones that roughly correspond to archaeological micro-regions (Fig. 1). The main zones are 1) the Baikal basin, including the lake itself, the coastal areas as well as the Little Sea area enclosed by Ol’khon Island; 2) the drainage of the upper and middle Angara River bounded by the Eastern Sayan Mountains to the west and the Central Siberian Plateau to the east and extending north towards Bratsk; 3) the upper Lena river basin cutting through the Central Siberian Plateau as it heads northwards; and 4) the Tunka region covering a sizeable valley running south of the Eastern Sayan Mountains and broadly connecting the southwestern tip of Lake Baikal to Lake Khovsgol in Mongolia. The upper and middle sections of the Angara River flow through Mesozoic and Quaternary deposits, with expected $^{87}\text{Sr}/^{86}\text{Sr}$ values in the range of 0.705–0.712. The upper Lena watershed and the surrounding Central Siberian Plateau are dominated by Cambrian and Precambrian limestones, with expected values fairly tightly clustered around 0.709 (Haverkort et al., 2008, Huh et al., 1994). The Baikal basin includes the Primorski and Baikalski mountain ranges and is characterized by relatively high $^{87}\text{Sr}/^{86}\text{Sr}$ (~0.720–0.735) due to the presence of Archean and Proterozoic granites (Galazii, 1993). Bedrock of similar ages occurs around the southwestern shores of Lake Baikal and drainages adjacent to the Eastern Sayan Mountains, however our preliminary data on distribution of biologically available strontium isotopes in the Cis-Baikal region, provided by examination of modern plant and water samples, indicate that these two regions have quite different $^{87}\text{Sr}/^{86}\text{Sr}$ values (Scharlotta, 2012). Both Archean and Proterozoic zones (Little Sea and Tunka Region) overlap $^{87}\text{Sr}/^{86}\text{Sr}$ ranges of neighboring regions (e.g., Angara Drainage), while only the Little Sea area exhibits values above 0.720. Further clarifications of the distinction between these two zones of similar age will be possible upon completion of regional sampling efforts. Overall values for Lake Baikal water are reported as 0.7085 (Kenison Falkner et al., 1992).

3. Khuzhir-Nuge XIV cemetery

The KN XIV cemetery is located on the west coast of the Little Sea micro-region of the Lake Baikal basin, near the southern end of Ol’khon Island and c. 3 km southwest of the mouth of the Sarma River (53° 04’ 58” N, 106° 48’ 21” E). It occupies the southeast slope of a hill rising from a shallow bay (Fig. 2). With 79 graves and a total of 89 individuals unearthed, KN XIV is the largest Early Bronze Age hunter-gatherer cemetery ever excavated in the entire Cis-Baikal region (Weber et al., 2008, Weber et al., 2007). All the graves were only c. 30–60 cm deep sub-rectangular pits filled with rocks and loamy sand, and covered by surface structures built of stone slabs still visible on the surface prior to archaeological excavation. Most graves contained single inhumations, seven were double, and two were triple interments.

The north-south orientation of Grave 7 is consistent with the Late Neolithic Serovo culture of the Ol’khon region, while all the other graves show clear similarities with the mortuary tradition of the Early Bronze Age Glazkovo culture (Goriunova and Mamonova, 1994; Konopatskii, 1982; Weber et al., 2002). The most diagnostic Glazkovo characteristics include the generally west-east orientation of the burials and such grave goods as copper or bronze objects (rings, knives, needles, and bracelets), kaolinite beads, and rings and discs made of white nephrite or calcite (Weber et al., 2008). Analysis of approximately 80 $^{14}\text{C}$ dates indicates that the KN XIV cemetery was used continuously by Glazkovo peoples for a maximum of 700 years between ~4650 and 3950 cal. BP but the majority of the burials (70%) date to between ~4450 and 4250 cal. BP (Weber et al., 2005). However, more recent reassessment of the radiocarbon evidence suggests much shorter use of the cemetery, perhaps not exceeding 100 years (Weber, 2011). Since the analysis...
did not reveal any obvious temporal trends in mortuary attributes, it seems justified to treat the cemetery, with the exception of the much earlier Grave 7, as one analytical unit (McKenzie, 2006; Weber et al., 2005).

To date, a sample of 25 individuals from KN XIV were analyzed for strontium isotope ratios and compared with 79 faunal specimens collected throughout Lake Baikal and the Cis-Baikal region (Haverkort et al., 2010, Haverkort et al., 2008, Weber et al., 2003). The human materials included 20 adult individuals for which three molars (M1, M2 and M3) and a femur sample were available and 5 subadult burials with only M1 and M2 crowns completed. The six femur samples used for the current study came from this pool of previously tested individuals (Table 1).

This past work allowed to expand the possible applications of strontium isotope research and to identify an interesting general pattern present in several mobility profiles within the sample of KN XIV individuals. Broadly speaking, it appears that there was a significant amount of movement of individuals during their lifetime, whereby people buried at KN XIV were frequently not born in the Little Sea region, but only migrated there as subadults or adults (Haverkort et al., 2010, Haverkort et al., 2008, Weber and Goriunova, 2012). There appears to be significant variability within the cemetery itself as to the place of origin, the timing of migration to the Little Sea, dietary preferences, mortuary practices and burial details, and cemetery spatial organization that is the subject of ongoing research (cf. Weber and Goriunova, 2012; Weber et al., 2011).

