PALAEOECONOMY OF THE EURASIAN STEPPE: BIOMOLECULAR STUDIES

Submitted for the degree of Doctor of Philosophy
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- I beg you! Be so kind!
Just favour me and taste it!
- Neighbour, I pray you, do not press me!
- Change your mind.
Another spoonful; do not waste it;
This fish-soup is the thing, 'tis luscious, capital.
- I've swallowed now three portions.
- What of that? No matter,
Come now, no foolish chatter,
Think of your health, and eat it all.

- from "Demyan's Fish Soup" by Ivan Andreevich Krilov

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ABSTRACT

The Bronze and Iron Age inhabitants of the Eurasian steppe region of the former USSR have long been considered dependent upon their domestic stock as the basis for their diet and economy. Research presented by O'Connell et al. (2000) first proposed that freshwater fish played an important and possibly even dominant role in the diets of central Eurasian steppe groups. This set of studies tests the hypothesis that freshwater fish were a major dietary staple for central Eurasian steppe and border-steppe humans during the Bronze and Iron Ages. Evidence for the frequent consumption of freshwater animals is provided by a large-scale palaeodietary study involving the carbon and nitrogen isotope analysis of human and faunal individuals obtained from central Eurasian archaeological sites. The palaeodietary evidence for high-freshwater fish diets is supported by the findings that the frequent consumption of dairy products is unlikely to lead to the highly elevated human $\delta^{15}N$ values observed in many Bronze and Iron Age humans. Sulphur isotope analysis of human and faunal remains from two Eurasian steppe sites indicate that $\delta^{34}S$ analysis can provide limited information by which to distinguish humans with a mainly freshwater from those with a mainly terrestrial diet. The criteria by which bone collagen may be assessed for sulphur isotope analysis are discussed. The analysis of archaeological food residues demonstrates the potential to identify vessels used to prepare fish in antiquity. Residue analysis of vessels from the archaeological site of Chicha suggest that dairying was a subsistence practice carried out at the site, in addition to fishing and the management of domestic herds for meat. A revision of the dietary and economic profile of central Eurasian steppe peoples is proposed, with added emphasis upon the importance of freshwater fish for many groups.

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CHAPTER 1

INTRODUCTION

1.1. *BACKGROUND*

This thesis arose out of a pilot project conducted by O'Connell *et al.* (2000), in which the stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) for 158 humans from Mesolithic through Iron Age Eurasia were compared with values of 41 terrestrial herbivores and 8 (modern) freshwater seals from Lake Baikal. The study by O'Connell *et al.* reported original data in addition to data from Lillie and Richards (2000), Bonsall *et al.* (1997) and Katzenberg and Weber (1999). These studies and a further report by Lillie *et al.* (2003) represented all published stable isotope analyses of archaeological central Eurasian populations. The humans considered by O'Connell *et al.* (2000) exhibited $\delta^{15}N$ values that seemed too highly elevated to indicate a diet based principally upon terrestrial herbivore protein. The authors concluded that the high human $\delta^{15}N$ values could be explained by a diet in which fish provided up to 50% of dietary protein.

This high-fish diet proposed by O'Connell *et al.* for central Eurasian steppe humans was met with scepticism by a number of archaeologists (S. Olsen and L. Koryakova, personal communications). The cultures represented by the humans studied (particularly Bronze and Iron Age communities) are traditionally regarded as fundamentally pastoral, and dependent upon domestic terrestrial herbivores for nearly all of their dietary protein intake. Notwithstanding the scepticism with which O'Connell's research was received, these new isotopic data were embraced by some archaeologists who felt that the role of fish in the diet of ancient Eurasian communities had been consistently overlooked due to biases at sites that favoured the preservation and recovery of large bones over small, and due to historical (e.g., Herodotus

4:2, 4:46), ethnographic (e.g., Rudenko 1970) and linguistic (see Jones 2003) reflections on Eurasian nomadic pastoral economy (H. Parzinger and B. Hanks, personal communications).

The data reported by O'Connell *et al.* could not, however, decisively resolve the controversial issue that they had raised. Though the humans analysed had all come from prehistoric Eurasian steppe and border-steppe sites, the samples covered a vast geographical spread and ranged in age from the Mesolithic to the Iron Age. More recent research by Lillie *et al.* (2003) contributed stable isotope data for 21 Epipalaeolithic humans from the site of Vasilyevka III, Ukraine. From all central Eurasian palaeodietary studies, the maximum number of humans represented at a single site in a single time period was 29 (the Mesolithic site of Vlasac, reported by Bonsall *et al.* 1997), but the majority of the sites were represented by ≤ 5 humans. Samples from the Early Bronze Age included 25 humans and 5 terrestrial herbivores from 6 sites, and the Iron Age samples comprised 4 humans and 7 horses from 3 sites. O'Connell *et al.* (2000) concluded, "Further isotopic analysis is needed of more human and animal samples from a wider range of sites in order to confirm that the results of this study are truly representative of ancient central Eurasian diets" (307).

This statement provided the foundation for the research reported in this thesis. The work discussed herein was designed to test the hypothesis raised by O'Connell *et al.*—that is, that fish were an important dietary staple for prehistoric central Eurasian communities. A dietary emphasis on fish, either in conjunction with or instead of terrestrial animal products, would reflect a dedication of significant time, effort and resources toward the procurement of fish. The confirmation of frequent fish consumption would therefore require a

reassessment of the economic basis of these communities. Peoples acquiring fish by trapping and/or trading in sufficient quantities to be considered a dietary staple could not be considered to have an economy entirely focused upon their domestic herds.

1.2. *TEMPORAL AND GEOGRAPHIC FOCUS*

In order to ascertain the importance of freshwater fish in the diets of central Eurasian populations, the main research conducted for this thesis involved a large-scale palaeodietary analysis of human and faunal remains from sites throughout the central Eurasian steppe (chapter 3). The temporal focus of this research was narrowed from the time range reported in O'Connell *et al.* to concentrate on Bronze Age and Iron Age groups. An extensive palaeodietary investigation focused on the Bronze and Iron Ages was required to supplement the few existing analyses representing this period. Furthermore, the issue of frequent fish consumption was most controversial with regard to the 'pastoralist' human groups that populated the central Eurasian steppe in the Bronze Age through the Iron Age.

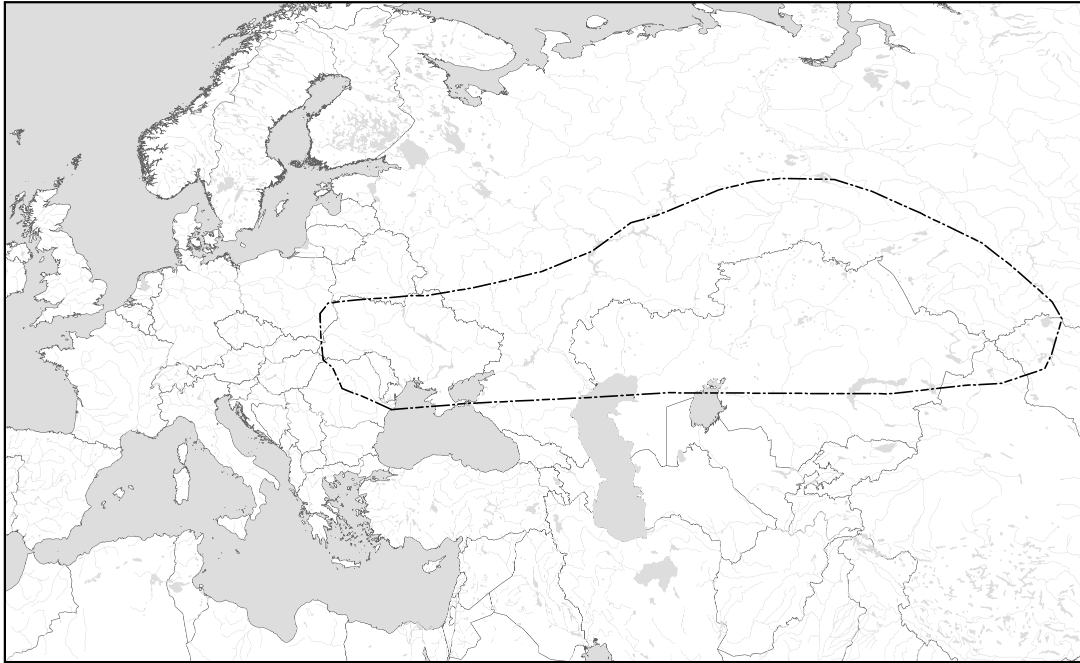

Figure 1.1. The general area from which samples were obtained for research (indicated by the broken line). This region includes steppe, forest-steppe and mountain-steppe areas within Ukraine, the Urals and western Siberia, the Altai region of Russia and the site of Chicha in southwestern Siberia.

Material was acquired for palaeodietary analysis from various sites throughout the central Eurasian steppe and border-steppe regions (i.e., forest-steppe and mountain-steppe). The general geographical area from which samples were obtained is shown in figure 1.1. For the purposes of this thesis, references to the 'Eurasian steppe' should be taken to refer to the area indicated in figure 1.1, extending from the North Pontic region in the west, across the Urals and northern Kazakhstan, to southwestern Siberia and the Altai region of Russia.

1.3. *THESIS STRUCTURE AND GOALS*

In chapter 2, I present an overview of stable isotope analysis and its use in palaeodietary research. The purpose of the chapter is to provide information about palaeodietary theory and methodology, as a background

against which the research presented in future chapters may be viewed and interpreted. Chapter 3 may be regarded as the central chapter of the thesis, in which I test the hypothesis that freshwater fish were an important dietary staple for Bronze and Iron Age Eurasian steppe communities, via the stable carbon and nitrogen isotope analysis of human and faunal bone collagen.

The three chapters that follow comprise supplementary research designed to test the palaeodietary conclusions drawn in chapter 3. These chapters are methodological in focus and each involves an element of research relevant to palaeodietary study not previously conducted. The results discussed in chapters 4 through 6 have important implications not only for the study of Bronze and Iron Age central Eurasian human diet, but also for palaeodietary research in general.

Chapter 4 tests the assumption that highly elevated human $\delta^{15}N$ values in the absence of high $\delta^{13}C$ values can only be caused by a diet high in freshwater animal protein. Chapter 5 probes the utility of sulphur stable isotope analysis in the detection of freshwater fish consumers. The final main chapter diverges from the focus on bone collagen analyses and presents a study of food residues extracted from archaeological potsherds obtained from the southwestern Siberian site of Chicha. The residue analysis provides a complementary assessment of resource exploitation at Chicha, which can be compared with the palaeodietary information derived from bone collagen analyses.

When viewed as a whole, the various strands of research presented in this thesis contribute to the construction of a detailed picture of ancient Eurasian steppe diet and economy. Due to the large number of samples analysed, the palaeodietary data can be used to compare the average diets of

humans from different cultures, regions and time periods. The research described in chapters 4 through 6 lends weight to the conclusions drawn in chapter 3, and adds further to the assessment of the contribution of freshwater animals (e.g., fish) to human diet in general.

CHAPTER 2

STABLE ISOTOPES AND DIET: BACKGROUND AND ARCHAEOLOGICAL APPLICATIONS

2.1. *INGESTED FOOD AND BODY COMPOSITION*

"Tell me what you eat, and I will tell you what you are."
- Anthelme Brillat-Savarin, *Physiologie du Gout* (1825)

The analysis of ancient diet through stable isotope analysis is based on the principle that 'you are what you eat'. That is, the food consumed by an animal is broken down and partially incorporated into the animal's body tissues. Thus, the biochemical composition of an animal's tissues is partially dependent upon the composition of the plants or animals it has consumed. Even the molecular makeup of plant tissues is determined in part by the air and soil from which plants derive their nutrients.

Our knowledge of the behaviour and distribution of stable isotopes in the environment allows archaeological scientists to address issues of palaeodietary reconstruction that are otherwise difficult or impossible to study using traditional archaeological techniques. The distinctive distributions of carbon and nitrogen isotopes in various ecosystems give us a framework within which we can identify dietary trends in ancient societies as well as differences between the diets of groups or individuals within a certain population. The examination of samples spanning a broad geographical or temporal range can reveal a dynamic picture of regional subsistence, which may have social, economic and political implications for the ancient populations concerned.

This chapter presents a discussion of the utility of stable isotope analysis in the study of the diets of modern and ancient animals. I mention the tissues commonly used in dietary analyses, tissue turnover, and the particular use of bones in palaeodietary research. The bulk of this chapter focuses on the

distribution of carbon and nitrogen stable isotopes in the biosphere, as these elements are of central importance to most current palaeodietary analyses, including the analysis of Bronze Age and Iron Age Eurasian steppe remains covered in this thesis (Chapter 3). Examples of previous archaeological applications of dietary analysis are described where applicable.

2.2. *TISSUES USED IN STABLE ISOTOPE ANALYSIS*

2.2.1. ISOTOPIC VARIABILITY

Dietary intake is reflected in the isotopic composition of an animal's body tissues, which incorporate ingested elements as they are formed and remodeled. Research has shown overall carbon and nitrogen isotope values to be essentially homogenous within a species, between males and females of a single species or within a body tissue type such as the liver or bone collagen (DeNiro and Schoeninger 1983; Lovell *et al.* 1986; Schoeninger *et al.* 1997). On the other hand, some degree of isotopic variability exists between the carbon and nitrogen atoms of different tissues of an individual, different molecule types within a tissue (e.g., proteins, lipids, carbohydrates), different amino acids and even atoms within the same molecule (Hare *et al.* 1991; Ambrose 1993; Lajtha and Marshall 1994; Keeling *et al.* 1999).

2.2.2. TISSUE TURNOVER

The rate of formation and remodeling also varies between tissues. The more metabolically active tissues (e.g., fat) have a turnover rate of a few days, while other tissues have a turnover on the order of weeks (e.g., skin) (Tieszen

et al. 1983). These tissues reflect the isotopic character of an individual's most recent diet. Bone collagen turnover occurs on a much longer timescale, on the order of years rather than months (Libby *et al.* 1964; Stenhouse and Baxter 1979; Ambrose 1993), and thus reflects the average isotopic composition of an individual's diet over that time. Hair protein does not undergo turnover, but instead is formed by linear deposition over time (Saitoh 1969). Hair thus preserves an isotopic record of an individual's diet over time, with the most recent diet reflected in the hair closest to the scalp. Proteins present in teeth undergo virtually no reworking once they are initially deposited, and so preserve an isotopic record of the diet consumed at the time of tooth formation (Koch *et al.* 1994; Balasse 1997).

2.2.3. DIETARY RECONSTRUCTION

Scientists can use isotopic data from tissues subject to rapid, intermediate and slow turnover to produce a profile of an individual's diet over the last years of his or her life. In rare cases where soft tissues are preserved in an archaeological context, examination of an individual's stomach contents coupled with hair, skin and bone isotopic analysis can reveal how the last meal ingested compares with the individual's short- and long-term dietary history. Such comprehensive, multi-tissue analysis was performed on the frozen remains of 'Otzi', a 5,300-year-old human male found in the Tyrolean Alps. Bone and hair isotope analysis revealed that Otzi had subsisted on a broad range of foods (meat & plant products, but not fish), while colon content analysis provided specific evidence for foods consumed

just before the time of death such as meat, barley and einkorn wheat (Dickson *et al.* 2000).

2.2.4. COLLAGEN AND DIETARY RECONSTRUCTION

Collagen is an attractive candidate for palaeodietary isotopic analysis for many reasons. It is the most abundant structural protein in the body (accounting for the majority of skeletal protein), and the compact structure of collagen makes it resistant to contamination and diagenetic alteration. Collagen is particularly useful in elucidating trends in protein consumption, as dietary protein tends to be routed into the formation and repair of body proteins, causing proteins such as collagen to reflect dietary protein intake (Ambrose and Norr 1993). The structure and composition of collagen have been extensively researched and are now well understood. Post-mortem alteration of in vivo collagen isotope values can be identified using a range of criteria that address the total yield and the carbon and nitrogen content of the collagen extracted from archaeological bone (DeNiro 1985; Ambrose 1990, 1993). These techniques used to test the integrity of biogenic isotope values are employed in section 3.6.1. Because the turnover rate of collagen is on the scale of years, its isotopic values reflect one's average, long-term dietary input. In research concerned with assessing differences within and between populations on an everyday (i.e., not ritual) level, the analysis of bone collagen can be extremely helpful in revealing long-term dietary trends.

2.3. *STABLE ISOTOPES*

Isotopes are atoms of the same element (i.e., with the same number of protons) that have different numbers of neutrons in the nucleus and therefore have different atomic masses. Whereas radioactive isotopes are unstable and degrade over time (e.g., ^{14}C), stable isotopes do not undergo decay and—as their name suggests—remain stable indefinitely.

Stable isotope ratios are measured relative to internationally recognised standards, which vary according to the element and material analysed. These ratios are reported in units permil (‰), or parts per thousand, as a delta (δ) value as follows: δX (‰) = $(R_{sample}/R_{standard} - 1) \times 1000$, where X is the heavier of the two isotopes measured and R is the ratio of the heavier to the lighter isotope (Hoefs 1997).

2.3.1. FRACTIONATION

As isotopes cycle through the environment, they undergo fractionation at various stages. Fractionation is a change in an isotope ratio (e.g., $^{15}N/^{14}N$) resulting from discrimination against one isotope in favour of the other during a chemical or physical process. Although isotopes of the same element are fundamentally alike, their subtle differences in mass cause them to behave slightly differently in some situations.

Fractionation can be classified as either thermodynamic or kinetic. Kinetic fractionation involves unidirectional reactions in an open system, such as diffusion, evaporation and photosynthesis. These processes yield reaction products that are isotopically lighter than the reactants (Hoefs 1997). During

enzyme-mediated reactions such as photosynthesis, the higher energy required to break molecular bonds formed by heavier isotopes causes the lighter isotopes to be preferentially incorporated into the reaction products. In the case of evaporation, liquid water molecules at the same temperature (energy) will change to a gaseous state at different rates, depending upon their mass. Lighter water molecules ($H_2{}^{16}O$) have a higher velocity and will evaporate preferentially over heavier $H_2{}^{18}O$ molecules, producing water vapor with a lower $^{18}O/^{16}O$ ratio than the liquid water from which the molecules evaporated.

Thermodynamic fractionation is concerned with reversible reactions that operate when a system is at equilibrium, and is sometimes referred to as equilibrium fractionation. Thermodynamic fractionation occurs because the molecules containing heavier isotopes tend to concentrate in the phase of lowest energy. In the equilibrium system that exists between atmospheric CO_2 and the carbonate and bicarbonate ions in surface ocean water, a differential proportion of ^{13}C and ^{12}C isotopes are present in the atmospheric CO_2 ($\delta^{13}C$ = ~ -8‰) above the ocean and the dissolved $HCO3^-$ and $CO_3{}^{2-}$ ($\delta^{13}C$ = ~ 0‰) in the surface ocean water.

2.3.2. CARBON

Carbon exists naturally in three main isotopic forms: ^{12}C, ^{13}C and ^{14}C or radiocarbon. The most common isotope, ^{12}C, accounts for 98.89% of environmental carbon, while ^{13}C and ^{14}C have a natural abundance of 1.11% and 1.0^{-10}%, respectively (Weast 1974).

In contrast to dating techniques that center on the detection of radioactive atoms and/or their decay products (^{14}C dating, U-series dating), palaeodietary studies of carbon isotopes focus entirely on the ^{12}C and ^{13}C stable isotopes. Because the amount of ^{12}C and ^{13}C in a sample remains constant over time (excluding diagenetic effects), the $^{13}C/^{12}C$ ratio of the sample theoretically remains static. If an archaeological sample has not undergone significant biogeochemical degradation, a scientist may take a measure of its $^{13}C/^{12}C$ ratio as a reflection of the sample's original isotopic composition.

2.3.2.1. *Photosynthetic Fractionation*

Plants are known to discriminate against ^{13}C to varying degrees during the photosynthetic process (O'Leary 1981, 1988). The degree of fractionation depends upon the metabolic pathway by which the atmospheric carbon (as CO_2) is initially fixed for photosynthesis. Most terrestrial plants such as trees, legumes, wheat and rice are known as C_3 plants because they fix atmospheric CO_2 by the carboxylation of ribulose 1,5-bisphosphate, which then produces two three-carbon molecules of 3-phosphoglycerate. C_3 plants have a $\delta^{13}C$ range of -35 to -21‰ (mean ~ -28‰), in comparison with the $\delta^{13}C$ of atmospheric CO_2 at ~-7‰ (O'Leary 1988; Hoefs 1997; Pate 1994).

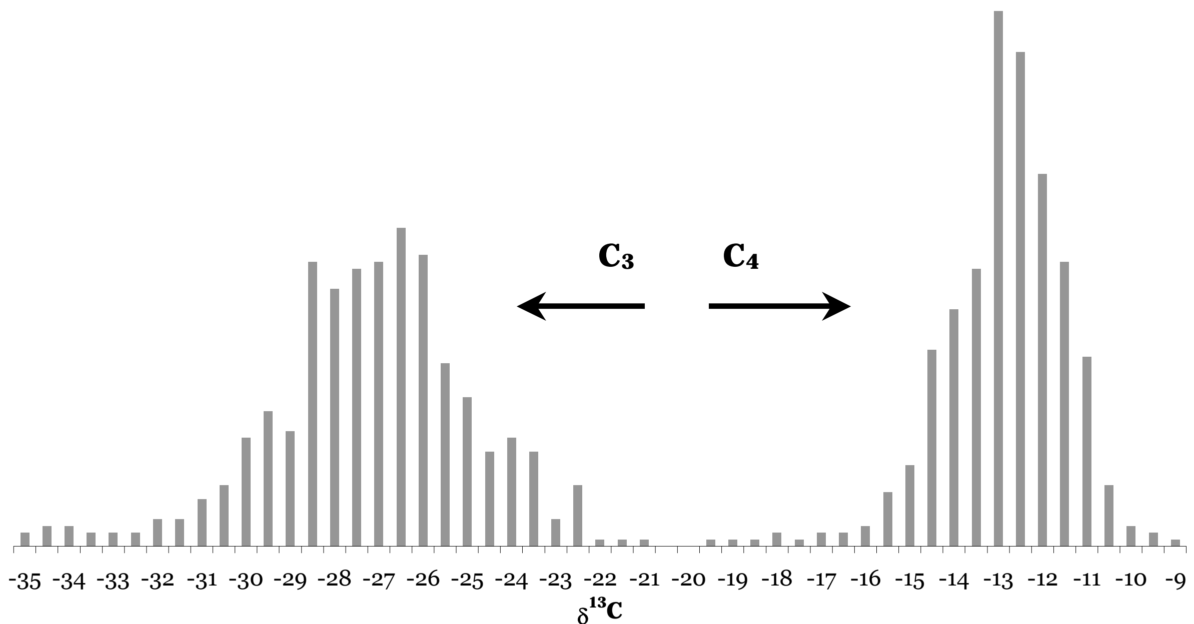

Figure 2.1. Histogram of $\delta^{13}C$ values recorded for C_3 and C_4 plants (after O'Leary 1988, approximately 1000 plant samples represented).

C_4 plants are named for the way in which they fix atmospheric carbon by the carboxylation of phosphoenolpyruvate, producing the four-carbon molecules malate and/or aspartate. These plants include tropical grasses, millet, maize and sugarcane. C_4 plants have mechanisms for water conservation and CO_2 concentration that C_3 plants lack, and are thus better adapted to function efficiently at higher temperatures and lower moisture levels. These adaptations are reflected in the geographical concentration of C_4 plants in areas with hotter, drier climates (Taiz and Zeiger 1991:241; see figure 2.2).

The C_4 photosynthetic pathway discriminates less against ^{13}C than the C_3 pathway; this results in a less negative $\delta^{13}C$ value for C_4 plants. C_4 plants exhibit $\delta^{13}C$ values of approximately -20 to -9‰ (mean ~ -14‰) (O'Leary 1988; DeNiro 1987). The $\delta^{13}C$ ranges of C_3 and C_4 plants do not overlap (O'Leary 1988; Pate 1994); similarly, the geographical ranges of these two plant types are fairly distinct.

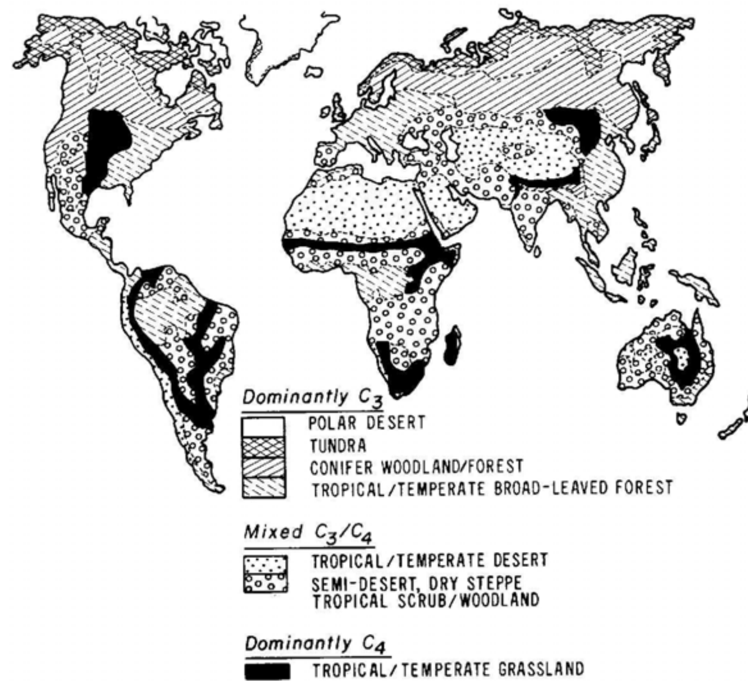

Figure 2.2. A map of the global distribution of C_3 and C_4 plant types, according to the isotopic composition of plant-derived soil carbonates (from Cerling and Quade 1993).

A group of plants known as CAM plants (Crassulacean Acid Metabolism) carry out some photosynthetic processes with mechanisms used by both C_3 and C_4 plants. The dynamics of CAM metabolism change according to environmental influences such as water availability, temperature and light. This metabolic variation causes differential discrimination against ^{13}C (in atmospheric CO_2), and is reflected in the wide spread of CAM $\delta^{13}C$ values, which lie on a continuum between C_3 and C_4 $\delta^{13}C$ values (O'Leary 1981, 1988). CAM photosynthesis is tailored to maximize water-use efficiency, making CAM plants particularly suited to desert environments (Taiz and Zeiger 1991:241).

Archaeological scientists have used $\delta^{13}C$ analysis of ancient human bone to investigate issues related to the introduction of maize cultivation and consumption in the Americas. In their study of human skeletal remains from pre-contact highland Ecuador, Ubelaker *et al.* (1995) used $\delta^{13}C$ data to show

that high-status individuals consumed much higher levels of maize (a C_4 plant) than did their low-status contemporaries. Research by Katzenberg *et al.* (1995) used $\delta^{13}C$ values obtained from archaeological human bone (in conjunction with palaeobotanical information) to determine the timing and intensity of maize consumption in the southern Ontario region of North America. In combining their isotopic information with those of other studies, Katzenberg *et al.* observed marked differences in both the timing of the adoption of maize horticulture/agriculture and the intensity of maize consumption across North America.

2.3.3. NITROGEN

Like carbon, nitrogen exists in two main stable forms. ^{14}N makes up 99.63% of total nitrogen, while ^{15}N accounts for 0.37% of nitrogen in the environment (Weast 1974). In most plants, nitrogen is incorporated into plant tissues from the soil in the form of nitrate and ammonium ions. Other plants, namely legumes and blue-green algae, usually rely upon symbiotic bacteria for their nitrogen requirements. These bacteria fix atmospheric nitrogen, which is then transferred to the legumes. Appreciable fractionation does not occur during nitrogen uptake into plants, but plants that take in atmospheric nitrogen (legumes) are depleted in ^{15}N relative to plants that utilize soil nitrogen, as nitrogen-containing compounds in the soil have a higher $\delta^{15}N$ value than atmospheric nitrogen (DeNiro 1987). Nitrogen-fixing plants have a typical $\delta^{15}N$ range of –2 to +2‰, while non-nitrogen fixers exhibit $\delta^{15}N$ of about 0-6‰; however, the $\delta^{15}N$ gap between these two groups of plants may narrow with different soils and the use of fertilizers in plant cultivation

(Virginia and Delwiche 1982; Pate 1994; DeNiro and Epstein 1981). Marine phytoplankton do not fix atmospheric nitrogen, but utilise nitrate and ammonium dissolved in ocean water as their nitrogen source. These organisms are more enriched in ^{15}N, on average, than terrestrial plants; but the inclusion of blue-green algae (marine 'legumes') causes the nitrogen isotope ratios of marine and terrestrial primary producers to show a great deal of overlap (DeNiro 1987).

2.4. THE TROPHIC LEVEL EFFECT

When an animal ingests and metabolises any type of food, some of the food's constituent molecules are retained in the body of the animal, and are used to build and repair body tissues. Nitrogen- and carbon-containing molecules make their way through the food chain from plants to herbivores to carnivores to secondary carnivores as they are incorporated into the body of each consumer.

Analysis of $\delta^{15}N$ values in animals at different levels of the food chain has led to the identification of a 'trophic level effect'. The $\delta^{15}N$ value of an animal's body proteins has been observed to be enriched in ^{15}N by approximately 3-5‰ relative to its dietary protein (Minagawa and Wada 1984; Schoeninger and DeNiro 1984; Schoeller *et al.* 1986; Ambrose 2000). Thus, the $\delta^{15}N$ of a herbivore's bone collagen is about 3-5‰ more positive than the $\delta^{15}N$ of the plants it consumes, and the $\delta^{15}N$ of omnivore/carnivore bone collagen is elevated by ~3-5‰ relative to the $\delta^{15}N$ of the animals and/or animal products consumed. This increase in $\delta^{15}N$ through the food chain is believed to be due

to the excretion of isotopically light waste products, such as urea (Steele and Daniel 1978; Ambrose and DeNiro 1986a&b; Macko *et al.* 1986, 1987).

There is no difference in $\delta^{15}N$ between the different types of protein obtained from the same animal, such as flesh, eggs or milk (Webb *et al.* 1980; Schoeller *et al.* 1986; Katzenberg and Krouse 1989; Minagawa 1992). Research by O'Connell and Hedges (1999) confirms that the hair keratin of individuals consuming any type of animal protein (both meat-eaters and ovo-lacto vegetarians) show a trophic level elevation in $\delta^{15}N$ over herbivores (vegans).

Crucial to the interpretation of isotope values in terms of palaeodietary research is the issue of the frequency with which an individual must consume animal protein in order to produce a detectable elevation in his/her body $\delta^{15}N$ (relative to average herbivore $\delta^{15}N$). Little work has been done to relate frequency of consumption to $\delta^{15}N$ values, but studies by O'Connell and Hedges (1999) indicate that consumption of animal protein at least once or twice per week produced elevated $\delta^{15}N$ in the hair keratin of individuals sampled, while less frequent consumption of animal protein does not cause an individual's $\delta^{15}N$ to differ significantly from that of vegans.

The magnitude of the trophic level effect is still a point of contention in the scientific community with regards to carbon. While some empirical data have shown the $\delta^{13}C$ of a consumer to be 1-4‰ or even 5‰ higher than that of its food (Koch *et al.* 1994), other studies have identified an almost negligible (≤1‰) enrichment in $\delta^{13}C$ per trophic level (Schoeninger and DeNiro 1984; Schoeller *et al.* 1986). Research by O'Connell and Hedges (1999) agrees with data collected by Webb *et al.* (1980), which show that the $\delta^{13}C$ values of vegans (herbivores) are statistically indistinguishable from the $\delta^{13}C$ values of

individuals whose diets include any amount of animal protein (i.e., omnivores and ovo-lacto vegetarians consuming animal protein less than once a week to once or more per day).

2.4.1. THE NURSING EFFECT

When an animal is born, its body tissues exhibit essentially the same isotopic values as those of its mother, reflecting her diet and trophic level. Immediately after birth the infant begins to nurse and its diet consists entirely of protein derived from its mother. An infant is thus one trophic level above its mother until it is weaned, and the tissues formed during the nursing period exhibit $\delta^{15}N$ values elevated ~3‰ relative to those of the infant's mother (Balasse *et al.* 1997; Schurr 1997; Fogel *et al.* 1997).

When a child is weaned, the diet of maternal milk is supplemented and ultimately replaced by other foods, and the child begins to occupy a lower place in the food chain. As the child consumes foods more depleted in ^{15}N than its mother's milk, new body tissues are formed that exhibit its lower ^{15}N intake. Eventually the child's $\delta^{15}N$ will reflect its new diet and trophic level (Schurr 1997; Balasse 1997). Between birth and the age at which a child's tissues no longer reflect a nursing diet, its $\delta^{15}N$ depends on a number of factors, including the rate of tissue synthesis, the age and rate at which new foods begin to replace maternal milk, and the isotopic composition of the child's diet when weaning is complete (Schurr 1997).

The age at which a child is weaned is determined by a combination of biological and cultural factors, and has direct consequences for child health and survival (Katzenberg *et al.* 1996; Fogel *et al.* 1997; Wright and Schwarcz

1998). An accurate assessment of weaning age in ancient societies can help archaeologists to interpret infant mortality patterns and to address issues of general health and population dynamics within and between different groups.

In their isotopic analysis of infant remains from the English medieval site of Wharram Percy, Fuller *et al.* (2001) determine that the children buried at this site had all been weaned between the ages of 1 and 2 years. The authors further suggest that infants were probably not weaned from maternal milk directly to adult foods, but rather to solid foods comprising a higher proportion of plant products than the average adult diet (Fuller *et al.* 2001; Richards *et al.* 2002).

2.5. *$\delta^{13}C$ AND $\delta^{15}N$ IN MARINE AND FRESHWATER ECOSYSTEMS*

Marine fish and mammals tend to show significant enrichment in the heavier ^{13}C and ^{15}N isotopes relative to terrestrial animals (Chisholm *et al.* 1983; Richards and Hedges 1999). Humans with a predominantly marine diet also have carbon and nitrogen isotopic values much higher than those with a terrestrial diet (Schoeninger *et al.* 1983). The ~8‰ difference between mean marine and terrestrial animal $\delta^{13}C$ can be traced back to the comparable difference in the $\delta^{13}C$ of atmospheric CO_2 (~ -7 to -8‰) and the $\delta^{13}C$ of dissolved CO_2 in the ocean (~0 to +1.5‰), which are taken up by plants and blue-green algae at the base of the food chain (Pate 1994; Hoefs 1997).

At the base of the terrestrial and freshwater food webs, plants and aquatic algae both fix carbon in the form of atmospheric CO_2. This shared carbon reservoir is reflected in the considerable overlap in $\delta^{13}C$ exhibited by aquatic and terrestrial foods, though the higher variability of CO_2 availability

in freshwater systems (relative to terrestrial) contributes to a wider observed $\delta^{13}C$ range in aquatic species (O'Leary 1988). Main sources of carbon also vary considerably within and between freshwater ecosystems in terms of type and magnitude of input, and include soluble organics such as sugars and amino acids, dissolved carbon dioxide, bicarbonate and carbonate. Particulate organics taken up into freshwater food webs may be derived from local bank vegetation, upstream debris and internal plant/algae production. A survey of the isotopic literature reflects the diversity of global freshwater fish $\delta^{13}C$ values, ranging between -42‰ and -6‰ (Fry 1991; Katzenberg and Weber 1999; Dufour *et al.* 1999; Vaz *et al.* 1999; White *et al.* 2001). In their study of modern Eurasian lacustrine fish isotope values, Dufour et al. (1999) measured $\delta^{13}C$ ranges of up to 6.9‰ for fish caught within the same lake.

The marine and aquatic food chains are considerably longer than the terrestrial food chain, with many fish occupying a trophic position at least two or three levels above primary producers (Schoeninger *et al.* 1983; Gu *et al.* 1996; Pauly *et al.* 1998). According to the ^{15}N enrichment observed with ascendancy through the food chain, we would expect many freshwater and marine foods to have higher $\delta^{15}N$ values than common terrestrial foods (i.e., plants, herbivores, and omnivores)—and this is indeed the case. The $\delta^{15}N$ values of some freshwater fish and marine animals overlap with the $\delta^{15}N$ values of terrestrial plants and animals, but the nitrogen isotope values of other marine and freshwater animals are significantly enriched in ^{15}N relative to terrestrial animals (Dufour *et al.* 1999:623; see figure 2.3). The additional mean ^{15}N enrichment of marine foods ($\delta^{15}N \approx 14‰$) over freshwater foods ($\delta^{15}N \approx 8‰$) is probably due to differential nitrogen fractionation at the base of the two food chains (Schoeninger and DeNiro 1984; France 1995).

Carbon and nitrogen isotope ratios have been studied as indicators of the presence and/or frequency of marine foods in the diets of ancient populations (Chisholm *et al.* 1982; Schoeninger *et al.* 1983; Pate 1997). Tauber (1981) has separately shown that the adoption of agriculture in coastal areas of Denmark during the Neolithic was reflected by a distinctive change in human bone $\delta^{13}C$ from a 'marine' to a 'terrestrial' signal. While late Mesolithic populations in coastal Denmark exhibited isotopic values greatly enriched in ^{13}C, Neolithic individuals had a markedly lighter isotopic profile, indicating the importance of terrestrial resources in their diet.

The highly elevated $\delta^{13}C$ and $\delta^{15}N$ values obtained from six ~5,700 year old human bone specimens from a small Hebridean island (Scotland) reflected the dependence of these individuals upon marine resources (Richards and Mellars 1998). However, one individual's lower $\delta^{15}N$ and more negative $\delta^{13}C$ suggested that s/he relied upon terrestrial foods for a significant proportion of his/her diet. In an archaeological context, these data could be used to draw important conclusions about the mobility of the Hebridean island dwellers and the issue of seasonal site use during the period concerned. Richards and Mellars concluded that the individual exhibiting lower isotope values probably spent a considerable time living inland or on a large island, where terrestrial foods would have been a more important part of the diet. The individual may have moved from an inland setting to the small island, or s/he may have occupied coastal and inland areas alternatively, on a seasonal basis.

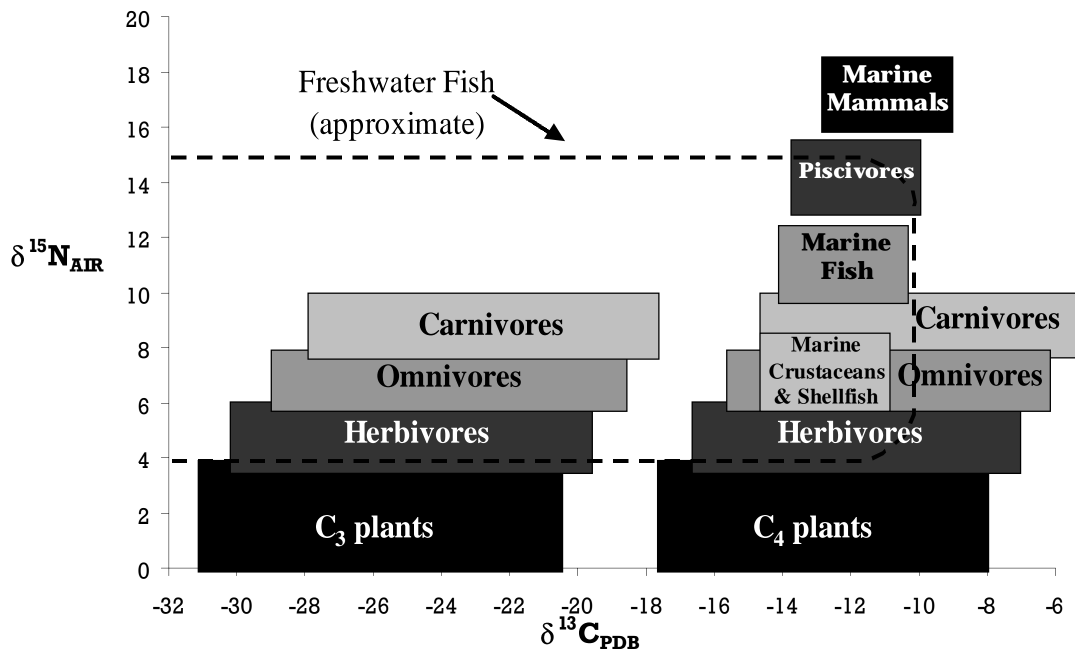

Figure 2.3. A plot of approximate $\delta^{13}C$ and $\delta^{15}N$ ranges in terrestrial and aquatic food chains.

2.6. *EFFECT OF CLIMATE ON $\delta^{15}N$ AND $\delta^{13}C$*

In addition to diet and trophic level, scientists have identified another possible factor influencing the $\delta^{15}N$ of animals. In arid regions such as southern Africa, central Australia and the Sahara Desert, the $\delta^{15}N$ of humans and other animals seems to increase with decreasing rainfall (Heaton 1987; Gröcke *et al.* 1997; Fogel *et al.* 1997). As a drought resistance mechanism, some animals prevent water loss during periods of little or no rainfall by excreting more isotopically light urea (Ambrose and DeNiro 1986). The high observed $\delta^{15}N$ of animals in arid environments may also be a function of soil processes involving nitrogen. It has been speculated that in times of drought, isotopically lighter volatile molecules may be preferentially evaporated from the soil, producing soils and therefore plants enriched in ^{15}N (Schwarcz *et al.* 1999). Furthermore, microbial nitrification and denitrification reactions within soil have been shown to influence the $\delta^{15}N$ of both NO_3^- nitrogen and of

whole soil, though it is unclear whether the dominance of denitrification processes does in fact result in increased overall soil $\delta^{15}N$ (Handley *et al.* 2001).

These metabolic effects on $\delta^{15}N$ in plants and soil are probably reflected in the elevated $\delta^{15}N$ of animals in areas plagued by drought. Heaton *et al.* (1986) have shown that animals in areas that receive less than ~400mm of rain per year can yield isotope values so elevated in ^{13}C and ^{15}N that their diet could be incorrectly interpreted as marine-based.

Some studies have suggested that a similar change in carbon fractionation occurs in plant tissues in proportion to differing climatic variables, albeit to a smaller extent than the climate-dependent fractionation of nitrogen. A comprehensive analysis of the $\delta^{13}C$ values of thousands of archaeological wood, charcoal and bone samples from Europe and the Middle East revealed a positive correlation between plant and animal $\delta^{13}C$ and average regional temperature, and an inverse relationship between biogenic $\delta^{13}C$ and average humidity and precipitation (vanKlinken *et al.* 1994). The authors note that a distinct (though less than 2‰) ^{13}C depletion can be traced along a temperature cline from warmer Mediterranean areas toward cooler Scandinavian regions across Europe. Fogel *et al.* (1997:280) suggest that the identification of drought stress in ancient human populations could be used to test anthropological hypotheses regarding population growth and mortality. Richards *et al.* (1998) use the observed temperature dependency of carbon fractionation to speculate about the presence of immigrants in a population sample from an Iron Age/Post-Roman cemetery in Dorset, England. Two individuals sampled showed elevated (less negative) $\delta^{13}C$ values than the other specimens, but did not exhibit the elevated $\delta^{15}N$ values that could have

indicated a marine component in their diet. Because C_4 foods were not present in this area during the time in question, Richards *et al.* propose that the two anomalous individuals were immigrants to England from a warmer region, such as the Mediterranean.

2.7. *THE 'CANOPY EFFECT'*

Some studies suggest that forested environments produce more negative $\delta^{13}C$ values than open environments (van der Merwe and Medina, 1991). This observed correlation between depleted $\delta^{13}C$ values and closed, forested environments has been called the 'canopy effect' (Vogel 1978). Although some researchers ascribe differences in $\delta^{13}C$ values between animals to their feeding patterns in open or closed environments (Iacumin *et al.* 1997; Drucker *et al.* 2003), variations wider than those attributed to the 'canopy effect' have been observed within animal populations with a highly restricted geographical, environmental and dietary range (Stevens *et al.* in prep.). Similarly, the influence of forest cover on $\delta^{15}N$ is still a matter of much debate (Rodière *et al.*, 1996; Handley *et al.*, 1999).

2.8. *PHYSIOLOGICAL EFFECTS ON $\delta^{15}N$ AND $\delta^{13}C$*

As well as plants, animal $\delta^{15}N$ can also be affected by physiological factors (Sealy *et al.* 1987; Ambrose 1991; Koch 1997; Gröcke *et al.* 1997). In addition to drought stress, pregnancy and lactation may have an effect on a female's body isotopic composition. A female's metabolism is accelerated during pregnancy and lactation, and her dietary requirements change during

this time (Truswell 1992). Altered metabolic rate and dietary intake during pregnancy and lactation may lead to enhanced representation of the pregnancy-lactation diet in a female's body tissues—a diet which may be significantly different from a female's normal diet (Parkington 1991).

2.9. *ATMOSPHERIC $\delta^{13}C$ VARIATION OVER TIME*

Changes in plant $\delta^{13}C$ over time are not only influenced by physiological factors, but also by the isotopic composition of atmospheric CO_2 available for plant uptake. Scientists have determined that atmospheric CO_2 has a variable $\delta^{13}C$ value, exhibiting decade- and century-scale isotopic changes of up to 1.5‰ (Becker *et al.* 1991; Beerling 1996; Heaton 1999). The main implication of these isotopic variations in atmospheric CO_2 in the context of palaeodietary studies is that the isotopic data gathered from non-contemporary individuals should be compared with caution, lest small isotopic differences due to atmospheric $\delta^{13}C$ fluctuations be attributed to differences in the dietary habits of the individuals.