4. Microbial biodeterioration

Microbial attacks on buried bone are either fungal or bacterial in nature which only rarely co-occur in the same bone (Jans et al., 2004). Marchiafava et al. (1974) noted that invading fungal hyphae contained mineral in solution, whilst the tunnels they created held no redeposited minerals, suggesting that the dissolved mineral was likely transported out of the bone. Fungal alteration of bones forms tunnels up to 250 μm in diameter, dissolving and removing mineral from the bone, and crossing cement lines without apparent difficulty. Yet, it is unclear whether this is the process of harvesting nutrients directly from the bone or the fungi are using the bone as a medium (Hackett, 1981; Jans, 2008). Cement lines have a high sulfur content and low calcium and phosphorous content, though a high Ca/P ratio, making them different enough in content and structure to inhibit bacterial progression (de Ricqles et al., 1991; Jackes et al., 2001; Martin and Burr, 1989). Bacteria appear to produce pores in bone, causing substantial degradation as they spread from blood vessels and fill ostecanes until they reach a cement line or another area of bacteria (Bell, 1990; Hackett, 1981; Katzenberg et al., 2009). This produces biodeterioration with a complex morphology while mineral is dissolved to expose collagen and redeposited as a hypermineralized rim at the edge of bacterial foci, reorganized rather than removed (Jans, 2008). It is unknown whether the rarity of co-occurrence of fungal and bacterial attacks is due to competition between the organisms, a lack of remaining nutrients after an initial alteration, or whether such alteration creates environmental incompatibility for further alteration (e.g., intrusive phosphate minerals such as francolite or brushite deposited by bacteria cannot support fungi as a nutrient source or as a medium) (Child, 1995; Jackes et al., 2001; Jans, 2008; Jans et al., 2004; Katzenberg et al., 2009; Smith et al., 2007).

Trace element assay of archaeological bone has long been an important aspect of research on bone diagenesis (e.g., Gilbert, 1975; Katzenberg et al., 2009; Nielsen-Marsh and Hedges, 2008b; Pate et al., 1989; Price, 1989; Pye, 2004; Radosевич, 1993; Trueman et al., 2008; Tuross et al., 1989). Much of this work has focused on what are the expected “normal” and “diagenetic” values for human skeletal tissues in a fashion similar to the use of C/N ratios as a measure of data integrity in dietary isotope studies (e.g., Burton et al., 2003; Cucina et al., 2007, Cucina et al., 2011; Grue, 1998; Hedges, 2002; Koenig et al., 2009; Lappalainen et al., 1981; Maurer et al., 2011; Molleson, 1988; Vrbic et al., 1987). Clear relationships in chemical composition exist between the biogeochemical environment of an individual’s lifetime on the one side and their skeletal remains on the other. However, many researchers have written off the potential utility of trace element data to provide any useful information about human behavior (e.g., mobility, see Cucina et al., 2007; Cucina et al., 2004, Cucina et al., 2011; Knudson and Price, 2007) and favor the use of such data strictly for identification and removal of possibly diagenetically altered tissues. Debates over whether trace element concentrations relate to diagenetic or anthropogen processes have quieted in recent years as improved laboratory techniques (e.g., Garvie-Lok et al., 2004; Koch et al., 1997; Nielsen-Marsh and Hedges, 2000b; Pate, et al., 1989) have enabled researchers to understand the nature of diagenetic changes and remove any mineral structures resulting from interaction with the burial environment. Two caveats, however, remain to be addressed: first, sample pretreatments may not remove all diagenetic alterations or may overcorrect for hypothesized alterations and thus bias the resultant data; and second, such approaches operate on the assumption, typically implicit, that sample homogenization is an appropriate means to acquire anthropogenic and behaviorally meaningful geochemical information. One possible approach to alleviate both of these potential problems is to micro-sample bone structure, thus avoiding altogether bone structures that exhibit diagenetic effects.

5. Materials and methods

As mentioned, femur samples analyzed in this study represent six individuals from the KN XIV cemetery (Table 1) all of which have been previously examined by Haverkort et al. (2008). Samples were mounted in epoxy, thin-sectioned, mounted on slides and ground to a thickness of ~100 μm to enable observation of internal structures. All samples were analyzed for elemental composition using LA-ICP-MS and for 87Sr/86Sr ratios using LA-MC-ICP-MS. All sample preparation and analyses were conducted at the Radiogenic Isotope Facility of the Department of Earth and Atmospheric Sciences at the University of Alberta, Edmonton.

5.1. Trace element analysis

Laser ablation for elemental concentrations was conducted using the Perkin Elmer Elan6000 quadrupole ICP-MS coupled to a UP213 nm laser ablation system (New Wave Research, USA). The instrument was optimized utilizing the NIST SRM 612 international glass standard reference material (RF power 1200 W, peak hopping acquisition, 50 ms dwell time). Individual osteones were sampled in a serial fashion using a 6 μm beam, with a repetition rate of 20 Hz and an energy density of ~13 J cm⁻², beginning at the Haversian canal and progressing outwards towards the cement line to examine the nature and extent of useful intra-osteon geochemical variability (Fig. 3).

Experiments were conducted in a mixed He/Ar atmosphere (ratio of 0.5:0.1 L min⁻¹) within the ablation cell, and mixed with Ar (1.03 L min⁻¹) prior to entering the torch assembly. The laser ablation cell was flushed with a higher flow rate of He (up to 0.9 L min⁻¹) for approximately 1 min between laser ablation runs to ensure adequate particle washout. The NIST SRM 612 glass standard was used as the external calibration standard. Quantitative results for 58 elements (Li, Be, B, Na, Al, Si, P, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Ni, Co, Cu, Zn, Ga, Ge, As, Rb, Sr, Y, 90Zr, 91Zr, Nb, Mo, Ag, Cd, In, Sn, Sb,
Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, Re, Au, Tl, Pb, Bi, Th, and U) were obtained and normalized to $^{24}\text{Mg}$, as the internal standard using the GLITTER®/C210 (XP version, Macquarie University) laser ablation software (Supplementary Material 1). Mg, measured by solution analysis, was used instead of Ca in order to assess variation in calcium concentrations in these samples.

5.2. Osteon formation and sampling

As noted, individual osteons generally have a lifespan of no more than 20–25 years and form themselves by cutting through and replacing old bone material (Frost, 1963). Osteons appearing intact and showing variable levels of alteration were sampled in this manner to observe the compositional changes associated with microbial deterioration. During bone remodeling, osteoid will be deposited at a rate of approximately 0.3–1.0 µm per day (Frost, 1963; Parfitt, 1979, 1984). This is followed by a minimum lag time of one week before mineralization will begin. Thus, each laser ablation line (represented by a single data point within each series) could reflect less than a month during the individual’s life. This, however, is difficult to gauge precisely given several factors, such as the age and sex of the individual, their state of health, and nutrition, that can impact the growth and size of individual osteons (Parfitt, 1979, 1984). It is more likely that a given ablation line will approximate closer to a span of six months to one year of the individual’s life. Individual elements have different residence times in the body and will vary in their sensitivity to changes resulting from movements or other external factors (Beard and Johnson, 2000; Britton et al., 2009; Schweissing and Grupe, 2003).

Progressive lines were spaced roughly 5–10 µm apart in order to ensure that individual laser lines did not represent overlapping periods of time (Fig. 3). The size and geometry of osteones is correlated with the age, sex, and weight of individuals during life; however, many researchers have noted broad temporal trends rather than refined spans of time (e.g., Currey, 1964; Iwamoto et al., 1978; Kerley, 1965, 1969; Kerley and Ubelaker, 1978; Koenig et al., 2009; Landerso and Frost, 1964; Pye, 2004; Scharlotta, 2012; Yoshino et al., 1994). The external lamella of an osteon forms first and the growth progresses inward from the periphery to the Haversian canal, with additional lamellae visible as an osteon ages. Histological analysis is necessary to confirm visually that osteons are indeed of similar age; however, estimates can be made based on visible voids left from osteocytes. Osteons with similar geometry and visible lamellae are likely to be of similar age but, as mentioned, this can be only be verified through separate histological or geochemical analysis. Chemical composition of bones produced and mineralized during the same interval will be the same, thus forming patterns in the microsampled data, reflective of different environments or periods of specific chemical interactions that will result from intra-osteonal analysis. The age of individual osteons can be externally verified, but this may not be necessary. Using a series of osteons, any geochemical patterning present will become apparent and the relative age of specific osteons can be determined by wiggle matching these patterns. In this way, the goal of finding useful patterning from intra-osteonal micro-sampling also serves to provide a buffer against potential time gaps between individual osteons being sampled.

5.3. Strontium isotope analysis

Laser ablation for isotopic analysis was conducted using a UP213 nm laser system coupled to the Nu Plasma HR MC-ICP-MS with the sample-out line from the desolating nebulizing
introduction system (DSN-100 from Nu Instruments) to allow for simultaneous aspiration of a 2% HNO₃ solution. At the beginning of each analytical session, parameters for the introduction system and the ion optics were optimized by aspirating a 100 ppb solution of the NIST SRM 987 Sr isotope standard. Experiments with the laser size and power quickly highlighted a problem with a replication of the analyses conducted for elemental composition. Similar spot sizes were unable to yield high enough Sr signals to gain useable ⁸⁷Sr/⁸⁶Sr ratios.

In order to determine the minimum spot size that could be effectively used on these bone samples, a size range experiment was conducted on Durango Apatite. The results showed that a spot size as small as 60 μm, yielding signal strengths of 1–1.5 V on ⁸⁶Sr (100% laser power; 20 Hz repetition rate; ~15 J cm⁻² energy density) could be effectively used, however, more reliable signals (3–V) were gained from analyses using sizes greater than 100 μm. Strontium levels are lower in most human samples than in Durango Apatite, so stable signals as low as 0.5 V were accepted. Analyses with questionable signals were repeated to ensure stability. Not all Apatite, so stable signals as low as 0.5 V were accepted. Analyses with questionable signals were repeated to ensure stability. Not all osteons are large enough to be able to hold a 100-μm laser beam, so the majority of analyses were conducted using an 80 μm beam, with 100 μm applied to a few particularly large osteons. This large beam size limits temporal assessment within the bone to the age of a given osteon, thus to the last years of life of a given individual. More sensitive equipment than available for this study would be required to conduct intra-osteon micro-sampling for measuring Sr isotope ratios.

5.4. Determination of diagenetic alteration

Altered portions can be visually identified, but determining the extent of alteration, or the factors impacting the osteon, can only be assessed via chemical analysis. Diagenetic alteration was assessed by visual inspection of changes in the osteon's physical structure. Such changes include discolorations, changes in material structure, cracking, and the deposition of intrusive materials. Burial 45 was used to experiment with different number of ablation lines. Some of these lines intentionally crossed the cement line and into areas of bone that were identified visually as altered. The progression of chemical compositional differences between intact and diagenetically altered bone was previously unknown. Specific attributes of different diagenetic processes have been discussed in the literature, but the exact history of diagenetic alteration for the current samples had to be determined. Sequential sampling of materials appearing intact and grading into areas that visually appear to be altered provides some insights into the chemical changes that corresponded with the changes in appearance. Based on these guidelines, trace element analysis of the remaining specimens employed five ablation lines for each of four intact osteons (Supplementary Material I). Furthermore, within each bone sample, nine intact osteons were analyzed for strontium isotope ratios along with five osteons that were suspected to have been impacted by diagenetic processes. This allowed assessment of the direction and potential extent of diagenetic alteration of intact osteons (Supplementary Material II).