2.10. *OTHER ISOTOPES AND PALAEODIET*

Carbon and nitrogen are the two basic elements commonly analysed in palaeodietary studies; but other elements are emerging as promising targets for food web analysis. Deuterium/hydrogen ($^2H/^1H$) isotope analysis has previously been used in much the same manner as $\delta^{18}O$ analysis, in studies of ancient climate and mobility. New work by Birchall (2003) shows that δD analysis may also be used as a correlate to $\delta^{15}N$ studies in modern and ancient

food web studies, as an indicator of an animal's level of carnivory. Similarly, $\delta^{34}S$ analysis, which has been most commonly used for modern pollutant and geographical tracer studies, is now being pursued as a potential complement to $\delta^{13}C$ and $\delta^{15}N$ analysis in palaeodietary studies (Richards *et al.* 2001; see also chapter 5). A number of factors have previously hindered the widespread use of alternative isotopic analyses in food web research, such as the large amounts of sample required for analysis, technical problems with running $\delta^{34}S$ and δD samples, and analytical cost per sample. A general lack of understanding of the mechanisms by which sulphur and hydrogen isotopes pass through food webs, and the dynamics of these isotopes in post-depositional conditions (as concern archaeological scientists) also restrict the utility of sulphur and hydrogen isotopes in modern and archaeological food web analysis. As sample preparation and analysis becomes increasingly easier and less expensive, required sample size continues to decrease, and our understanding of the behaviour of these isotopes in food webs grows; archaeological scientists will become more and more effective at employing δD and $\delta^{34}S$ analysis in palaeodietary studies.

2.11. *ALTERNATIVE METHODS OF PALAEODIETARY ANALYSIS*

2.11.1. TRACE ELEMENTS

In addition to stable isotope ratio measurements, trace elements have been used as dietary indicators. Minor elements such as strontium, calcium, vanadium and zinc are present in groundwater, which is absorbed by plants or directly ingested by animals. These elements are incorporated into the

mineral matrix of bone by the ionic replacement of other elements (e.g., strontium replacement of calcium). Because animal physiological processes discriminate against strontium, manganese and vanadium, the concentrations of these elements in animal tissues decreases with increasing trophic level, while elevated concentrations of zinc and copper are indicative of high meat (and/or nut) consumption (Hatch and Geidel 1983).

There is currently much debate on the subject of trace elements as dietary indicators and their use in palaeodietary research. Data collected by Elias (1980) indicate that among humans strontium content (of teeth) cannot be used to differentiate between vegetarian and non-vegetarian individuals. The relative effects of different dietary components (e.g., plant vs. animal and marine vs. terrestrial foods), physiological and geographic variability, diagenesis and sample treatment on the trace element composition of bones and teeth are still poorly understood (Pollard 1993; Pate 1994; Larsen 1997). Nevertheless, some palaeodietary studies using trace element analyses have occasionally been used to supplement stable isotope data (White and Schwarcz 1989; Schutkowski *et al.* 1999).

The measurement of strontium isotopic ratios ($^{87}Sr/^{86}Sr$, $\delta^{87}Sr$) in bones and teeth has also been employed in studies of human behaviour (Ezzo *et al.* 1997; Beard and Johnson 2000). However, $\delta^{87}Sr$ analysis is concerned with issues of migration rather than diet and therefore is not discussed further.

2.11.2. FOOD RESIDUES

Vessels used for the transport, storage or preparation of foods in antiquity are often found at archaeological sites. Scientists are able to extract

and identify many of the residues remaining in unglazed archaeological ceramic vessels, and relate the residues to foods consumed by prehistoric populations (Evershed *et al.* 2002; Craig *et al.* 2000). Protein residues adsorbed to ceramic surfaces can be released for analysis by treatment with hydrofluoric acid and identified by reaction with highly specific monoclonal antibodies (Craig and Collins 2000). This method of protein analysis is so specific that it can distinguish between milk and blood protein residues, and between bovine and ovine milk residues. Craig *et al.* (2000) have used such analysis to demonstrate the existence of dairying on the Atlantic coast of Scotland as early as the Iron Age.

In the analysis of lipid residues, lipid molecules adhering to the ceramic matrix of archaeological pots can be extracted with organic solvents and analysed by GC and GC-MS. If the lipid residues are sufficiently well preserved, they may display profiles characteristic of particular foods, such as meat, milk or plant foods (Evershed 1993; Dudd and Evershed 1998; Evershed *et al.* 2002). In one such study, residues extracted from late Saxon and medieval vessels from the British site of West Cotton were found to contain lipids characteristic of a plant of the *Brassica* genus, indicating that foods such as cabbage or kale had been prepared (and presumably consumed) by the inhabitants of West Cotton (Evershed *et al.* 1991). Lipid residues may also be isotopically ($\delta^{13}C$) analysed by combined gas chromatography-combustion-isotope ratio mass spectrometry, in order to further refine the identification of the extract (Mottram *et al.* 1999; Evershed *et al.* 2002).

Food residue analysis is a complementary palaeodietary technique to the stable isotope analysis of human and faunal remains. Whereas stable isotope analysis of archaeological bone provides a semi-quantitative picture of each

individual's average diet, residue analysis of archaeological pottery yields qualitative data reflecting an unknown number of specific cooking, transport and/or storage events related to the foods utilised by an ancient community.

In the next chapter, I present my isotopic analysis of human and faunal archaeological bones from various sites throughout the Eurasian steppe. In the chapters that follow, I will investigate and discuss the utility of residue analysis and sulphur isotope analysis in shedding further light upon our picture of the dietary habits of Bronze Age and Iron Age Eurasian steppe peoples.

Chapter 3

Carbon and Nitrogen Stable Isotope Analysis of Humans and Fauna from Bronze and Iron Age Eurasian Steppe Sites

3.1. *ECONOMY AND DIET IN BRONZE AND IRON AGE EURASIA*

> Researchers have been in a thrall to a pervasive image—that of mounted nomads and volatile Pontic tribes. This image is so powerfully documented historically that it is hard for prehistorians to think beyond it. Thus researchers with an interest in the earlier periods, during which the steppe expanses were first conquered, write prehistories populated by warlike, horse-riding nomadic pastoralists, driving their flocks from place to place, and terrorizing their neighbours (Rassamakin 1999:59).

In this passage, Rassamakin describes a problem of great relevance to this thesis. The works of historians and ethnographers, who over the centuries documented the exploits of the tribes that spread across the Eurasian steppe, depict vivid and romantic images that have greatly affected the way that archaeologists approach and interpret the archaeological record. It is virtually impossible to find archaeological literature that deals with the prehistory of the Eurasian steppe that does not reflect the influence of the historical and ethnographic images of the steppe peoples, even when dealing with periods centuries before Herodotus set about writing his *Histories*.

Ethnographic research has documented much about the food and daily subsistence practices of steppe tribes since the sixteenth century; but how much of this information can be extrapolated back and applied to the Bronze and Iron Age communities of the same region? Historians such as Herodotus gave accounts of tribes such as the Scythians and Sarmatians; but stories such as these are peppered with fantasies, inaccuracies, and tend to focus on military adventures, remarkable events and the exploits of rulers rather than the minutiae of daily life.

In terms of elucidating the everyday life of the ancient peoples of the Eurasian steppe, we may turn to archaeology for answers; yet archaeology

cannot entirely fill in the blanks left by the historical and ethnographic records. Archaeological research is also burdened with limitations and can only reveal so much about the lives, diets and economies of ancient steppe communities.

The aim of the research described in this chapter is to investigate ancient human diet and subsistence to reveal information beyond the reach of archaeological, ethnographic and historical records. I use stable isotope analysis of human and faunal bones to investigate the average, daily diets of various archaeological communities throughout the Eurasian steppe, dating primarily from the Bronze Age through the Iron Age. This research is done in the context of previous archaeological, historical and ethnographic studies, and seeks to clarify our picture of the daily subsistence behaviour of the groups represented.

In this chapter, I briefly discuss the ethno-historical context around which the image of the Bronze and Iron Age Eurasian steppe peoples has been built. A background of the archaeological evidence for the diet and economies of Eurasian steppe communities then follows, with a focus on the Bronze and Iron Ages. I then give a full description of the stable carbon and nitrogen isotope analysis of human and faunal remains from archaeological Eurasian steppe sites, and discuss the results from this study as they pertain to the dietary and economic trends of the Bronze and Iron Age communities concerned.

3.2. HERODOTUS' *HISTORIES*

Herodotus is the best-known and most often cited ancient historian to document the economic pursuits, military adventures and environmental context of the contemporary tribes of the Eurasian steppe, particularly the Scythians. In *The Histories*, Herodotus details events from the mid-sixth century to the early fifth century BC. Although the main thrust of Herodotus' text centres on the kingdom of Persia and its military conflicts with Greece and various non-Greek tribes, his writing takes on an ethnographic character as he describes the activities and customs of the peoples encountered by the Persians and Greeks.

The tribes described most at length by Herodotus, and most relevant to the material analysed in this chapter, are the Scythians and Sarmatians. Of the Scythians, Herodotus says:

> Since they have no towns or strongholds, but carry their homes around with them on wagons, since they are all expert at using their bows from horseback, and since they depend on cattle for food rather than on cultivated land, how could they fail to be invincible and elusive? (IV:46)

He depicts the Scythians, whose territory ranges north of the Black Sea roughly through the area of modern Ukraine, as nomadic herders who have no need of permanent settlements and do not practice agriculture. However, Herodotus later describes various Scythian groups including the Borysthenites, the Callippidae and the Alizones, whom he refers to as agricultural peoples.

According to Herodotus' account, the animals most central to the economic and dietary practices of the Scythians were horses and cattle. He

mentions that pigs are not only never used as sacrificial animals; "In fact they prefer not to keep them in their country at all" (IV:63). Notably, Herodotus does mention the exploitation of freshwater resources by the Scythians, whose land "does not really have remarkable features except for the size and number of its rivers" (IV:82).

> Apart from the Nile, the Borysthenes [Dnieper] is the most productive river. It not only provides wonderful, lush meadows for cattle, but outstandingly fine fish as well, in very large quantities...The river is home to large invertebrate fish called antakaioi, which the Scythians preserve by salting, and to many other remarkable creatures (IV:53).

Although Herodotus mentions fish consumption by the Scythians in his *Histories*, he does not place as much importance on fish as a dietary staple as his fellow historian Strabo (1969). Strabo, writing in the first century BC, refers to the Scythian way of life as one based on sheep and fish (II.viii:6-7).

The Sauromatians, Herodotus tells us, were descendants of Scythian fathers and Amazonian mothers, who inhabited the lands between the northern areas of the Black and Caspian Seas. He depicts the Sauromatians as a military-based society, where the status of women as well as men rested on their skill in battle. Regarding their overall economy, Herodotus describes the Scythians and Sarmatians as militant, mobile pastoralists, who raise their domestic stock for food and as commodities. Their valued horses provided the Scythians and Sarmatians with transport and were also commonly used as a source of milk, which was churned to produce a beverage (IV:2). These pastoralists obtained necessary or desired items such as agricultural products, vessels or ornaments from neighbouring groups by commercial and military means.

3.3. *ETHNOGRAPHIC EVIDENCE FOR DIET AND ECONOMY*

It may be said that Herodotus and his colleagues were the first ethnographers to probe the daily lives of the inhabitants of the Eurasian steppes. Russian and Soviet ethnographers followed Herodotus' lead and the tradition of ethnographic study remains strong in Russia and the other post-Soviet republics.

In trying to ascertain the nature of ancient human diet in the Eurasian steppe region, archaeologists often attempt to draw parallels between the behaviour of archaeological cultures and the groups subject to ethnographic studies in the (now former) USSR. These ethnographic investigations, carried out as early as the sixteenth century, purport to record the details of techniques of food acquisition, commonly consumed resources, and traditional dishes (among other aspects of daily life) of groups such as the Tatars of western Siberia, the Bashkirs of the South Urals and the Chuvash of western Russia.

Reports on the types of foods consumed by the communities in question consistently emphasise the primary importance of domestic animal products in the diets of those communities. According to the ethnographic literature, dairy products and meat traditionally occupy the position of highest importance in the diets of many ethnic groups found within the area of the former USSR, while plant products, fish and wild game generally occupy positions of lower dietary importance. In his description of the Bashkir diet in the eighteenth to twentieth centuries, Rudenko (1955) depicts a hierarchy of foods, the most important of which are domestic animal (sheep & horse) meat, followed by dairy products, fish and plant products, domestic fowl, eggs and

wild game. This hierarchy is maintained with minor variations in reports on other ethnic groups of the Russian steppe (e.g., Tolstova 1963; Pimenov 1988; Akhmetova 1995).

Fish are always mentioned in ethnographic reports on Eurasian steppe diet, but in twentieth century reports their dietary and economic importance is consistently overshadowed by that of domestic animal products. The Bashkir apparently refer to fish as "sacred" and consume a variety of species, though fish are generally regarded as food to be consumed by the poor or in hard times by Bashkir and Kazakhs (Rudenko 1955; Tolstova 1963). Fish may be prepared in a stew or by boiling, roasting, drying or salting (Rudenko 1955; Tomilov 1980; Akhmetova 1995).

In reading a number of ethnographic studies, I came across descriptions of certain dietary trends that I did not expect to find, as they are not highlighted by archaeologists in their interpretation of ancient human subsistence. Firstly, some ethnographers report that the frequency of meat consumption varies between communities and within a community on a seasonal level, whereas milk products are consistently important as a central dietary staple of many Eurasian steppe groups throughout the entire year (Tolstova 1963; Akhmetova 1995). Among the Kirgiz peoples of Russia, Tolstova (1963) observed that meat was only frequently consumed by the wealthy upper class, whereas common people only ate meat on special occasions.

Another feature of these ethnographic accounts that caught my attention was the emphasis that some researchers put on the dynamic nature of observed dietary trends. In their descriptions of traditional dishes or the relative importance of different foods, ethnographers often restrict their

discussion to specific time periods. By acknowledging that the subsistence strategy of Tomsk Tatars did not remain the same between the seventeenth and the nineteenth centuries, for example, Tomilov (1980) highlights a major limitation of the use of ethnographic information to deduce the habits of archaeological communities. Interestingly, in conjunction with this affirmation of dietary change over time, Tomilov and others (Rudenko 1955; Akhmetova 1995) mention the decline in importance of fish as a dietary staple over the centuries. It seems that, before the eighteenth century, fishing was much more widely and frequently practiced as a means to obtain food throughout the Eurasian steppe than in subsequent years.

3.4. THE ARCHAEOLOGICAL BACKGROUND: *CURRENT VIEWS ON THE DIET & ECONOMY OF THE BRONZE AGE & IRON AGE PEOPLES OF THE EURASIAN STEPPE*

However influenced by the ethnographic literature, archaeologists formulate their views on the diet and subsistence strategies of Bronze and Iron Age Eurasian steppe groups primarily with regard to material excavated from burial and settlement sites of those periods. Sites of temporary or permanent settlement have been discovered throughout the steppe, but most of the archaeological record for the Bronze and Iron Ages consists of burial sites, of which thousands have been identified and excavated since the early twentieth century. In this section I briefly discuss the current views on the subsistence and economy of the Bronze and Iron Age cultures represented by the sites from which I obtained material for isotopic analysis. I have chosen to focus on views regarding general subsistence trends in Bronze and Iron Age Eurasia rather than dwell on the subtle variability between cultural groups.

Readers may inquire as to the evidence provided by butchery marks on faunal bones, or the presence of small faunal bones on archaeological sites. It is not possible to provide an adequate account of the contributions that these finds have made or may make to the archaeological record at this time. There is no established tradition in Soviet and post-Soviet archaeology for the documentation of cut marks, nor is the recovery of small bones or bone fragments by sieving a common practice. Fish bones in particular are apparently considered with such indifference that some archaeologists have been observed to consciously discard fish bones recovered at an archaeological site (Bryan Hanks & Hermann Parzinger, personal communications). The tendency to excavate only those bones considered to be large and/or important enough (both subjective qualities) along with the lack of sieving has undoubtedly resulted in a catalogue of faunal data biased toward medium and large terrestrial herbivores. Therefore, when examining the available archaeological evidence for diet and economy, it is important to keep in mind that such evidence is gleaned primarily from faunal bone counts recovered from ritual, funerary deposits without sieving.

3.4.1. THE BRONZE AGE

In the Bronze Age (~18th to 12th centuries BC), the Sintashta, Petrovka, Alakul, Andronovo and Srubnaya (or Timber Grave) cultures occupied the steppe of the Trans-Urals, southwestern Siberia and modern-day northern Kazakhstan. Archaeological evidence suggests that these groups had very similar social and economic practices; sites are often attributed to cultural complexes identified as a combination of cultural labels such as 'Alakul-

Srubnaya', etc. The Bronze Age samples from the Urals/western Siberia region in this palaeodietary study all pertain to this general cultural complex.

Settlements attributed to the Bronze Age cultures of the Urals and western Siberia indicate a significant degree of sedentism for at least a portion of the community. Settlements of the Sintashta type were fortified, with domestic units and metallurgical facilities enclosed by a wooden palisade and moat (Gening *et al.* 1992; G. Zdanovich 2000). The overwhelming presence of cattle, sheep, horses and dogs in steppe Bronze Age burials have contributed to the depiction of these communities as primarily pastoralist, with the presence of chariots, horse-riding gear and weaponry in some burials suggesting an element of mobility and aggressiveness (Gening *et al.* 1992). Artefacts such as grindstones, fishhooks, sickles and pestles indicate that some metallurgical and agricultural activities, as well as fishing and hunting, also contributed to the economy of these Bronze Age pastoralists, though these pursuits are generally regarded as minor or incidental (*ibid*). Koryakova (2000) describes the economy of the Bronze Age Srubnaya and Andronovo cultures as "mixed", consisting of "pastoral stock-breeding, primitive cultivation in the river valleys, metallurgy, hunting and fishing, and some kinds of domestic craft."

A similar economy, "certainly based on horse and cattle breeding [as well as sheep and goat]" has been proposed for the settlement site of Chicha, located on the edge of a lake approximately 1100km east-northeast from the main Sintashta culture sites, in the Baraba steppe region of south-western Siberia (Molodin *et al.* 2001:123; region 4, figure 2). The form and style of Chicha ceramics indicated a chronological association with the transition from Bronze Age to Iron Age (~8th to 7th centuries BC), but new radiocarbon dates

show that osteological material analysed in this palaeodietary study pertains to the Middle Bronze Age (14th to 12th century BC, unpublished dates; J. Görsdorf, Deutsches Archäologisches Institut). The initial Chicha site report concluded (on the basis of the osteological data) that livestock breeding dominated the Chicha economy during the period of site occupation and that "hunting and fishing did not play an important role in the economy" (Molodin *et al.* 2001:124). However, subsequent work at the site has uncovered layers containing thousands of fish bones (carp, perch and pike), which has forced the excavators to reconsider the importance of fish in the diet of the Chicha inhabitants (Molodin *et al.* 2002).

A number of the archaeological cultures that inhabited the area of western Siberia to the Urals also spread to the steppe and forest-steppe of the Ukraine. The Srubnaya culture existed in the Ukraine from approximately the sixteenth to the thirteenth centuries BC. Preceding the Srubnaya culture in Ukraine was the Yamnaya (Pit Grave) culture (~late 4th – late 2nd mil. BC) and KMK (or Multiroller Ceramic) culture (~18th – 16th cent. BC); material from the later Sabatinovka culture is contemporary with the later stages of the Srubnaya culture (~14th – 13th cent. BC). Building on the increasing trend of animal domestication in the preceding Mesolithic, Neolithic and Eneolithic periods, the economy of the Yamnaya in Ukraine

> …was characterised by ovicaprid pastoralism, to the extent that ritual burials of sheep and goats were found among the funerary equipment…Subsequently the nomadic form of ovicaprid and bovine husbandry extended over the steppes north of the Black Sea across Eastern Europe" (Matyushin 2000:251-252).

According to the evidence obtained from funerary deposits Shilov (1989:124) concludes that

> Animal husbandry, hunting and fishing were the principal activities in the economy of the Bronze-age population...the role of animal husbandry increased considerably between the age of the Pit-grave culture and the Chamber culture...It can be said that in the economy of the Pit-grave culture and subsequent cultures animal husbandry was the predominant form of economic activity. Hunting and fishing did not play a prominent part.

Notwithstanding the compelling evidence from grave deposits, we must bear in mind that faunal remains deposited in a ritual context cannot necessarily be relied upon as a direct reflection of the economic and/or dietary importance of the species represented. As Rassamakin (1999:131) states in his discussion of Yamnaya burial sites, "We cannot, on the basis of faunal evidence from burials, make any detailed claims about the nature of the herd or about the predominance of any given species among the Yamnaya tribes." Rassamakin points out that the rarity of one species may relate to its economic value, while the bones of another species may have been deposited in a functional context irrelevant to the species' economic value (*ibid.*).

Nevertheless, the evidence from faunal bones excavated from some Ukrainian Bronze Age settlement sites does support the picture of a diet and economy based on domestic animals. Settlement sites exhibit a uniform dominance of domesticated cattle (NISP and MNI), followed by ovicaprids, pigs, horses, dogs and wild fauna (Zhuravlev *et al.* 1985; Zhuravlev *et al.* 1989; Zhuravlev 2000). Zhuravlev *et al.* (1989) hypothesise that the wild species encountered at archaeological sites would be used not only for meat, but also for fur, thus further de-emphasising the dietary importance of wild fauna. Zhuravlev limits his discussion of the economic uses of wild fauna to the few

medium to large species most commonly recovered from Ukrainian archaeological sites: beaver, red deer, roe deer and boar. Wild species such as fish, hare and turtle are presumably too rare, too small or too insignificant to merit mention of their place in the economy of Ukrainian Bronze Age and Iron Age archaeological communities.

Material analysed in this study associated with the Sintashta/Petrovka/Alakul/Andronovo cultures comes from the steppe and forest-steppe regions of the south Urals and trans-Urals. Remains associated with the Srubnaya culture were obtained from sites in both the Urals and the Ukraine. Further material collected from the Ukraine includes remains from Yamnaya, KMK and Sabatinovka culture sites. Late Irmen material was obtained from the southwestern Siberian site of Chicha.

3.4.2. THE IRON AGE

Climatically, the transition from the Bronze Age to the Iron Age in the few centuries around the transition from the second to first millennium BC was marked by an increase in aridity and a decrease in annual temperature throughout the steppe (Christian 1998). These climatic changes are often cited as a major contributor to the economic shift toward increased mobility (and in some cases, complete nomadism) through the first millennium BC. The Iron Age sites included in this palaeodietary study belong to the most dominant cultural complexes of the Iron Age steppe and forest-steppe—the Sauro-Sarmatians, Scythians and Sargat. Each of these 'umbrella' complexes represent a conglomeration of many cultures and sub-cultures, whose

similarities in the archaeological record reflect such similarities in economy, society and belief system that they are often referred to as a unit.

The Sauro-Sarmatian cultural complex includes the Sauromatians, the Sarmatians and a number of other closely related cultures that ranged from the Black Sea steppes to southern Siberia from about the sixth century BC to the fourth century AD. This area was also occupied by the Scythians from the seventh century BC to approximately the third century AD, and the maximum range of the Scythians reached from the Balkans to northern China.

Artefactual and historical evidence of these Iron Age cultures depict mobile and militant tribes, with the notable identification of female Sauromatian warrior graves (Melyukova 1990). In his description of the Pazyryk Scythians of the Altai, Rudenko (1970) concludes that "Stock-breeding was...the basic occupation, and the food of the inhabitants of the [Altai] consisted mainly of milk products and meat" (60). In order of descending economic importance, Rudenko lists the animals raised/used by the Scythians as horse, sheep, cattle; and describes hunting and fishing collectively as a "subsidiary occupation" at best (59). The similar "military-nomadic" economy of the Sauromatians and Sarmatians is evidenced by the prevalence of weaponry interred with the deceased (Dvornichenko 1995:106; Moshkova 1995a&b).

The peoples of the Sargat Culture, who inhabited the region from the Urals forest-steppe to the steppe of southern Siberia from approximately the seventh/sixth centuries BC to the third/fifth centuries AD, based their economy on livestock breeding (Koryakova and Daire 2000). The presence of open and fortified settlements attributed to the Sargat culture suggests a degree of sedentism in these communities, in contrast to the Scythian-

Sarmatian-Saka ‘nomadic world’ (Koryakova 2002). Nevertheless, within the Sargat society the upper strata are considered to have been generally nomadic in character (Koryakova and Daire 1997:167). The Gorokhovo culture, which was incorporated into the Sargat culture in the middle Iron Age (~5th cent. BC), seems to have occupied settlements on a seasonal basis, according to Matveeva (2000). The archaeological evidence indicates that at least a part of the population were mobile stockbreeders, maintaining the domestic stocks that formed the basis of the Gorokhovo economy; supplementary economic activities included cottage industries, hunting and fishing (*ibid.*).

Material from Sargat and Gorokhovo sites collected for this study comes from the Urals forest-steppe region. Sarmatian material collected for isotopic analysis has been obtained from sites throughout the south Urals steppe. Scythian samples have been obtained from the north Pontic steppe region of the Ukraine and from the eastern Scythian range of the Tuvan and Altayan mountain steppe. Contemporary material from the Greek settlement of Olvi’ia, located on the north coast of the Black Sea, was obtained for comparison with the Ukrainian Scythian material.

3.5. *MATERIALS AND METHODS*

This section comprises a description of the acquisition, selection, preparation and analysis of human and faunal bones from Bronze Age and Iron Age Eurasian steppe archaeological sites. The biochemical principles behind the procedure used are discussed briefly.

3.5.1. NOMENCLATURE

The majority of the human bone samples obtained had been labeled by the excavators in terms of the site of origin and kurgan, grave, and individual number (if more than one individual was found in a grave). Animal bones had commonly been assigned a dual number (i.e., in the format of #a/#b) related to the excavation/site number (#a) and the individual bone number (#b). In some cases, animal bones were identified solely as coming from a particular archaeological site. All of the original classification and labeling of samples was noted, but a more simple style of nomenclature was adopted for this study. Samples in this study are coded in the following fashion: XXX#, where XXX = letters representing the site of origin and # is the number of the individual analysed (human or faunal), assigned by the author.

3.5.2. BONE SELECTION

A list of archaeological bone samples analysed for this thesis is given in appendix 1. Locations of all sites from which samples were taken are shown in appendix 2. Bone samples were obtained from the Royal Belgian Institute of Natural Sciences, Brussels; the University of Ghent, Belgium; the Institute of History and Archaeology, Urals Division of the Russian Academy of Sciences, Ekaterinberg, Russia; the Urals Institute of Ecology, Ekaterinberg, Russia; the Deutsches Archäologisches Institut Eurasian Research Unit, Berlin, Germany; the Donetsk Regional Museum, Ukraine; and the Institute of Archaeology, Ukrainian National Academy of Sciences, Kiev. In addition to Bronze Age and Iron Age material, faunal samples from the Ukrainian Eneolithic sites of

Molyukhov Bugor (pre-Yamnaya culture) and Bil'shivtsi (Tripolye B1/B2 culture) were also sampled due to the number and variety of species available from the site. Where available, the femoral shaft was sampled from each individual. When the femur could not be sampled, a sample from another long bone was taken. Any other bone was sampled in the absence of long bones, including horn cores (particularly for goats).

Faunal bones were identified to genus and to species where possible by Pavel Kosintsev (Urals Institute of Ecology), Bryan Hanks (then of the University of Cambridge, currently at the University of Pittsburgh), Norbert Benecke (Deutsches Archäologisches Institut) and Oleg Zhuravlev (Institute of Archaeology, Ukrainian National Academy of Sciences). The only samples not identified to genus or species level are the fish from Molyukhov Bugor and Bil'shivtsi.

As mentioned above, there is evidence for climatic and environmental changes occurring throughout the Eurasian steppe region around the turn of the first millennium BC. Although some scholars contend that significant climatic and environmental transformations did not occur at this time (Shilov 1989), palaeodietary interpretations of isotope values should take into account that isotopic change may mirror climatic change (see section 2.7). To this end, faunal samples were obtained from sites as close in age and location to the human samples as possible; in many cases, human and faunal material was obtained from the same site.

Samples of terrestrial herbivore species and wild and domestic pigs were taken to represent possible dietary resources for humans. Other terrestrial omnivore and carnivore species were sampled to provide data against which to compare the human sample results. Samples of freshwater herbivore and

omnivore species (including fish) were taken as representative of potential freshwater dietary resources available to the archaeological humans.

All human and faunal samples were obtained from adult individuals. For humans, 'adult' is taken to mean individuals aged at least approximately fifteen years according to osteological indicators of age (Bass 1995). Faunal samples were identified as 'adult' by the faunal specialists named above; degree of epiphysial fusion, bone size and tooth eruption and wear patterns were considered when determining individual age. Juvenile individuals were not sampled for this thesis, as the isotopic composition of juvenile bones may reflect a mixture of pre-weaning, weaning and post-weaning dietary signals (see section 2.4.1).

3.5.3. SAMPLING

Approximately 0.3-1.0g of bone was obtained from each individual sample. If an appropriate sized piece of bone was not already available, a piece of bone was broken off by hand or sawn off from the bone using a handsaw. Areas of bone marked with ink were avoided when sampling when possible; bones treated with conservants and areas treated with glue were not sampled. Sample pieces were then transferred into individual, sealed plastic bags labeled with the sample information (sample name, provenance, age and sex information if available). Samples were kept at room temperature and brought back to the Research Laboratory for Archaeology and History of Art (Oxford, UK) for cleaning, collagen extraction and isotopic analysis.

3.5.4. CLEANING

The surfaces of the bone pieces were cleaned by shotblasting with fine aluminium oxide powder (Swam-Blaster, Crystal Mark, Inc., Glendale CA, USA; Airbrasive Powder No.1, REG Abrasonics, Ltd., Dartford, UK). This process removes ink and other surficial contaminants that have adhered to the bone.

Some collagen extraction protocols include a procedural step involving the treatment of samples with sodium hydroxide in order to remove humic and lipid contaminants (DeNiro 1985; Cannon *et al.* 1999). I did not include this step in light of evidence from experiments by Pollard *et al.* (1991), van Klinken and Hedges (1995) and Liden *et al.* (1995), which show that NaOH treatment does not significantly improve the quality of the isotopic data obtained from archaeological bone samples. Liden *et al.* (1995) also emphasise that this extra step causes a notable decrease in final collagen yield.

3.5.5. COLLAGEN EXTRACTION/PREPARATION

For the collagen extraction procedure, I followed the modified Longin method used by Richards (1998:32) with minor alterations, as described below.

3.5.5.1. *Demineralisation*

Most samples were broken up into smaller pieces in order to increase the surface area exposed to the reagents. These samples were either broken into

pieces using a clean percussion mallet or wrapped in aluminium foil and broken up with a hammer.

Bone fragments were placed in 10ml glass test tubes, which were then filled with 0.5M HCl and stored at -4˚C. The acid was drained and replenished every few days until demineralisation was complete. Treatment with acid dissolves the apatitic mineral matrix of the bone, as well as other acid-soluble contaminants (such as non-collagenous proteins).

The acid interferes with the cross-links and salt-bridge interactions that bind the collagen fibers, breaking some of them into their constituent fibrils and molecules. The extremities of the collagen molecules are also attacked by the acid, causing the ends of the α-helical strands to fray. The compact structure of the collagen triple helix prevents the protein from complete dissociation in the acid solution. The strong hydrogen bonds within each collagen molecule that hold the three α-helices together are not significantly affected by the acid solution. Thus, the physical arrangement of collagen is affected to some degree by treatment with cold HCl, but the fundamental chemical composition of the collagen remains unchanged.

At this point, the collagen has lost its distinctive macromolecular arrangement, and can no longer be considered collagen in the strictest sense. Thus, when referring to this compound after its initial acid treatment, I follow the convention of calling it 'collagen' to distinguish it from collagen in its true three-dimensional form (DeNiro and Weiner 1988).

As the mineral fraction dissolved, the appearance of a sample was not altered significantly; a 'replica' pseudomorph of the original fragments remained. When all or most of a sample became translucent and/or soft, the

supernatant was poured off. The residue was then washed with Milli-Q water (5 times) in order to get rid of any excess HCl and to remove remaining water-soluble impurities. When necessary, samples were stored in Milli-Q water at -4˚C for up to two weeks before gelatinisation.

3.5.5.2. *Gelatinisation*

Once samples were drained and washed, sample tubes were filled with pH3 water (Milli-Q water + HCl), capped and placed in hotblocks at approximately 75˚C for 48 hours. Heated above 60˚C, the α-chains of the collagen triple helix dissociate and the polypeptide chains dissolve into solution. Once removed from incubation, the liquid fraction was isolated using an Ezee filter (8μm polyethylene filter separator, Elkay Laboratory Products Ltd., Basingstoke, UK), leaving behind the acid-insoluble residue.

The filtrate was poured into clean plastic test tubes, then frozen at -40˚C. Once frozen, samples were supercooled with liquid nitrogen and lyophilised for approximately 48 hours to produce the final 'collagen' product. If a sample did not dry well (i.e., had a sticky rather than fluffy texture), it was redissolved in 3-4ml of Milli-Q water, refrozen, cooled with liquid nitrogen and lyophilised.

3.5.6. MASS SPECTROMETRIC ANALYSIS

Three 2.5-4.0mg portions of each 'collagen' sample were weighed out for mass spectrometric analysis. Samples with insufficient material for at least two replicate analyses were not analysed. Each replicate portion was weighed

into a tin capsule and loaded into a sample carousel for analysis. Samples were run through an automated carbon and nitrogen elemental analyser (Carlo Erba EA1108) coupled with a continuous-flow isotope ratio-monitoring mass spectrometer (Europa Geo 20/20).

A 'collagen' sample (containing C, N, S, O and H) is dropped into a 1000˚C combustion furnace in an atmosphere of oxygen. The tin capsule surrounding the sample burns exothermically, elevating the temperature to ~1800˚C and oxidizing the collagen sample. At this point, the carrier gas is changed from oxygen to helium. To aid oxidation, the temperature is maintained at 1000˚C and the sample is passed through a bed of Cr_2O_3 that readily gives up the oxygen needed. A layer of copper oxide ensures the oxidation of nitrogen oxides, and the sample is carried through silver wool in order to remove sulphur. The oxidized sample then enters a 600˚C copper-containing reduction furnace where NO_x is converted to molecular dinitrogen and excess oxygen is removed from the sample. Water is removed by passing the sample through a trap containing anhydrous magnesium perchlorate, leaving behind only CO_2 and N_2. These two gases enter a gas chromatograph where they are separated due to their differences in mass. 2% of the gas stream is then bled into the mass spectrometer.

The N2 enters the spectrometer first. The gas is ionized and passed through a magnetic field, which separates the gas molecules by mass into $^{14}N_2$, $^{14}N^{15}N$ and $^{15}N_2$ (amu 28, 29 and 30, respectively). The mass difference of the molecules causes them to hit different collectors, which measure the beam current and calculate the sample's $^{15}N/^{14}N$ ratio. Before the CO_2 stream enters the detector, the parameters are changed to detect samples of amu 44, 45 and 46 ($^{12}C^{16}O_2$, $^{13}C^{16}O_2$ and $^{12}C^{16}O^{18}O$, respectively). $^{13}C^{18}O_2$ is considered so rare

as to be negligible. A Craig correction is automatically applied by the instrument's software to account for the presence of ^{17}O (Craig 1957).

The $\delta^{13}C$ (VPDB), $\delta^{15}N$ (AIR) and C:N ratios were automatically calculated for each sample at the end of each sample run. Results were automatically drift-corrected according to nylon reference samples of known isotopic composition ($\delta^{13}C$ = -26.1‰, $\delta^{15}N$ = -1.8‰), and additional nylon and alanine ($\delta^{13}C$ = -26.9‰, $\delta^{15}N$ = -1.4‰) samples were used to check the integrity of the results for each run. The analytical error (±1σ) for the results is ±0.3 for carbon and ±0.2 for nitrogen.

3.5.7. ULTRAFILTRATION

A study of the use of ultrafilters in purifying extracted 'collagen' samples was conducted in the Research Laboratory for Archaeology and History of Art and University of Oxford Radiocarbon Unit in 1999. The results suggested that ultrafiltration improves the quality of 'collagen' (M. Richards, unpublished data). In order to test whether or not ultrafiltration would significantly improve the quality of the samples analysed for this thesis, 9 samples were selected at random, representing material from various species, sites and ages. After the selected samples were analysed by mass spectrometry in triplicate as described above, they were redissolved in Milli-Q water and ultrafiltered using 10kD ultrafilters (Amicon Ultra-15, Millipore, Bedford, MA, USA) that had been rinsed through twice with Milli-Q water. The recovered >10kD product was frozen at -40˚C, lyophilised and isotopically analysed in triplicate as described in sections 3.5.5.2 and 3.5.6.

C:N, $\delta^{13}C$ and $\delta^{15}N$ results for ultrafiltered and non-ultrafiltered samples are compared below (3.6.3).

3.6. *RESULTS*

3.6.1. SAMPLE PRESERVATION

3.6.1.1. *C:N Ratios*

The ratio of carbon atoms to nitrogen atoms in a sample of bone collagen is believed to be a good gauge of bone preservation and isotopic integrity. Fresh collagen has a C:N ratio of approximately 3.2 (Ambrose 1993). Diagenetic processes can significantly alter the C:N ratio of collagen to as low as 1.0 or as high as 15.0 (Koch *et al.* 1994). Samples with a C:N ratio of 2.9-3.6 are accepted as sufficiently well preserved to yield reliable $\delta^{13}C$ and $\delta^{15}N$ values (DeNiro 1985), though a slightly wider range has been accepted (Ubelaker *et al.* 1995).

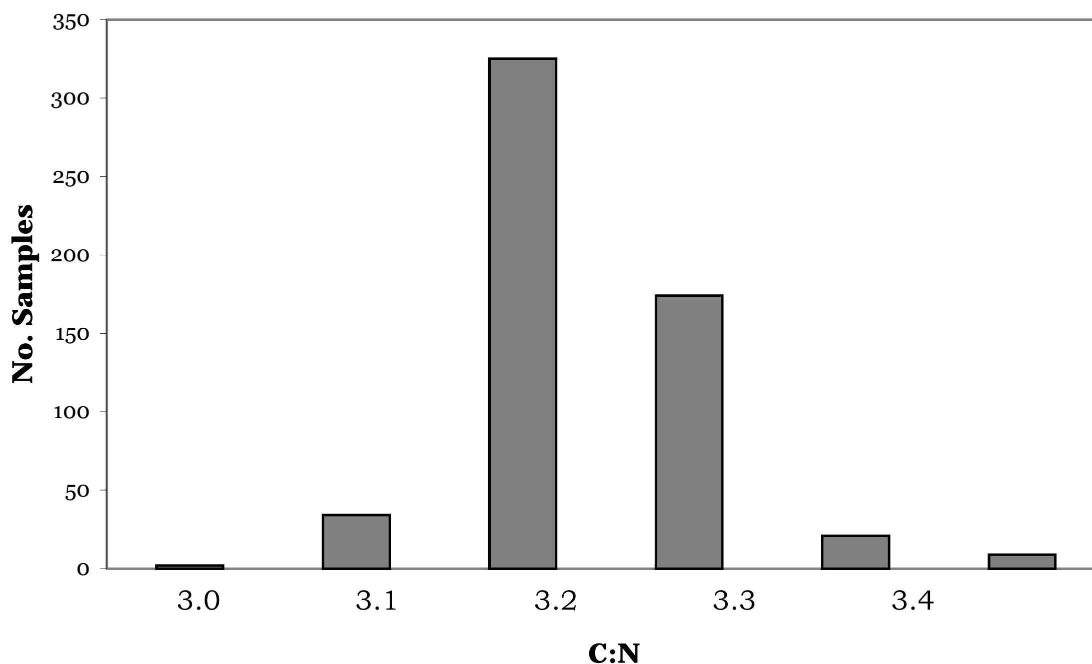

Figure 3.1. Frequency of C:N values for all samples reported in this study.

The isotope values of samples with C:N ratios outside the range of 2.9-3.6 were discarded and are not reported. The C:N ratios calculated for the remaining samples were between 3.1 and 3.6, indicating good collagen preservation (figure 3.1).

3.6.1.2. *%C and %N Yield*

Ambrose (1990) found that well preserved collagen produces final carbon weight percent yields of >13% and nitrogen yields of >4.8%, while %C and %N yields of less than 4.5 and 1.0, respectively, are indicative of poor collagen preservation. %C and %N values for all samples are listed in appendix 1. Every sample tested yielded >13% carbon and ≥4.8‰ nitrogen with the exception of VOZ 1, which has a carbon content of 11.6% and a nitrogen content of 3.9%. Many of the samples exhibited %C and %N yields in the range of modern human bone collagen (Ambrose 1990). In conjunction with the C:N calculations, the carbon and nitrogen yield data support the conclusion that the isotopic data presented here is from well preserved samples.

3.6.2. ULTRAFILTRATION EFFECTS

Ultrafiltered and non-ultrafiltered isotopic results for 'collagen' samples are presented in figure 3.3. A systematic shift can be observed between pre-ultrafiltered and post-ultrafiltered samples. The $\delta^{13}C$ values of pre-ultrafiltered samples are generally slightly enriched relative to post-ultrafiltered values of the same samples (average $\delta^{13}C$ difference of 0.1‰).

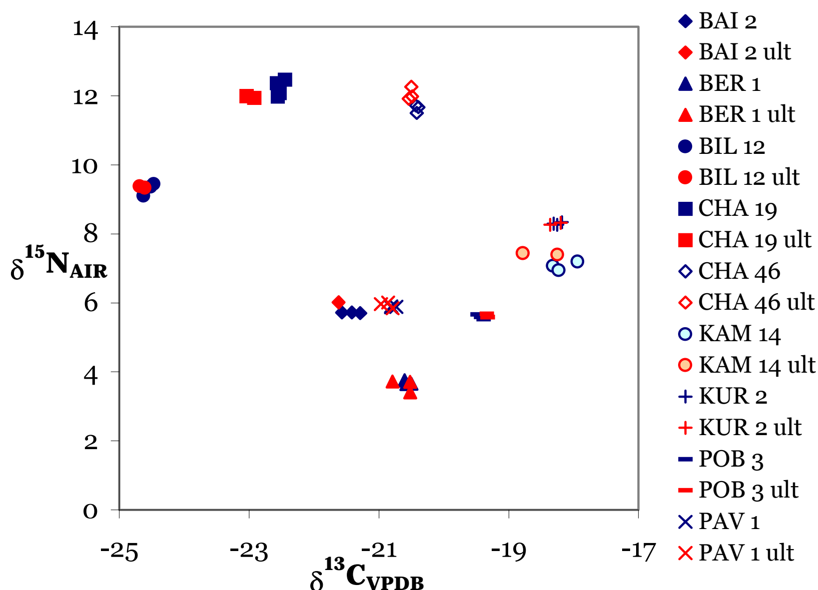

Figure 3.2. Ultrafiltered and non-ultrafiltered bone collagen sample δ^{13}C and δ^{15}N values.

Since it is impossible to ascertain the 'correct' isotopic result for extracted bone collagen, C:N values of pre- and post-ultrafiltered samples were compared in order to determine whether ultrafiltration was helpful in improving the condition of samples. As shown in figure 3.4, C:N values did not improve with ultrafiltration, but were often negatively influenced.

The correlation of poorer C:N values and of δ^{13}C depletion with ultrafiltration indicates that the 'collagen' carbon isotope values are being affected as a result of contamination from the ultrafilter itself rather than the removal of contaminants by ultrafiltration. The filter membranes are made of regenerated cellulose and therefore may contribute exogenous carbon to a sample. Colleagues at the University of Bradford now clean ultrafilters with NaOH before use to get rid of potentially contaminating carbon in the filters (Gundula Müldner, personal communication). Since the samples processed without ultrafiltration exhibited C:N values indicative of good collagen quality, and ultrafiltration did not seem helpful in improving collagen quality (in fact, it appeared to contaminate), no further samples were subjected to

ultrafiltration treatment. That is, the isotope values discussed in the following sections were obtained from non-ultrafiltered samples.

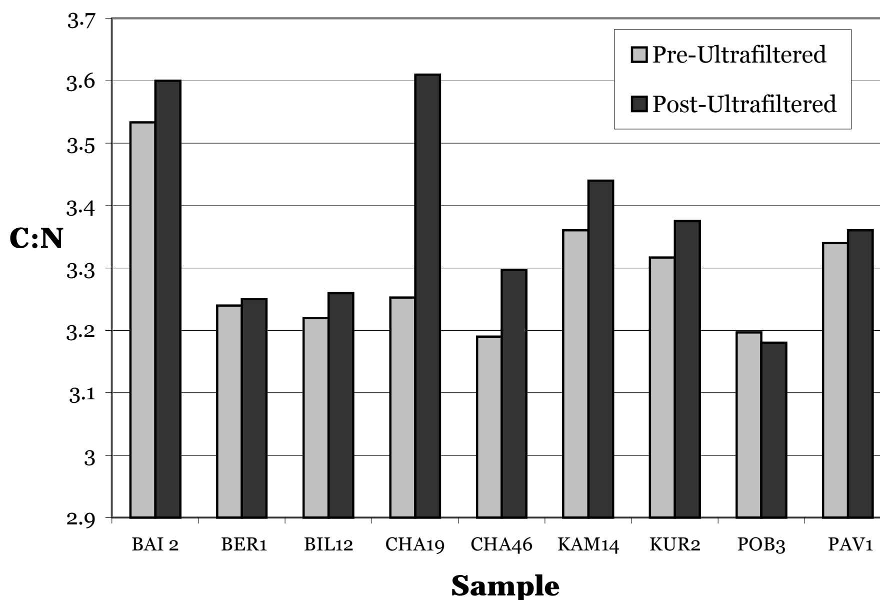

Figure 3.3. Ultrafiltered and non-ultrafiltered bone collagen sample C:N values.