6. Results and discussion

Our method of bone micro-sampling and the resulting trace element and strontium isotope data provide new and important insights on the following three points: middle Holocene hunter—gatherer mobility in Cs-Baikal, the process of bone diagenesis in archaeological settings, and applicability of laser ablation as an introduction method for examination of trace elements and strontium isotope ratios.

6.1. Hunter—gatherer mobility

A number of conclusions can be drawn from the elemental and isotopic results. Areas of bone visually assessed as structurally deteriorated or altered showed changes in trace element and isotopic profiles as compared to intact areas. That obvious signs of structural deterioration are coupled with skewed chemical data is less surprising than the documentation of this process progress. It appears that chemical changes actually precede the visual changes and that the chemical data are a better indicator of the bone mineral integrity as well as the nature and extent of diagenetic alteration in process in any given sample than the visual characteristics alone. Foremost, the femur samples appear to suffer primarily from bacterial attack as opposed to fungal attack. Biodeteriorated regions are visible and show the characteristics described for bacterial attack in the literature such as mineral redistribution and redeposited phosphate with significantly lower Ca values (e.g., Jackes, et al., 2001; Jans, 2008; Jans et al., 2004). Also note that analyses begin at the Havercan channel with lower Ca values and that where the laser approaches or crosses the cement line, Ca values spike down (Fig. 4). For Burial 45, data series B–F (each reflecting sampling within a single osteon) correspond with osteons shown in Fig. 3. Six osteons within a single femur sample from Burial 45 were analyzed for trace elements to examine inter- and post-osal analytical differences (Fig. 4). The results from Burial 45 showed that trace elemental composition was strongly correlated between intact portions of osteons or diagenetically altered portions inform primarily on localized microscopic changes to the bone structures. The remaining burials were analyzed for four osteons each after it was determined that similar patterning was present and additional osteonal sampling would provide redundant data (Supplementary Material I). The six osteons of Burial 45 were of similar size, but, as noted, factors such as age and health can impact the formation, size, and mineralization of osteons. Three osteons were more extensively sampled to investigate the bounds of useful data, and provide guidelines for what to expect in chemical compositional data when either altered portions of bone are encountered or features such as the cement line are crossed. Series B, C, and D, in Fig. 4 represent sampling beyond intact areas, into deteriorated areas, and past the cement lines (B, C). Series C (top right in Fig. 3) shows how, while the majority of the osteon is intact, where the biodeterioration begins (whitish or cloudy discoloration) an intrusive phosphate mineral is already being formed and the cement line is no longer visible in the compositional data. In this case, the laser’s entry into the hypermineralized intrusive phosphate looks very similar to the composition of the cement line of other osteons. The presence of intrusive material is clearly shown in the Ca content of series D (Fig. 4). It is important to be able to identify the type of microbial action and/or other diagenetic processes in action in any given sample to ensure that appropriate measures are taken into consideration prior to interpretation of the results.

Sr values display an interesting pattern, being present as a structural component and a trace element (i.e., floating in mineral traps within the matrix). Fully biodeteriorated portions demonstrate a loss of Sr in addition to a redistribution of the remaining portion present in the intrusive phosphates (Fig. 5). Along with the stable Sr:Ca values (Fig. 6), this suggests that the form of the intrusive phosphate is more akin to brushtine than to francolite (Garvie-Lok et al., 2004, Prohaska et al., 2002), though this is difficult to assess conclusively from a limited sampling area. Prior to complete dissolution, Sr does appear to be resistant to diagenetic effects (cf. Radosevich, 1993). Ba shows an interesting aspect of trace element uptake within the osteon (Fig. 7). Ba can act as a mineral replacement for Ca or Sr, yet those points closest to the
Haversian canal suggest diagenetic Ba values and that trace elements were not secured into the mineral matrix at the time of death and that the Ba of the most recently deposited bone matrix remained soluble upon burial.

Burials 35-2 and 39 (Figs. 8 and 9) show an interesting division in barium values. Recent research (e.g., Swanston, et al., 2012), has suggested that differences in trace elements values relate to the age of individual osteons, providing insight into the chemical environment of the individual closer to the time of death. The rate and extent of mineralization likely plays an important role in this differential uptake of trace elements, with older osteons still connected to the Haversian system, but less likely to incorporate elements such as barium in a structural capacity, limiting uptake of these older and more fully mineralized structures to flaws within the mineral matrix. The transitional values noted in Burial 35-2 (Fig. 8, series A) indicate such differences are progressive, as the pathway to full mineralization and reduced incorporation of higher Ba concentrations trends from high values at the Haversian Canal to lower values closer to the cement line. Such a difference could be a useful discriminator when attempting to identify older versus younger osteons within a sample and also provide additional evidence for movements in the last few years of life.