3.6.3. δ^{13}C AND δ^{15}N VALUES

In total, 535 of 614 bone samples (87%) yielded enough 'collagen' for multiple replicate analyses and exhibited C:N, %C and %N values indicative of well-preserved collagen. The values of those 535 samples are reported in appendix 1. Appendix 1 also includes the δ^{13}C and δ^{15}N values obtained from 16 Bronze Age Srubnaya/Alakul/Sintashta/Petrovka human samples from Urals/western Siberian sites, provided by the Oxford Radiocarbon Accelerator Unit; these sample data are included in the results and analysis discussed below.

Means and standard deviations for each species type are plotted in figure 3.2. The δ^{13}C and δ^{15}N values obtained for both humans and terrestrial

herbivores show a wider isotopic range than was previously reported for Eurasian steppe archaeological material (see O'Connell *et al.* 2000).

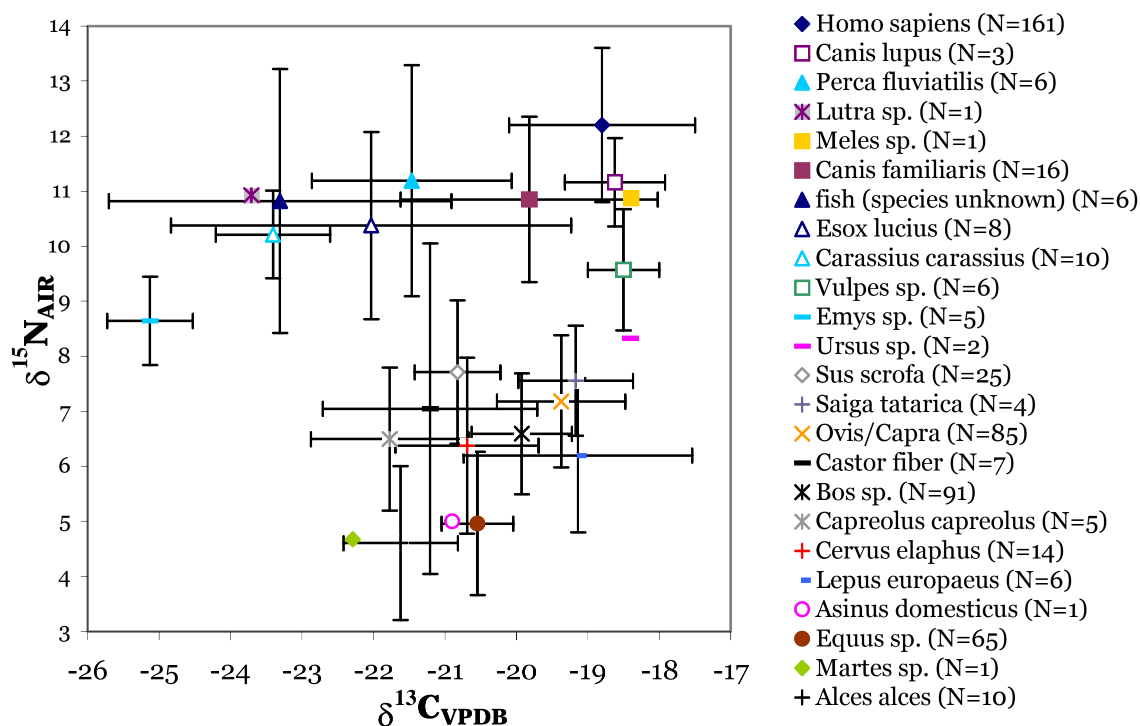

Figure 3.4. Plot of $\delta^{13}C$ and $\delta^{15}N$ results by species (mean $\pm 1\sigma$).

3.6.4. FAUNAL ISOTOPE DATA

Overall, faunal isotope values range from –26.5 to –15.9‰ for carbon and from 2.4 to 13.8‰ for nitrogen. Figure 3.5 shows the means $\delta^{13}C$ and $\delta^{15}N$ values ($\pm 1\sigma$) of terrestrial herbivores, terrestrial omnivores/carnivores, freshwater herbivores, freshwater fish (omnivores) and 1 freshwater carnivore. Domestic pig samples are omitted from this data plot since, though officially omnivores, their diets may be highly influenced towards herbivory or omnivory due to their management by humans. Species included in this trophic level plot are the following:

Terrestrial Herbivores: *Alces alces, Asinus domesticus, Bos sp., Capreolus capreolus, Cervus elaphus, Equus sp., Lepus europaeus, Ovis/Capra sp., Saiga tatarica*

Terrestrial Omnivores/Carnivores: *Canis sp., Martes sp., Meles sp., Sus scrofa (ferus), Ursus sp., Vulpes vulpes*

Freshwater Herbivores: *Emys sp., Castor fiber*

Freshwater Omnivores (Fish): *Carassius carassius, Esox lucius, Perca fluviatilis*, freshwater fish of indeterminate species

Freshwater Carnivore: *Lutra sp.*

$\delta^{13}C$ values seem to vary according to ecosystem (i.e., terrestrial or freshwater). The mean carbon isotope values of freshwater herbivores and fish are similar (-22.8 and –22.6‰, respectively); as are average $\delta^{13}C$ values for terrestrial herbivores and omnivores/carnivores (-20.0 and –19.7‰, respectively). For the samples analysed, freshwater species are ^{13}C-depleted on average relative to terrestrial fauna. The $\delta^{13}C$ values obtained for the freshwater species in this study do not necessarily reflect those for freshwater species throughout Eurasia, due to the high variability of $\delta^{13}C$ observed within and between freshwater environments (see section 2.5).

Terrestrial omnivores/carnivores exhibit an average elevation in $\delta^{15}N$ of +3.2‰ over terrestrial herbivores. The difference between the $\delta^{15}N$ of herbivores and omnivores of freshwater species shows a similar pattern: the omnivorous fish average at 10.6‰ in $\delta^{15}N$, 2.9‰ above the freshwater herbivore average $\delta^{15}N$ value. The single freshwater carnivore sample is elevated 3.2‰ above the freshwater herbivore average nitrogen isotope value.

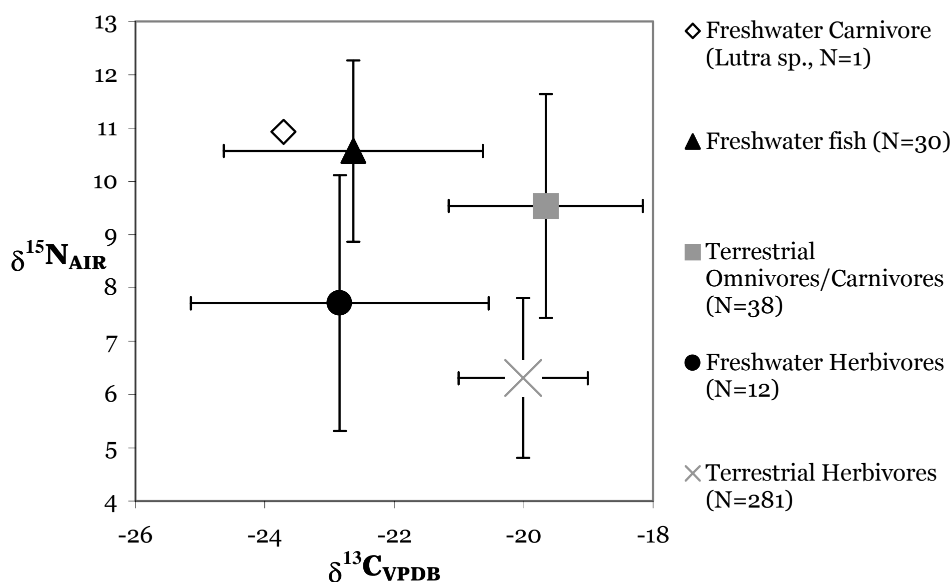

Figure 3.5. $\delta^{13}C$ and $\delta^{15}N$ values for species by trophic level type (mean ±1 σ).

The patterns exhibited by these data are in agreement with current stable isotope models of food webs (see sections 2.4 & 2.6). The isotopic relationships between herbivores and carnivores shown here suggest that the carbon isotope values of consumers and their food are broadly similar (possibly with slight consumer $\delta^{13}C$ enrichment relative to food), and that the average nitrogen isotopic distance between trophic levels within an ecosystem is approximately 3‰.

3.6.4.1. *By Species*

In figure 3.6, the $\delta^{13}C$ and $\delta^{15}N$ values of terrestrial herbivores/domesticates (i.e., herbivores and pigs) are plotted by species. Distinct isotopic differences exist between the average values of different species. The species with the lowest average $\delta^{15}N$ values are *Alces alces* and equids, while pigs and ovicaprids exhibit the highest average nitrogen isotope values. Samples of cattle and wild species including *Capreolus capreolus* and

Cervus elaphus produced intermediate $\delta^{15}N$ values of approximately 6.5‰ on average.

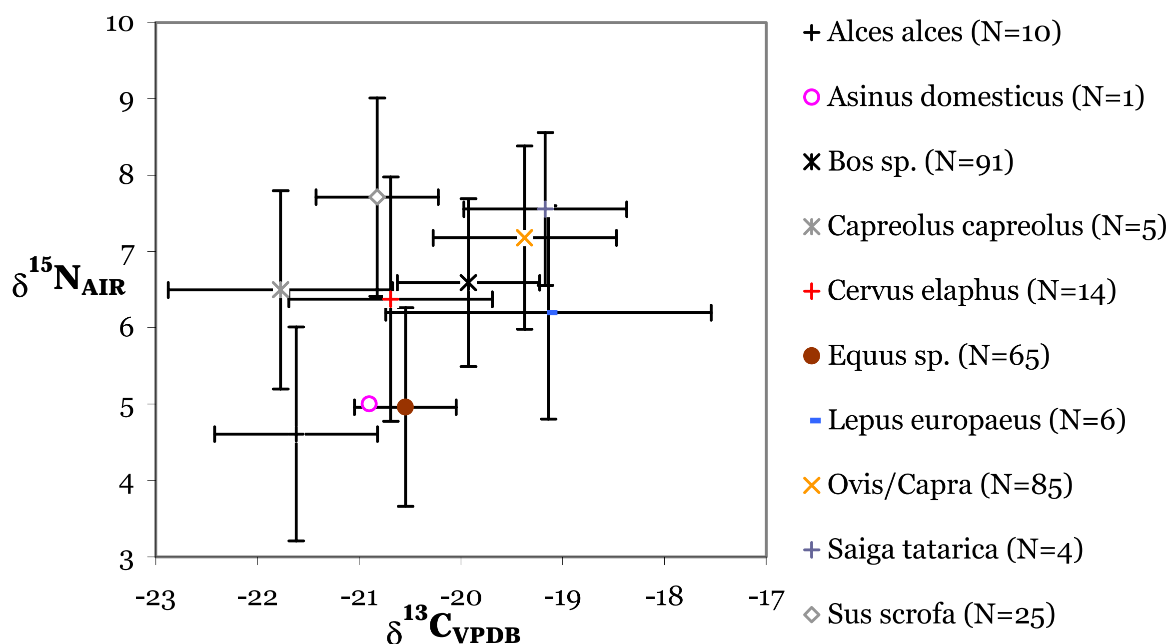

Figure 3.6. Terrestrial herbivore $\delta^{13}C$ and $\delta^{15}N$ values plotted by species (mean ±1σ).

The isotopic range of average intra-species nitrogen isotope values of 3.1‰ is on a similar scale to the spread of average $\delta^{13}C$ values obtained for terrestrial herbivores and pigs (2.7‰). There does not seem to be a significant correlation between $\delta^{15}N$ and $\delta^{13}C$ values amongst these faunal species; the species with the highest and lowest average $\delta^{13}C$ values (*Capreolus capreolus* and *Lepus europaeus*) have very similar average $\delta^{15}N$ values in the centre of the terrestrial herbivore $\delta^{15}N$ range.

3.6.4.2. *By Environmental Type*

Most of the faunal samples analysed in this study were from the Ukraine or Urals/western Siberia regions. In order to identify any isotopic variation in

material collected from different environmental zones, the isotopic values of terrestrial herbivores from forest-steppe sites were compared against those from steppe sites for each geographic region (figures 3.7 and 3.8). Berg (1950) defines the steppe environment as

> an area which is more or less level, unforested…and covered throughout the entire vegetative season with a more or less dense herbaceous vegetation… There are no trees except in the river valleys (90).

In some areas, the forest-steppe and steppe regions may be very similar. The forest-steppe is described as

> a zone of transition between the forest on the north and the steppe on the south. In the typical forest-steppe landscape large masses of forest alternate with vast sections of steppe, or there are coppices scattered in patches over a background of steppe (Berg 1950:68).

Within both regions, the mean isotope values of material from sites located in the steppe were enriched in both carbon and nitrogen relative to those from forest-steppe sites. However, these average isotopic differences are small and not statistically significant. Average carbon isotope values were heavier for all species groups (*Bos*, *Cervus*, *Equus* and *Ovis/Capra*) at steppe sites, enriched from 0.1 to 0.7‰ (by species) relative to forest-steppe sites.

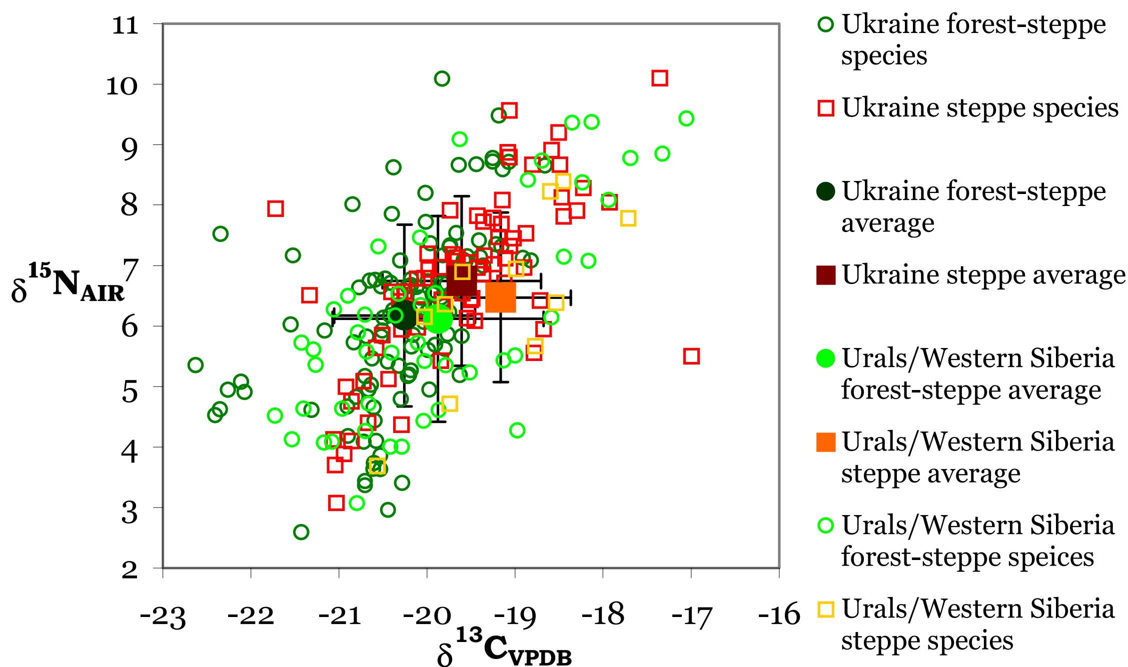

Figure 3.7. Steppe and forest-steppe herbivore species δ^{13}C vs. δ^{15}N.

Although nitrogen isotope values follow the same pattern as carbon isotope values on average, *Cervus* mean δ^{15}N values are higher at forest-steppe sites; *Ovis/Capra* steppe and forest-steppe δ^{15}N averages are identical.

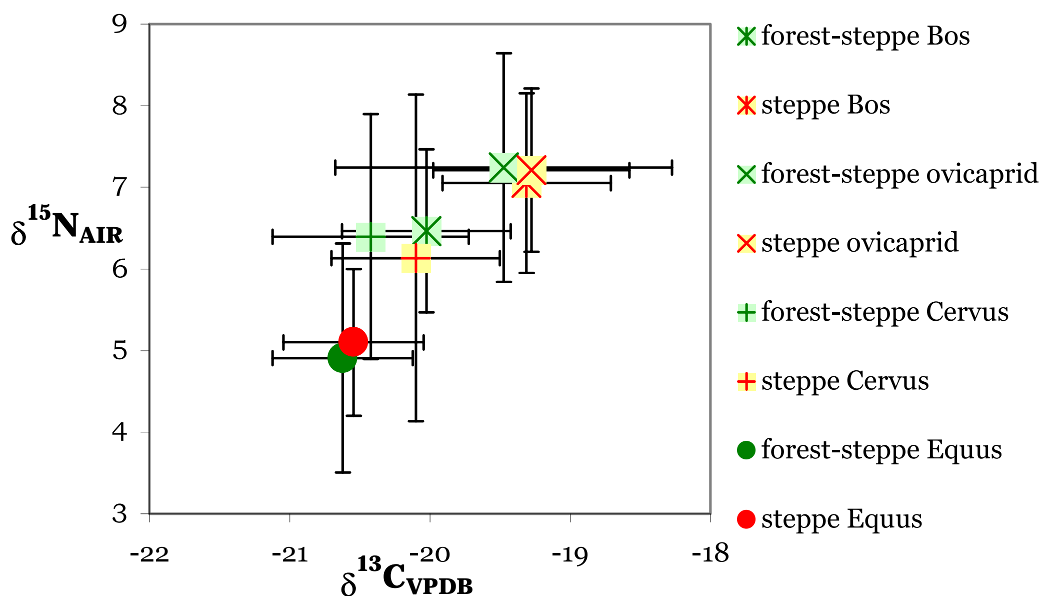

Figure 3.8. Steppe and forest-steppe herbivore δ^{13}C and δ^{15}N values, plotted by species.

3.6.4.3. *By Time Period*

Figures 3.9 and 3.10 show changes in carbon and nitrogen isotopic values between the Bronze Age and the Iron Age, for samples from the Ukraine and the Urals/western Siberia. $\delta^{13}C$ values for all major faunal species (*Bos, Equus* and *Ovis/Capra*) increase from the Bronze Age to the Iron Age in the Ukraine, while the same species show a consistent decrease in $\delta^{13}C$ over time in the Urals through western Siberia. On average, from the Bronze Age to the Iron Age Ukrainian faunal $\delta^{13}C$ values exhibit an enrichment of 0.5‰; faunal $\delta^{13}C$ values from Urals/western Siberian sites show a depletion of 1.2‰. These changes in $\delta^{13}C$ over time may be due to regional climatic variations from the Bronze Age to the Iron Age in the two geographical areas studied (see section 2.6).

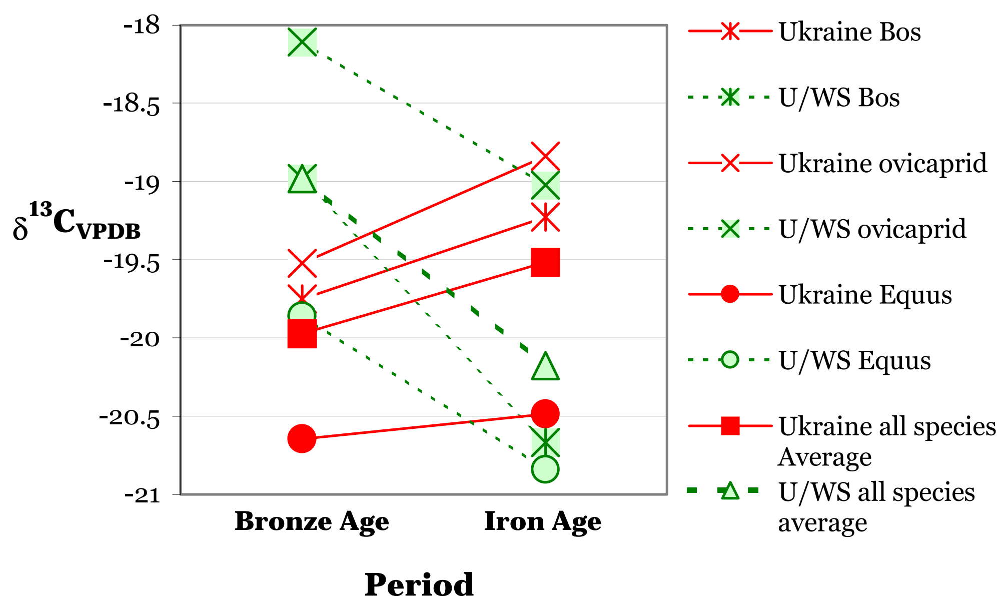

Figure 3.9. Average $\delta^{13}C$ of faunal species over time, grouped by region (Ukraine and Urals/western Siberia).

The average faunal nitrogen isotope values follow a pattern similar to that of the carbon isotope values over time, with a slight increase in $\delta^{15}N$ (0.3‰) in Ukraine and a decrease of equal magnitude in the Urals/western

Siberia. However, increasing and decreasing trends in $\delta^{15}N$ are not consistent for all species within the same area.

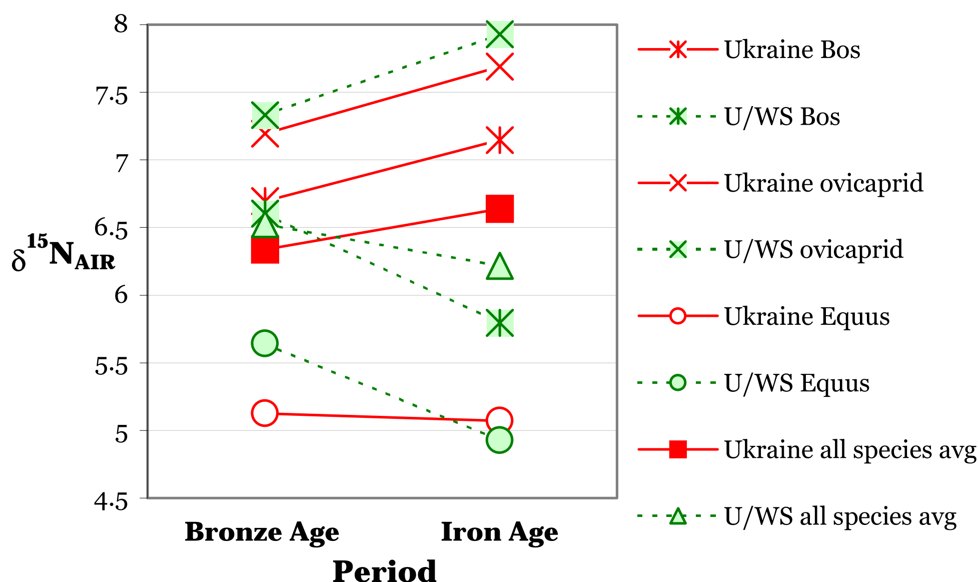

Figure 3.10. Average $\delta^{15}N$ of faunal species over time, grouped by region (Ukraine and Urals/western Siberia).

3.6.5. HUMANS

Human isotopic values obtained from Bronze Age and Iron Age sites is reported in this section. These data relate to the cultural communities described in section 3.4.2. Isotopic data obtained from humans at the Greek settlement site of Olvi'ia are not included in the following discussions of overall human dietary trends, but are considered further on in comparison to Iron Age Ukrainian isotope values.

Human $\delta^{13}C$ values obtained for this study range from -22.8‰ at the Trans-Uralian forest-steppe site of Murzino to -14.1‰ at the site of Ordzhonikidze in the north Pontic steppe. Aside from one outlier at 5.6‰, the nitrogen isotope values of the humans analysed all fall within the range of 9.5 to 15.9‰. As the inclusion of this individual outlier (POB3, from the site

of Pobyeda) could prevent calculated average values from reflecting the bulk of the data, this sample is omitted from the following reported average human values. The presence of a single sample with $\delta^{15}N$ value so different from all other human samples emphasises the importance of drawing palaeodietary conclusions from as large a set of data as possible. Sample POB3 draws attention to the potential to misinterpret the diet of a human group through the analysis of a single human believed to be representative of the group.

Due to the small isotopic difference between fauna from sites of different environments or time periods, human $\delta^{13}C$ and $\delta^{15}N$ values are compared against an isotopic baseline of all fauna analysed in this study, averaged by species.

3.6.5.1. *Freshwater Fish Consumption: the Isotopic Evidence*

The issue of the degree to which Bronze Age and/or Iron Age Eurasian steppe peoples relied upon freshwater fish as a dietary staple may be approached as a matter of assessing the relative dietary dependence of human groups upon two main food types: freshwater fish and terrestrial herbivores/domesticates. Humans are one trophic position above their food, and thus human samples will be isotopically enriched (by 3-5‰) relative to their major food sources.

In assessing the trophic position of humans in relation to terrestrial herbivores/domesticates and fish, it is useful to refer to figure 3.5, in which the isotopic averages for herbivores and omnivores/carnivores from terrestrial and freshwater ecosystems are plotted, using data obtained from Bronze and Iron Age Eurasian steppe sites. In figure 3.5, the two main potential food

sources for humans (terrestrial herbivores and fish) have an average $\delta^{15}N$ value of 6.3 and 10.6‰, respectively. The data shown in figure 3.5 suggest that animals consuming mainly terrestrial herbivores should have a $\delta^{15}N$ value around 9.5‰. Keeping in mind that the main terrestrial domesticates (cattle, pigs and sheep/goats) have an overall average $\delta^{15}N$ value of 7.0‰, humans relying primarily on their domesticated ovicaprids and cattle for food should exhibit a higher $\delta^{15}N$ value than 9.5‰. The frequent consumption of cattle, ovicaprid and pig products would lead to human $\delta^{15}N$ values to reach at least 10‰.

Figure 3.11 shows the total isotopic data obtained for humans in this study, relative to average faunal isotopic values. Aside from an individual with anomalously low $\delta^{15}N$, the lowest $\delta^{15}N$ value of the human samples analysed in this study is 9.5‰. For the human samples that produced the lowest $\delta^{15}N$ values, it is possible to interpret their isotopic values as indicative of a diet based exclusively on the consumption of terrestrial domesticates. However, most of the human $\delta^{15}N$ values fall above the maximum value obtained for a wild terrestrial carnivore (*Canis lupus*, 11.7‰). The average $\delta^{15}N$ value of Eurasian steppe humans included in this study is 12.2‰, which is 5.2‰ above the average $\delta^{15}N$ value of the terrestrial domesticates. The prevalence of high $\delta^{15}N$ human values, markedly elevated above those of contemporary terrestrial omnivores/carnivores, strongly suggests that many of the Bronze and Iron Age humans analysed in this study must have frequently consumed foods more enriched in $\delta^{15}N$ than their domesticated animals.

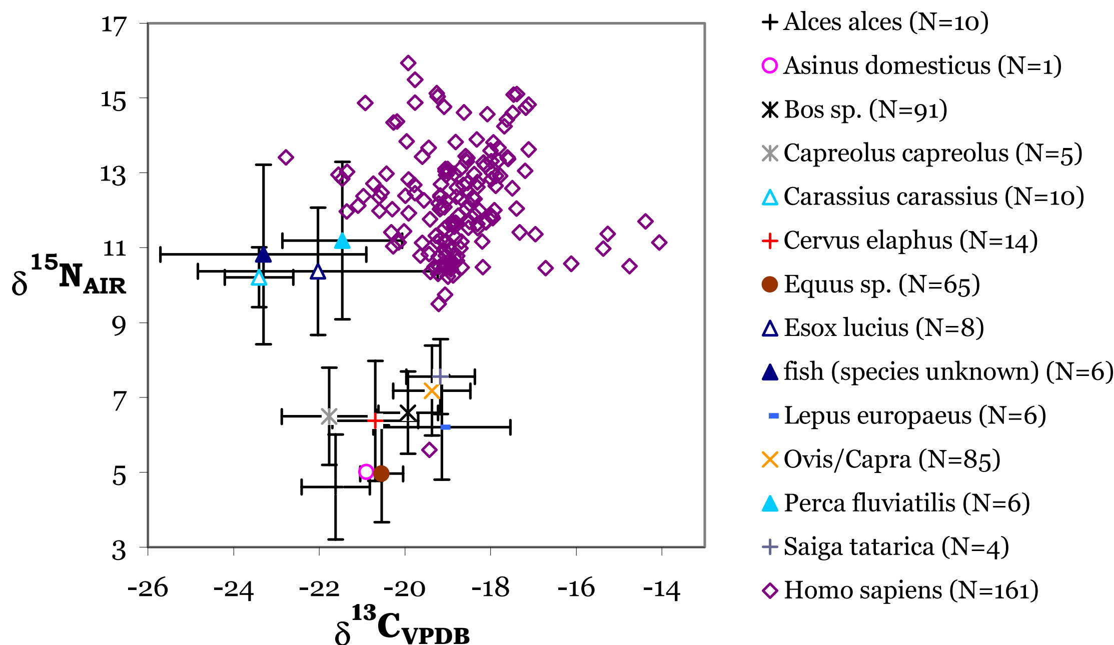

Figure 3.11. Human and faunal $\delta^{13}C$ and $\delta^{15}N$ values. Fauna are plotted as mean ±1σ, humans as individual values.

Since the frequent consumption of terrestrial omnivores or carnivores is unlikely, the only other food source likely to be consumed as a staple by the humans in question is freshwater animals, such as fish. According to the data obtained from this research as well as other studies (see section 2.4), it is clear that the frequent consumption of freshwater fish by humans would produce consumer $\delta^{15}N$ values higher than those expected as a result of terrestrial faunal consumption. Trends observed in the palaeodietary literature, as well as the trophic isotopic pattern illustrated in figure 3.5, indicate that animals principally consuming freshwater fish should exhibit $\delta^{15}N$ values of ~13.6‰ or more. According to empirical studies of food-consumer $\delta^{13}C$ enrichment (see section 2.4), carbon isotope values for freshwater fish consumers in this study may be expected to range from values similar to that of the fish analysed up to a maximum of approximately -17.6‰. Carbon isotope values are not particularly useful in determining relative dietary dependence upon freshwater and terrestrial resources, as most of the carbon isotope values of

the human samples may be explained by exclusive dietary reliance upon freshwater resources, terrestrial domesticates, or a mixture of the two. Furthermore, the fish samples analysed in this study are not from all of the geographical areas covered by the human samples. Freshwater fish $\delta^{13}C$ values are known to vary considerably within and between geographic regions and bodies of water (see Dufour *et al.* 1999 and section 2.5), and the fish bones sampled for this study may not reflect the full range of freshwater fish isotopic values that can be obtained throughout the Eurasian steppe and forest-steppe. For the human groups studied in this project, human $\delta^{15}N$ values appear to be a more useful indicator of diet when comparing freshwater fish *versus* terrestrial animal consumption.

Few human samples in this study have a $\delta^{15}N$ value over 13.6‰, suggesting that few humans consumed fish almost exclusively. Nevertheless, the nitrogen isotope values obtained for the majority of humans in this study fall somewhere between the $\delta^{15}N$ values expected for freshwater and terrestrial omnivores/carnivores. Therefore, the isotopic data indicate that most of these Bronze Age and Iron Age humans must have consumed variable proportions of freshwater fish and terrestrial herbivore protein as a basis of their diet.

3.6.5.2. *Evidence for Millet Consumption*

As mentioned above, most of the human $\delta^{13}C$ values can be explained by a dietary dependence upon freshwater resources, terrestrial domesticates, or a combination of the two. However, a small number of human samples exhibit highly enriched $\delta^{13}C$ values—up to -14.1‰—that cannot be explained solely by a reliance on freshwater animals or terrestrial domesticates as protein sources.

The consumption of marine foods would lead to highly enriched human $\delta^{13}C$ values as are exhibited by some humans, but high $\delta^{13}C$ values due to marine food consumption are accompanied by high $\delta^{15}N$ values. The humans in this study with the most enriched $\delta^{13}C$ values do not have correspondingly high $\delta^{15}N$ values; in fact, their $\delta^{15}N$ values are all below the total human average.

Carbon isotope values much less negative than those of contemporary terrestrial fauna yet not high enough to be representative of marine food consumption may be explained by the frequent consumption of another food type, moderately low in $\delta^{15}N$ but high in $\delta^{13}C$. Various species of C_4 grasses are native to the Eurasian steppe, and one C_4 plant—millet—has been recovered from archaeological sites in the steppe as early as the fifth millennium BC (Sériadès 2000). As a C_4 crop, millet has a high $\delta^{13}C$ value (between -18 and -8‰). The frequent consumption of millet could therefore explain the most highly enriched human $\delta^{13}C$ values. The Bronze and Iron Age humans included in this study may have obtained millet by cultivating it themselves, or through commerce with neighbouring agricultural groups.

The large difference between the human and faunal carbon isotope values suggests the direct consumption of C_4 agricultural products (i.e., millet) by the humans, rather than the acquisition of a C_4 dietary signal through the consumption of animals raised on C_4 pastures and/or fodder. If the enriched $\delta^{13}C$ values of the humans were derived from the consumption of the products of C_4-fed animals, the human and faunal carbon isotope values would be more similar than those observed in this study.

3.6.5.3. *Bronze Age vs. Iron Age*

Following the survey of average isotopic values and high-δ^{13}C outliers, it is of interest to explore dietary trends within and between time periods. The data may be examined chronologically to investigate whether the shift from a primarily sedentary lifestyle in the Bronze Age to a more mobile pastoral economy in the Iron Age is reflected in dietary changes detectable by stable isotope analysis. The average isotopic values of Bronze Age humans are compared with those of Iron Age humans in figure 3.12.

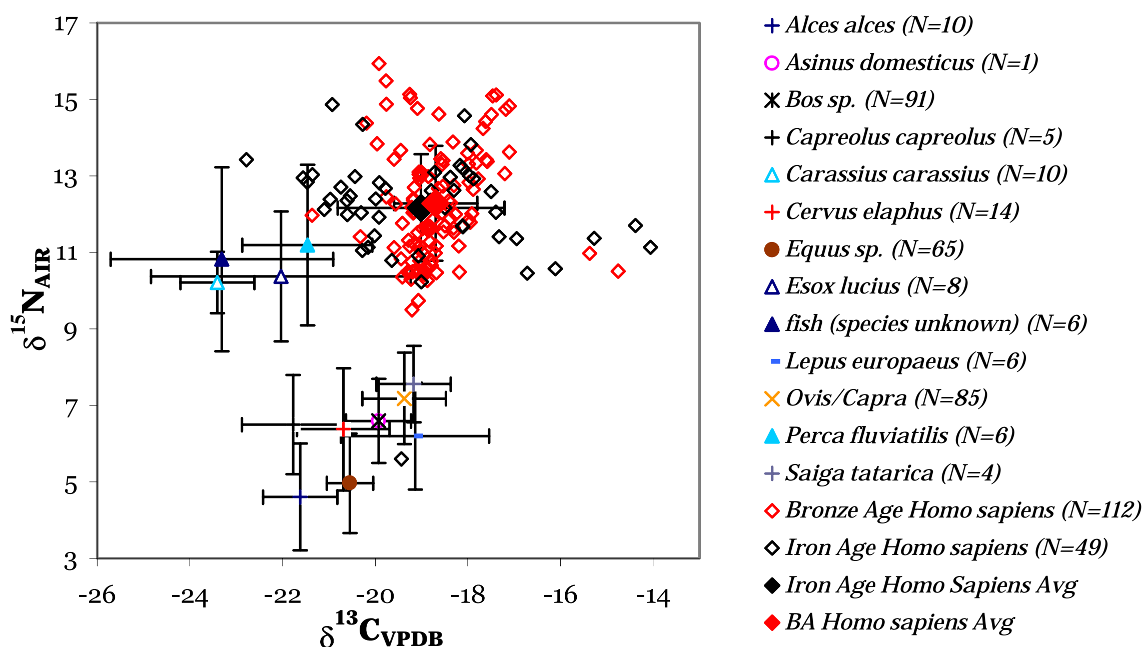

Figure 3.12. Bronze Age and Iron Age human samples plotted individually and by mean ±1σ. Fauna plotted by species mean ±1σ.

The isotopic averages of Bronze Age and Iron Age human samples are very similar. The average carbon isotope value of the Bronze Age humans (-18.7‰) is enriched by 0.3‰ relative to the Iron Age human average δ^{13}C (-19.0‰). The average δ^{15}N value of the Bronze Age humans (12.3‰) differs from that of the Iron Age humans (12.2‰) by only 0.1‰. The isotopic ranges of the human Bronze Age and Iron Age samples are also similar. Iron Age

human $\delta^{13}C$ values exhibit an isotopic spread from -22.8 to -14.1‰; the Bronze Age human $\delta^{13}C$ range falls inside the Iron Age range from -21.4 to -14.8‰. Human samples from Bronze Age contexts produced $\delta^{15}N$ values from 9.5 to 15.9‰, with the Iron Age human samples exhibiting a smaller nitrogen isotopic range of 10.2 to 14.9‰. The average isotopic data highlight the similarities in human diet between the Bronze Age and the Iron Age, and reflect no major dietary shifts between these two periods on the whole.

However, if we divide the human isotopic data into cultural groups by period and region, certain dietary patterns become apparent. Amongst the Bronze Age samples (figure 3.13), a degree of stratification can be observed in the $\delta^{15}N$ values. Alakul/Srubnaya humans from sites in the Urals through western Siberia yield consistently low $\delta^{15}N$ values relative to the human $\delta^{15}N$ average, while all of the humans from the southwestern Siberian site of Chicha exhibit $\delta^{15}N$ values above the total human average. Almost all of the samples obtained from Bronze Age sites in the Ukraine produce a tight clustering of intermediate $\delta^{15}N$ values; in contrast, $\delta^{15}N$ values for Sintashta/Petrovka humans from Urals/western Siberian sites range widely from 9.5 to 14.8‰.

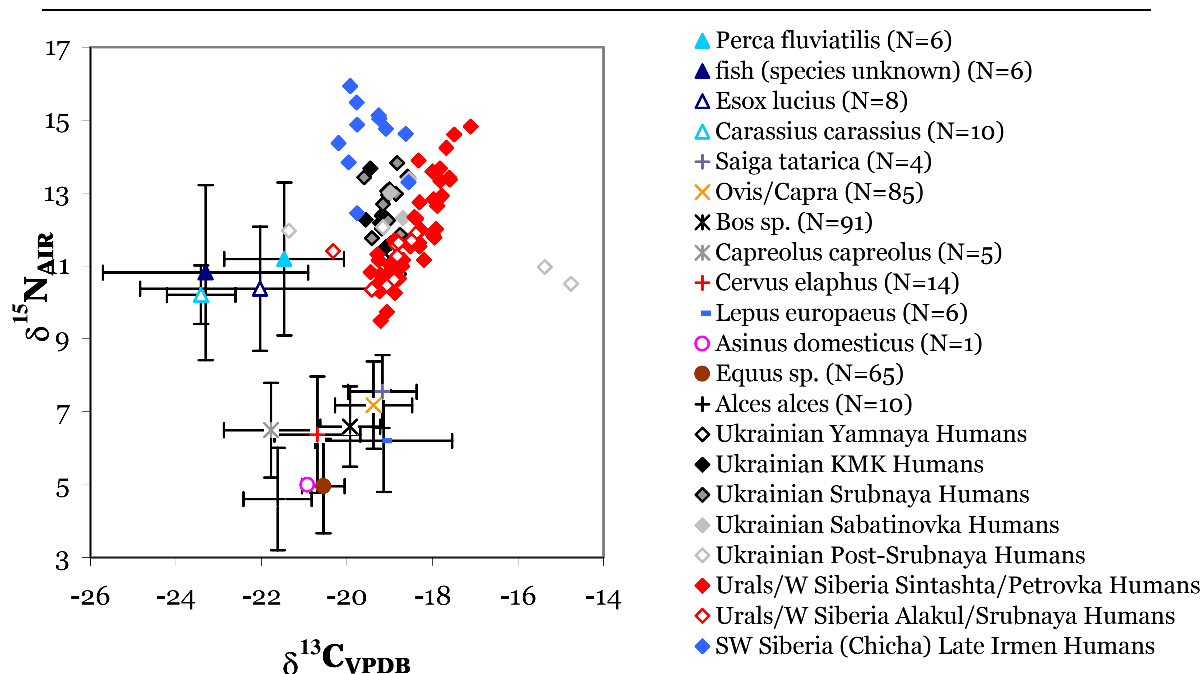

Figure 3.13. Bronze Age humans plotted by cultural groups. Bronze and Iron Age fauna plotted by species mean ±1σ.

These isotopic patterns suggest that freshwater resources were relied upon most heavily as a dietary staple by the inhabitants of Chicha during the Bronze Age, while Ukrainian Bronze Age peoples depended relatively more on terrestrial domesticates for food. The palaeodietary data for Chicha correspond well with the archaeological evidence for the exploitation of fish at the site as an important food resource. Of all archaeological sites from which samples were obtained for this study, Chicha exhibited the strongest archaeological evidence for high fish consumption by humans, due to the high number of fish bones recovered from the site (see Molodin *et al.* 2002). Few, if any, fish bones were recovered from the other Eurasian archaeological sites considered in this study.

Alakul/Srubnaya humans were most extreme in their dietary distance from the inhabitants of Chicha, in that their isotopic values may be explained by frequent consumption of terrestrial domesticates with little freshwater animal dietary input. The base of the Sintashta/Petrovka diet cannot be

defined as mainly freshwater or mainly terrestrial, according to the isotopic evidence. The wide $\delta^{15}N$ spread of the Sintashta/Petrovka humans indicates that the diet of these people was diverse, and varied between that typical at Chicha (high in freshwater fauna) and that of Alakul/Srubnaya groups (high in terrestrial domesticate products).

A striking pattern apparent in the $\delta^{13}C$ values of Bronze Age humans is the extremely wide spread exhibited by the samples from the post-Srubnaya levels of the settlement of Glubokoe Ozero in Ukraine. While other cultural groups display a limited $\delta^{13}C$ spread of less than 2‰, the four Glubokoe Ozero humans yield a $\delta^{13}C$ range of nearly 7‰, from -21.4 to a markedly high -14.8‰. As mentioned in the preceding section, the highly enriched $\delta^{13}C$ values exhibited by two of the Glubokoe Ozero individuals may be explained by the frequent consumption of millet by these humans. These individuals were recovered from a settlement site, and may have consumed millet grown at or near the site by members of their own community.

Amongst the Iron Age isotopic data (figure 3.14), the most highly enriched $\delta^{13}C$ values also belong to humans from Ukrainian sites. These individuals represent both Scythians from the burial sites of Ordzhonikidze and Bazavluk, and Greeks from the settlement site of Olvi'ia. The Iron Age Ukrainian humans all exhibit values indicative of frequent millet consumption. The non-Greek humans exhibiting the most enriched $\delta^{13}C$ values may have belonged to semi-agricultural Scythian groups; alternatively, these individuals could have obtained millet through trade with Greeks and other sedentary peoples in the north Pontic steppe.

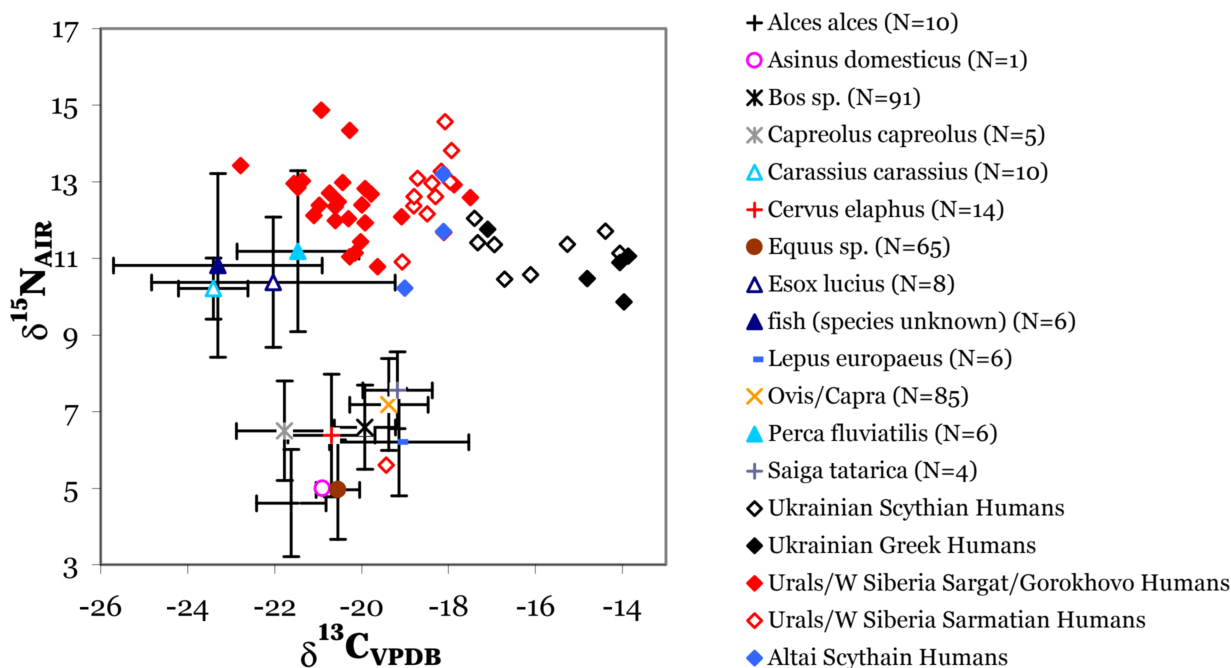

Figure 3.14. Iron Age humans plotted by cultural groups. Bronze and Iron Age fauna plotted by species mean ±1σ.

Although the Scythian individuals from the Ukraine are more closely related in terms of burial style and cultural affiliation with the other 'nomadic pastoralist' groups involved in this study, their isotopic values do not overlap with those of any other Scythian, Sarmatian or Sargat humans analysed. Instead, the Ukrainian Scythians exhibit a range similar to that of the isotope values obtained for the Greek humans from the Olvi'ia settlement. The dietary similarity between the Ukrainian Scythians and Greeks suggests that geographical or commercial ties may have influenced human diet more than broad cultural affiliations.

The $\delta^{13}C$ values obtained for the rest of the Iron Age humans in this study further support the idea that geographical differences correlate with dietary differences during this period. Scythians and Sarmatians from steppe sites outside the Ukraine (Altai and Urals/western Siberia) show a marked carbon isotopic depletion relative to the Ukrainian Scythian humans.

Individuals from Urals forest-steppe sites, affiliated with the Gorokhovo or Sargat cultures, exhibit a further decrease in average $\delta^{13}C$ from the Scythian and Sarmatian humans, though their overall $\delta^{13}C$ ranges do overlap on the more depleted end of the Sargat/Gorokhovo data.

In conjunction with the carbon isotopic patterns present in the Iron Age data, an increase in average $\delta^{15}N$ can be observed from the Ukrainian humans to the Sarmatians and Altai Scythians to the Gorokhovo and Sargat individuals of the Urals/western Siberian forest-steppe.

3.6.5.4. *Human Data by Region*

By arranging the data obtained for humans by region, we can examine trends in average group diet over time, through the Bronze and Iron Ages. By studying isotopic averages, it is also possible to observe similarities and differences in average group diet.

Among the Bronze Age groups of the Urals/western Siberia (represented by filled diamonds in figure 3.15), the average isotope values of the cultural groups are all very similar, with the exception of the Sintashta sample set. If the mean isotope values of the Sintashta human samples (N=40) are compared with those of the other Bronze Age humans of the region (N=39), there is a significant difference in average $\delta^{13}C$ and $\delta^{15}N$ values between the two datasets ($p<0.001$ for $\delta^{13}C$, $p=0.001$ for $\delta^{15}N$; independent samples t-test). The more highly elevated mean $\delta^{15}N$ exhibited by the Sintashta samples indicates that the Sintashta diet incorporated relatively more freshwater animal protein than the other Bronze Age groups in the region, on average. It is interesting to note that, while the individuals from Sintashta-Petrovka sites

are considered to be culturally related to Sintashta individuals (e.g., by way of similarities in burial rite, artefact typology and chronology), the isotopic evidence indicates that the Sintashta-Petrovka humans were more similar—at least in their dietary habits—to the other Bronze Age cultural groups.

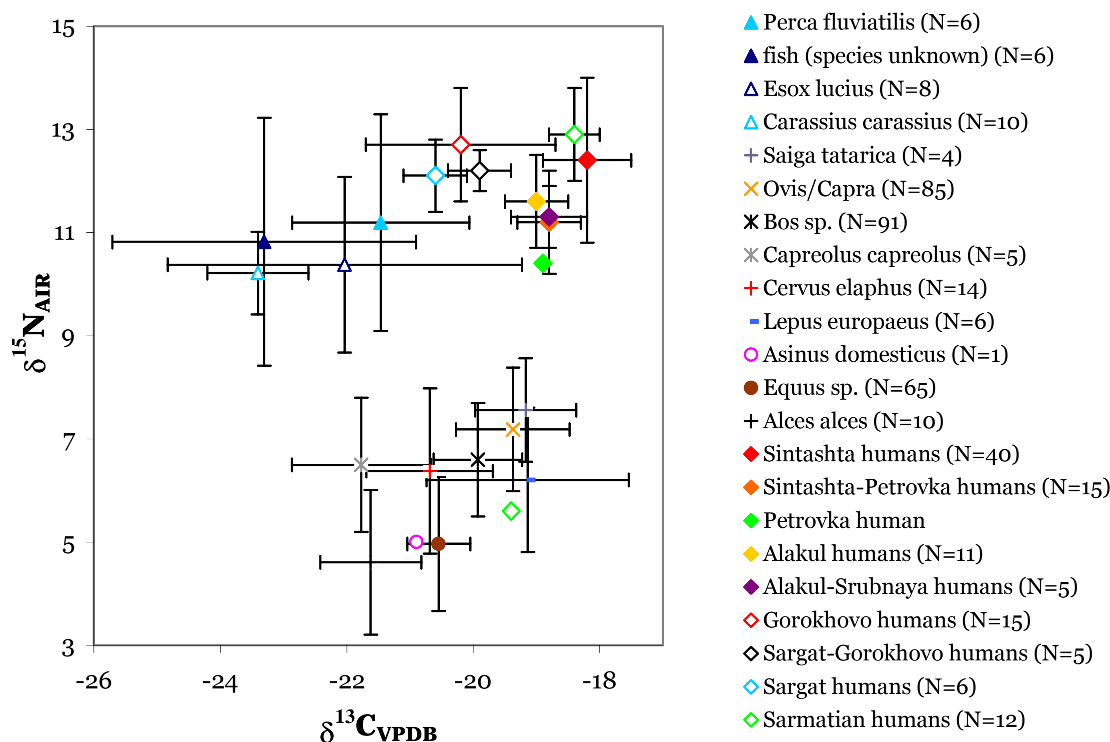

Figure 3.15. Plot of Urals/western Siberian Bronze Age and Iron Age human data by cultural group (±1σ); the faunal data is from all regions, by species (±1σ).

Samples associated with most of the Iron Age cultural groups of the Urals/western Siberia region exhibit depleted δ^{13}C values on average, relative to the Bronze Age human samples. However, this trend is not uniform, and Sarmatian humans (N=12) exhibit an average δ^{13}C value significantly different from those of other Iron Age humans (N=26) from the region ($p<0.001$; independent samples t-test). The mean Sarmatian human isotope values (both δ^{13}C and δ^{15}N) are very similar to those obtained from the Bronze Age Sintashta humans. These two cultural groups (Sintashta and Sarmatian) are chronologically separated by at least 1000 years, so their apparent dietary

similarity does not necessarily reflect continuity over time from one culture to the next. In contrast to the other Iron Age cultural groups included in this study, which come from the forest-steppe regions of the trans-Urals, the Sarmatian samples were taken from sites in the south Urals steppe. Sintashta material was also obtained from the steppe region of the south Urals. As I have shown above (section 3.6.4.2), differences between steppe and forest-steppe environments do not lead to significant isotopic differences between the fauna obtained from those two environments. Therefore it appears that the isotopic differences between human groups in the steppe and forest-steppe reflect differences in average human diet. The apparent distinction of Sarmatian average diet from that of other Iron Age groups as well as its similarity to the Sintashta average diet may be a reflection of the adaptation of subsistence strategies to the steppe environment.

Among the Ukrainian samples, there is an increase in average group $\delta^{15}N$ value over time, from the earlier Yamnaya period to the KMK period to the Srubnaya and Sabatinovka period (see figure 3.16). The general increase in average nitrogen isotope values from group to group may reflect an increase in the dietary importance of freshwater animals during these time periods.

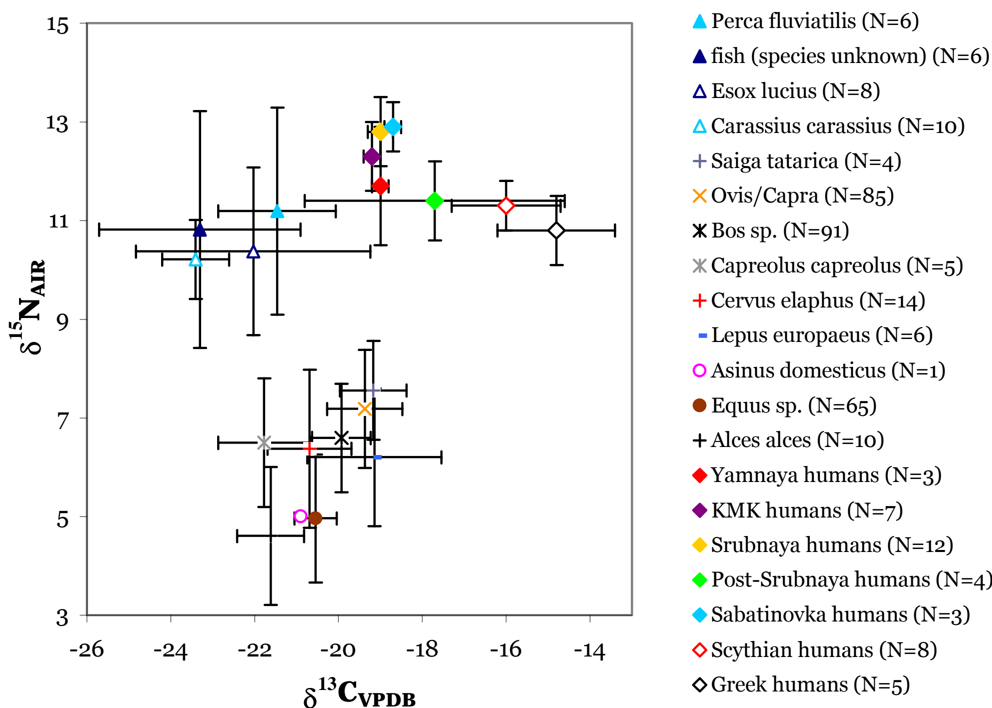

Figure 3.16. Plot of Ukrainian Bronze Age and Iron Age human data by cultural group (±1σ); the faunal data is from all regions, by species (±1σ).

However, the trend in increasing $\delta^{15}N$ with time does not continue through the whole of the Late Bronze Age. The samples associated with the later Ukrainian cultural groups (i.e., from approximately the 13th cent. BC), from the Bronze Age Post-Srubnaya to the Iron Age Scythian and Greek samples, exhibit a distinctly lower $\delta^{15}N$ average value relative to the earlier Bronze Age groups. As mentioned above, the lower $\delta^{15}N$ values of these later groups correspond to less negative $\delta^{13}C$ values—an isotopic profile indicative of an average diet based on C_4 plants and terrestrial animal protein. While the Post-Srubnaya isotopic data (see also, figure 3.13) show that C_4 plants were not a consistent dietary staple for this group, the Scythian and Greek isotopic values suggest the frequent consumption of C_4 plants in addition to terrestrial animal protein among these Iron Age Ukrainian groups. The Ukrainian isotopic data indicate that millet was not a major part of the average human diet before the Late Bronze Age. It seems that millet began to play an

important role in the diets of people in this area in the Late Bronze Age, and that this resource increased in importance in the Iron Age, becoming a dietary staple.

By examining the human isotopic data from Bronze Age and Iron Age sites by period, cultural affiliation and region, it has been possible to identify dietary patterns not evident in the average Bronze Age and Iron Age human values. Distinct isotopic groupings of individuals correlate with geographical regions in the Iron Age, and with both geographical and cultural divisions in the Bronze Age. According to the prevalence of high $\delta^{15}N$ values among humans in both periods, it is possible to suggest that fishing was a major subsistence activity practiced by many Bronze Age and Iron Age peoples of these Eurasian steppe regions. However, it is necessary to acknowledge the apparent decrease in the importance of fish in Ukrainian diets from the Bronze Age to the Iron Age.

Even as we may stress the importance of fishing in the subsistence economies of these Eurasian steppe peoples, the isotopic data indicate that agricultural products (notably, millet) were also an important element in the diets of many communities, particularly in the Ukraine during the Iron Age. The data presented here indicate that the current image of ‘nomadic pastoralists’ does not convey an accurate picture of the economy and diet of all of the groups involved in this study.

A further isotopic trend of note is the manner in which the human data obtained for this study are in conflict with how palaeodietary researchers are used to looking at the $\delta^{13}C$ and $\delta^{15}N$ of samples. Carbon isotopic elevation is usually coupled with higher $\delta^{15}N$ values, as is shown in all $\delta^{13}C$ and $\delta^{15}N$

trophic level diagrams (e.g. see figure 3.5). The Bronze and Iron Age human data in this study exhibit a completely contrary trend—that of higher $\delta^{15}N$ values corresponding to lower $\delta^{13}C$ values (e.g., figure 3.16). When dealing with economies and environments as complex as those in the Eurasian steppe in the Bronze and Iron Ages, where potential food sources are widely varied and include domesticated and wild terrestrial fauna, aquatic animals and cultivated plants, isotopic data cannot be expected to follow usual trends.

3.7. *A Revision of Bronze Age and Iron Age Eurasian Steppe Diet: Conclusions*

The enriched $\delta^{15}N$ values exhibited by most of the humans included in this study support the hypothesis that freshwater fauna (e.g., fish) were indeed a major staple in the diet of many Bronze Age and Iron Age humans that inhabited the Eurasian steppe. The isotopic data presented here reinforce the conclusion drawn by O'Connell *et al.* (2000), that freshwater fish continued to be an important element in the diets of Eurasian steppe peoples beyond the Mesolithic and Neolithic periods, throughout the Bronze and Iron Ages. The information obtained from this isotopic study suggests that archaeologists have not appropriately acknowledged the significance of fishing as a major subsistence technique among Bronze and Iron Age Eurasian steppe peoples, and that currently accepted descriptions of prehistoric Eurasian pastoralist economies need to be revised.

That is not to say that the current picture of the subsistence strategies of Bronze and Iron Age Eurasian steppe peoples must be entirely rewritten. It may still be valid to say that "The basis of their prosperity lay in their large

herds" (Abetekov and Yusupov 1994:25); but to this statement we should now add an emphasis on the acquisition of fish as a pursuit central to daily subsistence for many Bronze Age and Iron Age groups. The image of a pastoralist economy based on domestic animals need not be exclusive of a strong element of fishing for subsistence purposes. The main conclusion of this isotopic research is that fish played an important role in the regular diets of many Bronze and Iron Age Eurasian steppe groups; and that the importance of fishing as a subsistence activity should be remembered and emphasized rather than downplayed or ignored.

On a more specific level, this research has revealed the varying degrees to which the different groups studied relied upon freshwater fish, terrestrial herbivores/domesticates and cultivated crops as dietary staples. Of all the Bronze Age humans analysed, those from the southwestern Siberian site of Chicha depended most heavily upon freshwater fish as a food resource, while individuals from Alakul and Srubnaya culture sites seem to have consumed little fish and a great deal more terrestrial herbivore/domesticate products than the Chicha humans. In contrast to the fairly uniform, terrestrially-based diet reflected by Alakul/Srubnaya isotopic values, the values produced by Sintashta/Petrovka humans suggest a diet ranging anywhere from mainly freshwater to mainly terrestrial in nature. Bronze Age humans from all sites in the Ukraine, except the post-Srubnaya site of Glubokoe Ozero, exhibit isotope values indicative of a diet intermediate to that of the Chicha and the Alakul/Srubnaya humans.

Beginning with the human data from the Late Bronze Age settlement of Glubokoe Ozero (Post-Srubnaya culture), we begin to see the isotopic evidence

for the important role of millet in the diets of certain Ukrainian groups. The frequent consumption of millet is only reflected in the diets of two groups among the Iron Age samples. However, the dietary similarities of these two groups—Scythians and Greeks—highlights the fact that dietary patterns in the Iron Age correlate to geographical regions as well as cultural affiliations. The isotopic data suggest that the diets of Scythians and Sarmatians from steppe sites beyond the Ukraine are markedly different to the diets of Ukrainian Scythians, which indicates that the economic and commercial practices of these Scythian sub-groups was also significantly different. Further correlations between diet and environment are apparent in the Urals/western Siberian data, where humans from steppe sites, regardless of cultural affiliation, exhibit distinct overall dietary trends relative to forest-steppe groups. These results from the Urals/western Siberian region complement the patterns exhibited by the Ukrainian Iron Age data, which indicate that peoples from similar environments maintained similar diets, and perhaps practiced very similar subsistence strategies.

This study has shown how $\delta^{13}C$ and $\delta^{15}N$ analysis can play a major role in revising our picture of daily life in the Bronze and Iron Age Eurasian steppe. But there are still issues that lay beyond the reach of what carbon and nitrogen isotope analysis can reveal. Currently we must rely almost exclusively upon relatively high $\delta^{15}N$ values as an indicator of frequent freshwater fish consumption. But is there a way to definitively distinguish freshwater- and terrestrial-based diets? Also, can we learn more about Eurasian steppe diet from the analysis of residues extracted from archaeological vessels?

In the following chapters, I seek go beyond carbon and nitrogen bone collagen analysis to answer these questions by testing the utility of sulphur isotopes as an indicator of freshwater fish consumption, and by analysing lipid residues extracted from archaeological vessels recovered from the Bronze Age site of Chicha. By utilising these additional analytical techniques to reveal ancient dietary trends in various sites throughout Eurasia, I hope to lend further weight to the conclusion that freshwater fish were an important dietary staple for the Bronze and Iron Age peoples of the steppe.

CHAPTER 4

DAIRY PRODUCT ANALYSIS

4.1. *A POTENTIAL ALTERNATIVE EXPLANATION FOR HIGH HUMAN $\delta^{15}N$ VALUES*

The data presented in the previous chapter indicate that many Bronze and Iron Age humans throughout the Eurasian steppe region relied upon freshwater animals (e.g., fish) as a dietary staple. The individuals most heavily reliant on freshwater animals as a dietary resource are denoted by their highly elevated $\delta^{15}N$ values. According to our present understanding of the behaviour of isotopes through the food chain, the only possible explanation for highly enriched human $\delta^{15}N$ values (relative to associated herbivore values) in the absence of high $\delta^{13}C$ values is the frequent consumption of freshwater animal protein. The use of faunal bone isotope values as a proxy for all possible animal-derived dietary protein for humans is based upon the principle that all such consumable proteins are isotopically indistinguishable (Katzenberg and Krouse 1989; Minagawa 1992; O'Connell and Hedges 1999). However, there is no information to date that addresses the issue of the possible alteration of food isotope values with processing, including the fermentation and ageing of milk in the manufacture of dairy products.

Milk fermentation involves the metabolism of lactose (milk sugar) and various other nutrients by microorganisms. These microorganisms consume a number of nitrogen-containing compounds present in milk (e.g., proteins, peptides, free amino acids) and together with peptide-decomposing enzymes release nitrogenous compounds including urea, ammonia and aroma compounds (Dellaglio 1989; Law 1997). A $\delta^{15}N$ increase from food to consumer has been documented for a wide variety of organisms from

nematode worms (Boag *et al.* 1998) to aquatic animals (Fry *et al.* 1999; Dufour and Gerdeaux 2001) and humans (Minagawa 1992; Minagawa and Wada 1984). This enrichment in ^{15}N is thought to occur during amino acid metabolism, when deaminated amino acids release ^{15}N-depleted ammonia (which is converted to urea in mammals) and an organism incorporates ^{15}N-enriched amino acids into its own body proteins. It is possible that such a consumer $\delta^{15}N$ enrichment could occur in some lower organisms such as those used to produce fermented dairy products. A combination of $\delta^{15}N$ enrichment from milk to microbial consumers, coupled with the loss of volatile (and isotopically light) metabolic products such as ammonia and aroma compounds could cause fermented dairy products to be significantly enriched in $\delta^{15}N$ relative to the original milk.

If a significant isotopic difference (i.e., ≥ +1‰ $\delta^{15}N$) were detected between milk and its fermented dairy products, a revision of current and past palaeodietary data would certainly be necessary. Where freshwater animal consumption had been the only interpretation of high $\delta^{15}N$ values (without correspondingly high $\delta^{13}C$ values), fermented dairy products would in some cases need to be considered as an alternative palaeodietary interpretation.

Physiologically speaking, it is not unreasonable to suggest that dairy products may have been consumed in a high enough frequency and amount as to influence an individual's body isotope composition. Yoghurt and kefir contain approximately 8 and 14g of protein per 240g (a modern 'serving'), respectively; cheese contains about 14g of protein in a 60g serving. Thus, with each equivalent serving of fermented dairy products an adult individual could consume approximately 10-20% of his or her daily protein requirement (IoM 2002); five such servings would provide 50-100% of required daily protein.

Dairy products have a similar protein content per serving to meats (e.g., beef or mutton). Grain-based foods have a lower protein content per serving (e.g., wheat or oat porridge contains ~5-6g protein per 240g serving; one 46g piece of bread contains ~4g protein).

It is extremely important to examine whether or not milk and its derived dairy products are isotopically equivalent, particularly in the context of this thesis, which is concerned with the dietary habits of peoples that may have consumed dairy products as a regular dietary staple (see below). If the hypothesis that milk and its derived products are isotopically similar is not tested, then the question of the cause of highly elevated human $\delta^{15}N$ values included in this thesis could not be considered unquestionably settled as freshwater fish consumption. In this chapter, I explore the textual, archaeological and ethnographic evidence that suggests that dairy products may have been an important staple in the diets of the archaeological groups analysed in this thesis, and follow with an isotopic study of milk and dairy product composition.

4.1.1. SCYTHIAN DAIRY PRODUCT PREPARATION AND CONSUMPTION IN HERODOTUS

The consumption of fermented milk products such as kumys (fermented mare's milk) and cheese by peoples of the Eurasian steppe has been documented from as early as Scythian times to the present day. The Greek historian Herodotus (5th century BC) is the first known person to report on milking and the manufacture of dairy products by Eurasian steppe peoples. In

his detailed description of the Scythians and associated groups in his *Histories*, Herodotus gives an account of the milking of mares and the processing of the milk by slaves into different fractions for consumption:

> The plan they follow is to thrust tubes made of bone, not unlike our musical pipes, up the vulva of the mare, and then to blow into the tubes with their mouths, some milking while the others blow... The milk thus obtained is poured into deep wooden casks, about which the blind slaves are placed, and then the milk is stirred round. That which rises to the top is drawn off, and considered the best part; the under portion is of less account (Book IV, ii)

It is generally accepted that the above passage is a description of the churning involved in the production of kumys, a drink of fermented mare's milk still consumed throughout the Eurasian steppe region (e.g., West 1999; the LoveToKnow website 2003).

4.1.2. ARCHAEOLOGICAL AND ETHNOGRAPHIC EVIDENCE FOR DAIRY PRODUCT CONSUMPTION

In addition to Herodotus' ancient records, the excavations and observations of nineteenth and twentieth century archaeologists and ethnographers have yielded evidence supporting regular dairy consumption by peoples throughout the lands of the former Soviet Union. In his book on the Iron Age Pazyryk tombs of Siberia, Rudenko (1970) describes a large number of organic remains that remained preserved in the cold burial environment of the tombs, including large amounts of cheese (of 'fresh' or 'cottage' type) and vessels "that had undoubtedly been filled originally with milk products" (*ibid.* 60). Rudenko concludes that "the food of the inhabitants of the area consisted mainly of milk products and meat" and cites the presence of cheese and various vessels in the Pazyryk burials as "indisputable proof" of the important

role that dairy products played in the diet of the Iron Age Scythian people (*ibid.*). In 1993, another well-preserved frozen tomb was found, belonging to a female of the Scytho-Siberian Pazyryk culture (dated to the 5th cent. BC). This burial yielded further evidence for the importance of dairy products to the ancient peoples of the Eurasian steppe, with the discovery of a wooden vessel containing the "remains of a dairy product, maybe yoghurt" (Polosmak 1994:97).

Both Polosmak and Rudenko consider ethnographic observations as well as archaeological finds when determining the importance of dairy products in Scytho-Siberian life in the Iron Age of the Altai Region. Invoking a comparison with the Kazakh, Kirgiz and Altaian pastoralists of Siberia, Rudenko hypothesises that the two most important animals raised by the Scythians would have been horses and sheep—both of which would have provided the Scythians with milk that could be used to make products such as fermented milk and cheese (Rudenko 1970). Polosmak's study of the ethnographic literature contributes to her conclusion that the peoples represented by the Pazyryk burial subsisted on "mostly boiled meat, bouillon and dairy products such as cottage cheese and koumiss", and that the Pazyryk burial rite included feasting near the dead body, where the family would drink kumys in honour of the deceased (1994:102).

In a recent ethnographic study conducted by Levine (1998), the author reiterates that the Kazakhs of the Eurasian steppe have depended heavily upon kumys as a dietary staple for generations. According to another ethnographer, this beverage of fermented horse milk is a fundamental part of Kazakh life, being to Kazakhs "what bread is to Russian peasants", and is sometimes consumed as "their only food" (Toktabaev in Levine 1998:93).

One informant described to Levine (1998) the social role of kumys as a standard offering to guests, and the common use of kumys as sustenance on long journeys, where up to 40 litres of the drink may be consumed in a single day's ride.

The use of ethnographic observations as an aid for determining the behaviour of archaeological groups is a strategy employed commonly in Eurasian steppe archaeology. Archaeologists studying Bronze Age and particularly Iron Age cultures often draw heavily from the ethnographic record when interpreting the archaeological record. In my doctoral research, colleagues from the Ukraine and Russia have directed me to ethnographic literature such as Tolstova (1963), Tomilov (1980) and Akhmetova (1995) as evidence for their view of the traditions of ancient Eurasian steppe societies. These works all include references to the frequent consumption of dairy products by Kazakh, Kirgiz and Tatar tribes in the former Soviet Union.

4.2. *OBSERVATIONS OF MODERN COW MILK AND CHEESE $\delta^{15}N$*

As a preliminary test of milk and derived dairy product isotope values, milk and cheese from two dairies were sampled, lyophilised and isotopically analysed (SH & GH Keen and EFJ Gould & Co. dairies, Somerset, UK). The $\delta^{15}N$ value of milk obtained from EFJ Gould & Co. was compared to the isotopic value of the cheeses obtained from the same dairy, and likewise for samples from the SH & GH Keen dairy. Analysis revealed a $\delta^{15}N$ difference of approximately +1.5‰ between the milk and cheese from each dairy (figure 4.1). The existence of an isotopic difference between milk and cheese from the same dairies suggested the need for further analysis of a milk-dairy product

$\delta^{15}N$ difference. Isotopic variability on the scale observed may be attributed to seasonal fluctuations in dietary $\delta^{15}N$ composition, because of changes in fodder/pasture source through the year and/or due to climatically-influenced change in pasture $\delta^{15}N$ (see section 2.7). Furthermore, the cheeses analysed were not produced from the milks analysed, but from milk produced months before. These sources of uncertainty mean that it is possible that the milk from each dairy does not appropriately represent the isotopic composition of that used to produce the respective cheeses. Thus, further work was required to establish whether or not fermentation (and/or ageing) alters the isotopic composition of milk, because the comparison of simultaneously sampled milk and cheese samples could not be considered conclusive.

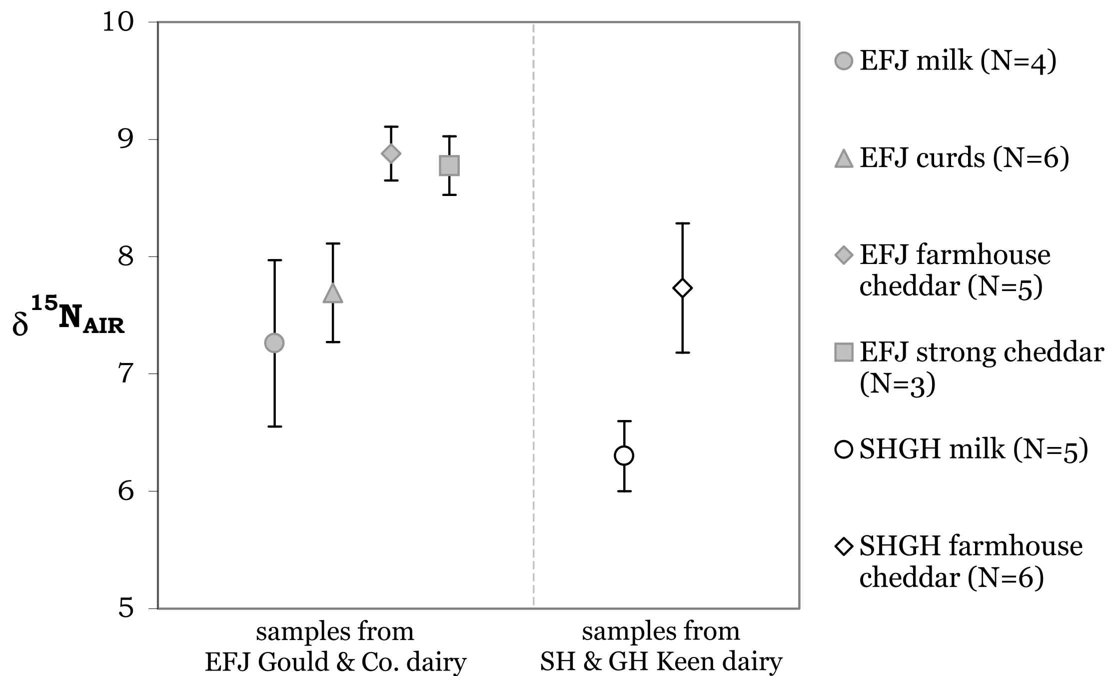

Figure 4.1. $\delta^{15}N$ values for dairy products obtained from two Somerset, UK dairies (SH & GH Keen, Wincanton, UK; EFJ Gould & Co., Shepton Mallet, UK). Analytical error is ±0.2‰.

4.3. *δ^{15}N ANALYSIS OF MILK AND ITS DERIVATIVE DAIRY PRODUCTS*

4.3.1. SELECTION OF FERMENTED DAIRY PRODUCTS

In order to test the isotopic character of milk and its derived dairy products, I made cheese, yoghurt and kefir from milk of known isotopic composition. Kefir and yoghurt are consumed as beverages, while the whey is drained from the cheese curds to make the solid final product. Both fresh and aged (2, 8 & 14 mo.) cheeses were analysed, as well as the curds and whey of the cheese. Archaeological and ethnographic studies mentioning dairy product consumption by the inhabitants of the Eurasian steppe (see above) most often list 'fresh' (i.e., not extensively aged) dairy products such as kefir, kumys and 'fresh' or 'cottage' cheese as common dietary resources. Due to the primary focus of this research on prehistoric Eurasian steppe communities, I concentrated on fresh rather than aged dairy products in the study reported here.

4.3.2. PREPARATION OF CHEESE, KEFIR & YOGHURT

Fresh, whole, non-homogenised milk was supplied by Unigate Dairies (Oxfordshire, UK), and cheese, yoghurt and kefir starter cultures were obtained from the New England Cheesemaking Supply Company (Ashfield, MA, USA). One batch of milk was used to produce the cheese (a hard, 'cheddar' type), while another batch was used to produce both the kefir and yoghurt. A sample of fresh milk was taken from each batch of milk before any culture was added, to serve as a δ^{15}N reference.

Yoghurt was made by adding the bacterial culture to milk heated to 85˚C then cooled to 43˚C. This mixture was kept warm by storage in a thermos for approximately 24 hours, at which time the yoghurt was ready for consumption. To produce Kefir, starter culture was added to a jug of milk heated to 85˚C then cooled to 22˚C. The mixture was allowed to sit until ready for consumption (approximately 24 hours). A sample of kefir and a sample of yoghurt were taken once the products were made.

Cheese was made by heating the milk to 32˚C in a large pot, adding the bacterial culture and maintaining the temperature for 45 minutes. Rennet was added and the mixture stirred. A solid mass formed in the pot, surrounded by the whey. Curds were cut with a knife to a size of approximately 2-3cm^3, and the whey was drained away. Salt was mixed in with the drained curds, which were then wrapped in a cheesecloth and pressed into a cheese mould. The cheese was pressed into the mould for 15 minutes on each side under 5kg of pressure, then for one day on each side under a weight of 9.5 kilos to produce the 'fresh' cheese. This cheese was divided into three parts, which were each coated with wax in an effort to keep out unwanted mould that might ruin the cheese. The wax-coated pieces of cheese were then kept at room temperature until they were sampled.

During the cheesemaking, a portion of discarded whey was kept aside for analysis when the curds were strained; a sample of curds was retained for analysis as well. Once the cheese had been pressed and formed, a sample of fresh cheese was removed for analysis. One of the wax-coated cheese pieces was sampled at 2 months, another at 8 months, and the final piece at 14 months, avoiding the mould that had grown through the cheese despite the wax coating.

Upon sampling each kefir, yoghurt and cheese sample was stored at -18˚C. Samples were then lyophilised before analysis.

Table 4.1. Dairy products made and analysed, and the composition of the cultures used to produce them

Product	Bacteria	Yeasts
Farmhouse Cheddar Cheese (fresh)	*Streptococcus sp.* *Lactobacillus sp.*	
Farmhouse Cheddar Cheese (aged 2 months)	*Streptococcus sp.* *Lactobacillus sp.*	
Yoghurt	*Streptococcus salivarius subsp. thermophilus* *Lactobacillus delbrueckii subsp. bulgaricus*	
Kefir	*Lactobacillus caucasus* *Leuconostoc sp.* *Acetobacter sp.* *Streptococcus sp.*	*Saccharomyces kefir* *Torula kefir*

The active organisms in each product's culture are listed in table 4.1. No dairy product incorporating mould was included in this study. Because the main concern was the nitrogen isotopic composition of the products, the removal of carbon-containing fats from each sample was not necessary, and I have focused on the $\delta^{15}N$ values of the milk and derived products.

4.3.3. Sample Preparation and Mass Spectrometric Analysis

Lyophilised samples were weighed into tin capsules and loaded into an autosampler for analysis by IRMS. 10-15 replicates of each sample were analysed. Isotopic analysis was performed on an automated carbon and nitrogen elemental analyser (Carlo Erba EA1108) coupled with a continuous-flow isotope ratio-monitoring mass spectrometer (Europa Geo 20/20). Nylon of known isotopic content was used as a standard, and alanine and bovine liver samples of known isotopic composition were included in each sample run

to monitor analytical accuracy. $\delta^{15}N$ values were automatically calculated and reported with reference to the AIR standard. The analytical error on all individual isotope measurements in this study is ±0.2‰ for nitrogen.

4.4. *RESULTS: $\delta^{15}N$ VALUES*

Nitrogen isotope values for each sample are shown in figure 4.2. An average difference of less than 0.3‰ $\delta^{15}N$ exists between the fresh, two month aged and fourteen month aged cheeses and their original milk; the same pattern holds for the isotopic values of the second batch of milk and its derived yoghurt and kefir. The only final product tested that shows any significant isotopic ($\delta^{15}N$) enrichment relative to the original milk from which it was made is the cheese aged for eight months, and this average milk-product difference is 1.1‰. If the $\delta^{15}N$ value for the eight month old cheese were elevated due to the evolution and release of volatile N-containing compounds such as ammonia, the nitrogen content of the sample should be lower than that of the younger cheeses; likewise the C:N value for the sample should be higher than that of the younger cheeses. Neither of these trends can be observed when comparing the %N, C:N and $\delta^{15}N$ values for the samples analysed (figure 4.3). It is possible that the apparently enriched nitrogen isotope value of the eight month old cheese may be a result of the inclusion of mould when the sample was taken; although efforts were made to prevent mould from growing in the cheese samples, some mould did grow and may have been accidentally sampled along with the eight month old cheese.

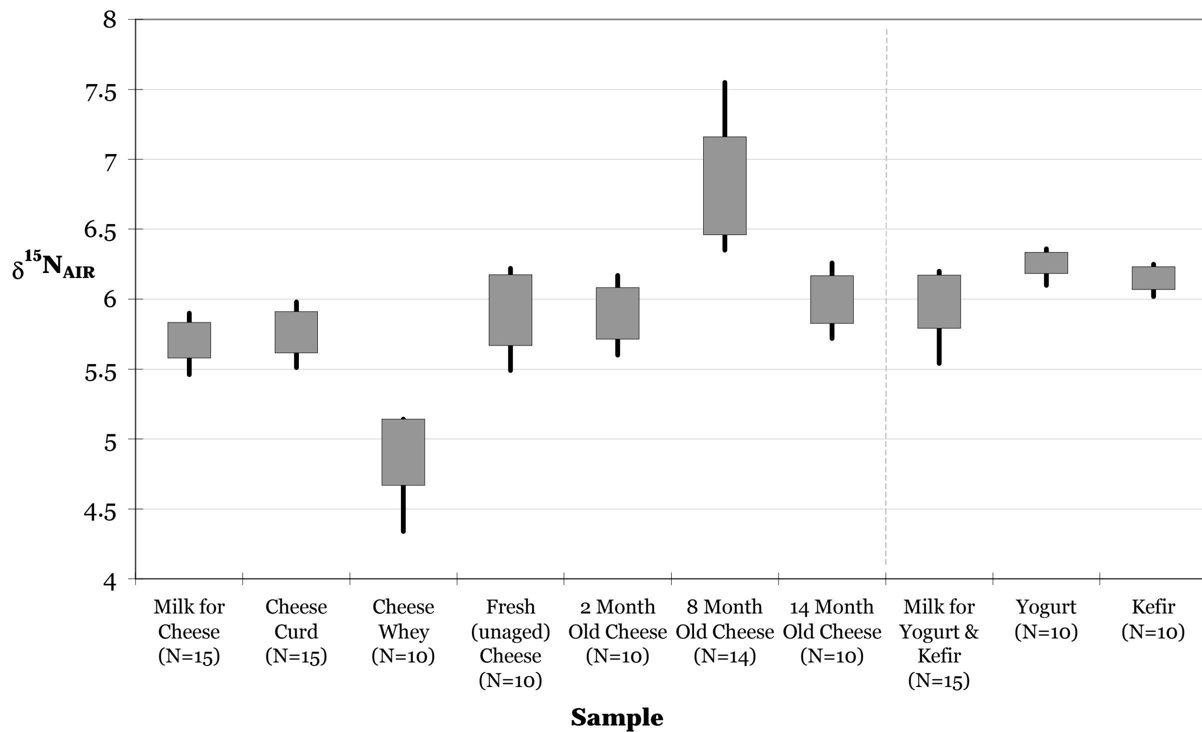

Figure 4.2. $\delta^{15}N$ for all milk, cheese, yoghurt and kefir samples. Grey boxes reflect average $\delta^{15}N$ ±1σ, black lines represent the $\delta^{15}N$ range for each sample.

In contrast to the other dairy samples, the whey discarded in cheesemaking shows a notable isotopic depletion (-0.8‰ average) relative to the milk from which it was derived. Whey proteins previously have been shown to be isotopically lighter than casein proteins (retained in the cheese curds) (Kornexl *et al.* 1997). The disposal of isotopically light whey proteins during cheesemaking should not greatly alter the overall isotopic composition of the cheese because the amount of nitrogen present in the whey fraction only accounts for ~17% of the nitrogen present in milk and varies by approximately 1-2‰ from casein $\delta^{15}N$. This isotopic continuity between milk and curds is confirmed in the values obtained in this study.

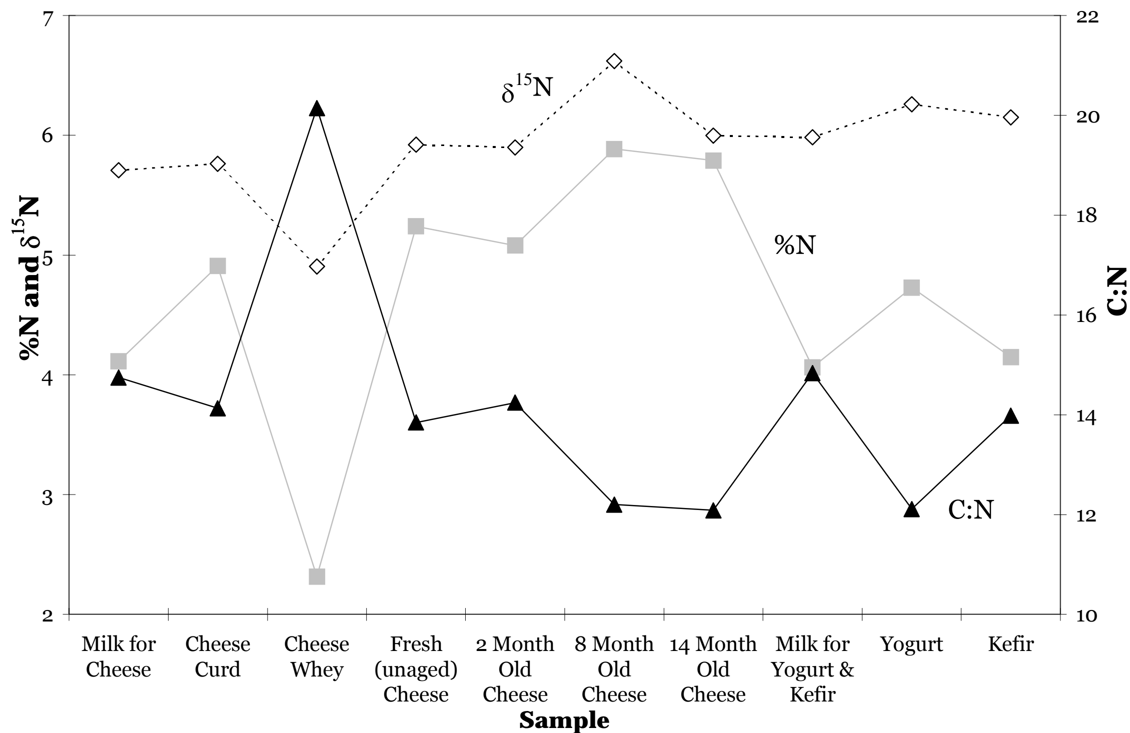

Figure 4.3. Average $\delta^{15}N$, %N and C:N values for dairy samples. %N and $\delta^{15}N$ values are plotted against the left vertical axis; C:N values are plotted against the right vertical axis.

4.5. CONCLUSIONS

Although the range of dairy products used in this study are by no means comprehensive, I believe that they represent main types of fermented dairy products that archaeologists and ethnographers believe may have been an important part of human diet in Bronze and Iron Age Eurasia. The results presented here suggest that there is no reason to believe that the regular consumption of fresh fermented dairy products can be isotopically distinguished from the regular consumption of other body proteins derived from the same type of animal. These data suggest that fermentation does not cause a significant alteration from milk $\delta^{15}N$ values. The fresh and two month old farmhouse cheddar analysed had undergone both the removal of the whey fraction and fermentation (as well as some aging). For other types of cheese that are consumed without substantial aging as well as those formed by simple

acid precipitation of the casein fraction, the data indicate that no significant isotopic difference would exist between the milk and these dairy products.

The results of this study allow little to be said about the effect of extensive aging (1+ yr.) upon the isotopic composition of cheese. From the fourteen month old cheese $\delta^{15}N$ values, it seems that $\delta^{15}N$ does not consistently increase with age; but further work particularly designed to address issues of ageing would be required before any concrete conclusions could be reached regarding the isotopic character of extensively aged cheeses. Nevertheless, I believe it is unlikely that a high level of consumption of aged cheeses is a possible dietary scenario for most archaeological populations. In the Eurasian steppe area today, fresh dairy products are readily available and frequently consumed, while aged dairy products are rarely eaten. I present these data from fresh liquid and solid fermented dairy products as most representative of possible dairy products consumed with any regularity in prehistory.

4.6. *IMPLICATIONS FOR PALAEODIETARY RESEARCH*

The data presented here greatly reduce the feasibility of explaining high human $\delta^{15}N$ values by a dietary reliance on fermented milk products. Thus, these data lend support to the conclusion that humans from Bronze Age and Iron Age Eurasia with highly enriched $\delta^{15}N$ values that cannot be explained by the consumption of terrestrial or marine animals must reflect the regular consumption of freshwater animal resources. In the case of the Bronze Age and Iron Age humans from the Eurasian steppe with highly elevated $\delta^{15}N$

values (as those discussed in section 3.6.5.1), the isotopic evidence cannot support the theory that these individuals relied almost exclusively upon their terrestrial domesticates for their dietary protein, whether in the form of meat, milk or dairy products. The new data from this study indicate that in an archaeological context where there is no evidence for the frequent consumption of other high-^{15}N foods such as terrestrial omnivores or marine foods, we must consider the possibility of freshwater animal consumption by ancient humans. There is no evidence that could lead us to invoke the consumption of fermented dairy products as an explanation of highly elevated human $\delta^{15}N$ values.

Chapter 5

Sulphur Isotope Analysis

5.1. *SULPHUR ISOTOPE RESEARCH IN FOOD WEB STUDIES*

The analysis of sulphur stable isotopes has been employed in ecosystem food web studies for nearly two decades. Due to the very small isotopic offset between food and consumer (-1 to +2‰), $\delta^{34}S$ values can be used to identify an organism's dietary sulphur source(s) (Mektiyeva *et al.* 1976; Peterson *et al.* 1985 & 1986; Kennedy and Krouse 1990). Sulphur isotope analysis is often combined with the measurement of carbon and nitrogen isotopic ratios, which have also been shown to reflect the average isotopic composition of an animal's dietary intake (DeNiro and Epstein 1978 & 1981; Minagawa and Wada 1984; O'Connell and Hedges 1999). The analysis of sulphur isotopes has been employed in estuarine food web studies where there is a pronounced difference between the isotopic composition of potential sulphur sources at the base of the food chain (i.e., marine vs. freshwater/terrestrial sulphate) (e.g., Peterson *et al.* 1985; Hesslein *et al.* 1991), as a geographical tracer used to determine the provenance of individuals or animal products (Katzenberg and Krouse 1989; Rossman *et al.* 1998), and in studies monitoring industrial pollution (Faure 1977; Peterson and Fry 1987).

5.2. *SULPHUR ISOTOPIC VARIATION IN THE ENVIRONMENT*

The Canyon Diablo Troilite (CDT), a FeS meteorite, is the internationally recognised standard for sulphur isotope analysis, with a designated $\delta^{34}S$ of 0‰. Ocean water sulphate (SO_4^{2-}) has a fairly uniform value of ~+21‰ (Rees *et al.* 1978), with oceanic primary producers exhibiting $\delta^{34}S$ values from approximately +17 to +21‰ (Peterson and Fry 1987; Krouse and Herbert 1988). Continental geological

formations comprise both uplifted marine sediments, pyrite and sulphur-containing evaporites, which exhibit a wide range of $\delta^{34}S$ values that vary over different areas of bedrock between –19 and +30‰ (Peterson and Fry 1987; Krouse *et al.* 1987; Holser *et al.* 1989). Terrestrial plants have reported values averaging around -7 to +8‰ (Nriagu and Coker 1978; Chukhrov *et al.* 1980), with the exception of plants in coastal areas, which can exhibit $\delta^{34}S$ values close to that of seawater, due to the effect of sea spray or precipitation high in marine sulphur (Kusakabe *et al.* 1976).