Remaining three burials, i.e., 27, 35 and 57 (Figs. 10−12), indicate more stable levels of Ba throughout the lives of the sampled osteons. This would suggest that these individuals remained in the same biogeochemical area for many years. Nonstructural elements (i.e., Fe) show significant variations in concentrations, marked redistribution associated with biodeterioration and reduced predictability in terms of uptake (Fig. 13). As an essential nutrient, this could relate to episodes of nutritional abundance and stress, could

Fig. 4. Calcium distribution (in ppm. concentrations divided by 1000) of six osteons of Burial 45 beginning at the Haversian canal and ending at or past the cement line. The series or path names (B−F) correspond with the osteons shown in Fig. 3. Shorter series came close to, but did not cross the cement line at their ends. Longer series crossed the cement line approximately where noted. Osteon C was specifically chosen as a partially impacted osteon to investigate the relationship between visual and chemical compositional changes. The six osteons were of similar size, but three osteons were more extensively sampled to investigate the bounds of useful data. Series B, C, and D, represents sampling beyond intact areas, into deteriorated areas, and past the cement lines but likely do not represent greater spans of time in terms of useful data.

Fig. 5. Strontium distribution (in ppm. concentrations) of six osteons of Burial 45 beginning at the Haversian canal and ending at or past the cement line. The series or path names (A−F) correspond with the osteons shown in Fig. 3. Shorter series came close to, but did not cross the cement line at their ends. Longer series crossed the cement line approximately where noted. Osteon C was specifically chosen as a partially impacted osteon to investigate the relationship between visual and chemical compositional changes.
indicate interactions with the biogeochemical environment, or could be an artifact of diagenetic changes, but are difficult to separate at present.

It is difficult to tell whether trace elements were deposited in the bone from the soil or if alteration lowered the Mg values used to calibrate the data. Analysis of trace elements and $^{87}$Sr/$^{86}$Sr on teeth from the six individuals analyzed herein and several other individuals from KN XIV demonstrated a relationship between certain trace elements and the mobility data traditionally provided by isotope analysis (Haverkort et al., 2008; Scharlotta, 2012; Scharlotta et al., 2011).

Scharlotta et al. (2011) demonstrated that within this population, Re replicated Sr isotope data, with higher Re concentrations in areas with higher Sr isotopic ratios. Thus, variability, as well as repeated patterns between osteons, in Re values for Burial 45 suggest that there were at least three major movement events (the patterning of peaks and valleys) in this individual’s life within the Little Sea region. When the sampled osteons were initially formed, the individual lived in an area of low Re values, shown by sampling points near the cement line (Fig. 14). This was followed by a movement from this area into a significantly different Re area (Peak X, Fig. 14). Then there was movement out of the high Re area, producing Valley Y (Fig. 14). The third movement showed a return to higher Re values indicated by sampling nearer to the Haversian Canal. It is possible that this final movement represents a return to a higher Re area of the Little Sea near the time of death or transportation of the body after death, if the elevated Re values in the Haversian Canal represent local soil conditions and/or if the cemetery was used to inter more than just individuals who died near the location. That similar patterning, of peaks and valleys, is seen across multiple osteons of the same individual (Burial 45) suggest this to be anthropogenic and not diagenetic alteration. To see such

![Fig. 6. Strontium to calcium ratio distribution of six osteons of Burial 45 beginning at the Haversian canal and ending at or past the cement line. The series or path names (A–F) correspond with the osteons shown in Fig. 3. Shorter series came close to, but did not cross the cement line at their ends. Longer series crossed the cement line approximately where noted. Osteon C was specifically chosen as a partially impacted osteon to investigate the relationship between visual and chemical compositional changes.](image1)

![Fig. 7. Barium distribution (in ppm concentrations) of six osteons of Burial 45 beginning at the Haversian canal and ending at or past the cement line. The series or path names (A–F) correspond with the osteons shown in Fig. 3. Shorter series came close to, but did not cross the cement line at their ends. Longer series crossed the cement line approximately where noted. Osteon C was specifically chosen as a partially impacted osteon to investigate the relationship between visual and chemical compositional changes.](image2)

![Fig. 8. Barium distribution (in ppm concentrations) of four osteons of Burial 35-2 beginning at the Haversian canal and ending inside the cement line, each data series (A–D) reflects one osteon. Note the divisions between groups of osteons.](image3)
duplication in diagenetic processes in multiple locations, each with unique history of preservation and potential alteration would be highly unlikely.

The preservation of Re signatures is somewhat easier to see in other individuals. Burial 35-2 (Fig. 15) has three series that are fairly well correlated and one that appears to be offset. Series C has a valley at ablation line 2, while the other series show a small peak. Then at ablation line 3, there is a peak while the others have a valley. The beginning and ending of this series may have been slightly offset relative to the span of time represented by other osteons. The placement of laser ablation lines relative to the Haversian Canal and spacing was consistent within each bone sample, likely indicating that this osteon is either older or younger than the other osteons sampled. The pattern of beginning in a low Re area, moving to a higher Re area, and then back again would suggest three or possibly four movements. Intriguingly, whereas Burial 45 showed elevated Re values near the Haversian Canal, Burial 35-2 shows a decrease. This raises the possibility that either extremely localized changes in the soil chemistry are impacting diagenetic alterations within the KN XIV cemetery, or that one of these two individuals was transported to the cemetery after death. The relatively smaller peaks and valleys could indicate that these movements are taking place across less geologically diverse areas of the Little Sea, or that these movements are part of a multi-year pattern of relocation and return to the same or similar areas.

Burial 39 (Fig. 16) also appears to have osteons of two different ages. Series A and D show elevated Re values at ablation line 4, while series B and C show reduced values. Again at ablation line 3, series A is quite low while series C is high. Together, there still appears to be a combination of peak and valley, or valley and peak, followed by a final reduction in Re values near the Haversian Canal, suggesting that the individual likely made three movements during life.

Burial 57-2 (Fig. 17) also likely shows three movements. Re values decrease from near the cement line at ablation line 5 to ablation line 3. This is followed by a peak at ablation line 2 and return to lower Re values at ablation line 1 near the Haversian Canal.
Canal. There is some divergence at ablation lines 3 and 4 that could suggest differences between the mineralization rate and Re intake, or perhaps shorter term movements in between areas of higher and lower Re that are not equally represented by the ablation lines in all osteons.

Burial 35-1 (Fig. 18) shows two major movements during their life. There are possible small peaks or valleys present at ablation line 3 and 4, though the clearest trend is a reduction in Re values from ablation line 5 to ablation line 2, with a subsequent increase at

Burial 45 suggests that there were at least three major movement events in this individual’s life within the Little Sea region. When the sampled osteons were initially formed, the individual lived in an area of low Re values, shown by sampling points near the cement line (Fig. 14). This was followed by a movement from this area into a significantly different Re area (Peak X, Fig. 14). Then there was movement out of the high Re area, producing Valley Y (Fig. 14). The third movement showed a return to higher Re values indicated by sampling nearer to the Haversian Canal. It is possible that this final movement represents a return to a higher Re area of the Little Sea near the time of death or transportation of the body after death, if the elevated Re values in the Haversian Canal represent local soil conditions and/or if the cemetery was used to inter more than just individuals who died near the location.
ablation line 1. Multiple smaller movements may have occurred, but the data only show two clear movements between significantly different areas of Re concentration.

Individual 27-1 (Fig. 19) may not have moved between geologic zones, or was possibly more contaminated than other samples.
Values near the cement line and Haversian Canal are similar. There are clear peaks and valleys in the Re values, but there is not a consistent pattern between multiple osteons, making it likely that localized microscopic changes could be influencing the data, and would require further analysis to clarify the matter based on Re data alone.

Another element that showed strong promise for anthropogenic data was cesium, with similar correlations between strontium isotope ratios and Cs concentrations (Scharlotta et al., 2011). Individual 27-1 appears to have made three major movements in their life, in contrast with the Re results (Fig. 20). This inference is based on transitions between low and high concentrations with two peaks, and one trough clearly observed in two osteons, and very similar in a third. The initial peak at ablation line 2 shows higher concentrations than ablation line 1, closest to the Haversian canal and most likely to reflect the composition of the burial environment. Using Re alone, the data did not clearly indicate three movements as multiple ablation lines produced divergent data. With Cs supporting multiple movement events in an unambiguous fashion, a re-examination of the Re data is in order. As noted, there are clear peaks and valleys in the Re data, with points of relative convergence at ablation lines 2 and 4. These points could indicate low Re values with higher Re values along both the cement line and the Haversian Canal. Ablation line 3 presents the real mystery in this instance, indicating either stable, low values of Re, or significantly elevated Re values. It is possible that a shorter duration movement occurred in this period of time and that differences in the mineralization of different osteons altered the incorporation of Re during this time, with some osteons readily accepting Re, while others only registered the relatively lower values. Ultimately, multiple lines of evidence would tend to reinforce one another and support that movement events have occurred with all uncertainty in the nature or locations of these events.

Based on Cs data, individuals 35-1 (Fig. 21), and 57-2 (Fig. 22) appear to have only made two major movements and so likely occupied area with a more even geochemical gradient as opposed to the sharp shifts noted in Re. The results for Burial 35-2 (Fig. 23), Burial 39 (Fig. 24), and Burial 45 (Fig. 25) also suggest three major movements, though with varying degrees of clarity. Individual 45 showed concentrations of cesium close to the Haversian canal that were higher than the other individuals examined. It is possible that

Fig. 20. Cesium distribution (in ppm. concentrations) of four osteons of Burial 27-1 beginning at the Haversian canal and ending inside the cement line each data series (A–D) reflects one osteon. Individual 27-1 appears to have made three major movements in their life. This inference is based on transitions between low and high concentrations with two peaks, and one trough clearly observed in two osteons, and very similar in a third. The initial peak at point 2 shows higher concentrations than point 1, closest to the Haversian canal and most likely to reflect the composition of the burial environment.

Fig. 21. Cesium distribution (in ppm. concentrations) of four osteons of Burial 35-1 beginning at the Haversian canal and ending inside the cement line each data series (A–D) reflects one osteon. Cs values show a general trend from high values near the cement line to low values at ablation line 2. Intermediate peaks and valleys may represent smaller scale movements or other environmental changes, but are not correlated between all sampled osteons. There was a second movement, back to a high Cs area indicated by ablation line 1.

Fig. 22. Cesium distribution (in ppm. concentrations) of four osteons of Burial 57-2 beginning at the Haversian canal and ending inside the cement line each data series (A–D) reflects one osteon. Burial 57-2 shows a gradual trend of increasing Cs values between ablation line 4 and ablation line 2. There is a small decrease between ablation lines 5 and 4, but it not clear if such a small change should be inferred as a clear movement, or if this could be explained by other factors. The sharp decrease between ablation lines 2 and 1, on the other hand, more clearly indicates that a significant movement event has occurred.
this is a diagenetic signal indicating an extremely localized difference in soil chemistry. It is also possible that this individual arrived in the Little Sea region near their time of death and did not inhabit the region long enough for new bone to mineralize. Cesium does not precisely replicate strontium isotopic values, but rather suggests greater degrees of mobility within geological areas with similar signatures.