The isotopic difference between sulphur sources in marine and non-marine environments has allowed scientists to distinguish between marine and non-marine consumers by their $\delta^{34}S$ values (Krouse and Herbert 1988; Kennedy and Krouse 1990; Weber *et al.* 2002). Archaeological scientists have taken advantage of this differentiation between marine and terrestrial diets using sulphur isotope analysis, correlating high $\delta^{13}C$ and $\delta^{15}N$ values indicative of marine resource consumption with 'marine' $\delta^{34}S$ values close to +20‰ (Leach *et al.* 2001). The body of sulphur stable isotope data has led Richards *et al.* (2001) to suggest a revision to this marine-*versus*-terrestrial dietary interpretation, proposing that inhabitants of coastal regions with non-marine diets could also be identified by low $\delta^{13}C$ values coupled with 'coastal' (rather than strictly 'marine') $\delta^{34}S$ values around +20‰.

The potential benefit of sulphur isotope research at inland archaeological sites has previously focused upon the identification of migrants in archaeological populations by the association of regionally-dependent $\delta^{34}S$ values to ancient human $\delta^{34}S$ values (Krouse *et al.* 1987; Katzenberg and Krouse 1989; Richards *et al.* 2001). The consideration of freshwater $\delta^{34}S$ values in inland continental ecosystems adds a further element of complexity to the study of sulphur isotopes in archaeological populations, due to the extreme variability in $\delta^{34}S$ found in freshwater sediments and

organisms, and the identification of freshwater resource consumption has only been briefly mentioned in the palaeodietary literature to-date (Richards *et al.* 2001).

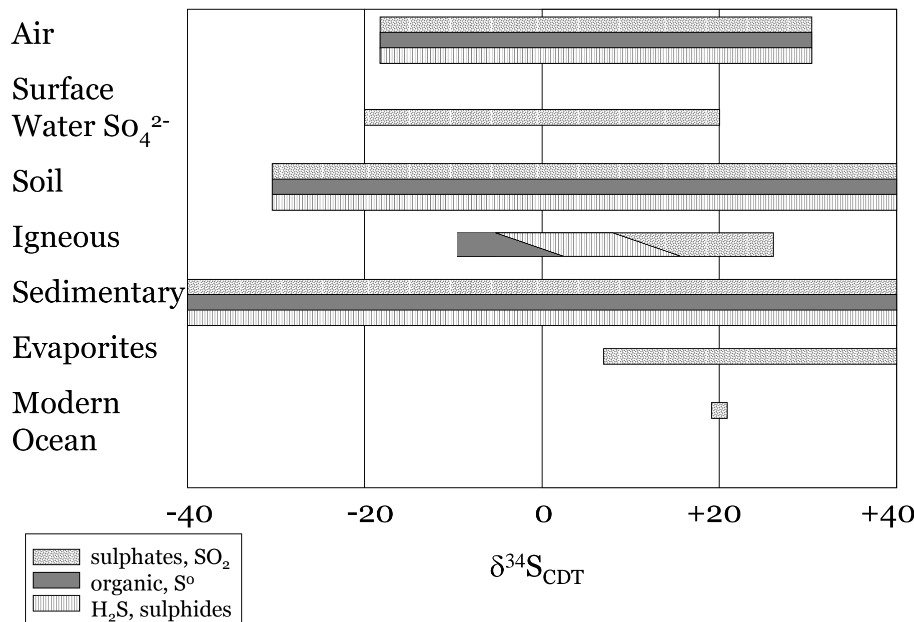

Figure 5.1. A diagram of approximate $\delta^{34}S$ ranges for various reservoirs (after Krouse 1980, figure 11-1).

Sulphur isotope values for freshwater sulphate can range widely between –22 to +20‰, and microbially-mediated sulphate-sulphide reduction in freshwater systems can produce H_2S with $\delta^{34}S$ values as low as -62‰ (Krouse 1980; Peterson and Fry 1987). As mentioned above, land plants have recorded values of approximately +2 to +8‰. In geographical situations where the ranges of $\delta^{34}S$ values of aquatic and terrestrial primary producers are distinct, it should be possible to quantify dietary contributions from terrestrial and freshwater sources among archaeological humans (suggested in Richards *et al.* 2001). In archaeological contexts where marine dietary input is not a consideration, high human $\delta^{15}N$ values together with 'aquatic' $\delta^{34}S$ signatures could identify humans whose diet was based primarily on freshwater resources. The ability to supply isotopic evidence either supporting or refuting the conclusion of high freshwater fish consumption would be of great benefit to

palaeodietary analysis, which at this time relies solely on highly enriched $\delta^{15}N$ values as evidence of a high fish diet.

The purpose of the $\delta^{34}S$ research described here is twofold. This study seeks to test the utility of sulphur isotope analysis for palaeodietary research, specifically regarding the detection of freshwater animal dietary intake among archaeological humans. And more importantly, this survey of both modern and archaeological $\delta^{34}S$ values sheds light upon the variability of sulphur content in modern samples, which is crucial to our interpretation of the sulphur isotopic integrity of archaeological samples.

5.3. *MATERIALS AND METHODS: $\delta^{34}S$ ANALYSIS*

5.3.1. SAMPLE SELECTION

In order to test the utility of sulphur isotope analysis in the detection of dietary input from freshwater resources in archaeological populations, extracted bone collagen of humans and fauna from two inland archaeological sites in the Eurasian steppe was analysed. The site of Bil'shivtsi lies near the Dneister River and is attributed to the Eneolithic Tripolye culture of western Ukraine (3520 ± 110 BC). The site of Chicha is located in south-western Siberia and has been dated to the Late Bronze Age/Early Iron Age (12th-14th cent. BC). The area around Chicha is crossed by small rivers and dotted with freshwater and saline lakes up to ~50km in diameter. Bil'shivtsi and Chicha are located over 580 and 1440 km from the nearest sea, respectively, and there is no archaeological evidence at either site for the consumption of marine foods. Carbon and nitrogen isotopic analysis of bone collagen

samples from these sites indicates that the humans at Chicha consumed a moderate to high degree of freshwater fish protein (see section 3.6.5.3 and figure 5.2), while the human from Bil'shivtsi had a diet based on terrestrial resources (see figure 5.2). Samples of human, terrestrial faunal and fish bones were taken from each archaeological site for analysis.

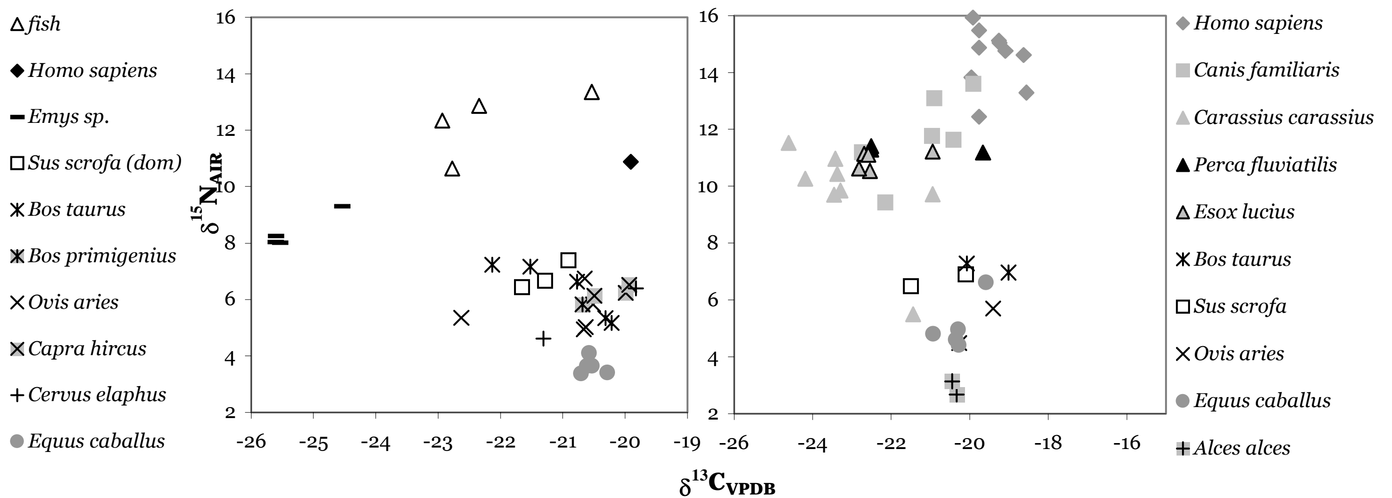

Figure 5.2. Carbon and nitrogen stable isotope values for humans and fauna from Bil'shivtsi (left) and Chicha (right). Analytical error is ±0.2‰ for both δ^{13}C and δ^{15}N. The high δ^{15}N values of the Chicha humans (12.5 to 15.9‰) indicate a diet high in fish, while the relatively low Bil'shivtsi human δ^{15}N value (10.9‰) indicates a low- or no-fish diet.

Twenty-two samples of modern bone were also selected as reference material, against which archaeological %S, C:S and C:N values could be compared. The sulphur content, C:S and C:N ratios of modern samples is used here to check the quality of bone collagen for δ^{34}S analysis. The use of these quality indicators is not routinely employed in δ^{34}S studies. Two samples of modern fish (*Carassius carassius*) were obtained from the area of the Chicha archaeological site (Jens Schneeweiβ and Norbert Benecke, DAI, Berlin, Germany). Samples of modern human bone collagen from three different individuals were provided for analysis by Tamsin O'Connell (RLAHA, Oxford, UK). Bone collagen from one cow raised on a British organic farm was obtained from the stores of the RLAHA, Oxford, UK.

Sixteen samples were taken from distinct sea and estuarine fish caught in the Sandon River Estuary, NSW, Australia in January 2001.

5.3.2. SAMPLE PREPARATION

All modern samples were de-fatted with chloroform:methanol (2:1) except the modern Carassius samples, which were de-fatted with acetone. De-fatted samples were rinsed ten times with milli-Q water before collagen extraction in order to remove any residual organic solvent. Samples TOC NOC 1, 4 and 11 were provided for this analysis as non-ultrafiltered, dried collagen. The TOC NOC samples were thus prepared as described below from the point of rehydration and ultrafiltration.

Samples were prepared for stable isotope analysis by a modified Longin method as described in section 3.5. Collagen extraction produces sulphur for analysis primarily in the form of methionine, the major sulphur-containing amino acid in type I bone collagen (0.9% by mass); the other sulphur-containing amino acid in collagen is cystine (0.1%, Leach *et al.* 2001). A small amount of sulphur-containing proteoglycans may also be present in the collagen extract, but their concentrations and affects on $\delta^{34}S$ are poorly understood; they are generally considered to have similar isotope values to the bone collagen (*ibid*).

After collagen extraction, samples were lyophilised and portions of dried 'collagen' were weighed into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analysis (see figure 5.1). Archaeological samples met the criteria for %C, %N and C:N representative of well-preserved collagen as described in section 3.6.1. Samples not satisfying these criteria for well-preserved collagen would not be considered for further isotopic study. For $\delta^{34}S$ analysis, dry 'collagen' samples were then redissolved in water and ultrafiltered (Amicon 10kD Ultra-15, Millipore, Bedford, MA, USA) to remove highly degraded

molecules, salts and remaining contaminants such as residual exogenous pyrite (FeS). Individual, undamaged collagen α-chains have a molecular weight of approximately 140kD. The >10kD residue recovered from the ultrafilters was frozen and lyophilised before analysis. Approximately 10mg of dried, ultrafiltered 'collagen' was required for each replicate $\delta^{34}S$ analysis; sulphur analysis was conducted in duplicate for 69 of 92 samples (see Appendix 3 for details). Insufficient material for analysis or instrument failure prevented the duplicate analysis of 23 samples. Two samples with sufficient collagen remaining after primary $\delta^{34}S$ analysis were redissolved in milli-Q water, re-ultrafiltered using 30kD ultrafilters (Amicon), lyophilised and submitted for $\delta^{34}S$ analysis a second time. The samples ultrafiltered at 10kD are referred to as BIL 45 and CHA 6; the 30kD ultrafiltered results for the same samples are given for BIL 45 ult2 and CHA 6 ult2.

5.3.3. MASS SPECTROMETRIC ANALYSIS

The $\delta^{34}S$ values reported here were measured relative to a standard calibrated against CDT, and so are reported as VCDT. Sulphur isotope analysis was conducted by EA-IRMS (Europa elemental analyser & mass spectrometer) at Iso-Analytical (Cheshire, UK). Sulphur isotope values were calculated relative to reference standard NBS-127 (barium sulfate, $\delta^{34}S_{VCDT}$ = +20.3‰, distributed by IAEA, Vienna, Austria). IAEA standards NBS-127, IAEA-S-1 (silver sulfide, $\delta^{34}S_{VCDT}$ = -0.3‰) IAEA-S-3 (silver sulfide, median value $\delta^{34}S_{VCDT}$ = -31.4‰) were included in the isotopic analysis as samples for calibration and correction. Analytical error is ±0.4‰ for $\delta^{34}S$. Sulphur percentage (by mass) was also reported for each sample by Iso-Analytical. The precision of %S values is ±15% of reported value.

5.4. *RESULTS I: COLLAGEN QUALITY ASSESSMENT*

5.4.1. SULPHUR CONTENT

The two sulphur-containing amino acids in collagen are methionine (~1.935mg/g in collagen) and cystine (~0.267mg/g in collagen; Leach *et al.* 2001). The theoretical sulphur content of bone collagen is approximately 0.22% (*ibid*). Most samples had a sulphur content above 0.22% (see figure 5.3). The modern samples ranged in sulphur content from 0.19 ± 0.03% (cow bone 'SMBG') to 0.60 ± 0.09% (human bone 'TOC NOC 4'), excluding sample TOC NOC 11. All of the archaeological samples exhibited sulphur content within the range exhibited by the modern samples, when measurement error is considered. The archaeological sample with the lowest sulphur content of 0.17 ± 0.02% was BIL 26; CHA 22 yielded the highest sulphur content of the archaeological samples with 0.63 ± 0.09% sulphur. The apparent sulphur content of samples BIL 45 and CHA6 increased after 30kD ultrafiltering from 0.20 to 0.36% and from 0.25 to 0.26% (± 15% of values).

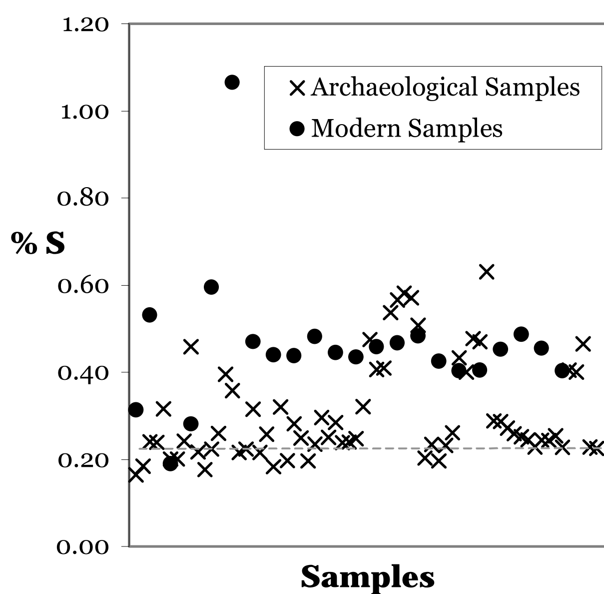

Figure 5.3. Percent sulphur content of modern and archaeological bone collagen samples. Theoretical content of bone collagen (0.22%, Leach *et al.* 2001) indicated by broken line.

5.4.2. C:S AND N:S VALUES

Human bone collagen (type I) has an approximate molecular C:S ratio of 780. Richards *et al.* (2001) proposed that C:S values of collagen samples could be used as an indicator of collagen quality, with a C:S over 780 indicative of poorly preserved and/or highly contaminated collagen, and C:S values under 780 suggestive of sulphur loss. Collagen loss would actually lead to an increase in the ratio of carbon to sulphur, while contamination by addition of exogenous sulphur would lead to a decrease in C:S value. Richards *et al.* (2001) further state that the content of methionine (the S-containing amino acid in collagen) of extracted bone collagen has been reported at approximately one-half of the original content, which they say would result in a decrease in C:S. While a significant amount of methionine may be lost during collagen extraction, such loss would produce a higher sample C:S due to sulphur loss.

The modern samples analysed in this study showed a high correlation between C:S and N:S values (figure 5.4). The modern cow bone exhibited the highest C:S and N:S ratios of 666 and 211, respectively. The fish bone sample 'Mangrove Jack 3' produced the lowest modern C:S value of 210, while the lowest modern N:S ratio (excluding sample TOC NOC 11) was exhibited by the human bone sample 'TOC NOC 4' (which also had the highest sulphur content). None of the modern samples yielded C:S values above 780, which according to the logic used by Richards *et al.* would have been expected for extracted modern bone collagen.

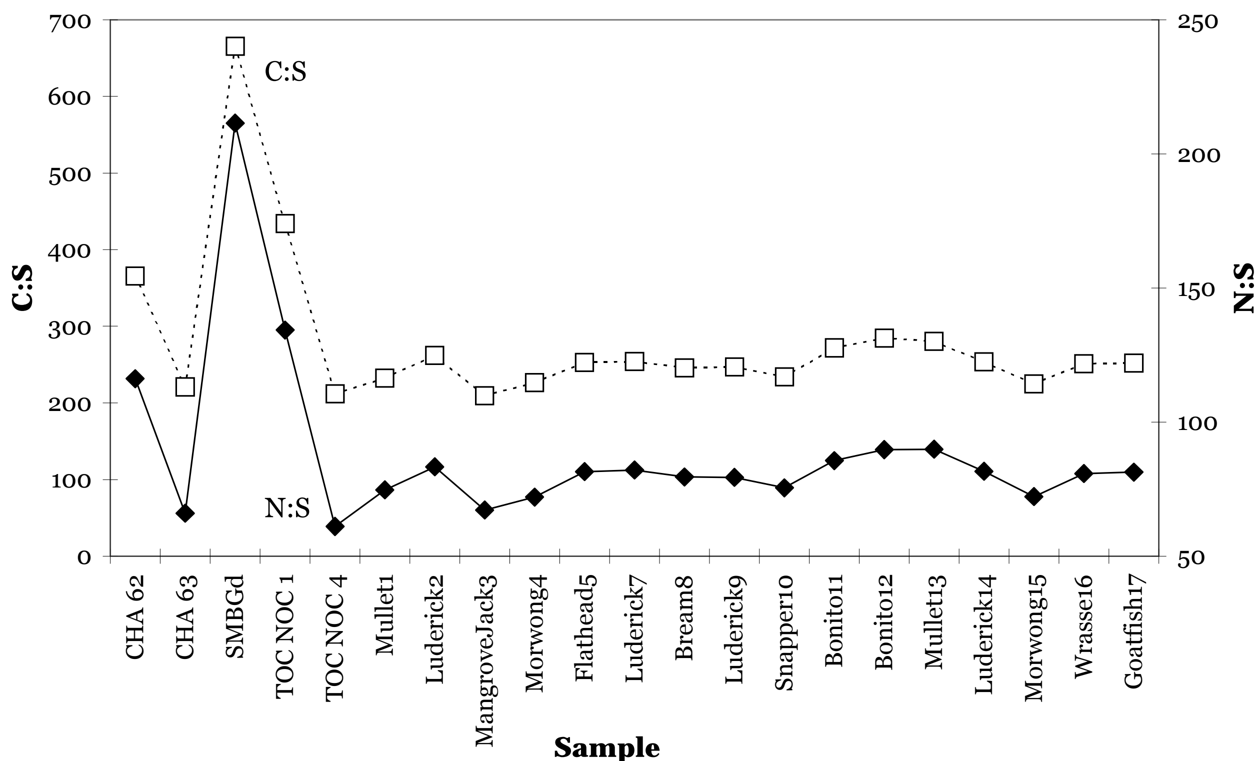

Figure 5.4. C:S and N:S values for modern samples.

Sample TOC NOC 11, identified as highly damaged or degraded collagen by its C:N ratio, yielded anomalously low C:S and N:S values and an anomalously high %S value compared to the rest of the modern samples. While no other modern samples exhibited a C:S value below 200 or a N:S value below 50, TOC NOC 11 produced a C:S value of 106 and a N:S value of 27. TOC NOC 11 had a sulphur content of 10.70 ± 1.61%, over 17 times higher than the maximum percentage sulphur of the rest of the modern samples, 0.60 ± 0.09%.

Seven of the archaeological samples analysed yielded a C:S value below the range of the modern samples: BIL 25, CHA 8b, CHA 22, CHA 26, CHA 28, CHA 29, CHA 30 and CHA 31. All of the samples with C:S below 210 were fish bone, representing all fish species analysed (*Perca fluviatilis*, *Esox lucius*, *Carassius carassius*, and unknown). The two archaeological samples with the lowest C:S values also had N:S values below the modern range: BIL 25 (55) and CHA 22 (50). None of the archaeological samples exceeded the upper C:S or N:S limit exhibited by the

modern samples, with BIL 26 yielding the maximum archaeological C:S and N:S of 622 and 187, respectively.

In parallel with apparently increased %S values, samples CHA 6 ult2 and BIL 45 ult2 exhibited decreased C:S and N:S values relative to CHA 6 and BIL 45, respectively (see figures 5.5 and 5.6). Notwithstanding the difference between pre- and post- 30kD ultrafiltered values for these samples, their C:S and N:S values were all within the observed modern range.

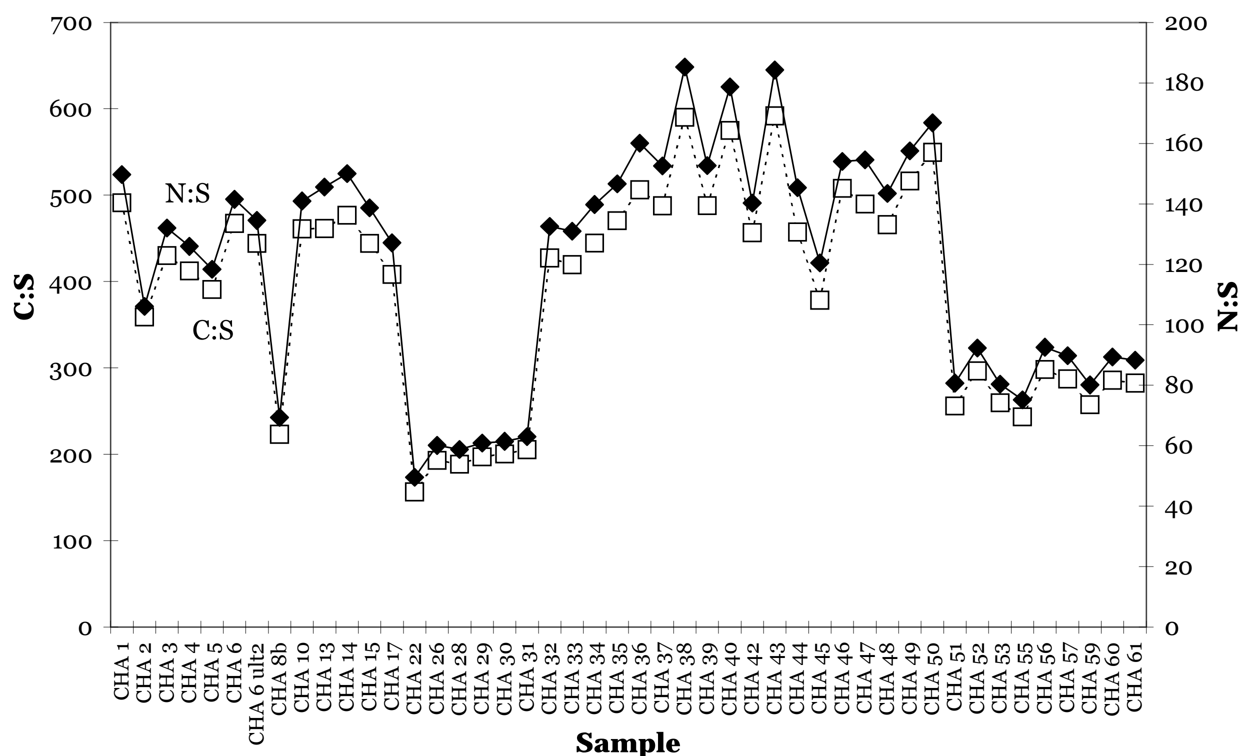

Figure 5.5. C:S and N:S values for archaeological samples from the site of Chicha.

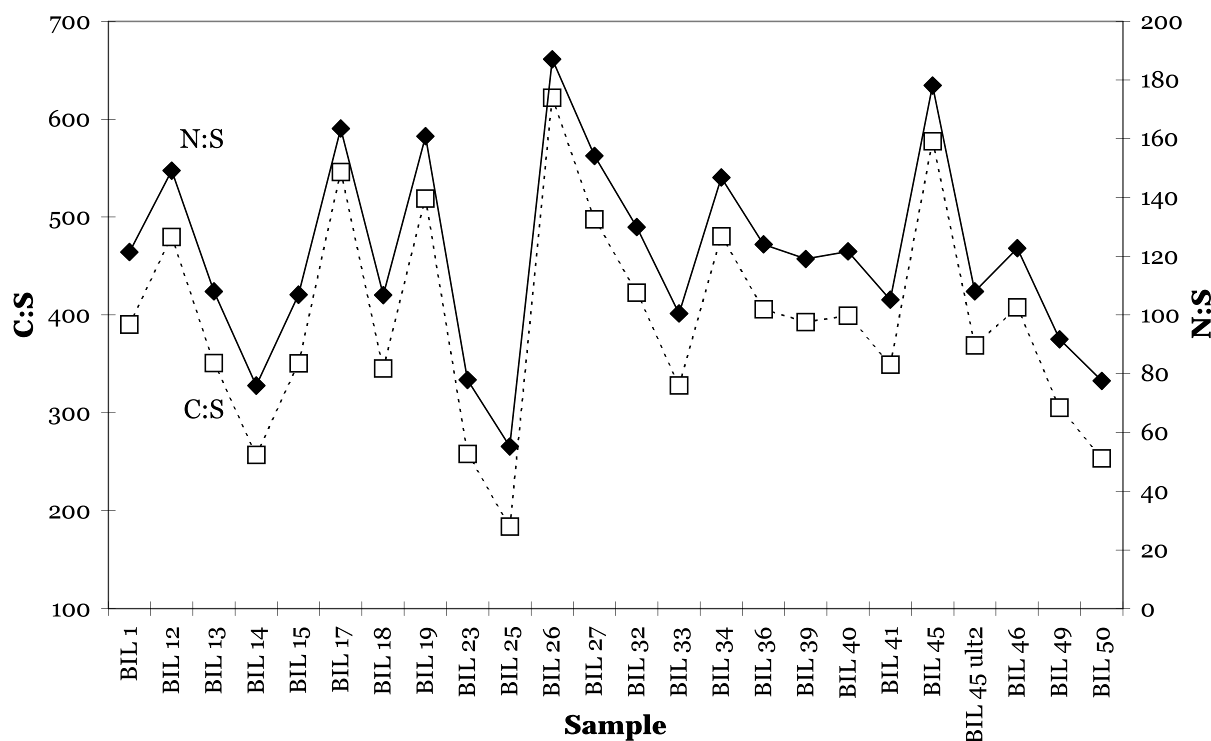

Figure 5.6. C:S and N:S values for archaeological samples from the site of Bil'shivtsi.

5.4.3. CONSISTENCY OF δ34S VALUES

Duplicate $\delta^{34}S$ values for individual samples varied up to 1.48‰. The samples re-analysed after ultrafiltration at >30kD (BIL45 and CHA6) exhibited differences (pre-post >30kD ultrafiltration) of 1.1 and 3.2‰, respectively. In both cases, the 'ult2' (post-30kD ultrafiltration) samples had lower $\delta^{34}S$ values than the same samples ultrafiltered at >10kD. While the results presented here do not indicate that the isolation of higher molecular weight molecules (>30kD vs. >10kD) necessarily improves the quality of an archaeological collagen sample, these isotopic discrepancies between samples treated in different ways is the cause for some concern. Further testing is required to effectively assess the error in measured $\delta^{34}S$ values of archaeological samples.

Whether the $\delta^{34}S$ values for the pre- or post-30kD ultrafiltered samples are used in the following assessment of the isotopic results from Bil'shivtsi and Chicha, the

trends observed remain consistent. In section 5.6, the $\delta^{34}S$ values for CHA6 and BIL45 are used.

5.5. *COLLAGEN QUALITY ASSESSMENT: CONCLUSIONS*

The high correlation of C:S to N:S (and carbon content to nitrogen content) exhibited by the samples analysed suggests that the ratio of nitrogen to sulphur is equally suitable in assessing the quality of a collagen sample as that of carbon to sulphur. However, as exogenous carbon contamination may be detected by anomalously high C:S values while unusually low N:S values may indicate very low protein content, it is of use to analyse both parameters. I propose that both C:S and N:S sample values should be used in tandem with percent sulphur values in order to assess collagen quality. This research suggests that a sample's $\delta^{34}S$ result should not necessarily be regarded as reliable if all of the following criteria are true: %S value above 0.60%, C:S below 200 and N:S below 60 (within the limits of precision). As further research is done into the sulphur content of modern, extracted bone collagen, these criteria may be somewhat modified.

Although the sulphur content of the majority of the archaeological samples was above the theoretical sulphur content of bone collagen (0.22%), this does not necessarily indicate that the samples were of poor quality; most of the modern collagen samples also exhibited %S values above 0.22. In order to further assess collagen quality for $\delta^{34}S$ analysis, it would be of particular benefit to obtain amino acid content information for all samples, as reported in Leach *et al.* (2001). Amino acid profiles would help to elucidate the causes of deviations of sample C:S, N:S and %S from expected values.

A major concern with the isolation of archaeological bone collagen is the presence of exogenous sulphur contamination, particularly in the form of pyrite, which can be deposited in archaeological bone after burial. Particulate pyrite should be removed by ultrafiltration. If sulphur content does increase (and C:S and N:S decrease) after the use of 30kD ultrafilters as the results from two samples indicate, then it seems that the 30kD ultrafilters are not aiding in the removal of sulphur-containing contaminants any more than the 10kD ultrafilters. Instead, it may be that the use of larger molecular size ultrafilters promotes the loss of smaller, fragmented but endogenous collagen strands of good isotopic integrity. Due to the small amount of sulphur present in collagen, loss of small collagen fragments is more likely to result in a loss of carbon and nitrogen rather than sulphur, producing increased sample %S values and lower C:S and N:S values. Whereas the use of ultrafilters in the routine $\delta^{13}C$ and $\delta^{15}N$ analysis of archaeological material may not always be worthwhile (see section 3.6.3), I would still recommend the use of ultrafilters for $\delta^{34}S$ analysis. However, according to the data presented here, ultrafilters of a smaller molecular size (>10kD) seem to be more useful in retaining collagen fragments while filtering out contaminants than larger (>30kD) molecular weight ultrafilters.

5.6. *RESULTS II: SULPHUR ISOTOPE VALUES*

The results of the sulphur isotope analysis of all of the archaeological samples are presented here. Sulphur pollution has been known to significantly affect the $\delta^{34}S$ values of modern plants and fauna (Faure 1977; Peterson and Fry 1987), so the values of the modern Chicha *Carassius* samples are not compared with the archaeological Chicha samples. Because of the variation of sulphur isotope values with geographic location, the values for the remaining modern samples are also not given. $\delta^{34}S$ values

of samples from the UK and Australia would not be appropriate for comparison with central Eurasian isotope values.

Sulphur isotope values for the archaeological samples are plotted versus $\delta^{15}N$ values in all cases. At inland sites, such as Chicha and Bil'shivtsi, nitrogen isotope values seem to be a better indicator of food chain relationships than $\delta^{13}C$ values (see sections 2.6 and 3.6.2). $\delta^{13}C$ values of different species at Chicha and Bil'shivtsi show a great degree of overlap and no distinct resolution that would greatly aid in supporting or refuting a particular hypothesis regarding human diet at these sites. Therefore, $\delta^{34}S$ vs. $\delta^{15}N$ plots should be more helpful in determining ancient human diet at Bil'shivtsi and Chicha than $\delta^{13}C$ vs. $\delta^{34}S$ plots.

5.6.1. CHICHA

When considering all of the sulphur isotope results from Chicha, there appears to be no distinction between the $\delta^{34}S$ values of freshwater and terrestrial faunal species at the site (figure 5.7). Terrestrial herbivores (*Bos*, *Ovis*, *Alces* and *Equus*) range from 4.2 to 12.3‰. The two pig (*Sus*) samples have very similar $\delta^{34}S$ values of 10.9 and 11.2‰, while the other non-human omnivore species analysed, *Canis familiaris*, exhibit a very wide $\delta^{34}S$ range of 6.2 to 29.5‰. The archaeological fish samples (*Carassius*, *Esox* and *Perca*) exhibit a range of 11.9 to 22.2‰ (14.8 to 21.8‰ excluding samples with N:S and C:S below the modern range). The modern *Carassius* samples have lower $\delta^{34}S$ values than the archaeological *Carassius* samples from Chicha, with the two modern samples yielding $\delta^{34}S$ values of 12.3 and 12.9‰.

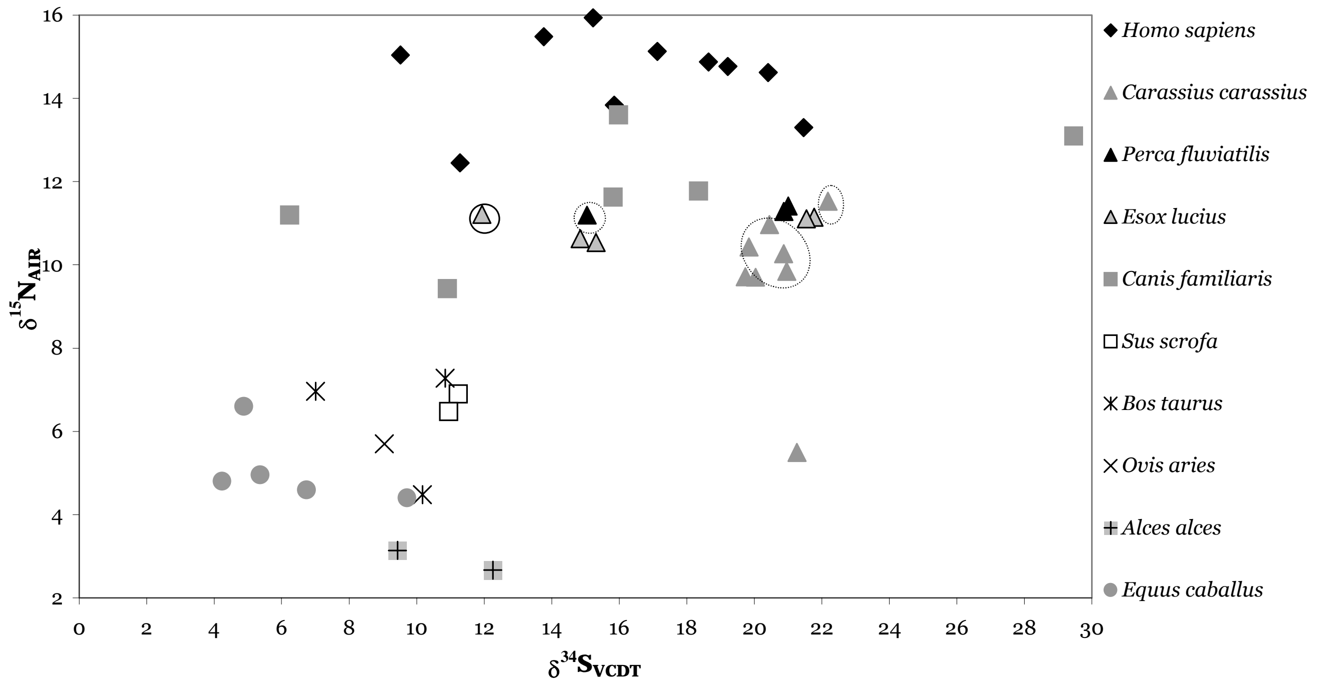

Figure 5.7. Plot of $\delta^{34}S$ vs. $\delta^{15}N$ for all Chicha archaeological samples. Samples with C:S values below the observed modern range are circled with a broken line; CHA22, with C:S and N:S below observed modern values, is circled with a solid line.

The archaeological data do not suggest that the Chicha humans consumed their domestic dogs as food (Molodin *et al.* 2001, 2002). If the *Canis* samples are excluded from consideration as a potential food species and samples with C:S and N:S below the observed modern values are also excluded, the archaeological data *do* show isotopic resolution between two groups of potential human food sources—terrestrial and freshwater species—at Chicha (figure 5.8). The terrestrial fauna considered then range up to a maximum $\delta^{34}S$ of 12.3‰, and the archaeological fish samples yield a minimum of 14.8‰.

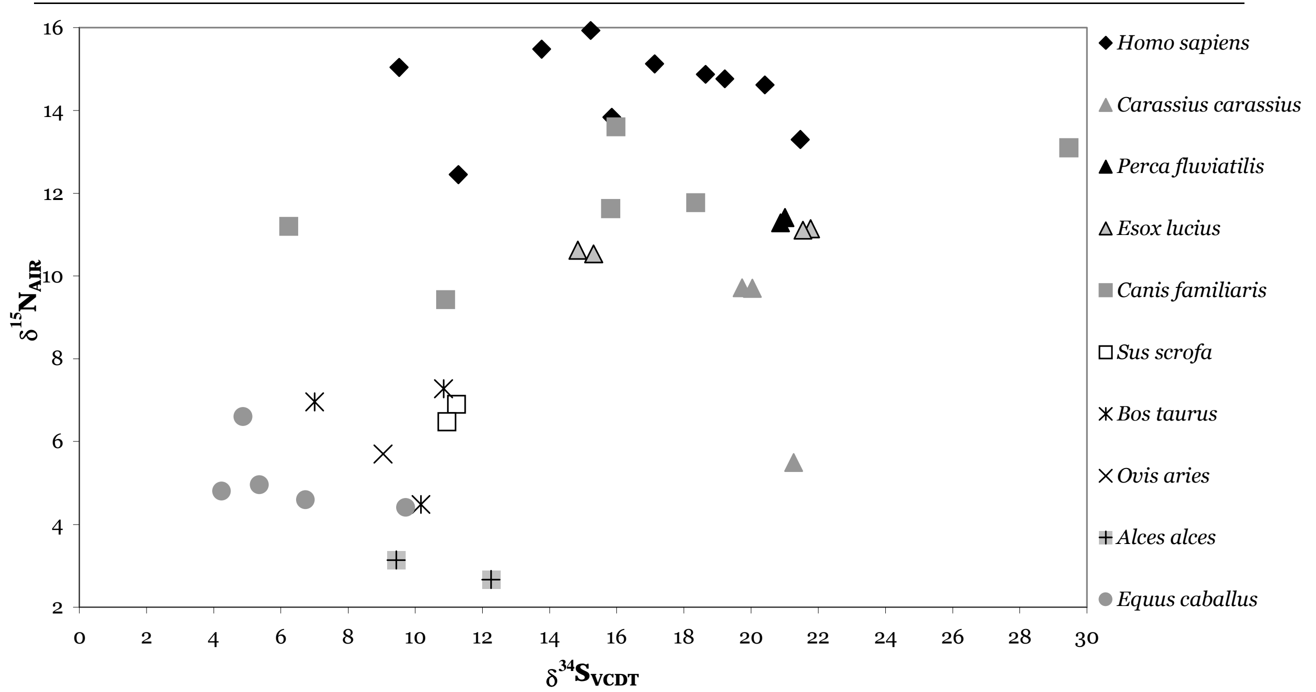

Figure 5.8. Plot of δ34S vs. δ15N for Chicha samples with C:S and N:S values within the observed modern range.

The human samples from Chicha range in δ34S from 9.5 to 21.5‰, with an average of 16.3‰. The human average δ34S is closest to that of the domestic dog samples (16.1‰) and the fish samples (19.2‰); it is highly 34S-enriched relative to the terrestrial faunal samples (excluding dogs), which have an average δ34S value of 8.6‰. The average δ34S value for the fish does not greatly change with the omission of the low-C:S and low-N:S samples (19.6‰).

The similarity in human and fish δ34S values reinforces the dietary interpretation based on the δ15N values of humans and fauna at Chicha. The human δ15N values are too highly elevated to reflect a diet based primarily upon the consumption of terrestrial fauna. Aside from the frequent consumption of domestic dog meat (which is unlikely given the archaeological evidence), the Chicha human δ34S and δ15N values can only be explained by the consumption of fish as a dietary staple.

5.6.2. BIL'SHIVTSI

The freshwater species at Bil'shivtsi show a wide range of $\delta^{34}S$ values that overlap almost completely with those of the terrestrial fauna (figure 5.9). Of the two fish sampled from Bil'shivtsi, one produced a $\delta^{34}S$ value of 3.2‰ and the other—with low C:S and N:S values—yielded a $\delta^{34}S$ value of 6.9‰. The freshwater turtle samples (*Emys sp.*) are ^{34}S-enriched relative to the fish samples, with $\delta^{34}S$ values of 10.0 to 17.3‰.

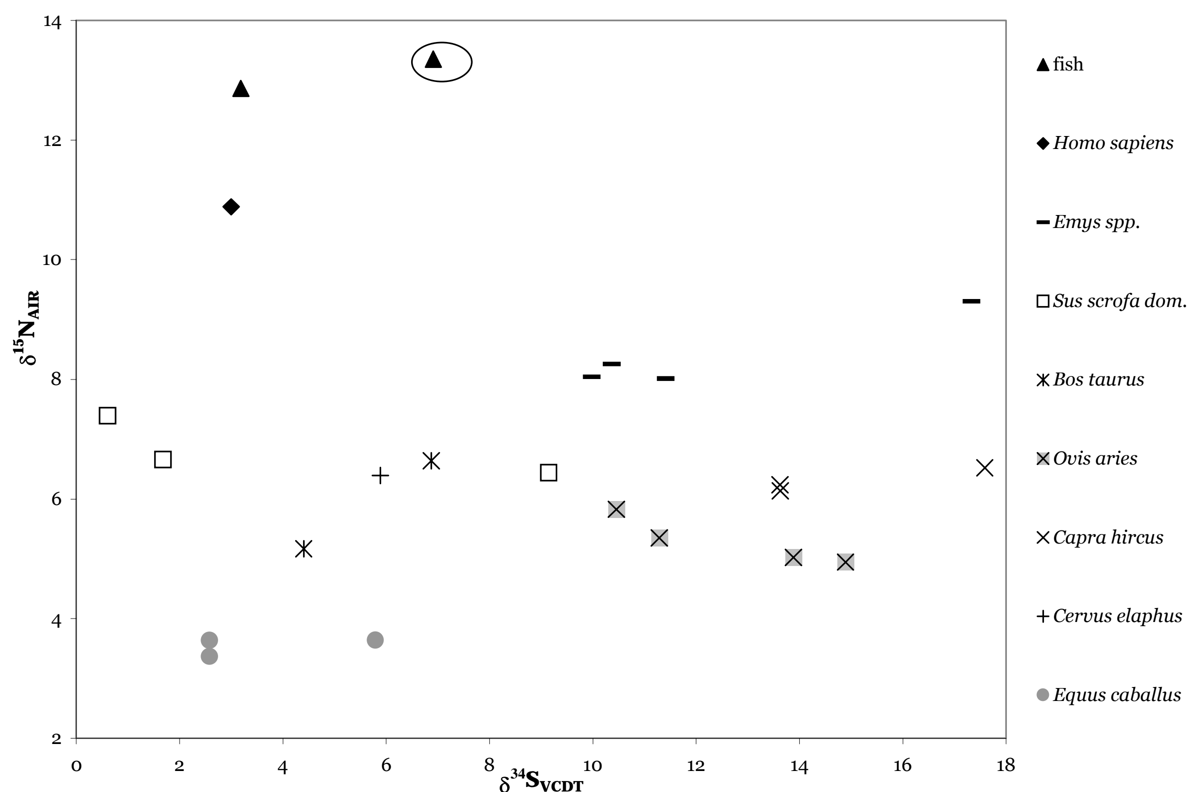

Figure 5.9. Plot of $\delta^{34}S$ vs. $\delta^{15}N$ for the Bil'shivtsi samples. The sample with C:S and C:N below observed modern values (BIL25) is circled.

Terrestrial faunal species at Bil'shivtsi cannot be separated by their $\delta^{13}C$ and $\delta^{15}N$ values, apart from horses, which exhibit consistently low $\delta^{15}N$ values (see figure 5.1). The Bil'shivtsi human values suggest a diet based on terrestrial products, but low in fish and low in horse meat/milk. The $\delta^{34}S$ values for the terrestrial fauna exhibit a different pattern to the $\delta^{15}N$ and $\delta^{13}C$ values obtained. The terrestrial faunal

$\delta^{34}S$ values fall into two main groups according to their mean and overall $\delta^{34}S$ values. *Equus*, *Sus*, *Bos* and *Cervus* samples exhibited relatively low $\delta^{34}S$ values: 3.7, 3.8, 5.6 and 5.9‰ on average, respectively. In contrast, the ovicaprid samples exhibit a relatively high $\delta^{34}S$ range of 10.5 to 17.6‰, with an average of 13.6‰.

The single human sample from Bil'shivtsi has a $\delta^{34}S$ value of 3.0‰. The human $\delta^{34}S$ falls closest to those of the fish and non-ovicaprid terrestrial faunal samples from Bil'shivtsi.

5.7. *$\delta^{34}S$ and the Identification of Freshwater Animal Consumption: Conclusions*

The results of this study of archaeological material from two sites in the Eurasian steppe show that sulphur isotope analysis alone cannot be relied upon as a reliable tool for the identification of freshwater resource consumption in ancient human diets. However, as a supplementary tool in addition to nitrogen and carbon isotope studies, the $\delta^{34}S$ analysis of archaeological remains can provide important supporting evidence for a dietary dependence upon freshwater animal protein.