Overall, these results indicate a lifestyle involving frequent movement by these six individuals between areas with much different geochemical signatures. Since three of the examined individuals were c. 8–10 years old (Burials A, B and C), one 18–20 years old (Bural D), and two 35–50 years old (Burials E and F), it is pretty much safe to say that such travel characterized any stage of life of these six persons and whoever else traveled with them. For the younger individuals the age of osteons cannot be greater than the age of the individual, thus analysis of individual osteons represents small spans of time in the last years of life. Patterning in movements between geochemical regions suggested that 2–3 movements were the norm, not for example 1 or 5 movements. This suggests a consistent strategy of movement every other year, though the meanings of these movements could be different depending on the age of the individuals and not represent the patterns of a cohesive group.

Individuals in this study reflect two dietary patterns, three individuals with a game-fish (GF) and three with a game-fish-seal (GFS) diet (Table 1; see Weber et al., 2011). Furthermore, only two of these six individuals (Burial A and B) had been identified as born locally (Weber and Goriunova, 2012). As a result, consistent patterning of movement suggests that dietary differences are not simply the result of location, or resource availability during life. Perhaps individuals in the region were closely knit into a larger network that permitted the fluid exchange of people between smaller groups in different areas. Since, in a different study (Scharlotta, 2012) we documented a substantial amount of mosaic in distribution of various geochemical traces across the Little Sea, west coast of Baikal, and Upper Lena micro-regions, it is impossible at this stage of our research to pinpoint more precise locations of these areas. In other words, it is too early to relate this information to the scale of movement which could be anything from relatively short distances to quite substantial. Further work on geochemical mapping of Cis-Baikal is in progress.
6.2. Bone diagenesis

Elemental compositional data also provide insights into the nature and extent of diagenetic alteration within samples that may appear visually intact and/or identical in preservation as well as engage in discussion as to the role and manifested nature of different elements within primary bone structure and diagenetic mineral counterparts in archaeological bone samples. Experimentation regarding the correlation between visible physical changes and chemical alteration suggested that portions of the bone that would be identified as altered or deteriorated in histological analysis also showed chemical changes related to the nature and extent of diagenetic factors. Perhaps most importantly from the perspective of this study, the elemental results can provide additional information on provenance and mobility of individuals during the latter portions of their lives as well as locality and dietary contributions in adult individuals during the years immediately prior to death, both aspects generally extending beyond the accessible record of molar formation. Further work with bone samples of young adults could help to elucidate problems or uncertainties with residence time of different elements within the body system as a whole and within specific tissues as there would be overlapping records of bone and teeth, accessible in micro-scale.

One additional point of interest with micro-sampling bones is that with the progression of microbial alterations, based upon observation of slides prepared for this research and locating intact osteons therein, there appears to be strong evidence that the periosteum harbors a disproportionate number of intact osteons in spite of the seemingly more direct contact with the burial environment. Consideration of this matter highlights one important aspect of bones, that they contain water themselves, and that their post-depositional alteration progresses with water contact. Regardless of the form of diagenesis, there are a limited number of ways in which the various effects can alter the composition of the bone matrix.

Microbial attacks are generally discrete intrusions that may or may not target collagen (Child, 1995; Hedges, 2002; Hedges and Millard, 1995; Jans, 2008; Jans et al., 2004; Landerso and Frost, 1964). Organic diagenesis regards the processes of collagen decay and loss, though the ability of certain humic substances to penetrate bone and attach to collagen is somewhat worrisome. Diffusion reactions and internal remodeling of the bone matrix control inorganic diagenesis. Newer lamellae are present in all living osteons, and will likely not be fully mineralized, still highly soluble and closest to the cavities that were Haversian canals until the organisms’ death. With specific note that the internal bone water is generally sufficient to maintain a closed system that requires the diffusion of ions either in or out; the remaining liquid-phase minerals on free-floating collagen fibrils will dissolve the partially mineralized structures of the young lamellae to create the saturated solution and the base material for any recrystallization (Hedges, 2002).

The patterning or layout of each individual osteons is unique, the result of cellular life history, as will be the progression of diagenetic factors. Caniculi connect all living osteocytes, however the diffusion capabilities, or liquid penetration, through these caniculi will be limited (Tuross et al., 1988). Caniculi generally lead to secluded and fully mineralized bioapatite portions of older lamellae. Saturated or partially saturated solutions will have difficulty in dissolving a largely insoluble mineral crystal through such limited pathways (Katzenberg, 2008). As such, the liquid penetration of diagenetic solutions will be limited within the complex microstructure of bones and there will be preferential dissolution of decaying mineral structures. Thus, even if in diffusion equilibrium with the external environment, it will be difficult for diagenetic ions to reach the mature mineral structures until microbial attack can break down the matrix and eliminate the collagen fibrils holding everything together (Hedges, 2002; Katzenberg, 2008; Katzenberg and Harrison, 1997; Nielsen-Marsh and Hedges, 2000a).

Furthermore, no materials cross the cement lines of osteons even when the systems are alive, thus penetration of the cement line will be difficult even if the protein structure does not grant any buffering effects (Kerley, 1965). Osteocytes have a limited lifespan (~25 years), and their mineralized structures are replaced as new osteons cut through the existing bone (Frost, 1963; Maximov and Bloom, 1957). As a result, the interstitial lamellae (bone matrix between the cement lines of living osteons) is mineralized material from dead osteons, thus any remaining matrix is not connected to any active caniculi and will be very hard pressed to be in dissolution contact with bone fluids post-mortem. Given the nature of the post-mortem bone liquid solution, it is unlikely that the ideal balance of ionic raw materials will be present to enable the precipitation of largely insoluble bioapatite mineral crystals rather than the precipitation of more soluble mineral forms, especially in the absence of guidance from nucleation or any of the protein/substance signals that control bone formation and mineralization during life (Hedges, 2002; Millard, 2001; Sillen, 1989).