At Bil'shivtsi, the $\delta^{34}S$ values for the fish lay within the sulphur isotope range obtained for the terrestrial species; this isotopic overlap precludes the characterization of any human $\delta^{34}S$ value obtained as representative of a mainly terrestrial or freshwater diet at this site. The patterns evident in the sulphur data obtained for the site of Chicha differ from those at Bil'shivtsi in that only a small degree of isotopic overlap exists between freshwater fish and terrestrial herbivores/pigs. At Chicha, sulphur isotope data can be considered useful as supporting evidence for a human diet high in freshwater fish and low in terrestrial animal protein.

A main problem with sulphur isotopic identification of freshwater fish consumption that is the degree of overlap between freshwater and terrestrial species cannot be easily predicted at any archaeological site. Therefore, for any palaeodietary study seeking to support or refute the theory of freshwater resource consumption, it is recommended that a preliminary sulphur isotope survey of freshwater and terrestrial material from the site be carried out in order to assess the potential for isotopically separating potential dietary sources. A preliminary study may show that $\delta^{34}S$ values are likely to provide resolution between freshwater and terrestrial resources; it may reveal an unpromising overlap in $\delta^{34}S$ ranges for freshwater and terrestrial species. In the latter case, a preliminary study would prevent the future waste of time and resources on a full-scale sulphur isotope study.

5.8. *THE BENEFIT OF SULPHUR ISOTOPE ANALYSIS IN PALAEODIETARY STUDIES*

Regardless of the utility of sulphur isotopes in resolving issues of freshwater fish consumption, the results of this study suggest that sulphur isotope analysis should be undertaken routinely in conjunction with $\delta^{15}N$ and $\delta^{13}C$ analysis. At sites where the identification of freshwater fish consumption is not an issue, or where isotopic resolution between freshwater and terrestrial species cannot be obtained, $\delta^{34}S$ data has the potential to refine palaeodietary profiles by lending support to conclusions based upon $\delta^{13}C$ and $\delta^{15}N$ values, or by excluding potential foods with very different $\delta^{34}S$ values relative to those of humans.

For example, the $\delta^{34}S$ data obtained from Bil'shivtsi does not allow separation of potential freshwater and terrestrial dietary sources by means of their isotope values. However, sulphur isotope analysis reveals a large difference between the ovicaprid and other terrestrial fauna that is not suggested by the $\delta^{13}C$ and $\delta^{15}N$ data. The

ovicaprid $\delta^{34}S$ values are so far removed from those of the human that sheep and goat protein must have been only rarely (if ever) consumed by the Bil'shivtsi individual. The separation of ovicaprid $\delta^{34}S$ values from those of other terrestrial fauna was not expected, but as a result of the sulphur isotope analysis, further refinement has been provided for the picture of human diet at Bil'shivtsi.

In addition to providing further details about ancient human diet, the distinct Bil'shivtsi ovicaprid values also raise questions about the mobility of the peoples at Bil'shivtsi and their trade and communication with other communities. As mentioned above, different geological formations can yield distinct sulphur isotope ratios, which are passed up the food chain and incorporated (relatively unchanged) into animal collagen. Sulphur isotopic variability with geographical location can allow us to distinguish between local animals and animals imported from or raised in other regions (of distinct $\delta^{34}S$). The enriched $\delta^{34}S$ values exhibited by the Bil'shivtsi ovicaprids indicate that they were not raised and maintained in the same general area as the other terrestrial fauna. The distinct Bil'shivtsi ovicaprid $\delta^{34}S$ values lead me to speculate that the animals may have been imported to Bil'shivtsi from another area. The purpose of their importation is not clear, as the isotope data suggest that ovicaprids were not major dietary resources for humans at the site. It is possible that the ovicaprids were used for their hair and wool, though there is no further evidence to support this hypothesis. Since the radiocarbon date quoted for Bil'shivtsi was obtained from the remains of the single human found, it is further possible that the faunal remains are not contemporary with the human, and that the ovicaprids were in fact imported for food, but during another period not represented by the Bil'shivtsi human. In order to test the hypothesis that the Bil'shivtsi ovicaprids were imported from another area, a more secure dating of the Bil'shivtsi samples and an assessment of $\delta^{34}S$ values from surrounding areas would be required. Once the sulphur isotope

profile of the surrounding region was established, it would be possible to determine possible geographic origins for the ovicaprids.

As is common in stable isotope analysis, one study answers few questions and raises many. But as I have shown here, sulphur isotope analysis promises to be a useful addition to the palaeodietary toolkit, and with continued research into the relationship between sulphur content and collagen quality, the technique may be further refined to aid in the reconstruction of past dietary and economic trends.

CHAPTER 6

RESIDUE ANALYSIS

6.1. *LATE BRONZE AGE DIET AT CHICHA*

The site of Chicha, comprising the archaeological remains of a fortified settlement and associated burial areas, occupies an area of over 50,000m^2 on the edge of a small lake in the Zdvisk region of the Russian province of Novosibirsk. Using traditional artefact chronologies, archaeologists initially identified the major period of occupation at Chicha to have coincided with the transition from the Bronze Age to the Iron Age (Molodin *et al.* 2001). Further excavation and new (currently unpublished) radiocarbon dates have revealed three main phases of occupation at the site: the Late Bronze Age (14th-12th cent. BC), the transitional period from the Bronze Age to the Iron Age (11th-9th cent. BC) and the Sargat Period (Iron Age, 50 BC-50 AD) (Jens Schneeweiβ, personal communication).

As mentioned in chapter 3, osteological analysis of the faunal remains recovered from Chicha during the first excavation season indicated that the ancient inhabitants of the site had an economy "based on horse and cattle breeding…and to a considerably lesser extent on sheep and goat" (Molodin *et al.* 2001:123). Artefactual evidence for the practice of metal processing, crop cultivation, weaving, hunting and fishing was discovered at Chicha, but these activities were viewed as relatively minor elements of the Chicha economy (Molodin *et al.* 2001). The discovery of a midden full of fish bones at the Chicha settlement during the second excavation season in 2001 subsequently led archaeologists to reconsider the role that fish might have played in the economy and diet of the inhabitants of Chicha (Molodin *et al.* 2002). According to the cumulative osteological data from all excavation years, the

main potential food species present at Chicha were fish, cattle and ovicaprids (Molodin *et al.* 2002).

In chapter three, I have presented the results of stable isotopic analysis of Late Bronze Age human and faunal remains from Chicha. The results of the carbon and nitrogen isotopic analyses contradict the dietary profile proposed for the inhabitants of Chicha in the first published site report (Molodin *et al.* 2001). The carbon and nitrogen isotopic data support the view that the average human diet at Chicha involved the frequent consumption of freshwater resources (e.g., fish). In fact, the $\delta^{13}C$ and $\delta^{15}N$ data indicate that the people of Chicha relied upon fish as a dietary staple to a greater degree than any of the other Bronze Age or Iron Age groups discussed in this thesis (see chapter 3). The sulphur isotope data presented in chapter 5 further supports the hypothesis that freshwater fish played an important role in the diet of the Chicha Late Bronze Age humans.

This chapter describes the analysis of lipid residues extracted from a number of archaeological vessels from Chicha. The primary goal of this research is to detect and identify residues representative of particular foodstuffs exploited at Chicha. The study was conducted in two phases: a primary, methodological portion, and a subsequent archaeological application. In the first phase, I analysed modern freshwater fish lipids in order to test whether or not freshwater fish residues may be distinguished from those of other animals by GC-C-IRMS. This avenue of inquiry has not yet been pursued in the archaeological scientific literature.
In the second phase of this study, the results of the modern fish study were used as a reference against which to compare archaeological residues. The

application phase of the study involved the analysis of lipid residues extracted from ceramic vessels found at Chicha, with the aim of attributing extracted archaeological fats to a specific food type.

6.2. *ANALYSIS OF ARCHAEOLOGICAL RESIDUES*

Up to this chapter, this thesis has dealt with stable isotope analysis of human bone collagen, a method that yields semi-quantitative information about an individual's diet. This chapter deals with the extraction and identification of pottery residues found at an archaeological site—a technique that can provide additional complementary, qualitative information about foods that were prepared, processed and/or stored at that site.

A number of distinct methods of analysis have been performed on ceramic vessels from central Eurasian archaeological sites in an effort to associate vessel residues with specific food types. The simplest technique involved the visual examination of residue scraped from the inner surface of a potsherd, using a light microscope (L. Guyduchenko, personal communication). Guyduchenko has classified residues analysed by this method by their appearance as deriving from either porridge, meat (or meat broth), dairy products or wine. Other possible foodstuffs were not identified.

Demkin (2000) claimed to identify foods that had been deposited with archaeological vessels by measuring the phosphate content of soils extracted from the inner portion of the vessels. Based on reference values of P_2O_5 content for foods such as millet, soy, pork, fish and milk, Demkin drew the connection between archaeological soil residue phosphate concentration and foods as follows: < 2 mg/100g = water; < 2 to 8 mg/100g = milk product

(kumys) or meat soup; > 8 mg/100g = porridge (2000:101). Although I am not aware of the exact contents of the author's earlier publications regarding phosphate analysis of ceramic residues, I can confirm that Demkin's 2000 paper does not discuss possible alternative sources for the detected phosphate; nor does the author appear to have ever analysed the phosphate content of soils found in association with—but not inside—the archaeological vessels. Phosphate does not tend to be highly mobile in soils, but can accumulate as a result of decomposing organics such as leaves or manure, or from the application of chemical fertilizers at the soil surface (Schuman 2001; Robinson 2003). Notwithstanding the shortcomings of Demkin's research, this type of analysis continues to be cited and replicated by other Eurasian steppe archaeologists (Koryakova and Daire 1997; Aleksandrovskii and Aleksandrovskaia 1999).

The most common type of residue analysis conducted on archaeological ceramics is based on a methodology generally regarded as sound by the scientific community. This technique involves the extraction of preserved, endogenous lipid residues from the interior surface of a potsherd and their analysis by combined gas chromatography-mass spectrometry (GC-MS; Evershed 1993). The presence of certain fatty acids and other, more complex molecules in a lipid extract forms a molecular profile that can sometimes be matched to that of a characteristic food type. The identity of molecules present in the extract can be verified by matching the mass spectrum of the unknown molecular peak to the mass spectra of known molecules. Analysis of potsherd lipid residues by combined GC and GC-MS has been conducted on archaeological materials since the early 1990s (Evershed *et al.* 1990).

Examples of residue types identified (at least in part) by GC-MS analysis of archaeological ceramics include *Brassica* sp. (Evershed *et al.* 1991), vegetable oil (Kimpe *et al.* 2001) and ruminant milk (Dudd and Evershed 1998).

The detection and classification of archaeological residues by GC-MS may be accomplished by the identification of individual molecules ('pure' biomarkers) and/or a combination of molecules ('impure' biomarkers) representative of a specific lipid type (Malainey *et al.* 1999b). Most studies focus on the latter in their classification of ancient food residues. Drawing from the above examples: ruminant dairy lipids can be identified in archaeological vessels in part by the abundance of short-chain free fatty acids and di- and triacylglycerides with short-chain acyl moieties (Evershed *et al.* 1992). Leaf wax components (alkanes, ketones and alcohols) extracted from British Late Saxon/medieval pots have been matched to those derived from leaves of the *Brassica* genus (Evershed *et al.* 1991). The presence of long-chain alcohols, β-sitosterol and sigmastanol in Roman oil lamps led Kimpe *et al.* (2001) to conclude that vegetable oil(s) had been used in the vessels as fuel.

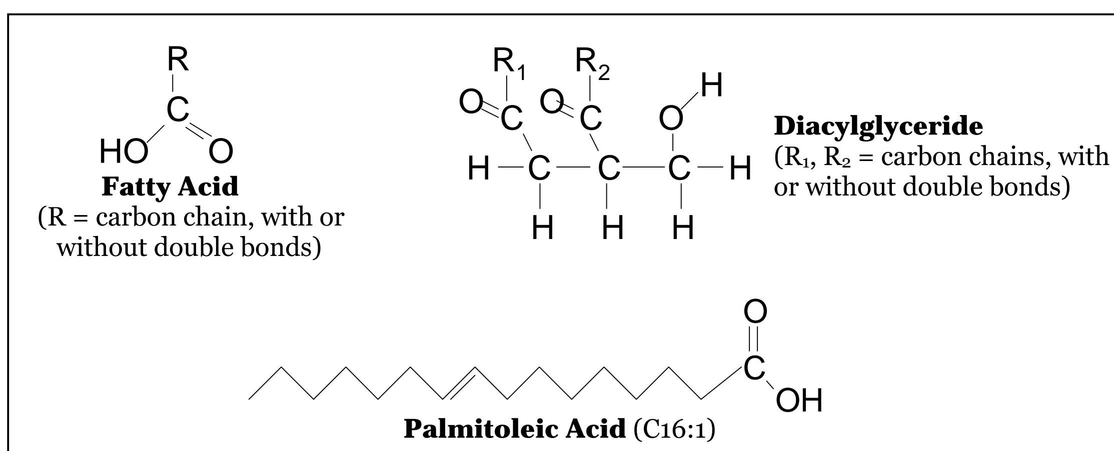

Figure 6.1. The basic structure of fatty acids and (di)acylglycerides, and the structure of palmitoleic acid. Acyl glycerides are formed by the condensation of fatty acids onto a glycerol $C_3H_5(OH)_3$ backbone. In monoacylglycerides, one -OH group is replaced with an -OCR (fatty acid) group on the glycerol backbone. In triacylglicerides, all three -OH groups of glycerol are replaced with -OCR groups. In this chapter, fatty acids are referred to by the notation 'Ca:b', where 'a' denotes the number of carbons in the molecular chain, and 'b' indicates the number of double bonds present. For example, C16:1 is a 16-carbon chain fatty acid with a single double bond.

In the 1990s, chemists began to isolate individual types of molecules from an extracted archaeological residue by gas chromatography and isotopically analyse them by isotope ratio mass spectrometry (GC-C-IRMS) (Evershed *et al.* 1994; see Meier-Augenstein 1999 for a review of this technique). The molecules most often targeted for this type of analysis are the C16:0 and C18:0 fatty acids, which are the major molecular species recovered from archaeological lipid residues of animal origin (Evershed *et al.* 2002). Using GC-C-IRMS, ruminant dairy fats can be distinguished from ruminant adipose fat, which can in turn be differentiated from non-ruminant adipose fat (including marine fish lipids), allowing the distinction between lipids derived from these different sources (Mottram *et al.* 1999; Evershed *et al.* 2002). The use of mixing lines constructed between the isotope values of two reference fats has also led to the identification of archaeological pots that had been used to process more than one type of animal fat (Evershed *et al.* 2002).

Archaeological scientists have taken advantage of the good preservation of lipid residues within archaeological ceramics to extract and identify lipid profiles characteristic of a wide range of foods in a variety of archaeological contexts. The hydrophobic nature of lipid molecules aids in their preservation, and the close association of organic molecules with a mineral matrix such as the fabric of a pot seems to guard against oxidative breakdown of the molecules (Evershed 1993). By extracting lipids absorbed to the ceramic matrix of a pot, we target material likely to be better preserved than the molecules that have been openly exposed to diagenetic contamination and degradation. Lipid profiles of soil surrounding sampled pots can be analysed in addition to pottery residues in order to ascertain the character and magnitude of possible lipid contaminants derived from the burial environment (Heron *et al.* 1991).

Published reports utilising the analysis of residues on archaeological pottery have generally limited the discussion of potential food sources to those of terrestrial origin (i.e., milk, terrestrial herbivore meat, pork, terrestrial plant foods), and have given little consideration for the potential contribution of fish lipids to archaeological residues. Only a small amount of research has been done to explore the possibility of distinguishing fish-derived lipids from other food lipids extracted from archaeological vessels (Malainey *et al.* 1999a&b; L. Brown and C. Heron, UK Archaeological Sciences 2003, poster; M. Forster and O. Craig, personal communications). With the notable exception of Malainey *et al.* (1999a&b), these projects have been limited exclusively to the study of marine fish residues. This study is therefore one of the first to investigate the potential of lipid residue analysis to identify

freshwater fish-derived residues in archaeological potsherds, and is the first to integrate GC-MS with GC-C-IRMS in such a project.

The recovery of fish lipids in archaeological vessels may seem unlikely, considering that fish may be prepared for consumption without the use of pots by salting, drying, frying or roasting. However, a survey of the ethnographic literature in the (now former) Soviet Union reveals that many ethnic groups commonly prepared fish by boiling, frying or in stew (Rudenko 1955; Akhmetova 1995). In fact, in his study of the Bashkir of the southern Urals region, Rudenko (1955) reports that fish were almost exclusively prepared by boiling, and were only rarely roasted. Therefore, it is reasonable to suppose that the people at Chicha may have used their ceramic vessels to cook freshwater fish.

6.3. *ANALYTICAL TECHNIQUES USED*

For this study, I have used a combination of GC-MS and GC-C-IRMS analyses to investigate the potential for the detection of freshwater fish lipid residues. GC-MS profiles can be used to detect compounds present in fish, but no reliable lipid biomarker has been determined to exclusively identify freshwater fish. The C22:1 fatty acid (cetoleic) that can be used to demonstrate the presence of marine fish is derived from the wax esters of marine calanoid copepods in the diet (Henderson and Tocher 1987), and therefore cannot be relied upon as a biomarker for freshwater species. In addition to cetoleic acid, the C20:1 fatty acid (gadoleic) has been proposed as a biomarker for fish (O. Craig, personal communication). However, the presence of the latter fatty acid in various plant and animal lipids (including

butterfat, see figure 6.2) precludes its use alone as a 'pure' biomarker indicative of freshwater fish lipid in archaeological residues. It is furthermore not feasible to use the high levels of polyunsaturated fatty acids present in freshwater fish as characteristic indicators, as these molecules are highly susceptible to oxidative and thermal breakdown (Malainey *et al.* 1999a) and are thus unlikely to survive in archaeological contexts. Despite these limitations, GC-MS analysis can provide evidence that can be used to support or refute the presence of particular food types in an archaeological ceramic extract.

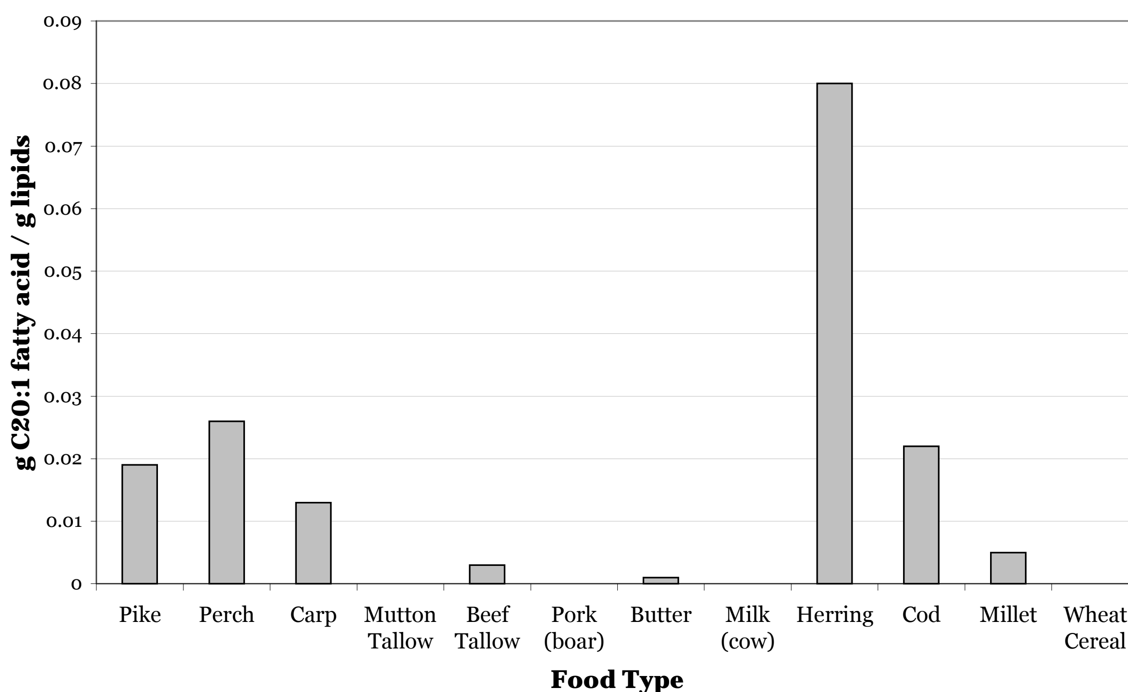

Figure 6.2. Amount of C20:1 fatty acid present in selected foods, in grams per gram lipids in raw food, except for whole wheat cereal cooked in water (data from online United States Department of Agriculture food contents database, www.nal.usda.gov/fnic/).

In the process of GC-MS analysis, the lipid profiles obtained from archaeological extracts are compared with those of modern reference fats. As referred to above, the effects of diagenetic processes on the nature and concentration of the lipids present in the archaeological samples must be

considered. For example, short-chain fatty acids are more likely to be lost by groundwater leaching than long-chain acids, due to their higher solubility. Unsaturated fatty acids are highly susceptible to oxidation at double bond sites, leading to their under-representation in archaeological extracts, relative to saturated fatty acids (Regert *et al.* 1998). Experimental research on lipid degradation in a burial environment has shown that the absolute concentrations and relative proportions of different molecular types extracted from potsherds can change over time to such a degree that the lipid profile of a degraded residue may not resemble that of the original source lipids (Dudd *et al.* 1998). The interpretation of the GC-MS profiles of the archaeological samples is given in section 6.4, with consideration given to contamination and diagenetic processes, and with a discussion of the relationship between archaeological and modern lipid profiles.

Studies have shown that the carbon stable isotope values of C16:0 and C18:0 fatty acids can be used to separate lipids from different species, and to distinguish milk from adipose lipids (Evershed *et al.* 1997). The observed differentiation in fatty acid isotopic composition between dairy and adipose fats and between species arises from a combination of differing fatty acid synthetic pathways and species dietary differences (Evershed *et al.* 2002). It is reasonable to hypothesise that freshwater fish will exhibit distinct C16:0 and C18:0 isotopic values from other types of animals, since the former differ from mammals and marine fish in their diet and in some aspects of their lipid metabolism (Cowey and Sargent 1979; Henderson and Tocher 1987). In this study, GC-C-IRMS analysis is used as a complementary tool to further confirm or contest the GC-MS evidence for the existence of freshwater fish residues in the Chicha archaeological vessels.

6.4. *MATERIALS & METHODS FOR ANALYSIS OF CHICHA CERAMIC RESIDUES*

6.4.1. SAMPLE ACQUISITION

27 samples from the 2000 and 2001 excavation seasons were selected for residue analysis by Jens Schneeweiβ (Deutsches Archäologisches Institut, Berlin, Germany). Potsherds were obtained from various contexts throughout the settlement, including areas identified as houses, pits, layers and ditches. The samples represent all three of the main phases of site occupation at Chicha (approximate dates of samples are given in table 6.1). Samples with charred surface residues were supposed to have been used for cooking, but no further information was available regarding the possible uses of the vessels analysed.

Three soil samples were also analysed in order to identify possible soil-derived lipid contaminants in sample extracts. A sample of virgin soil from the site was taken, as well as soil samples from two different pit fills. Samples were air dried and stored in plastic bags at room temperature before analysis[*]. All sample contexts are given in table 6.1. The locations from which the archaeological samples were taken are shown in appendix 4.

Modern fish samples were obtained in order to add to the published reference isotopic data on faunal C16:0 and C18:0 carbon isotope values, which currently do not include numbers for freshwater fish. One individual each of pike (*Esox lucius*), perch (*Perca fluviatilis*) and carp (*Carassius carassius*) were acquired from the freezer stores at the Research Lab for Archaeology and the History of Art, University of Oxford, UK. The modern

[*] Ideally, samples should be stored in acid-free paper to avoid phthalate contamination due to contact with plastic.

samples represent the three species of fish found at Chicha, though the samples themselves were all collected from sites in mainland Britain. The pike, carp and perch samples were labeled ci28a, ci29a and ci30a, respectively.

6.4.2. SAMPLING

All laboratory work described from this point, including software-assisted data analysis, was conducted at the Stable Isotope Unit of the University of Newcastle, UK, under the supervision of Dr. Oliver Craig and with the technical assistance of Ms. Gillian Taylor, Mr. Paul Donohoe and Mr. Ian Harrison.

Sherds ci0102, ci0104, ci0105, ci0112, ci21, ci22 and ci23 had visible charred residue on the inner surface of the sherd. For these samples, the visible surface deposit was removed by abrasion with a modeling drill bit and transferred to glass tubes for analysis. Once the surficial residue was removed, a few millimetres of the inner surface of each sherd was ground into a powder and transferred to a separate glass tube. For the sherds with no major visible surface residue, approximately 1mm of the inner surface of each sample was removed by drilling. 0.4 to 1.3g of the ceramic sub-surface of each sample was then removed, and the drilled powder collected in glass tubes. Drill bits were cleaned by ultrasonic washing with chloroform-methanol (x3) and dried before use. Approximately 1g of each soil sample was ground using an agate mortar and pestle (washed before each use with chloroform-methanol), then transferred to a glass tube for analysis. The first portion taken from each sherd or soil sample was designated as fraction 'a' (e.g.,

ci0102a). For samples with visible surface residue the 'a' fraction represents the residue removed from the surface of the sherd; 'b' represents the sub-surficial ceramic matrix. For samples with no visible surface residue (including soil samples), the first and only portion taken was designated as fraction 'a' (e.g., ci0127a). The fraction from the outer surface of sherd ci0112 was labeled 'ci0112c'.

For the modern samples, dissection scissors were used to remove ~0.5g of skin and flesh from the ventral region of each fish. The tissue was placed in a glass vial for lipid extraction.

Table 6.1. Sample information for Chicha potsherd & soil samples.
Material = description of the material analysed
Fragment = portion of the pot represented by sampled sherd
Context = location within the site from which sample obtained, if known
Period = LBA (Late Bronze Age, 14th-13th cent. BC), LBA-EIA (Late Bronze Age – Early Iron Age transitional period, 11th-9th cent. BC), Sargat (50BC – 50AD)
TLE Yield = total lipid extract yield calculated for each sample; samples with negative calculated yield indicated (-).
GC-MS = sample analysed by gas chromatography-mass spectrometry
GC-C-IRMS = sample analysed by gas chromatography-combustion-isotope ratio mass spectrometry

Sample	Material	Fragment	Context	Period/Age	TLE Yield (μg/g sample)	GC-MS	GC-C-IRMS
ci0102a	surface residue	rim	trench 6, house 3b	LBA	1422		
ci0102b	interior surface				923	x	x
ci0103a	interior surface	body	?		93		
ci0104a	surface residue	body	trench 5, house 8a	LBA-EIA	1199		
ci0104b	interior surface				372	x	x
ci0105a	surface residue	rim	trench 6, house 3a	LBA-EIA	1214		
ci0105b	interior surface				706	x	x
ci0106a	interior surface	body	trench 5, house 8a, pit 346	LBA-EIA	581	x	x
ci0107a	interior surface	body	?		-		
ci0108a	interior surface	rim	?	LBA-EIA	0	x	x
ci0109a	interior surface	rim	trench 8, layer 29/66	LBA	535	x	x
ci0112a	surface residue	rim?	?		1305		
ci0112b	interior surface				0	x	
ci0112c	exterior surface				-		
ci0119a	soil	n/a	trench 5, house 8a, pit 346		257		
ci0127a	soil (virgin)	n/a	beneath trench 6, house 3a		158		
ci0128a	soil	n/a	trench 6, house 3a		452	x	

Sample	Material	Fragment	Context	Period/Age	TLE Yield (μg/g sample)	GC-MS	GC-C-IRMS
ci7a	interior surface	rim & body	trench 1, ditch	LBA-EIA	-	x	x
ci8a	interior surface	rim	trench 1, ditch		0		
ci9a	interior surface	rim & body	trench 2, house 3, pit 305		743		
ci10a	interior surface	rim	trench 2, house 3, layer 2		-	x	
ci12a	interior surface	body	trench 2, layer 2	LBA-EIA	127	x	x
ci13a	interior surface	rim	trench 2, house 3, layer 1		-		
ci14a	interior surface	rim	trench 1, house 2, layer 3a	LBA-EIA	0	x	x
ci15a	interior surface	rim	trench 2, house 3	Sargat	83	x	
ci17a	interior surface	body	trench 2, pit 393		0		
ci18a	interior surface	rim	trench 1, house 2, layer 3	LBA-EIA	81	x	x
ci19a	interior surface	body	trench 3, horizont 2		107		
ci20a	interior surface	rim	trench 1, house 2	LBA-EIA	82	x	
ci21a	surface residue	rim	trench 1, house 2, layer 3b	LBA-EIA	0	x	x
ci21b	interior surface				174		
ci22a	surface residue	body	trench 2, house 3, layer 1		433	x	
ci22b	interior surface				0		
ci23a	surface residue	body	trench 2, house 3, layer 2		-	x	
ci23b	interior surface				73		

6.4.3. LIPID EXTRACTION

Lipid extraction of the potsherd residues was performed according to the standard protocol used in the Department of Civil Engineering and Geosciences, University of Newcastle, following the methods described in Charters *et al.* (1993) and Dudd *et al.* (1999). The initial mass of each sample was recorded, and 20μl of n-tetratriacontane was added to each tube as an internal standard. Lipids were extracted from the ground sample by ultrasonic treatment with a chloroform-methanol mixture (2:1v/v, 15min × 3). The chloroform-methanol extract was rotary evaporated to a volume of 1-3ml and transferred to a small glass vial. The remaining solvent was evaporated under nitrogen. The extract was then redissolved in 3ml hexane, sonicated and centrifuged to separate out any remaining particulate matter. The liquid

from each sample was transferred to a pre-weighed glass tube and dried under nitrogen. A portion of this extract was used for GC and GC-MS analysis, the remainder was stored at ~20˚C until saponified for GC-C-IRMS analysis.

Lipid extraction of the modern fish samples was performed by the Folch method, as described in Hamilton and Hamilton (1992:21-22). Samples were mashed with a glass stirring rod, then lipid extracted three times by mixing and ultrasonication (15min) with chloroform:methanol (2:1). Samples were filtered using Whatman #40 filter paper, and the filtrate collected in clean glass tubes. The filtrate was mixed with 2ml saturated aqueous sodium chloride solution; the upper layer that then formed was removed and discarded. A mixture of chloroform:methanol:water (3:48:47) was added gently, and the upper layer removed (this step repeated 3x). The remaining lipid extract was transferred into pre-weighed glass vials, then dried under nitrogen. A portion of each modern fish total lipid extract (TLE) was used for GC-MS analysis. The remaining TLE fractions of the samples were kept at ~20˚C for future analysis.

6.4.4. DERIVATISATION

The TLE portions reserved for GC and/or GC-MS analysis were reacted with 20µl-30µl N,O-bis(trimethylsilyl)trifluoroacetamide containing 1% v/v chlorotrimethylsilane for 1 hour at 70˚C to produce trimethylsilyl derivatives of the lipids present. Derivatisation reduces the polarity of the lipids while increasing their volatility, thus improving their transport through the GC column. The derivatised product was dried under nitrogen and dissolved in hexane for GC analysis.

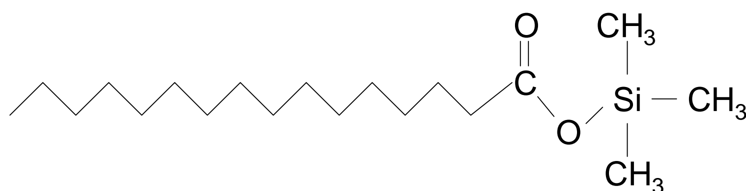

Figure 6.3. Structure of the trimethylsilyl derivative of C16:0.

6.4.5. GC AND GC-MS ANALYSIS

Extracts from all samples were analysed by high-temperature gas chromatography (HT-GC) in order to scan for the presence of lipids. Samples were individually auto-injected into a gas chromatograph (Hewlett Packard 5890) fitted with a bonded-phase fused-silica capillary column coated with 100% dimethylpolysiloxane stationary phase (DB-1HT column, 15 m × 0.32 mm, 0.1mm film thickness; J&W Scientific, Folsom, CA, USA). Samples were heated from 50° to 350°C at 10°C per minute, after a 2-minute isothermal hold at 50°C. Peak areas were compared against an internal standard, which was introduced to the sample prior to solvent extraction as described above (20μl C30 n-alkane). After GC analysis, samples were stored at -70°C until needed for GC-MS analysis.

Samples whose GC traces indicated the presence of lipids in sufficient quantities for analysis were then run by GC-MS (see table 6.1). For samples with 'a' and 'b' fractions, the GC traces for each fraction were very similar, so only one was run by GC-MS. One of the soil samples (ci0128a) was analysed by GC-MS as well, even though its GC profile indicated the presence of lipids only in very low concentration. In addition to ci0128a, sample ci0112c (outer surface of sherd) and nine other potsherd samples produced gas

chromatographs indicating very low lipid yield. The GC traces for low-yield samples are shown in appendix 5.

The potsherd extracts with substantial lipid content, one soil extract and the modern fish extracts were analysed by GC-MS using the same temperature program as above (5890 gas chromatograph, 5972 mass spectrometer, Hewlett Packard). The mass spectrometer was run on the following program: ion source 350°C, interface temperature 345°C, with spectral detection over the range m/z 50-850 at 1.55 scan/s.

6.4.6. SAPONIFICATION: FORMATION OF FAMES

After GC analysis, samples with high yields of C16:0 and C18:0 fatty acids were selected for GC-C-IRMS analysis (see table 6.1). The lipid extracts from the modern fish samples (ci28, ci29 and ci30) were also prepared for GC-C-IRMS analysis. For the potsherd extracts, a portion of the total lipid extract was prepared for GC-C-IRMS. A fraction of each of the remaining modern fish TLEs was used for GC-C-IRMS.

The lipid residue was saponified with 2ml NaOH (5% w/v) in methanol, heated for 1 hour at 70˚C. This process produces free fatty acid sodium salts from the lipids present, including the component fatty acids of acyl glycerides. The solution was acidified with 420μl 6M HCl to release the free fatty acids. The lipid fraction was extracted with hexane three times. The collected extract from each sample was dried under nitrogen, then redissolved in 1ml chloroform:methanol (2:1 v/v). 2ml BF3 (14% w/v) was added to each sample that was then heated for 1 hour at 70˚C, producing methyl esters of the fatty acids present. These methyl ester derivatives were extracted with hexane and

then dried under nitrogen. The fatty acid methyl esters (FAMEs) were redissolved in hexane for analysis by GC-C-IRMS.

6.4.7. ANALYSIS BY GC-C-IRMS

FAMEs were analysed using a gas chromatograph (Hewlett Packard 5890) attached to an isotope ratio mass spectrometer (Geo 20/20, PDZ Europa Ltd., Crewe, UK) via a combustion interface (Orchid II, PDZ Europa Ltd.). Analysis of all samples except the modern fish extracts and ci0109c was performed using a 30m × 0.32 mm, 0.25mm internal diameter fused-silica column coated with BPX70 stationary phase (immobilised 70% cyanopropyl polysilphenylene-siloxane; 0.25mm film thickness; SGE, Milton Keynes, UK). Modern fish extracts and ci0109c were run through a 60m column of otherwise identical specifications (SGE, UK). The temperature program was as follows: 130°C (2min); 130°-190°C at 4°C/min; 190°C (2min). Helium was used as the carrier gas at a head pressure of 66.19kPa. The combustion furnace was maintained at 860°C and the mass spectrometer source pressure was 0.80-1.01kPa. The values were corrected for the derivatisation process by comparison with simultaneously analysed fatty acid standards that had been previously analysed by bulk IRMS (PDZ, Europa). The standards were derivatised according to the protocol used for the samples. Purified subsamples of the standard FAMEs were measured by bulk IRMS as a further control. Carbon isotopes were measured relative to the PDB standard (Belemnitella Americana), and are reported as in previous chapters. Extracts from all samples were run at least in triplicate. Modern fish sample values were corrected for the presence of post-industrial carbon. The burning of

fossil fuels since the advent of the Industrial Revolution has led to a decrease in the $\delta^{13}C$ of atmospheric CO_2 by approximately 1.14‰ over the last 130 years (Fredli *et al.* 1986).

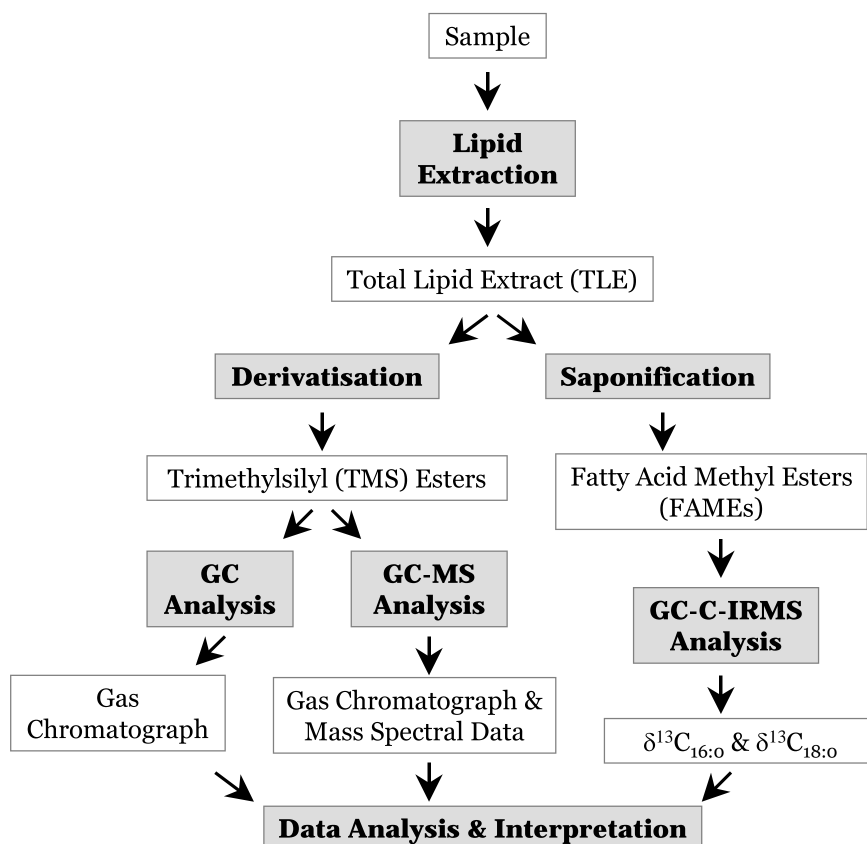

Figure 6.4. Procedure for archaeological and modern lipid analysis followed in this study. Shaded boxes represent preparatory or analytical procedures; white boxes indicate substances prepared and analysed and data obtained.

6.5. *RESULTS*

6.5.1. TOTAL LIPID YIELD

Evershed *et al.* (2002) considered total lipid extract of 100 to 1000 µg per gram of potsherd to be "appreciable quantities" of lipid worth analyzing. Of the 31 samples taken from the interior of a potsherd (residue or ceramic matrix), 15 were found to have a TLE yield of more than 100µg extract per gram original material (see table 6.1). However, many samples produced negative yield values, which is impossible as final mass for each sample was

calculated by subtracting the mass of the empty test tube from the combined TLE plus test tube mass. The apparent inaccuracy of the balance used to weigh the total lipid extracts made the quantification of sample yield difficult.

All extracts were initially analysed by gas chromatography, and those with very low lipid content were identified by their GC traces. Samples with low lipid content (listed in table 6.1) were not analysed further by GC-MS or GC-C-IRMS (with the exception of soil sample ci0128a) and are omitted from the following discussion.

6.5.2. GC-MS PEAK IDENTIFICATION

Chromatograms for all samples run by combined gas chromatography-mass spectrometry are shown in appendix 6. In most cases, only the portion of the total ion chromatogram (TIC) in which the major fatty acid and sterol peaks are present is shown. Parts of the TIC that have been omitted for most samples comprise the early section reflecting only rapidly-eluting solvents, and the segment late in the program where the baseline is extremely noisy and elevated. Major peaks are labeled on each sample's GC-MS trace.

Identifications of molecules were based on mass spectral data and elution order, with the assistance of the MS reference library held at the Department of Civil Engineering and Geosciences, University of Newcastle, UK. Characteristic MS peaks and peak distributions for trimethylsilyl derivatives of the main species identified (i.e., fatty acids, sterols, alcohols) are detailed in Stacey (1999). The most common molecular type present in the lipid extracts was fatty acids (present as trimethylsilyl esters). Figure 6.5 shows the mass spectrum obtained for the (derivatised) C16:0 fatty acid

extracted from sample ci12a, compared with the C16:0 trimethylsilyl ester reference spectrum. The peak representing the C16:0 molecular ion minus a methyl group (m/z 313) is highlighted. Ion peaks characteristic of fatty acids (m/z 117, 132, 145) are also indicated (see Stacey 1999). The molecular fragmentation and rearrangement that produce ions characteristic of fatty acids are illustrated.

The presence of acylglycerides was suggested by the GC traces of the archaeological lipid extracts obtained from two sherds (ci7a, ci0105a&b). Acylglycerides (particularly triacylglycerides) have been used in previous studies to identify the origins of archaeological lipid residues (Dudd *et al.* 1999, 2003). However, these molecules could not be identified by their mass spectra in this study. High baseline elevation obscured the portion of the chromatogram where triacylglycerides eluted, making it extremely difficult even to identify the molecules as acyl glycerides in many cases. In future, reference standards of known acylglyceride composition could be simultaneously analysed with archaeological extracts, in order to identify acyl glycerides by their elution times.

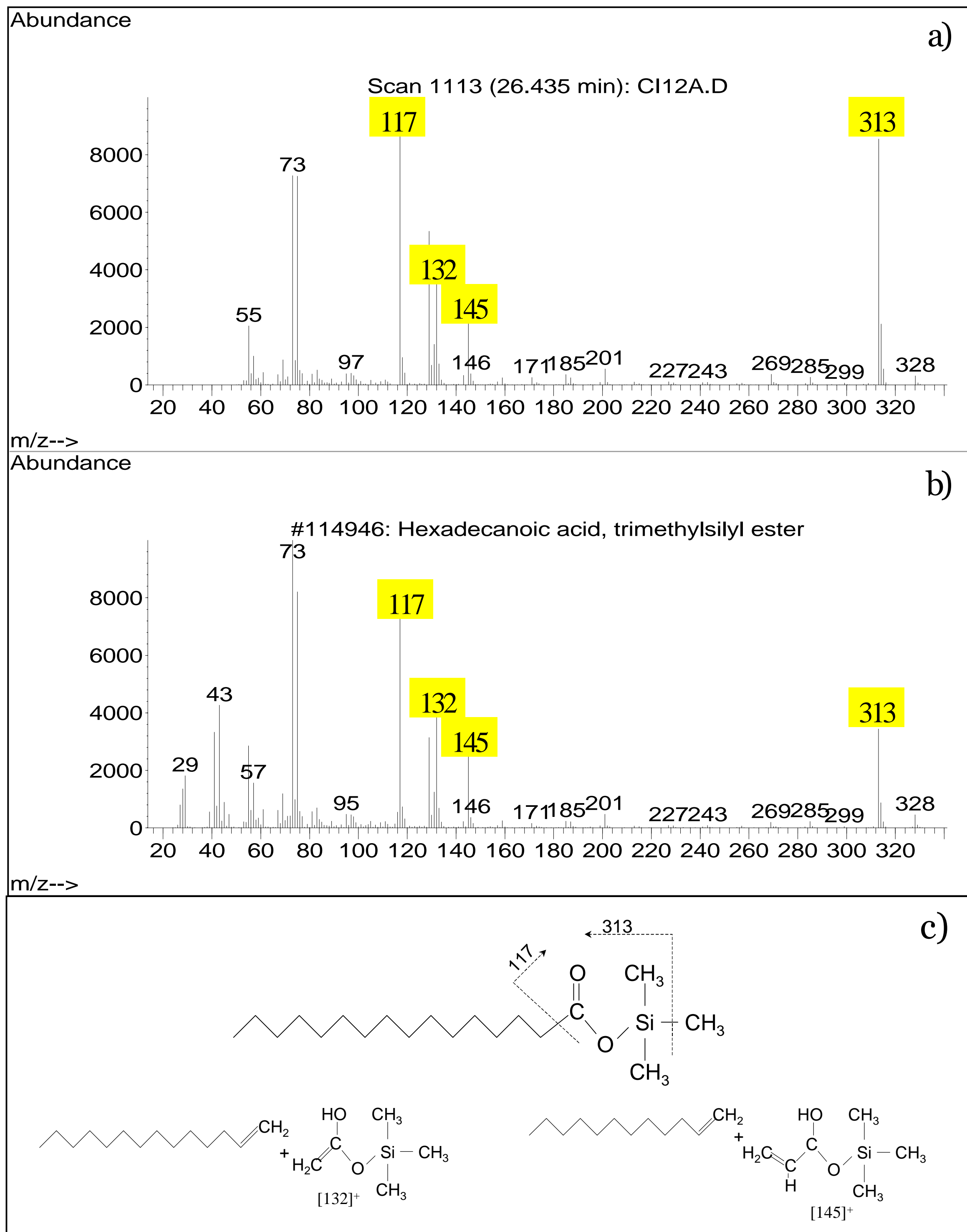

Figure 6.5. Mass spectrum of a molecular peak present in extract ci12a (a) identified as the trimethylsilyl ester of C16:0, compared with the reference spectrum for C16:0 TMS ester (b). The structure of the 16:0 TMS ester is also shown, with sites of fragmentation and resultant ions indicated (c). The m/z 132 and 145 peaks occur as the result of a McLafferty rearrangement of the fatty acid TMS ester (Evershed 1992).

All samples but one (ci0102b) contained phthalates in detectable quantities. Phthalates are compounds that are used in the manufacture of

plastics to impart qualities such as flexibility. These molecules are fat-soluble and can easily migrate into materials with which they come into contact (Kumar 1999). These contaminants could have derived from the plastic bags used to store the Chicha samples between their excavation and analysis, and/or from the plastic gloves that had to be used during parts of the analytical process. In some cases, phthalate peaks overlapped with the peak(s) of other molecules, hindering the identification of the specific molecular species. Analysis by GC-MS revealed that the major peaks identified in sample ci10a were phthalates. As a result, this sample is classified as low-yield and is not included in the assessment of samples analysed by GC-MS below.

6.5.2.1. *Soil*

In the single soil sample run by GC-MS (ci0128a), saturated fatty acids and long-chain alcohols (between C_{22} and C_{28}) account for most of the identifiable components. Long-chain saturated fatty acids and long-chain alcohols (C16 to C30) are two of the most abundant organic compounds present in soils (Morrison and Bick 1967; Jambu *et al.* 1993). The presence of these molecules in soil reflects the input of mainly plant-derived organic matter, with the long-chain alcohols principally derived from plant waxes (Jambu *et al.* 1993). The derivation of long-chain alcohols from the hydrolysis of plant wax ester linkages would explain the presence of free long-chain alcohols in the soil sample. Sitosterol is one of the two most abundant sterols occurring in higher plants (Rossell and Pritchard 1991), and its putative presence in the ci0128a extract is further suggestive of decaying plant

matter in the soil. Bacteria produce odd-numbered carbon straight-chain fatty acids and branched-chain fatty acid isomers (particularly of C15:0, C17:0 and C19:0; Jambu *et al.* 1993; Gunstone *et al.* 1994; Gharaibeh and Voorhees 1996). A degree of bacterial activity in the soil sample was evident from the presence of odd-numbered fatty acids (C15:0, C23:0, C25:0), though no branched-chain fatty acid isomers were detected. The odd-numbered straight-chain alcohols identified in ci0128a provided additional evidence for microbial activity in the soil, as these compounds can be formed by the bio-oxidation of soil alkanes (Jambu *et al.* 1993).

6.5.2.2. ***Potsherd Residues***

Extract ci0112b produced a distinct chromatogram from the other sherd extracts analysed. Hopanoids and even-numbered carbon chain alcohols were detected in the unique extract. In addition to odd- and branched-chain fatty acids and odd-numbered carbon chain alcohols, hopanoids are a biomarker indicative of bacterial activity. Hopanoids are a component of the cytoplasmic membranes of many bacteria, in which they are thought to take the place of steroids as structural stabilisers. The alcohols identified in the extract may be derived from a plant source, but the hopanoids indicate that this sample has been subjected to a high degree of microbial attack. Aside from samples ci0112b and ci0128a, no other samples exhibited identifiable levels of alcohols, hopanoids or sitosterol.

Cholesterol was found in eleven out of sixteen sherds whose extracts were analysed by GC-MS, excluding sample ci10a (see table 6.2). Cholesterol

is considered to be a reliable biomarker characteristic of animal fats. It is the main sterol present in animal fats, and is only found in trace amounts in some higher plants (Rossel and Pritchard 1991). Animal fats are further characterized by the presence of principally medium- to long-chain fatty acids, commonly saturated. C18:0 (stearic) fatty acid is a major component of animal fats, whereas it occurs only in small amounts in vegetable fats. C16:0 fatty acid is another widely occurring saturated fatty acid in animal fats, but is present in high levels in plant lipids as well. The dominance of the C18:0 fatty acid in all of the ceramic residues—with the exception of ci0112b—further demonstrates the animal origin of these residues.

Sample	Cholesterol	Short-Chain FA(s)	Odd-# C FA(s)	Branched-Chain FA(s)	C20:1 FA	Even-# C Alcohols	Hopanoids	Sitosterol
Sherds								
ci0102b	x	x	x	x	?			
ci0104b			x	x	?			
ci0105b			x	x				
ci0106a	x	x	x	x	?			
ci0108a	x		x					
ci0109a		x	x	x	x			
ci7a	x		x					
ci12a	x	x	x	x	x			
ci14a			x	x				
ci15a	x							
ci18a	x	x	x					
ci20a	x	x						
ci21a	x	x	x	x				
ci22a	x		x	x				
ci23a	x							
ci0112b						x	x	
Soil								
ci0128a			x			x		?

Table 6.2. The occurrence of major molecular species in the archaeological sample extracts. An 'x' indicates the presence of a particular molecular type; '?' represents uncertain identification. FA(s) stands for fatty acid(s).

In the extracts shown to have an animal source (or sources), a number of biomarkers are present that indicate a ruminant origin. Odd-numbered carbon saturated fatty acids (e.g., C15, C17 and C19) and branched-chain fatty acid isomers (e.g., C14, C15 and C17), commonly associated with bacterial

activity, can also be indicative of a ruminant fat source. These molecular species are produced by microbial metabolism in the rumen, and are incorporated into the body fats of the ruminant animals, including milk fats (Garton 1963; Gunstone *et al.* 1994). Twelve of the fifteen samples deemed to be of animal origin were found to contain odd-numbered carbon fatty acids and/or branched-chain fatty acid isomers (see table 6.2). Although the production of these molecular species by post-depositional bacterial attack cannot be ruled out, the high levels of C18:0 and C16:0 fatty acids—and in some cases the presence of cholesterol—support a primarily ruminant rather than a mainly bacterial origin (in contrast to the soil sample and residue ci0112b).

In distinguishing between ruminant adipose and milk fats, the presence of short-chain (i.e., C4-C10) saturated fatty acids is considered a marker for milk (de Man 1990; Evershed *et al.* 2002). Short-chain fatty acids are synthesized ***de novo*** from β-hydroxy-butyrate and acetate in the ruminant mammary gland, not by bacterial activity (Dils 1983). Of the samples that contained the molecular species representative of ruminant fats, seven also contained short-chain fatty acids (see table 6.2). The information provided by the main molecular species present in the sample extracts suggests that the majority of the vessels analysed had at one time been used to process ruminant fats, in the form of meat and/or milk.

The presence of fish lipids in the Chicha extracts is not clearly evident from the GC-MS data. The monounsaturate C20:1 fatty acid was positively identified in two samples (ci0109a and ci12a), and may also be present in the ci0102b, ci0104b and ci0106a extracts. As mentioned above (section 6.2), this fatty acid is found in fish oil as well as other lipids such as butterfat. The

presence of this fatty acid could thus only tentatively be interpreted as evidence for the processing of fish or a combination of fish and other animal fats. For those samples in which the C20:1 fatty acid was identified, ruminant dairy biomarkers were also detected, indicating a mixture of fish oils and milk fats or the sole presence of milk fats in the vessels. As expected due to their high susceptibility to oxidative degradation, no polyunsaturated fatty acids (i.e., those with two or more double bonds) were detected in any of the

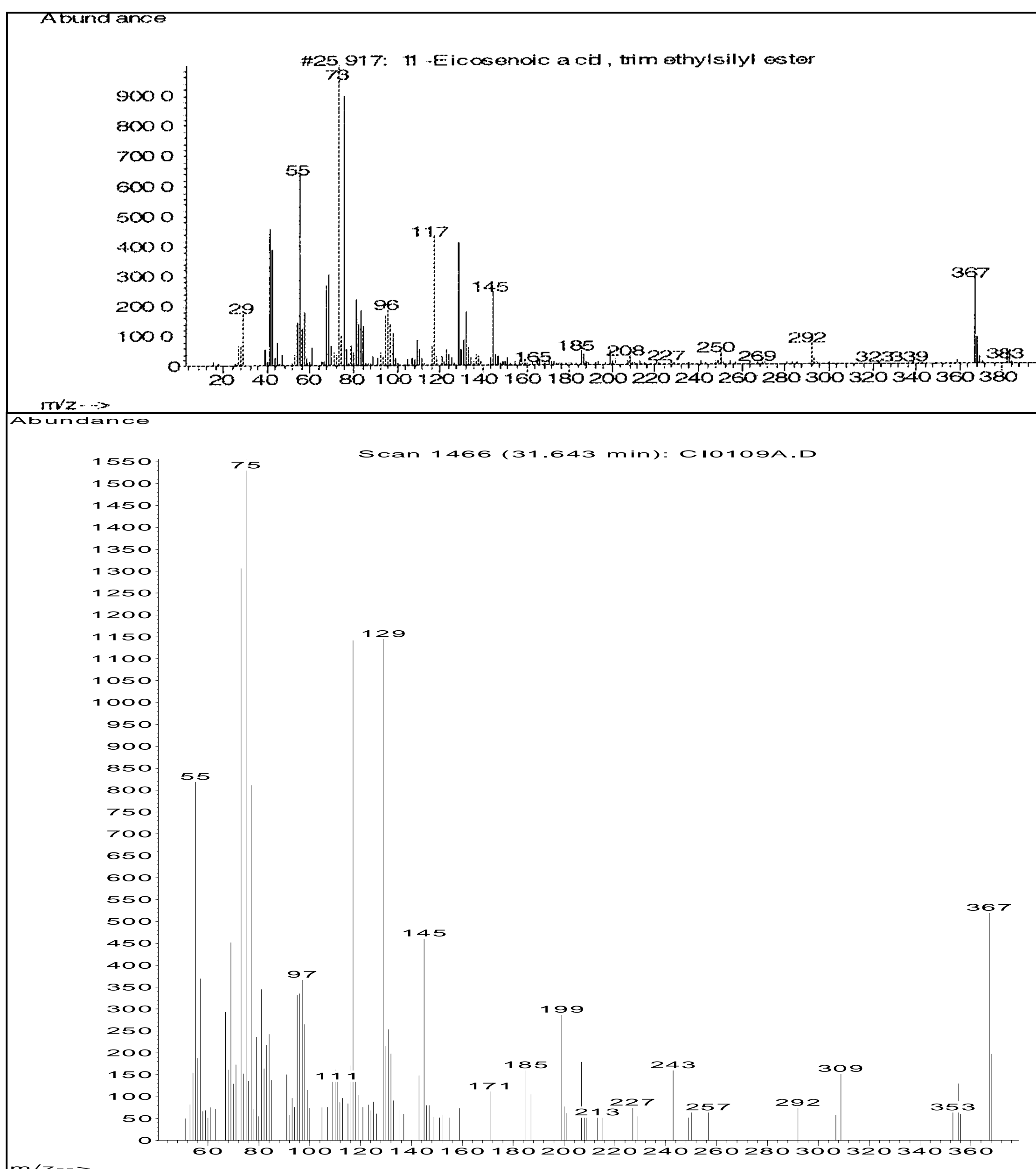

Figure 6.6. The reference mass spectrum for the C20:1 fatty acid (eicosenoic/gadoleic acid, top) and the mass spectrum obtained for a peak identified as C20:1 in the ci0109a extract from Chicha (bottom).

The concentration ratio of the C16:0 and C18:0 fatty acids has been used in previous studies to differentiate between the fat sources of archaeological extracts (e.g., ruminant *vs.* non-ruminant; Mottram *et al.* 1999). The concentrations of C16:0 and C18:0 fatty acids are calculated by taking into account free fatty acids and those bound up in acyl glycerides (most fatty acids in archaeological extracts are present in their free form). The C16:0 and C18:0 fatty acid profiles of archaeological extracts are likely to generally reflect the relative concentrations of the fatty acids in the original lipids deposited in the vessels, rather than the products of bacterial activity or the degradation of other molecular species (Dudd *et al.* 1998). C16:0 and C18:0 are hydrophobic and have low solubility in water. However, the relatively lower solubility of C18:0 relative to C16:0 makes the longer-chain fatty acid more resistant to groundwater leaching. The concentration ratio of the C16:0 and C18:0 fatty acids may thus be expected to decrease over time. C16:0 and C18:0 are not formed by the decomposition of other molecular species prevalent in modern animal fats, such as unsaturated fatty acids, and their ratio thus may be considered to be essentially unaffected by the decomposition of other lipids. The concentration ratio of C16:0 to C18:0 fatty acids in an archaeological extract may be compared to the corresponding concentration ratios of modern reference fats; but the likely decrease in this ratio over time must be kept in mind.

Concentration ratios for the Chicha archaeological samples were calculated by integrating the appropriate GC peak areas and then dividing the area calculated for the C16:0 fatty acid peak by that calculated for the C18:0 fatty acid peak. Integration was performed automatically by Atlas 2000 software (LabSystems, Thermo Electron Corp., Altrincham, UK) and visually

checked. C16:0 and C18:0 fatty acids have equivalent FID (detection) responses by GC (G. Taylor and O. Craig, personal communication; see Morrison and Bick 1967), so their peak areas may be compared with each other without the use of a correction factor. Concentrations ratios (in g C16:0 per g C18:0) for modern reference fats were obtained from the USDA food contents database, (www.nal.usda.gov/fnic/). Reference concentration ratios were multiplied by the molecular mass of the C18:0 fatty acid (254) and divided by the molecular mass of the C16:0 fatty acid (226) in order to obtain molecular ratios.

Most of the archaeological residues exhibited C16:0/C18:0 ratios similar to those given for modern reference ruminant fats (see figure 6.7). Samples ci12a and ci20a exhibited much higher C16:0/C18:0 values than the other samples, with values similar to those calculated for the modern freshwater fish reference fats. The values obtained for these samples indicate a non-ruminant fat source. None of the samples yielded C16:0/C18:0 ratios near the modern horse meat reference value. Considering the preferential loss of C16:0 relative to C18:0 over time, it is possible that the original source(s) of the archaeological lipid extracts contained relatively more C16:0 than indicated by the extract data. The proximity of the ci12a C16:0/C18:0 ratio value to those of freshwater fish, combined with the presence of the C20:1 fatty acid in the archaeological extract, suggests that the sample vessel may have been used to process freshwater fish as well as ruminant products.

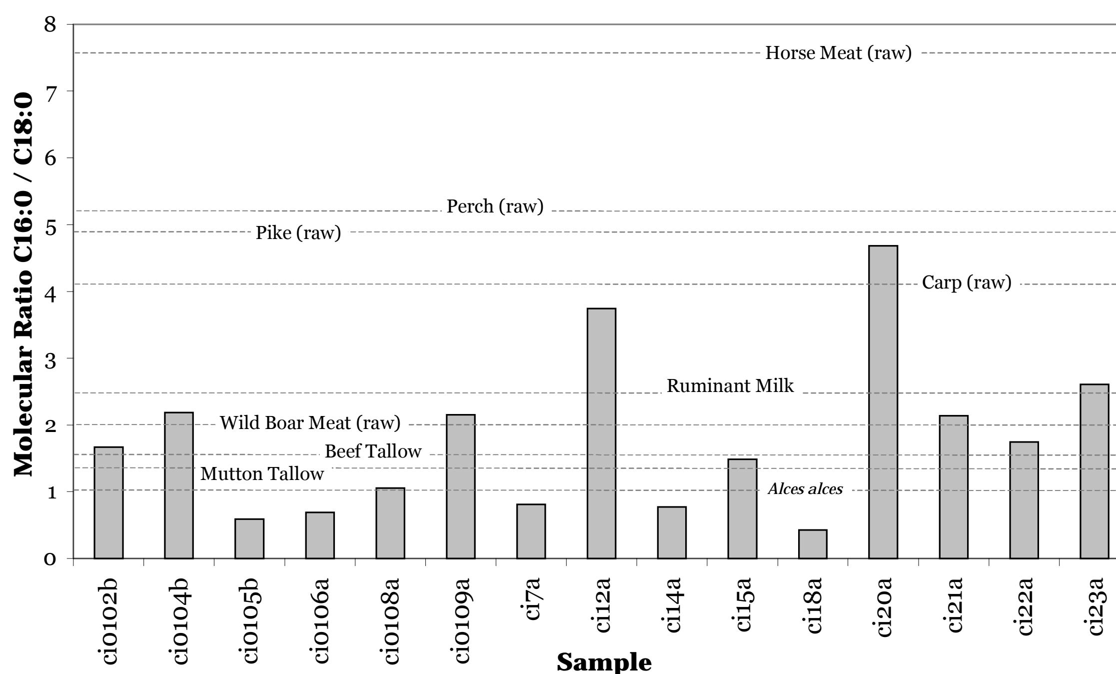

Figure 6.7. C16:0/C18:0 ratios for the archaeological extracts likely to be of animal origin. Reference ratio values for various potential animal food sources are given as dashed lines.

Although it may be useful to compare the ratios of certain molecular species between different food types, it is important to consider that the relative amounts of fatty acids in any plant or animal product are subject to variation, according to various factors such as diet and temperature. Therefore, the ratios discussed here should not be regarded as absolute, fixed values, but as approximate guidelines.

6.5.3. GC-C-IRMS

Samples ci0109a and ci14a were either not run or incorrectly named due to a label mis-reading in the laboratory before GC-C-IRMS processing. The GC-C-IRMS results for these two samples cannot be reported because isotopic values could not be assigned to either sample with certainty.

Modern and archaeological $\delta^{13}C$ reference values for C16:0 and C18:0 fatty acids from various species were compiled from data published by

Evershed *et al.* (2002) and data held by Dr. Oliver Craig at the University of Newcastle. Work by Evershed and colleagues (e.g., Evershed *et al.* 2002) has shown that the C16:0 and C18:0 $\delta^{13}C$ values of non-ruminants such as pigs and marine fish are heavier than those of ruminant species (e.g., see figure 6.8). While horse adipose fats, like ruminant fats, tend to be isotopically heavier in C18:0 relative to dairy fats, they can exhibit $\delta^{13}C$ values more depleted in C16:0 than ruminant milk and adipose fats on average. Causes for the observed isotopic differences between species are not certain, but probably include inter-specific differences in dietary preference and metabolism (Evershed *et al.* 2002).

In figure 6.8, the C16:0 and C18:0 fatty acid carbon isotope values for each archaeological sample are shown. Data from the archaeological extracts is plotted against the reference fat values of major potential food species. The $\delta^{13}C$ values of the modern references were corrected for the presence of post-industrial carbon; atmospheric CO_2 has undergone a carbon isotopic depletion of approximately 1.14‰ within the past 130 years (Friedli *et al.* 1986).

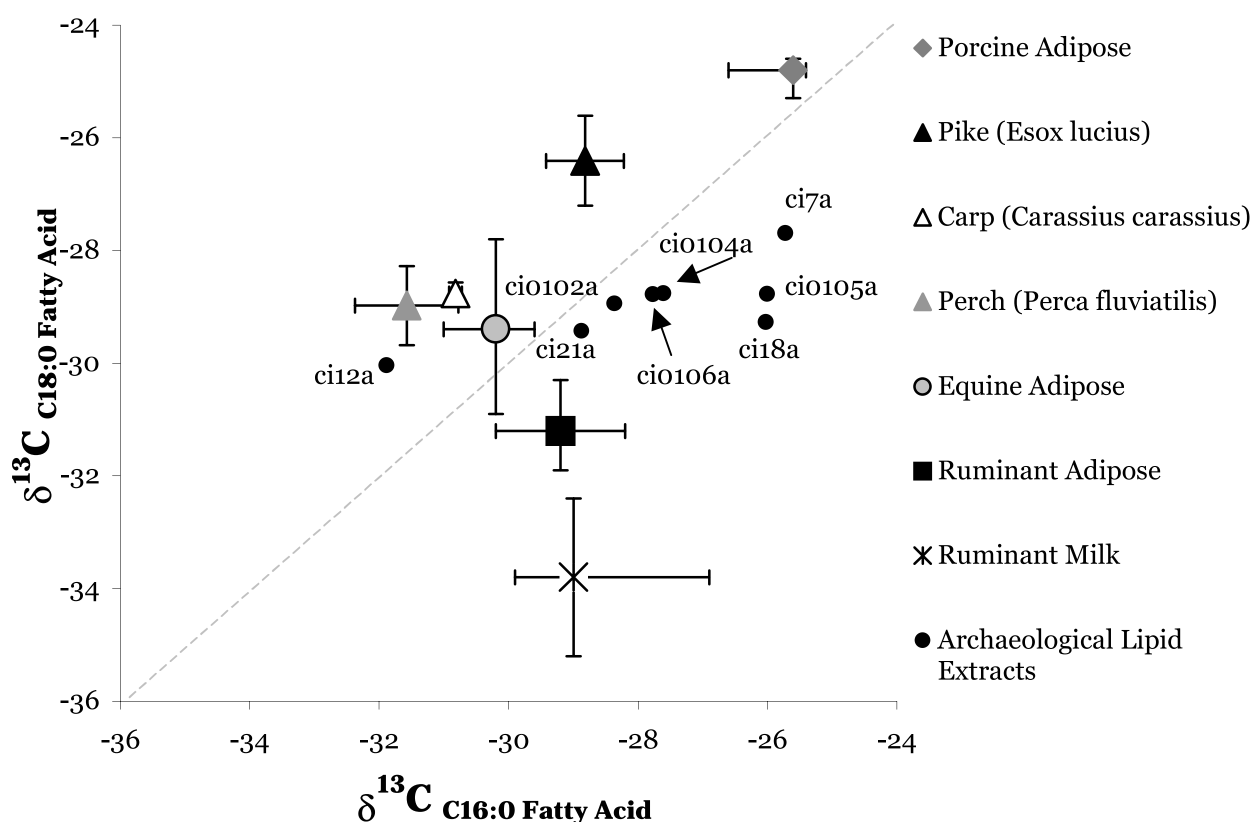

Figure 6.8. Plot of $\delta^{13}C$ values for C16:0 and C18:0 fatty acids recovered from the Chicha archaeological sherds. Modern reference sample values for porcine, ruminant adipose, horse adipose, ruminant milk and freshwater fish fats (including data from Evershed *et al.* (2002) and O. Craig, unpublished) are provided for comparison. The equation for the broken line is y=x. Error bars indicate ±1σ for the sample set. Analytical error is ±0.3‰ for both C16:0 and C18:0 $\delta^{13}C$.

The isotopic values of the archaeological extracts fall outside the 1σ ranges exhibited by the modern reference fats. Sample ci12a falls near the freshwater fish isotopic range (particularly perch), and ci21a is equally close to values for ruminant and equine adipose. The remainder of the archaeological samples exhibit isotopic values that could reflect a mixture of ruminant and nom-ruminant fats.

The data used as reference values in figure 6.8 were obtained from samples from northwestern Europe (including the UK), a temperate, C_3-dominated ecosystem. Craig *et al.* (in press) have found that lipids originating from animals fed on high-C_4 diets exhibit C16:0 and C18:0 carbon isotope values distinct from those originating from the same species fed on a C_3 diet.

This research shows that C16:0 and C18:0 $\delta^{13}C$ values of milk from cows fed a high-maize (C_4) diet are elevated (i.e., less negative) relative to those of C_3-fed cow's milk. The data indicate that C_4 dietary input enriches the $\delta^{13}C$ values of milk C16:0 and C18:0. Notably, regardless of dietary $\delta^{13}C$ composition, the average offset between C16:0 and C18:0 isotope values remains consistent.

A study by Evershed *et al.* on preserved archaeological lipids from the site of Ak-Alakha in the Altai mountain-steppe of southern Siberia obtained isotopic data similar to those of Craig, concluding that "comparison of the stable isotope data from the fats of animals raised in different geographic locations are not directly comparable" (2002:85). The study found that individual fatty acids obtained from the archaeological horse (skin) fat were ~2 to 3‰ less depleted than modern C_3 horse reference fats. The archaeological horse skin sample used by Evershed *et al.* as a reference horse fat (for comparison with archaeological ceramic vessel extracts) exhibited $\delta^{13}C$ values for the C16:0 and C18:0 fatty acids of -28.1‰ and -27.4‰, respectively.

The data from Craig *et al.* (in press) and Evershed *et al.* (2002) draw attention to a potential problem with interpreting the Chicha GC-C-IRMS data. According to Evershed *et al.* (2002), material from Botai, another Eurasian steppe site, could not be directly related to the reference isotope data generated from northern European material. However, regardless of provenance and diet, the isotopic evidence suggests that calculated C16:0-C18:0 $\delta^{13}C$ differences remain essentially the same for individuals of the same species. The average difference between $\delta^{13}C$ values for C16:0 and C18:0 fatty acids of horses raised on C_3 diets in northwestern Europe (-0.8‰) is similar to the C16:0-C18:0 $\delta^{13}C$ difference for the Evershed *et al.* (2002) Siberian

archaeological horse sample considered most likely to represent consumable horse fats (-0.7‰). The average isotopic difference between C16:0 and C18:0 $\delta^{13}C$ values is also conserved in the data from Craig *et al.* (in press) between C_3-fed and C_4-fed cow's milk (4.8‰ for both).

In the light of these studies, the isotopic data for the Chicha samples was arranged in a different fashion—as a single value for each sample, representing the difference between the carbon isotope values for the C16:0 and C18:0 fatty acids ($\Delta^{13}C = \delta^{13}C_{16:0} - \delta^{13}C_{18:0}$, see figure 6.9). In addition to the Chicha samples, $\Delta^{13}C$ average values were calculated for modern reference fats. Minimum $\Delta^{13}C$ values were determined by subtracting the maximum (mean+1σ) $\delta^{13}C_{18:0}$ value from the minimum (mean-1σ) $\delta^{13}C_{16:0}$ value for each reference set; maximum $\Delta^{13}C$ values were derived from the maximum $\delta^{13}C_{16:0}$ value minus the minimum $\delta^{13}C_{18:0}$ value. The data represented in figure 6.9 show that ruminant milk and adipose exhibit entirely positive $\Delta^{13}C$ values, while porcine and fish values fall below zero. On average, horse adipose exhibits a $\Delta^{13}C$ value of -0.8‰, but the calculated maximum range of horse adipose values overlaps with those of ruminant adipose, fish and porcine fats.

All but one of the archaeological extracts have $\Delta^{13}C$ values that fall in the range calculated for ruminant adipose and milk fats. Two samples, ci0102a and ci21a, exhibit values that also overlap with the reference horse adipose fat values. On the plot of $\delta^{13}C_{16:0}$ versus $\delta^{13}C_{18:0}$, ci21a was the sample with values nearest the horse reference values. Sample ci12a has a $\Delta^{13}C$ distinct from all other archaeological extracts. The $\Delta^{13}C$ value for ci12a is negative (-1.9‰), and falls within the range of all of the freshwater fish reference sample values. The ci12a $\Delta^{13}C$ value also lies within the maximum ranges calculated for the horse adipose and porcine fat reference values.

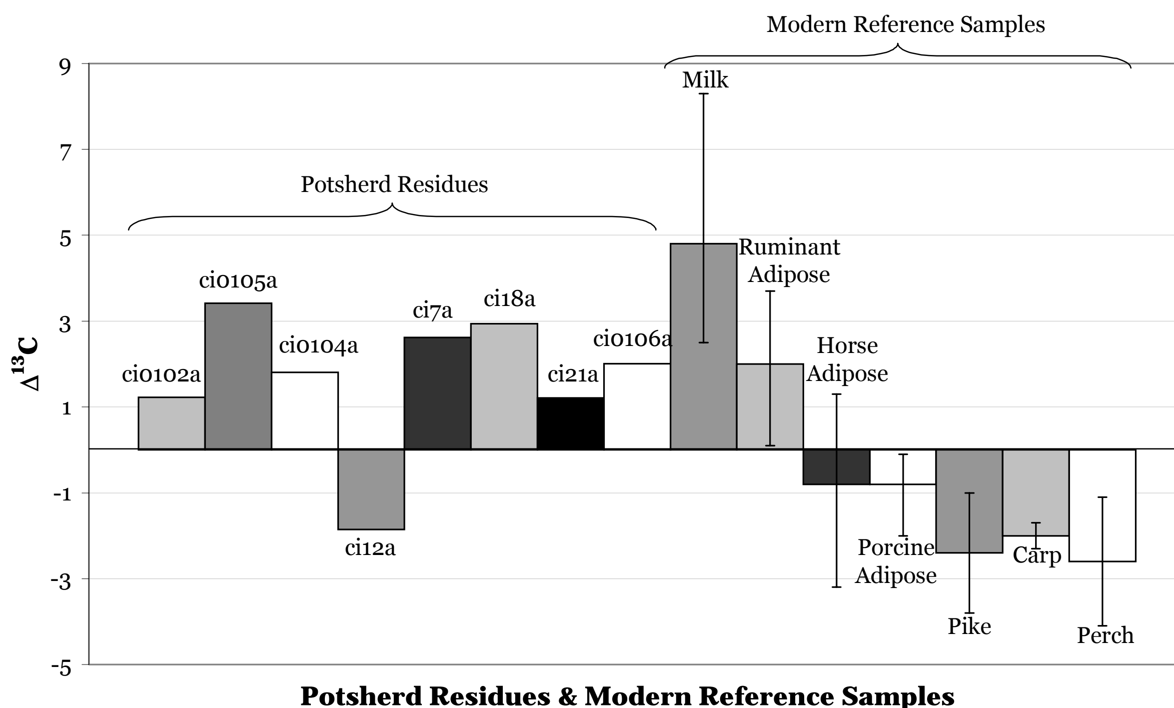

Figure 6.9. Plot of $\Delta^{13}C$ for modern references and all Chicha samples analysed by GC-C-IRMS. Maximum-minimum calculated error is shown for each reference.

6.6. *CONCLUSIONS*

The GC and GC-MS data show that the majority of the Chicha sherds analysed contained significant amounts of well-preserved lipid residues derived from the processing of animal products. The extract from the inner surface of ci0112 contained long-chain alcohols that could be indicative of a plant source. The soil lipid profiles as well as that of the outer surface of sherd ci0112 are distinct from the profiles of the inner surface extracts of the Chicha samples, demonstrating that the lipids were derived from the vessel contents rather than from the surrounding soil.

The lipid compositions of the majority of the archaeological extracts suggest a primarily ruminant fat origin, with seven samples yielding short-chain fatty acids indicative of a dairy source. The GC-C-IRMS data further support the conclusion that ruminant fats were processed at Chicha. For

those samples lacking short-chain fatty acids, the C16:0 and C18:0 fatty acid isotope values further support a ruminant over a non-ruminant origin. The prevalence of ovicaprid and cattle bone at Chicha (Molodin *et al.* 2001, 2002) corresponds to the residue data, indicating that these species were extensively exploited by humans at the site, probably in part for food.

The results of this study provide the only evidence to date for the practice of dairying at the site of Chicha. No research into kill-off patterns has yet been conducted on cattle and ovicaprid remains from the site, which could also provide useful information about the management of domestic herds at Chicha. The residue data suggest that the exploitation of ruminant animal products involved a significant amount of dairy production. The economic pursuits of the Late Bronze Age-Early Iron Age inhabitants of Chicha therefore included not only fishing and meat production from their domestic animals, but must have entailed some degree of dairy production as well.

C20:1 fatty acid (gadoleic, a possible fish biomarker) was identified in two of the archaeological extracts from Chicha (ci0109a and ci12a). These two extracts also exhibited short-chain fatty acids indicative of ruminant dairy products, although the ratio of C16:0 to C18:0 in extract ci12a is suggestive of a non-ruminant fat origin. Sample ci0109a, which dates to the Late Bronze Age, was not analysed by GC-C-IRMS. Though sample ci12a may also have been used for the preparation of ruminant (dairy) fats, the $\delta^{13}C$ values obtained for the sample strongly suggest that the principal source of the vessel lipids were derived from a non-ruminant source, possibly freshwater fish. This sample dates from the Bronze Age-Iron Age transitional period, for which no human bone stable isotope values are available.

The stable isotope analysis of human bone collagen at Chicha (see chapters 3 and 5) was performed using osteological material dated to the Late Bronze Age. Notwithstanding the high quantity of cattle and sheep bones at the site, the human palaeodietary analysis indicated a high fish diet for the Late Bronze Age inhabitants of Chicha. Potsherd samples ci0102b and ci0109a date from the Late Bronze Age. These samples contained short-chain fatty acids suggestive of ruminant milk processing, though both samples also show evidence for the presence of possible non-ruminant fats. Extract ci0109a has a similar GC-MS profile to that of ci12a, and also contains an identifiable amount of C20:1 fatty acid. If the isotopic data were available for this sample, its ruminant or non-ruminant origins could have been better identified. Sample ci0102b has a $\Delta^{13}C$ value that could be interpreted as indicative of either ruminant or horse fats.

The results of the vessel residue analysis and bone stable isotope analysis are not necessarily conflicting. The apparent dominance of ruminant fats in the Chicha vessels reflects the processing of such fats at one or more use events. Ruminant fats were obviously exploited to some degree at Chicha during the Late Bronze Age through the Early Iron Age. Whether this exploitation was conducted regularly and for what purpose (i.e., ritual or domestic) we cannot accurately tell from these analyses. The deposition of lipids on the archaeological vessels may have occurred as a single event or series of events, the frequency and context of which we cannot determine. However, the semi-quantitative dietary information provided by the bone stable isotope analysis indicates that the human diet at Chicha was not based exclusively or mostly upon ruminant products, but on freshwater resources, probably fish. The detection of ruminant fats in the Chicha vessels may reflect

the preferential processing of meat and milk in vessels, while fish were prepared in other ways. Since the majority of the residues date from a later period than the bone samples used for palaeodietary analysis, we must also consider the possibility that the dietary dependence upon freshwater resources waned over time at Chicha.

Looking beyond Chicha to the implications of this work for future residue studies, the data presented here suggest that freshwater fish lipids can indeed be isotopically distinguished from other animal fats, particularly those of ruminant adipose and milk. This work also highlights the challenges involved in positively identifying freshwater fish lipids in archaeological residues. Perhaps in future, lipid biomarkers specific to freshwater fish may be targeted. Furan-containing acids have been shown to occur in fish in quantities up to 20% of total lipids (Gunstone *et al.* 1978). Although these acids occur in both freshwater and marine species, their detection in residues at an inland site such as Chicha would undoubtedly be a useful indicator of freshwater fish lipids. The durability of such potential biomarkers over archaeological time spans must be investigated before they can be utilised in ancient residue studies.

As a complementary technique to lipid analysis for assessing the nature of food residues recovered from archaeological vessels, protein extraction and identification has proven to yield important complementary information about the presence of freshwater fish lipids. The DACIA technique employed by Craig and colleagues (Craig and Collins 2000; Craig *et al.* in press) has allowed them to positively identify bovine milk lipids in ceramic vessels up to 3000 years old. This technique involves the production of monoclonal

antibodies that bind exclusively to a site on a specified protein molecule (such as bovine α-casein). Using DACIA, one could target a protein specific to freshwater fish or specific freshwater species. The creation of monoclonal antibodies is a very expensive process, particularly for proteins that require sequencing before antibody production can be undertaken. Furthermore, this technique relies upon the survival of protein residues in archaeological vessels, which is generally poor relative to lipid preservation. Nevertheless, the extremely good preservation of the lipid extracts and bone collagen obtained from Chicha suggest that the additional expense and chemical processing involved in protein residue analysis would prove fruitful in revealing further details of the food resources exploited by humans at the site.

Notwithstanding the potential benefits of protein residue analysis by the DACIA method, the drawbacks of expense (for protein sequencing) and hydrofluoric acid extraction required for this technique may cause archaeological science to rely upon lipid residue analysis for some time. The techniques of lipid extraction and analysis are straightforward, and as this study has shown, further information can be gleaned from the already established analytical techniques regarding food sources previously unexplored.

CHAPTER 7

FINAL SYNTHESIS

7.1. *SYNOPSIS: MAJOR CONTRIBUTIONS OF RESEARCH PRESENTED*

This thesis was designed primarily to test the hypothesis that freshwater fish were an important dietary staple for the Bronze Age and Iron Age communities of the central Eurasian steppe regions. Through the direct analysis of ancient human diet by stable carbon and nitrogen analysis, the work presented here has demonstrated the frequent consumption of freshwater fish by many Bronze and Iron Age Eurasian steppe humans. Analysis of fermented dairy products has allowed me to conclude that such high $\delta^{15}N$ values as those observed for many Eurasian steppe humans must be due to the frequent consumption of fish rather than a diet high in dairy products. I have proposed a revision of the current view of human diet and economic practices of this period to include the major role of fish acquisition and consumption in the daily lives of many communities.

The large scale of the data set analysed for this research has allowed the examination of dietary trends within and between groups of different geographical areas, cultures and time periods. On average, Bronze Age and Iron Age human diet are very similar, based on a mixture of fish and terrestrial herbivore products. But within both the Bronze Age and the Iron Age, dietary differences can be observed between humans assigned to different cultural groups. A particularly interesting trend apparent in the isotopic data is the correlation between diet and environment. In the Urals and western Siberia, the isotope data indicate that a higher degree of dietary similarity existed between human groups from sites located in the same ecological region (i.e., steppe or forest-steppe) rather than between closely

associated cultural groups. Likewise, Ukrainian Scythian humans exhibited isotope values closest to those of humans from a Greek settlement in Ukraine, and entirely distinct from those of Scythian individuals from other regions (the Urals/western Siberia and the Altai).

In addition to the evidence provided by this study for the frequent consumption of fish amongst many Bronze and Iron Age Eurasian steppe groups, the isotope data obtained have raised the issue of millet consumption and the importance of millet in the diets of these peoples. The data presented show a shift toward less negative $\delta^{13}C$ values from the Late Bronze Age through to the Iron Age among humans from Ukrainian sites. The great isotopic distance between human and faunal $\delta^{13}C$ values indicates that these less depleted human $\delta^{13}C$ values are the result of the direct consumption of C_4 foods (i.e., millet) rather than the consumption of the products of animals fed a high-C_4 diet. The results suggest that millet was an important component in the human diet, at least for communities in the Ukraine during the Iron Age.

The sulphur isotope analysis of humans and fauna from the site of Chicha further supports the conclusion that the high $\delta^{15}N$ values exhibited by the Chicha humans is a reflection of high fish consumption. The $\delta^{34}S$ values obtained for the human and fauna from the site of Bil'shivtsi also draw attention to the potential benefit that sulphur isotope analysis may provide in palaeodietary studies. This research has shown that our ability to assess ancient human diet may be significantly enhanced by the addition of sulphur isotope analysis to the palaeodietary toolkit. Furthermore, the study of modern and archaeological bone collagen for $\delta^{34}S$ and sulphur content presented here has contributed to the limited body of knowledge relating to the general utility of sulphur isotopes in palaeodietary research. In addition to

criteria proposed by Leach *et al.* (2001) and Richards *et al.* (2000), I put forward a set of conditions that may be used to evaluate the quality of bone collagen and the validity of bone collagen $\delta^{34}S$ values.

In addition to the direct analysis of human diet by bone collagen stable isotope analysis, lipid residue analysis of modern samples and archaeological vessels has contributed to our picture of fish consumption and overall food resource exploitation at the site of Chicha. The bone collagen isotope data reflect the dominant importance of freshwater fish in the Late Bronze Age human diet at Chicha, but residue analysis indicates that dairying was also practised at the site during this period. The prevalence of ruminant (primarily milk) fat residues recovered from vessels dated to the subsequent Late Bronze Age-Early Iron Age transitional period suggests that ruminant products played a significant role in the diet of Chicha humans during that time. For the first time, this research has demonstrated that lipid residues derived from freshwater fish may be distinguishable from those derived from terrestrial and marine sources by a combination of GC-MS and GC-C-IRMS analyses. One vessel residue from Chicha exhibited a lipid profile and C16:0 and C18:0 $\delta^{13}C$ values suggestive of a freshwater fish source. The additional presence of dairy lipid biomarkers in the same vessel extract indicate that the vessel may have been used to process a number of different foods.

The results of the research presented here promote a re-evaluation of the everyday subsistence practices of the Bronze and Iron Age peoples of the central Eurasian steppe region. In contrast to a picture of uniform dependence upon terrestrial domesticates as a principal dietary resource, this thesis has provided extensive evidence for the frequent consumption of

freshwater resources (e.g., fish) by many Eurasian steppe groups. For the groups identified as subsisting heavily upon freshwater fish, the data presented here require us to consider that the acquisition of fish—whether directly by fishing or indirectly through trade—must have been a major economic pursuit. Further palaeodietary analyses (including $\delta^{34}S$ analysis) of Bronze Age and Iron Age individuals from this area will continue to enhance our understanding of the dietary and economic role of freshwater fish throughout the Eurasian steppe. Future food residue studies will provide additional information regarding resources exploited for consumption and about food processing techniques. The methodological developments described herein will be of use in any future palaeodietary projects (whether focused on Bronze and Iron Age central Eurasia) in which freshwater fish consumption is an important dietary and economic issue.

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APPENDICES

APPENDIX 1. *CHAPTER 3 SAMPLE INFORMATION*

Table A1.1. Iron Age samples pertaining to the Scythian culture, from mountain-steppe sites in the Altai region of Russia.

Site	Sample	Species	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Sebÿstei	SEB 96 K2a	*Homo sapiens*	-18.1	13.2	3.3	43.6	15.6
Sebÿstei	SEB 96 K1b	*Homo sapiens*	-18.1	11.7	3.2	47.8	17.3
Kizil	KZ 3a	*Homo sapiens*	-19.0	10.2	3.3	46.2	16.4
Kizil	KZ 2	*Equus caballus*	-20.3	2.4	3.3	49.0	17.3
Kizil	KZ 1	*Equus caballus*	-19.4	5.4	3.2	42.7	15.4

Table A1.2. Samples from the site of Chicha. All samples analysed belong to the the Late Bronze Age period of site occupation, associated with the Late Irmen culture.

Sample	Species	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
CHA 16	*Homo sapiens*	-20.2	14.4	3.2	41.9	15.4
CHA 37	*Homo sapiens*	-20.0	13.8	3.1	40.2	15.0
CHA 14	*Homo sapiens*	-19.9	15.9	3.2	43.6	16.0
CHA 13	*Homo sapiens*	-19.8	15.5	3.2	42.3	15.6
CHA 1	*Homo sapiens*	-19.8	12.5	3.3	42.2	15.0
CHA 33	*Homo sapiens*	-19.8	14.9	3.2	44.3	16.3
CHA 32	*Homo sapiens*	-19.3	15.1	3.2	45.0	16.5
CHA 15	*Homo sapiens*	-19.2	15.0	3.2	42.4	15.4
CHA 35	*Homo sapiens*	-19.1	14.8	3.1	42.2	15.7
CHA 34	*Homo sapiens*	-18.6	14.6	3.1	43.6	16.2
CHA 36	*Homo sapiens*	-18.6	13.3	3.2	46.9	17.3
CHA 17	*Alces alces*	-20.4	3.1	3.2	43.2	15.7
CHA 6	*Alces alces*	-20.3	2.7	3.3	43.7	15.4
CHA 4	*Bos taurus*	-20.3	4.5	3.3	45.8	16.3
CHA 10	*Bos taurus*	-20.1	7.3	3.3	40.6	14.5
CHA 12	*Bos taurus*	-19.8	9.2	3.2	42.0	15.1
CHA 43	*Bos taurus*	-19.0	7.0	3.2	47.0	17.3
CHA 45	*Canis familiaris*	-22.7	11.2	3.1	40.4	15.0
CHA 2	*Canis familiaris*	-22.2	9.4	3.4	43.2	14.9
CHA 48	*Canis familiaris*	-21.0	11.8	3.1	42.7	15.9
CHA 44	*Canis familiaris*	-20.9	13.1	3.2	43.1	16.0
CHA 46	*Canis familiaris*	-20.4	11.6	3.2	44.6	16.3
CHA 47	*Canis familiaris*	-19.9	13.6	3.1	40.0	14.9
CHA 28	*Carassius carassius*	-24.6	11.5	3.2	40.1	14.6
CHA 29	*Carassius carassius*	-24.2	10.3	3.2	43.0	15.5
CHA 59	*Carassius carassius*	-23.7	10.0	3.2	43.0	15.8
CHA 61	*Carassius carassius*	-23.5	9.9	3.2	43.7	16.1
CHA 26	*Carassius carassius*	-23.4	11.0	3.2	38.9	14.1
CHA 30	*Carassius carassius*	-23.4	10.4	3.3	43.0	15.4

Sample	Species	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
CHA 27	*Carassius carassius*	-23.4	10.5	3.3	41.9	14.8
CHA 31	*Carassius carassius*	-23.3	9.9	3.3	39.1	14.0
CHA 60	*Carassius carassius*	-23.2	10.0	3.2	41.7	15.2
CHA 9	*Carassius carassius*	-21.4	8.7	3.3	40.9	14.4
CHA 40	*Equus caballus*	-20.9	4.8	3.3	29.3	10.5
CHA 38	*Equus caballus*	-20.3	4.6	3.1	43.5	16.1
CHA 39	*Equus caballus*	-20.3	5.0	3.2	44.2	16.3
CHA 3	*Equus caballus*	-20.3	4.4	3.3	42.2	15.1
CHA 11	*Equus caballus*	-20.2	6.2	3.3	43.1	15.5
CHA 42	*Equus caballus*	-19.6	6.6	3.3	39.8	14.3
CHA 25	*Esox lucius*	-25.3	9.8	3.3	42.6	15.3
CHA 21	*Esox lucius*	-23.9	12.2	3.3	41.9	14.8
CHA 23	*Esox lucius*	-23.2	11.3	3.3	41.9	14.9
CHA 7c	*Esox lucius*	-22.8	8.4	3.3	40.8	14.3
CHA 24	*Esox lucius*	-22.1	12.4	3.3	40.0	14.3
CHA 7b	*Esox lucius*	-22.1	7.8	3.3	32.9	11.7
CHA 22	*Esox lucius*	-20.9	11.2	3.2	37.1	13.7
CHA 7a	*Esox lucius*	-15.9	9.9	3.2	34.0	12.3
CHA 5	*Ovis aries*	-19.4	5.7	3.3	33.4	11.8
CHA 20	*Perca fluviatilis*	-23.1	12.5	3.3	43.4	15.5
CHA 19	*Perca fluviatilis*	-22.5	12.3	3.3	39.0	14.0
CHA 18	*Perca fluviatilis*	-22.2	13.8	3.3	43.0	15.4
CHA 8a	*Perca fluviatilis*	-21.1	9.0	3.4	43.7	15.2
CHA 8c	*Perca fluviatilis*	-20.2	8.4	3.4	42.6	14.8
CHA 8b	*Perca fluviatilis*	-19.7	11.2	3.2	39.0	14.1
CHA 50	*Sus scrofa*	-21.5	6.6	3.2	39.4	14.5
CHA 49	*Sus scrofa*	-20.1	6.9	3.2	43.4	16.0

Table A1.3. Human Bronze Age samples from Urals and western Siberian sites.