Finally, proportions of available poorly mineralized bioapatite crystals for dissolution (i.e., hypercalcified bright lines and laminar bone) will be different for specific bone structures depending on how they are in contact with the bone liquid and depend on the age and species of the organism (Evershed et al., 1995; Glémcher, 1976). Thus there are number of reasons to believe that there will be significant variability and/or inequities within the bone matrix as it relates to the impact and effects of diagenesis, regardless of the form(s) in operation over any given period of time.

6.3. Laser ablation and strontium isotope analysis

There is an ongoing debate in archaeology and geochemistry regarding problems with isotopic analysis of strontium in calcium phosphate matrices using laser ablation as an introduction method, for some aspect of the laser ablation sample introduction leads to the formation of a polyatomic species that interferes with mass 87 and thus interpretation of strontium isotope ratios (Prohaska et al., 2002, Scharlotta, 2010, 2012, Scharlotta et al., 2011, Simonetti et al., 2008, Woodhead et al., 2005).

Strangely enough, even though there is great similarity in the mineral matrices that form teeth and long bones, there is no evidence for significant alteration of isotopic signals from bones. Looking at the elemental data, there appear to be similar formations of the related polyatomic species at mass 91, though with fairly high error terms and low concentrations all around, yet there is no clear interference as a result. This can be seen in Fig. 25; for each individual, the average 87Sr/86Sr value for the nine unaltered osteons fails close to that obtained for the same individual using solution-mode analysis. The values for the diagenetically altered osteons are, in contrast, clearly aberrant and cover a wide range of values (Figs. 26 and 27, Supplementary Material II).

As these samples were recovered from a high-87Sr/86Sr region, it is entirely possible that diagenetic contaminants were not fully removed from samples during solution preparation and purification, thus leaving an elevated result that could mask the presence of interference resulting from laser ablation. This seems unlikely in this particular case as several of the samples’ diagenetic portions yielded lower Sr isotope ratios, and only one sample (Burial 35-1) was suspected of having partial alteration on even intact osteons, and thus was likely to be almost completely compromised.
There are a few possible explanations for this, and likely others not considered here (see e.g., Radloff, et al., 2010). First, the problem may be with the solution data masking the presence of interference in the laser data. Second, perhaps there is enough difference in the structure of the mineral matrices that the laser interaction does not actually create the polyatomic interference noted in analyzing teeth and the elemental data are simply the result of large error factors on low concentrations. Third, what is being seen is actually a large coincidence, with a homogenized complex life history coming up with similar numbers as an altered laser averaging.

There are a few solution preparation procedures intended to remove diagenetic overprinting by eliminating the most soluble portions of the bone structure, namely those that were mobilized in the burial environment rather than life. However, there remains a degree of uncertainty as to what is being removed, what the true target data are (i.e., remaining solids after n leaches, or the nth leachate) and whether an efficient leaching has been achieved. This difficulty is further confounded by the complexity of bone structure and formation processes. Any long bone is a combination of material from potentially well over 30 years of an individual’s life. The lifespan of any given Haversian system is generally no more than 20–25 years; however, remnant portions of old Haversian systems, not fully removed by the cutting actions of visible osteons, frequently remain visible in histological slides. The true age of these remnants is often uncertain and they could thus contribute intact, diagenetically resistant bone material to the analytical result without much regard as to what its contribution is truly telling us about the individual as we do not have a good fix on the chronology of such portions. This matter is the focus of continued investigation to assess the intriguing source of this confusion.

7. Conclusion

Numerous laboratory procedures and a series of checks have been implemented to assess the quality of a sample and to counteract or remove the effects of biodeterioration on bone sections or powders (e.g., Garvie-Lok et al., 2004; Koch et al., 1997; Nielsen-Marsh and Hedges, 2000b). Such methods have proven effective and necessary, given the prevalence of biodeterioration, in accessing bio- and geochemical data obtained from archaeological bone. The downside of these techniques is that they all rely on homogenized sample tissue which makes it impossible to identify important movement or dietary events within individual life histories.

While microscopic observations of biodeterioration are not new (e.g., Bell, 1990; Jackes et al., 2001; Nielsen-Marsh and Hedges, 2000b), our data show that many chemical alterations reported in the literature can be avoided and/or monitored in tandem with chemical analyses of bones. Micro-sampling (i.e., laser ablation, micro-drilling) provides an avenue towards obtaining anthropogenic chemical data and, most importantly, individual life history events. Areas of biodeterioration can be avoided or corrections for specific alterations observed can be applied with greater finesse than bulk sampling approaches and potentially enable further use of archaeological materials that hold detailed data on diet, mobility, and health at different intervals of the entire individual life history.

In the Cis-Baikal region, micro-sampling of individual osteons strongly supports the hypothesis that these hunter-gatherers made numerous major movements during their lives. Without more detailed geochemical mapping of the region it is difficult to narrow down their exact locations during life, however, it certainly does appear that many individuals were moving into and out of the Little Sea region of Lake Baikal. Furthermore, individuals were making
major movements either shortly before their death, or were transported to the Little Sea post-mortem, both suggesting a strong cultural importance of either the area, or the Khuzhir-Nuge XIV cemetery in particular.

In terms of general application, the tandem use of strontium isotope and elemental compositional analysis using LA-ICP-MS seems very feasible and effective. At present, the isotopic signatures of whole osteons can be observed and micro-sampling can be conducted using elemental data for further enhancement of provenance determination. Perhaps with future improvements in equipment, a more direct approach may become possible, but with the equipment available today, major progress in the amount and nature of information available from bone samples is already feasible.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jas.2013.07.014.

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