Site	Sample	Culture	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Isiney I	ISI 4	Alakul	-18.4	11.9	3.2	25.7	9.3
Isiney I	ISI 5	Alakul	-20.3	11.4	3.2	39.0	14.2
Isiney I	ISI 3	Alakul	-19.1	10.5	3.2	40.5	14.7
Isiney I	ISI 1	Alakul	-18.8	11.6	3.3	41.6	14.8
Isiney I	ISI 6	Alakul	-18.8	11.6	3.2	43.6	16.0
Isiney I	ISI 2	Alakul	-18.8	11.3	3.2	44.0	16.0
Nikolaevka II	NIK 1	Alakul	-18.9	10.6	3.2	41.2	14.9
Novonikolskoye I	NOV 3	Alakul	-19.4	10.4	3.2	41.3	15.0
Solonchanka IB	SOLIB 2	Alakul-Srubnaya	-18.5	11.7	3.2	40.8	14.8
Bolshekaragansky	BKAR 4	Sintashta	-19.3	10.5	3.3	34.4	12.2
Bolshekaragansky	BKAR 12	Sintashta	-18.9	11.7	3.2	38.2	13.8
Bolshekaragansky	BKAR 14	Sintashta	-19.2	10.8	3.2	39.4	14.2
Bolshekaragansky	BKAR 6	Sintashta	-18.3	11.6	3.2	41.5	15.3
Bolshekaragansky	BKAR 13	Sintashta	-19.3	11.2	3.2	41.6	15.1
Bolshekaragansky	BKAR 7	Sintashta	-18.9	10.5	3.2	42.1	15.4
Bolshekaragansky	BKAR 8	Sintashta	-18.2	11.2	3.2	42.6	15.7
Bolshekaragansky	BKAR 1	Sintashta	-18.0	11.8	3.2	43.1	15.7
Bolshekaragansky	BKAR 9	Sintashta	-18.3	11.5	3.2	43.1	15.8
Bolshekaragansky	BKAR 3	Sintashta	-18.9	10.3	3.2	43.1	15.8
Bolshekaragansky	BKAR 11	Sintashta	-19.1	10.6	3.2	43.7	16.0
Bolshekaragansky	BKAR 10	Sintashta	-19.2	9.5	3.2	44.3	16.1
Bolshekaragansky	BKAR 2	Sintashta	-19.1	9.7	3.2	44.4	16.3
Bolshekaragansky	BKAR 5	Sintashta	-19.2	10.3	3.3	45.7	15.9
Kamenni Ambar V	KAM 19	Sintashta	-17.8	13.3	3.2	26.7	9.7
Kamenni Ambar V	KAM 37	Sintashta	-17.9	12.7	3.2	37.3	13.6
Kamenni Ambar V	KAM 28	Sintashta	-18.9	11.7	3.2	38.2	13.9
Kamenni Ambar V	KAM 22	Sintashta	-18.5	11.5	3.2	40.1	14.7
Kamenni Ambar V	KAM 32	Sintashta	-17.6	13.4	3.2	40.4	14.8
Kamenni Ambar V	KAM 24	Sintashta	-18.0	12.8	3.2	40.5	14.6
Kamenni Ambar V	KAM 36	Sintashta	-17.5	14.6	3.2	40.6	14.8
Kamenni Ambar V	KAM 38	Sintashta	-18.3	12.7	3.2	41.2	15.0
Kamenni Ambar V	KAM 26	Sintashta	-19.3	11.3	3.2	41.3	14.9
Kamenni Ambar V	KAM 34	Sintashta	-17.1	14.8	3.2	41.6	15.1
Kamenni Ambar V	KAM 25	Sintashta	-18.3	13.9	3.2	41.6	15.1
Kamenni Ambar V	KAM 21	Sintashta	-18.4	12.3	3.2	41.8	15.2
Kamenni Ambar V	KAM 33	Sintashta	-17.6	13.4	3.2	41.9	15.1
Kamenni Ambar V	KAM 35	Sintashta	-17.7	14.2	3.2	41.9	15.2
Kamenni Ambar V	KAM 29	Sintashta	-18.0	11.9	3.2	42.2	15.4
Kamenni Ambar V	KAM 30	Sintashta	-18.8	11.7	3.2	42.7	15.6
Kamenni Ambar V	KAM 23	Sintashta	-17.9	12.0	3.2	42.7	15.6
Kamenni Ambar V	KAM 27	Sintashta	-17.8	13.7	3.2	42.8	15.6
Kamenni Ambar V	KAM 31	Sintashta	-18.3	12.0	3.2	43.0	15.7

Site	Sample	Culture	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Bestamak	BES 8	Sintashta-Petrovka	-18.8	10.7	3.2	41.0	14.8
Bestamak	BES 16	Sintashta-Petrovka	-19.2	10.5	3.3	41.0	14.6
Bestamak	BES 13	Sintashta-Petrovka	-18.0	13.6	3.2	41.0	14.8
Bestamak	BES 11	Sintashta-Petrovka	-17.8	12.9	3.2	41.2	14.9
Bestamak	BES 17	Sintashta-Petrovka	-18.7	11.2	3.3	41.2	14.6
Bestamak	BES 9	Sintashta-Petrovka	-19.1	10.5	3.2	41.2	14.9
Bestamak	BES 15	Sintashta-Petrovka	-18.7	11.0	3.2	41.4	14.9
Bestamak	BES 14	Sintashta-Petrovka	-18.9	10.6	3.2	41.5	15.1
Bestamak	BES 12	Sintashta-Petrovka	-18.9	11.0	3.2	41.7	15.1
Bestamak	BES 18	Sintashta-Petrovka	-19.3	10.5	3.2	41.9	15.2
Bestamak	BES 6	Sintashta-Petrovka	-19.4	10.8	3.3	41.9	14.8
Bestamak	BES 10	Sintashta-Petrovka	-18.4	12.3	3.2	42.0	15.2
Bestamak	BES 19	Sintashta-Petrovka	-19.0	10.8	3.3	42.2	15.1
Bestamak	BES 7	Sintashta-Petrovka	-19.0	11.0	3.2	42.2	15.3

Table A1.4. Faunal Bronze Age samples from Urals and western Siberian sites.

Site	Sample	Species	Environment	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Berlik	BER 1	*Bos taurus*	steppe	-20.6	3.7	3.2	30.6	11.0
Berlik	BER 2	*Bos taurus*	steppe	-19.6	6.9	3.3	42.3	15.2
Bestamak	BES 3	*Bos taurus*	steppe	-18.5	8.4	3.3	40.4	14.5
Bestamak	BES 5	*Canis familiaris*	steppe	-19.0	10.5	3.3	42.3	15.0
Bestamak	BES 4	*Canis familiaris*	steppe	-18.5	10.1	3.3	42.4	15.2
Bestamak	BES 1	*Equus caballus*	steppe	-20.0	6.2	3.3	41.6	14.9
Bestamak	BES 2	*Ovis aries*	steppe	-17.7	7.8	3.3	41.4	14.6
Bolshekaragansky	BKAR 16	*Bos taurus*	steppe	-19.0	6.9	3.2	42.8	15.6
Bolshekaragansky	BKAR 15	*Equus caballus*	steppe	-19.7	4.7	3.2	43.6	15.8
Bolshekaragansky	BKAR 17	*Ovis aries*	steppe	-18.5	6.4	3.3	43.9	15.6
Bolshekaragansky	BKAR 18	*Sus scrofa (ferus)*	steppe	-21.2	6.4	3.2	44.0	16.0
Kamenni Ambar V	KAM 15	*Bos taurus*	forest-steppe	-19.0	5.5	3.2	42.2	15.3
Kamenni Ambar V	KAM 14	*Bos taurus*	forest-steppe	-18.2	7.1	3.4	43.1	15.0
Kamenni Ambar V	KAM 17	*Bos taurus*	forest-steppe	-19.1	5.4	3.2	43.4	15.9
Kamenni Ambar V	KAM 16	*Bos taurus*	forest-steppe	-18.4	9.4	3.3	43.5	15.6
Kamenni Ambar V	KAM 18	*Bos taurus*	forest-steppe	-18.6	6.1	3.2	43.9	16.0
Kamenni Ambar V	KAM 1	*Canis familiaris*	forest-steppe	-18.6	9.7	3.2	42.6	15.5
Kamenni Ambar V	KAM 2	*Canis familiaris*	forest-steppe	-18.7	8.1	3.2	44.4	16.2
Kamenni Ambar V	KAM 5	*Equus caballus*	forest-steppe	-19.8	5.4	3.2	40.2	14.6
Kamenni Ambar V	KAM 7	*Equus caballus*	forest-steppe	-20.0	4.4	3.2	40.8	15.0
Kamenni Ambar V	KAM 6	*Equus caballus*	forest-steppe	-20.1	6.3	3.2	42.7	15.4
Kamenni Ambar V	KAM 4	*Equus caballus*	forest-steppe	-19.9	6.5	3.1	43.8	16.4
Kamenni Ambar V	KAM 3	*Equus caballus*	forest-steppe	-19.5	5.2	3.2	43.9	16.0
Kamenni Ambar V	KAM 12	ovicaprid	forest-steppe	-18.4	7.1	3.2	38.6	14.0
Kamenni Ambar V	KAM 10	ovicaprid	forest-steppe	-17.9	8.1	3.2	39.9	14.7
Kamenni Ambar V	KAM 9	ovicaprid	forest-steppe	-17.1	9.4	3.2	41.3	15.0
Kamenni Ambar V	KAM 8	ovicaprid	forest-steppe	-19.0	4.3	3.2	41.8	15.3
Kamenni Ambar V	KAM 11	ovicaprid	forest-steppe	-17.3	8.8	3.2	42.4	15.5
Kamenni Ambar V	KAM 13	ovicaprid	forest-steppe	-18.2	8.4	3.2	44.4	16.1
Novonikolskoye I	NOV 1	*Equus caballus*	steppe	-19.8	6.4	3.2	41.8	15.4
Novonikolskoye I	NOV 2	*Ovis aries*	steppe	-18.8	5.7	3.2	40.9	14.9

Table A1.5. Data obtained from the Oxford Radiocarbon Accelerator Unit for human Bronze Age samples from Urals and western Siberian sites. Sample numbers are 'p' numbers assigned by ORAU.

Site	Sample	Culture	$\delta^{13}C$	$\delta^{15}N$
Kulevchi VI	14235	Alakul	-18.5	12.7
Kulevchi VI	14236	Alakul	-18.6	12.7
Kulevchi VI	14237	Alakul	-19.1	13.1
Peschanka 1	14233	Alakul-Srubnaya	-19.0	11.2
Peschanka 1	14234	Alakul-Srubnaya	-19.6	11.1
Ust'e	14222	Alakul-Srubnaya	-18.2	10.5
Ust'e	14223	Alakul-Srubnaya	-18.6	12.0
Kamenni Ambar V	14242	Sintashta	-17.2	13.1
Kamenni Ambar V	14243	Sintashta	-17.5	15.1
Kamenni Ambar V	14244	Sintashta	-17.4	15.1
Kamenni Ambar V	14245	Sintashta	-17.1	13.6
Kamenni Ambar V	14247	Sintashta	-17.2	14.7
Kamenni Ambar V	14248	Sintashta	-17.6	14.4
Sintashta	14254	Sintashta	-18.0	13.3
Krivoe Ozero	14251	Sintashta-Petrovka	-18.7	10.5
Ust'e	14221	Petrovka	-18.9	10.4

Table A1.6. Human Iron Age samples from Urals and western Siberian sites. Material from the site of Baitovo is associated with the transition from the Late Bronze Age to the Early Iron Age.

Site	Sample	Culture	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Kurtuguz I	KUR 10	Gorokhovo	-18.1	11.7	3.3	38.6	13.8
Kurtuguz I	KUR 4	Gorokhovo	-17.9	12.9	3.2	41.0	15.0
Kurtuguz I	KUR 5	Gorokhovo	-19.8	12.7	3.2	42.1	15.2
Kurtuguz I	KUR 6	Gorokhovo	-17.5	12.6	3.3	27.1	9.5
Kurtuguz I	KUR 7	Gorokhovo	-21.6	13.0	3.3	38.1	13.5
Kurtuguz I	KUR 8	Gorokhovo	-21.4	13.0	3.3	36.7	13.1
Kurtuguz I	KUR 9	Gorokhovo	-20.3	14.3	3.3	31.4	11.2
Murzino I	MUR 1	Gorokhovo	-20.5	12.5	3.3	39.8	14.0
Murzino I	MUR 2	Gorokhovo	-21.1	12.1	3.3	40.3	14.3
Murzino I	MUR 4	Gorokhovo	-20.4	13.0	3.4	42.9	14.9
Murzino I	MUR 5	Gorokhovo	-22.8	13.4	3.3	39.6	14.1
Murzino I	MUR 6	Gorokhovo	-20.9	14.9	3.2	39.7	14.6
Murzino I	MUR 7	Gorokhovo	-21.0	12.4	3.2	41.0	14.8
Skaty I	SKA 2	Gorokhovo	-19.6	10.8	3.2	43.3	15.8
Skaty I	SKA 3	Gorokhovo	-20.2	11.1	3.2	35.1	12.8
Shaidurikha	SHA 1	Sargat-Gorokhovo	-19.9	11.9	3.5	39.7	13.2
Shaidurikha	SHA 2	Sargat-Gorokhovo	-19.9	12.8	3.2	43.1	15.6
Shaidurikha	SHA 3	Sargat-Gorokhovo	-20.6	12.0	3.2	42.1	15.4
Shaidurikha	SHA 4	Sargat-Gorokhovo	-19.1	12.1	3.2	42.2	15.2
Shaidurikha	SHA 5	Sargat-Gorokhovo	-20.0	12.4	3.3	42.6	15.3
Gayovsky I	GAY 1	Sargat	-20.7	12.7	3.3	43.7	15.6
Gayovsky I	GAY 3	Sargat	-21.5	12.8	3.3	39.8	14.3
Gayovsky I	GAY 4	Sargat	-20.0	11.4	3.3	42.0	14.9
Gayovsky I	GAY 5	Sargat	-20.6	12.4	3.3	42.6	15.2
Gayovsky I	GAY 6	Sargat	-20.3	11.0	3.2	42.5	15.6
Gayovsky I	GAY 7	Sargat	-20.3	12.0	3.2	43.5	15.7
Pobyeda	POB 1	Sarmatian	-18.0	13.0	3.2	40.2	14.7
Pobyeda	POB 2	Sarmatian	-17.9	13.8	3.2	40.9	15.0
Pobyeda	POB 3	Sarmatian	-19.4	5.6	3.2	41.3	15.1
Pobyeda	POB 4	Sarmatian	-18.3	12.6	3.2	42.6	15.6
Pobyeda	POB 5	Sarmatian	-18.4	13.0	3.2	42.0	15.4
Pobyeda	POB 6	Sarmatian	-18.7	13.1	3.2	42.0	15.4
Pobyeda	POB 7	Sarmatian	-18.8	12.4	3.2	41.3	15.1
Pobyeda	POB 8	Sarmatian	-18.8	12.6	3.2	41.8	15.2
Solonchanka II	SOLII 1	Sarmatian	-18.1	14.6	3.2	40.5	14.9
Solonchanka II	SOLII 3	Sarmatian	-18.2	13.3	3.1	38.2	14.2
Staraya Mel'nitsa	STA 1	Sarmatian	-18.5	12.2	3.2	42.4	15.6
Varnenskiye	VAR 1	Sarmatian	-19.1	10.9	3.2	43.1	15.7

Table A1.7. Faunal Iron Age samples from Urals and western Siberian sites. Material from the site of Baitovo is associated with the transition from the Late Bronze Age to the Early Iron Age.

Site	Sample	Species	Environment	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Baitovo	BAI 1	*Bos taurus*	forest-steppe	-20.0	5.4	3.2	40.1	14.4
Baitovo	BAI 2	*Bos taurus*	forest-steppe	-21.4	5.7	3.5	22.7	7.5
Baitovo	BAI 3	*Bos taurus*	forest-steppe	-20.1	5.7	3.2	41.7	15.0
Baitovo	BAI 4	*Bos taurus*	forest-steppe	-20.7	6.2	3.3	39.5	13.9
Baitovo	BAI 7	*Equus caballus*	forest-steppe	-21.3	5.6	3.2	41.4	14.9
Baitovo	BAI 8	*Equus caballus*	forest-steppe	-21.5	4.1	3.3	41.7	14.7
Baitovo	BAI 9	*Equus caballus*	forest-steppe	-21.7	4.5	3.4	35.6	12.3
Kurtuguz I	KUR 1	*Alces alces*	forest-steppe	-20.8	3.1	3.5	41.6	13.9
Kurtuguz I	KUR 2	*Ursus sp.*	forest-steppe	-18.2	8.3	3.3	40.4	14.2
Kurtuguz I	KUR 3	*Ursus sp.*	forest-steppe	-18.6	8.4	3.3	41.8	14.7
Murzino I	MUR 9	*Bos taurus*	forest-steppe	-20.5	7.3	3.2	42.5	15.4
Murzino I	MUR 8	*Capreolus capreolus*	forest-steppe	-20.7	4.7	3.3	39.3	14.1
Pavlinova	PAV 1	*Bos taurus*	forest-steppe	-20.8	5.9	3.3	27.8	9.7
Pavlinova	PAV 18	*Bos taurus*	forest-steppe	-21.3	5.4	3.4	39.5	13.5
Pavlinova	PAV 19	*Bos taurus*	forest-steppe	-20.7	5.6	3.4	35.0	12.1
Pavlinova	PAV 2	*Bos taurus*	forest-steppe	-20.4	6.2	3.5	41.3	13.9
Pavlinova	PAV 3	*Bos taurus*	forest-steppe	-20.4	5.6	3.2	40.5	14.6
Pavlinova	PAV 4	*Bos taurus*	forest-steppe	-20.4	4.0	3.2	40.1	14.6
Pavlinova	PAV 5	*Bos taurus*	forest-steppe	-20.9	6.5	3.2	41.2	15.0
Pavlinova	PAV 10	*Equus caballus*	forest-steppe	-21.1	4.1	3.2	41.2	15.1
Pavlinova	PAV 20	*Equus caballus*	forest-steppe	-21.4	4.6	3.2	42.3	15.3
Pavlinova	PAV 21	*Equus caballus*	forest-steppe	-20.1	7.5	3.3	42.8	15.1
Pavlinova	PAV 6	*Equus caballus*	forest-steppe	-21.1	6.3	3.2	41.7	15.2
Pavlinova	PAV 7	*Equus caballus*	forest-steppe	-20.7	4.3	3.2	41.6	15.1
Pavlinova	PAV 8	*Equus caballus*	forest-steppe	-21.0	4.6	3.3	41.2	14.8
Pavlinova	PAV 9	*Equus caballus*	forest-steppe	-20.3	4.0	3.3	41.3	14.7
Pavlinova	PAV 11	*ovicaprid*	forest-steppe	-17.7	8.8	3.2	41.4	15.1
Pavlinova	PAV 12	*ovicaprid*	forest-steppe	-18.1	9.4	3.2	39.6	14.4
Pavlinova	PAV 13	*ovicaprid*	forest-steppe	-19.6	9.1	3.2	41.7	15.1
Pavlinova	PAV 14	*ovicaprid*	forest-steppe	-18.7	8.7	3.2	40.8	14.9
Pavlinova	PAV 15	*ovicaprid*	forest-steppe	-20.3	6.5	3.2	42.2	15.3
Pavlinova	PAV 16	*ovicaprid*	forest-steppe	-19.9	4.6	3.2	40.2	14.7
Pavlinova	PAV 17	*ovicaprid*	forest-steppe	-18.9	8.4	3.2	41.7	15.2
Skaty I	SKA 1	*Equus caballus*	forest-steppe	-21.2	4.1	3.2	40.7	14.8

Table A1.8. Human Bronze Age samples from Ukrainian sites.

Site	Sample	Culture	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Golovkovka	GOL 2	Yamnaya	-19.1	13.0	3.3	41.0	14.7
Golovkovka	GOL 4	Yamnaya	-18.8	10.8	3.2	41.3	15.0
Golovkovka	GOL 8	Yamnaya	-19.2	11.2	3.3	41.9	15.0
Beeva Mogila	BEE 1	KMK	-19.6	12.3	3.2	42.0	15.1
Khamush-Oba	KHA 1	KMK	-19.0	11.6	3.2	41.3	15.0
Khamush-Oba	KHA 2	KMK	-19.1	11.5	3.2	41.5	15.0
Krinichki	KRI 1	KMK	-19.2	12.2	3.2	37.4	13.7
Nizhniaia Krynka	NIZ 2	KMK	-18.6	13.4	3.2	39.5	14.4
Novoamvrosievka	NOV AMV 1	KMK	-19.2	12.4	3.2	38.9	14.3
Novo-Poltavka	NOV POL 1	KMK	-19.5	13.7	3.3	29.1	10.3
Ugledar	UGL 1	KMK	-19.2	12.4	3.2	41.0	15.0
Razdol'noe	RAZ 1	KMK/Early Srubnaya	-19.0	12.9	3.3	41.2	14.6
Aleksandrovsk	ALEK 1	Srubnaya	-19.0	13.1	3.2	43.8	15.8
Glubokoe Ozero II (lower)	GLU 58	Srubnaya	-19.0	12.3	3.2	39.8	14.5
Novoamvrosievka	NOV AMV 2	Srubnaya	-18.9	13.0	3.2	39.9	14.6
Novoamvrosievka	NOV AMV 3	Srubnaya	-19.4	11.8	3.3	38.9	14.0
Novoamvrosievka	NOV AMV 4	Srubnaya	-19.0	13.1	3.2	42.6	15.3
Novovasilievka	NOV VAS 1	Srubnaya	-19.2	12.7	3.2	40.5	14.8
Novovasilievka	NOV VAS 2	Srubnaya	-18.8	13.8	3.2	41.7	15.3
Novovasilievka	NOV VAS 3	Srubnaya	-19.6	13.4	3.2	41.6	15.3
Novozhelannoe	NOV ZHE 1	Srubnaya	-18.8	11.9	3.2	38.4	14.0
Novozhelannoe	NOV ZHE 2	Srubnaya	-19.2	12.0	3.2	34.1	12.3
Glubokoe Ozero II (upper)	GLU 1	Post-Srubnaya	-19.2	12.1	3.3	30.1	10.7
Glubokoe Ozero II (upper)	GLU 115	Post-Srubnaya	-14.8	10.5	3.2	42.6	15.5
Glubokoe Ozero II (upper)	GLU 2	Post-Srubnaya	-15.4	11.0	3.4	43.6	15.2
Glubokoe Ozero II (upper)	GLU 3	Post-Srubnaya	-21.4	12.0	3.3	39.4	13.9
Vinogradyi Sad	VIN 1	Sabatinovka	-18.5	13.4	3.2	44.2	15.9
Vinogradyi Sad	VIN 3	Sabatinovka	-19.0	13.0	3.2	41.7	15.0
Vinogradyi Sad	VIN 5	Sabatinovka	-18.7	12.3	3.2	39.0	14.0

Table A1.9. Faunal samples from the Ukrainian Eneolithic sites of Molyukhov Bugor and Bil'shivtsi.

Site	Sample	Species	Environment	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Bil'shivsti	BIL 8	*Bos primigenius*	forest-steppe	-20.7	5.8	3.3	24.9	8.8
Bil'shivsti	BIL 26	*Bos taurus*	forest-steppe	-20.2	5.2	3.3	38.5	13.5
Bil'shivsti	BIL 27	*Bos taurus*	forest-steppe	-20.8	6.6	3.2	34.4	12.4
Bil'shivsti	BIL 28	*Bos taurus*	forest-steppe	-21.5	7.2	3.4	39.7	13.5
Bil'shivsti	BIL 29	*Bos taurus*	forest-steppe	-20.3	5.3	3.4	39.4	13.4
Bil'shivsti	BIL 39	*Capra hircus*	forest-steppe	-20.5	6.1	3.3	35.5	12.6
Bil'shivsti	BIL 40	*Capra hircus*	forest-steppe	-20.0	6.2	3.3	35.9	12.7
Bil'shivsti	BIL 41	*Capra hircus*	forest-steppe	-19.9	6.5	3.3	41.4	14.6
Bil'shivsti	BIL 44	*Cervus elaphus*	forest-steppe	-21.3	4.6	3.3	18.7	6.5
Bil'shivsti	BIL 45	*Cervus elaphus*	forest-steppe	-19.8	6.4	3.2	34.9	12.6
Bil'shivsti	BIL 11	*Emys sp.*	forest-steppe	-24.4	9.6	3.3	34.1	11.9
Bil'shivsti	BIL 12	*Emys sp.*	forest-steppe	-24.5	9.3	3.2	36.2	13.1
Bil'shivsti	BIL 13	*Emys sp.*	forest-steppe	-25.6	8.3	3.2	31.9	11.5
Bil'shivsti	BIL 14	*Emys sp.*	forest-steppe	-25.6	8.0	3.3	38.2	13.6
Bil'shivsti	BIL 15	*Emys sp.*	forest-steppe	-25.5	8.0	3.3	28.5	10.2
Bil'shivsti	BIL 46	*Equus caballus*	forest-steppe	-20.5	3.6	3.3	27.0	9.5
Bil'shivsti	BIL 47	*Equus caballus*	forest-steppe	-20.3	3.4	3.3	41.5	14.5
Bil'shivsti	BIL 48	*Equus caballus*	forest-steppe	-20.6	4.1	3.3	40.9	14.4
Bil'shivsti	BIL 49	*Equus caballus*	forest-steppe	-20.6	3.6	3.3	25.6	9.0
Bil'shivsti	BIL 50	*Equus caballus*	forest-steppe	-20.7	3.4	3.3	24.7	8.8
Bil'shivsti	BIL 51	*Equus caballus*	forest-steppe	-20.6	3.7	3.3	35.9	12.8
Bil'shivsti	BIL 23	*fish*	forest-steppe	-22.3	12.9	3.3	38.3	13.5
Bil'shivsti	BIL 24	*fish*	forest-steppe	-22.9	12.3	3.6	14.9	4.8
Bil'shivsti	BIL 25	*fish*	forest-steppe	-20.5	13.4	3.3	24.7	8.6
Bil'shivsti	BIL 32	*Ovis aries*	forest-steppe	-20.7	4.9	3.3	35.4	12.7
Bil'shivsti	BIL 33	*Ovis aries*	forest-steppe	-22.6	5.4	3.3	38.8	13.9
Bil'shivsti	BIL 34	*Ovis aries*	forest-steppe	-20.5	5.8	3.3	38.9	13.9
Bil'shivsti	BIL 35	*Ovis aries*	forest-steppe	-20.6	6.7	3.3	40.5	14.5
Bil'shivsti	BIL 36	*Ovis aries*	forest-steppe	-20.6	5.0	3.3	39.3	14.0
Bil'shivsti	BIL 17	*Sus scrofa (dom)*	forest-steppe	-21.3	6.7	3.3	37.6	13.1
Bil'shivsti	BIL 18	*Sus scrofa (dom)*	forest-steppe	-21.7	6.4	3.2	42.3	15.3
Bil'shivsti	BIL 19	*Sus scrofa (dom)*	forest-steppe	-20.9	7.4	3.2	46.7	13.9
Molyukhov Bugor	MOL 36	*Alces alces*	steppe/forest-steppe	-21.4	7.5	3.4	37.1	12.7
Molyukhov Bugor	MOL 37	*Alces alces*	steppe/forest-steppe	-22.0	5.7	3.2	41.6	15.0
Molyukhov Bugor	MOL 49	*Bos primigenius*	steppe/forest-steppe	-21.3	6.1	3.2	41.2	14.9
Molyukhov Bugor	MOL 1	*Bos taurus*	steppe/forest-steppe	-21.3	8.0	3.5	43.5	14.6
Molyukhov Bugor	MOL 2	*Bos taurus*	steppe/forest-steppe	-20.6	7.0	3.2	38.8	14.2
Molyukhov Bugor	MOL 16	*Canis familiaris*	steppe/forest-steppe	-21.9	9.6	3.3	34.2	12.1
Molyukhov Bugor	MOL 39	*Capreolus capreolus*	steppe/forest-steppe	-21.8	7.8	3.5	44.3	14.6
Molyukhov Bugor	MOL 43	*Capreolus capreolus*	steppe/forest-steppe	-23.5	6.8	3.2	43.1	15.5
Molyukhov Bugor	MOL 44	*Capreolus capreolus*	steppe/forest-steppe	-21.4	7.6	3.3	41.1	14.6
Molyukhov Bugor	MOL 45	*Capreolus capreolus*	steppe/forest-steppe	-21.5	5.6	3.2	43.8	15.9

Site	Sample	Species	Environment	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Molyukhov Bugor	MOL 51	*Castor fiber*	steppe/forest-steppe	-22.5	4.8	3.3	40.4	14.5
Molyukhov Bugor	MOL 52	*Castor fiber*	steppe/forest-steppe	-22.3	4.4	3.2	38.8	14.0
Molyukhov Bugor	MOL 54	*Castor fiber*	steppe/forest-steppe	-21.8	8.0	3.3	41.8	15.0
Molyukhov Bugor	MOL 32	*Cervus elaphus*	steppe/forest-steppe	-22.1	7.9	3.3	31.7	11.1
Molyukhov Bugor	MOL 33	*Cervus elaphus*	steppe/forest-steppe	-22.7	7.4	3.3	36.7	12.9
Molyukhov Bugor	MOL 34	*Cervus elaphus*	steppe/forest-steppe	-21.5	4.7	3.3	36.3	12.9
Molyukhov Bugor	MOL 27	*fish*	steppe/forest-steppe	-26.5	10.4	3.2	41.5	14.9
Molyukhov Bugor	MOL 28	*fish*	steppe/forest-steppe	-26.1	8.1	3.4	43.8	14.9
Molyukhov Bugor	MOL 29	*fish*	steppe/forest-steppe	-21.5	7.8	3.3	39.6	13.8
Molyukhov Bugor	MOL 10	*Lepus sp.*	steppe/forest-steppe	-19.8	5.0	3.3	45.2	16.0
Molyukhov Bugor	MOL 15	*Lutra sp.*	steppe/forest-steppe	-23.7	10.9	3.4	45.6	15.5
Molyukhov Bugor	MOL 13	*Martes sp.*	steppe/forest-steppe	-22.3	4.7	3.2	45.9	16.9
Molyukhov Bugor	MOL 14	*Meles sp.*	steppe/forest-steppe	-18.4	10.9	3.4	43.2	14.8
Molyukhov Bugor	MOL 35	*ovicaprid*	steppe/forest-steppe	-19.7	5.8	3.2	41.5	15.0
Molyukhov Bugor	MOL 21	*Sus scrofa (ferus)*	steppe/forest-steppe	-20.4	8.3	3.3	39.4	14.1
Molyukhov Bugor	MOL 22	*Sus scrofa (ferus)*	steppe/forest-steppe	-20.6	9.1	3.2	41.9	15.2
Molyukhov Bugor	MOL 24	*Sus scrofa (ferus)*	steppe/forest-steppe	-19.9	6.5	3.3	38.4	13.8
Molyukhov Bugor	MOL 25	*Sus scrofa (ferus)*	steppe/forest-steppe	-20.8	5.3	3.3	36.8	13.1
Molyukhov Bugor	MOL 26	*Sus scrofa (ferus)*	steppe/forest-steppe	-21.2	8.8	3.6	38.6	12.7
Molyukhov Bugor	MOL 12	*Vulpes sp.*	steppe/forest-steppe	-19.2	7.9	3.2	43.4	15.7

Table A1.10. Faunal samples from Bronze Age Ukrainian sites. For the site of Glubokoe Ozero II, "L" stands for lower cultural layers, "U" represents upper cultural strata.

Site	Sample	Species	Environment	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Beeva Mogila	BEE 2	*Bos taurus*	steppe/forest-steppe	-20.2	4.9	3.2	41.6	15.2
Beeva Mogila	BEE 3	*Ovis aries*	steppe/forest-steppe	-19.6	6.5	3.2	41.1	14.8
Desyatiny	DES 4	*Alces alces*	forest-steppe	-22.1	5.1	3.3	39.9	14.3
Desyatiny	DES 5	*Alces alces*	forest-steppe	-22.4	4.6	3.3	26.7	9.5
Desyatiny	DES 6	*Alces alces*	forest-steppe	-22.4	4.5	3.3	27.4	9.7
Desyatiny	DES 7	*Alces alces*	forest-steppe	-22.3	4.9	3.3	38.2	13.5
Desyatiny	DES 8	*Alces alces*	forest-steppe	-22.1	4.9	3.3	38.3	13.6
Desyatiny	DES 15	*Bos taurus*	forest-steppe	-20.2	5.9	3.3	31.9	11.3
Desyatiny	DES 16	*Bos taurus*	forest-steppe	-20.0	6.7	3.2	40.5	14.6
Desyatiny	DES 19	*Bos taurus*	forest-steppe	-20.4	6.7	3.2	36.1	13.1
Desyatiny	DES 20	*Bos taurus*	forest-steppe	-19.7	7.5	3.3	33.7	12.1
Desyatiny	DES 21	*Bos taurus*	forest-steppe	-20.4	6.3	3.4	39.2	13.6
Desyatiny	DES 37	*Bos taurus*	forest-steppe	-20.4	8.6	3.3	39.1	13.9
Desyatiny	DES 38	*Bos taurus*	forest-steppe	-20.4	7.9	3.3	38.1	13.6
Desyatiny	DES 39	*Bos taurus*	forest-steppe	-20.5	5.9	3.2	37.8	13.6
Desyatiny	DES 40	*Bos taurus*	forest-steppe	-20.2	6.7	3.3	39.4	13.9
Desyatiny	DES 41	*Bos taurus*	forest-steppe	-20.2	6.1	3.3	36.8	13.0
Desyatiny	DES 42	*Bos taurus*	forest-steppe	-20.2	6.8	3.2	36.7	13.3
Desyatiny	DES 43	*Bos taurus*	forest-steppe	-19.8	6.3	3.3	39.4	14.0
Desyatiny	DES 44	*Bos taurus*	forest-steppe	-20.5	6.8	3.3	40.0	14.2
Desyatiny	DES 45	*Bos taurus*	forest-steppe	-19.7	7.3	3.3	37.1	13.1
Desyatiny	DES 46	*Bos taurus*	forest-steppe	-19.9	5.7	3.2	34.0	12.3
Desyatiny	DES 2	*Capra hircus*	forest-steppe	-19.8	6.7	3.2	38.5	13.9
Desyatiny	DES 3	*Capra hircus*	forest-steppe	-22.3	7.5	3.3	25.7	9.0
Desyatiny	DES 1	*Castor fiber*	forest-steppe	-21.8	3.3	3.3	40.7	14.3
Desyatiny	DES 14	*Cervus elaphus*	forest-steppe	-20.0	8.2	3.3	38.2	13.5
Desyatiny	DES 10	*Equus caballus*	forest-steppe	-21.4	2.6	3.3	23.1	8.1
Desyatiny	DES 11	*Equus caballus*	forest-steppe	-20.5	6.7	3.3	39.4	13.8
Desyatiny	DES 12	*Equus caballus*	forest-steppe	-20.8	4.8	3.3	41.1	14.6
Desyatiny	DES 13	*Equus caballus*	forest-steppe	-20.7	5.2	3.3	39.4	13.9
Desyatiny	DES 9	*Sus scrofa (ferus)*	forest-steppe	-20.9	6.2	3.2	35.8	12.9
Glubokoe Ozero II L	GLU 10	*Bos*	forest-steppe	-19.6	7.1	3.2	38.8	14.0
Glubokoe Ozero II L	GLU 7	*Bos*	forest-steppe	-20.0	5.0	3.2	41.3	15.0
Glubokoe Ozero II L	GLU 8	*Bos*	forest-steppe	-19.8	6.7	3.2	42.8	15.4
Glubokoe Ozero II L	GLU 9	*Bos*	forest-steppe	-19.3	8.8	3.2	41.6	15.3
Glubokoe Ozero II L	GLU 100	*Bos taurus*	forest-steppe	-20.0	7.7	3.3	42.2	14.9
Glubokoe Ozero II L	GLU 140	*Bos taurus*	forest-steppe	-20.2	5.2	3.2	40.3	14.5
Glubokoe Ozero II L	GLU 141	*Bos taurus*	forest-steppe	-20.3	7.1	3.2	43.7	16.0
Glubokoe Ozero II L	GLU 18	*Bos taurus*	forest-steppe	-19.4	7.4	3.2	42.1	15.2
Glubokoe Ozero II L	GLU 27	*Bos taurus*	forest-steppe	-20.0	5.6	3.2	42.3	15.6
Glubokoe Ozero II L	GLU 28	*Bos taurus*	forest-steppe	-19.6	7.1	3.2	41.7	15.1

Site	Sample	Species	Environment	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Glubokoe Ozero II L	GLU 29	*Bos taurus*	forest-steppe	-19.7	6.7	3.2	40.4	14.6
Glubokoe Ozero II L	GLU 30	*Bos taurus*	forest-steppe	-20.0	6.1	3.2	41.6	15.0
Glubokoe Ozero II L	GLU 69	*Bos taurus*	forest-steppe	-19.1	8.6	3.2	42.0	15.1
Glubokoe Ozero II L	GLU 70	*Bos taurus*	forest-steppe	-19.7	7.3	3.2	45.0	16.3
Glubokoe Ozero II L	GLU 71	*Bos taurus*	forest-steppe	-19.8	6.5	3.3	42.4	14.9
Glubokoe Ozero II L	GLU 98	*Bos taurus*	forest-steppe	-20.4	5.4	3.3	40.6	14.4
Glubokoe Ozero II L	GLU 38	*Capra hircus*	forest-steppe	-19.7	6.9	3.2	43.3	15.6
Glubokoe Ozero II L	GLU 64	*Capra hircus*	forest-steppe	-20.8	8.0	3.3	42.1	14.9
Glubokoe Ozero II L	GLU 95	*Capra hircus*	forest-steppe	-19.3	8.7	3.2	39.7	14.3
Glubokoe Ozero II L	GLU 23	*Cervus elaphus*	forest-steppe	-20.2	5.3	3.4	41.9	14.6
Glubokoe Ozero II L	GLU 136	*Equus caballus*	forest-steppe	-20.7	3.4	3.2	42.4	15.4
Glubokoe Ozero II L	GLU 16	*Equus caballus*	forest-steppe	-20.6	4.4	3.2	44.1	15.8
Glubokoe Ozero II L	GLU 22	*Equus caballus*	forest-steppe	-20.2	5.7	3.3	38.4	13.6
Glubokoe Ozero II L	GLU 25	*Equus caballus*	forest-steppe	-20.3	4.8	3.3	42.4	15.1
Glubokoe Ozero II L	GLU 26	*Equus caballus*	forest-steppe	-20.9	4.2	3.3	43.5	15.4
Glubokoe Ozero II L	GLU 59	*Equus caballus*	forest-steppe	-19.8	10.1	3.2	39.1	14.1
Glubokoe Ozero II L	GLU 91	*Equus caballus*	forest-steppe	-20.4	3.0	3.2	42.5	15.4
Glubokoe Ozero II L	GLU 92	*Equus caballus*	forest-steppe	-20.4	6.2	3.3	42.1	15.1
Glubokoe Ozero II L	GLU 12	*Ovis aries*	forest-steppe	-19.2	7.3	3.2	43.8	15.9
Glubokoe Ozero II L	GLU 134	*Ovis aries*	forest-steppe	-19.6	5.8	3.2	41.4	15.1
Glubokoe Ozero II L	GLU 20	*Ovis aries*	forest-steppe	-19.1	8.7	3.3	42.4	15.1
Glubokoe Ozero II L	GLU 96	*Ovis aries*	forest-steppe	-18.7	8.7	3.2	39.6	14.3
Glubokoe Ozero II L	GLU 14	*Sus scrofa (dom)*	forest-steppe	-21.1	7.6	3.5	41.6	13.9
Glubokoe Ozero II L	GLU 21	*Sus scrofa (dom)*	forest-steppe	-21.2	8.6	3.3	42.7	15.3
Glubokoe Ozero II L	GLU 93	*Sus scrofa (dom)*	forest-steppe	-21.1	9.3	3.2	41.6	15.1
Glubokoe Ozero II L	GLU 63	*Sus scrofa (ferus)*	forest-steppe	-21.1	9.3	3.2	42.3	15.2
Glubokoe Ozero II U	GLU 112	*Bos taurus*	forest-steppe	-19.6	6.5	3.2	41.4	14.9
Glubokoe Ozero II U	GLU 113	*Bos taurus*	forest-steppe	-20.0	6.3	3.3	43.3	15.5
Glubokoe Ozero II U	GLU 114	*Bos taurus*	forest-steppe	-19.8	5.6	3.2	40.9	14.9
Glubokoe Ozero II U	GLU 129	*Bos taurus*	forest-steppe	-19.4	7.0	3.2	42.6	15.6
Glubokoe Ozero II U	GLU 131	*Bos taurus*	forest-steppe	-19.8	5.9	3.2	40.5	14.9
Glubokoe Ozero II U	GLU 132	*Bos taurus*	forest-steppe	-19.4	7.1	3.2	43.3	15.8
Glubokoe Ozero II U	GLU 133	*Bos taurus*	forest-steppe	-19.5	7.2	3.2	43.2	15.6
Glubokoe Ozero II U	GLU 39	*Bos taurus*	forest-steppe	-19.4	8.7	3.2	39.7	14.3
Glubokoe Ozero II U	GLU 76	*Bos taurus*	forest-steppe	-20.5	3.9	3.3	42.6	15.2
Glubokoe Ozero II U	GLU 77	*Bos taurus*	forest-steppe	-19.7	6.2	3.3	43.1	15.4
Glubokoe Ozero II U	GLU 78	*Bos taurus*	forest-steppe	-20.6	6.8	3.5	30.5	10.2
Glubokoe Ozero II U	GLU 79	*Bos taurus*	forest-steppe	-20.1	6.5	3.3	41.9	14.9
Glubokoe Ozero II U	GLU 80	*Bos taurus*	forest-steppe	-20.1	6.6	3.4	40.7	14.1
Glubokoe Ozero II U	GLU 81	*Bos taurus*	forest-steppe	-20.2	6.7	3.2	42.1	15.3
Glubokoe Ozero II U	GLU 57	*Canis familiaris*	forest-steppe	-17.9	9.2	3.2	43.0	15.8
Glubokoe Ozero II U	GLU 54	*Capra hircus*	forest-steppe	-19.2	9.5	3.2	41.5	15.1
Glubokoe Ozero II U	GLU 126	*Cervus elaphus*	forest-steppe	-21.2	5.9	3.2	44.1	15.9
Glubokoe Ozero II U	GLU 52	*Cervus elaphus*	forest-steppe	-19.6	8.7	3.1	41.5	15.4
Glubokoe Ozero II U	GLU 53	*Cervus elaphus*	forest-steppe	-20.8	5.7	3.3	42.4	15.0

Site	Sample	Species	Environment	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Glubokoe Ozero II U	GLU 104	*Equus caballus*	forest-steppe	-20.9	4.7	3.3	39.8	14.2
Glubokoe Ozero II U	GLU 105	*Equus caballus*	forest-steppe	-20.7	4.1	3.2	41.0	14.8
Glubokoe Ozero II U	GLU 121	*Equus caballus*	forest-steppe	-20.6	5.5	3.2	43.3	15.8
Glubokoe Ozero II U	GLU 122	*Equus caballus*	forest-steppe	-21.5	6.0	3.3	41.9	14.9
Glubokoe Ozero II U	GLU 50	*Equus caballus*	forest-steppe	-19.8	7.2	3.3	38.5	13.7
Glubokoe Ozero II U	GLU 88	*Equus caballus*	forest-steppe	-20.6	4.7	3.2	42.4	15.3
Glubokoe Ozero II U	GLU 90	*Lepus europaeus*	forest-steppe	-19.6	5.2	3.2	43.7	16.1
Glubokoe Ozero II U	GLU 107	ovicaprid	forest-steppe	-19.2	7.3	3.2	43.1	15.6
Glubokoe Ozero II U	GLU 117	ovicaprid	forest-steppe	-19.6	6.5	3.2	44.7	16.4
Glubokoe Ozero II U	GLU 118	ovicaprid	forest-steppe	-20.0	7.4	3.3	39.4	14.2
Glubokoe Ozero II U	GLU 46	*Ovis aries*	forest-steppe	-18.8	7.1	3.2	36.2	13.1
Glubokoe Ozero II U	GLU 83	*Ovis aries*	forest-steppe	-18.9	7.1	3.1	43.3	16.1
Glubokoe Ozero II U	GLU 84	*Ovis aries*	forest-steppe	-19.9	6.3	3.2	44.1	16.3
Glubokoe Ozero II U	GLU 109	*Sus scrofa (dom)*	forest-steppe	-21.1	7.4	3.2	43.8	15.9
Glubokoe Ozero II U	GLU 123	*Sus scrofa (dom)*	forest-steppe	-22.2	7.9	4.1	39.4	11.3
Glubokoe Ozero II U	GLU 48	*Sus scrofa (dom)*	forest-steppe	-21.2	10.2	3.2	43.6	15.7
Glubokoe Ozero II U	GLU 85	*Sus scrofa (dom)*	forest-steppe	-20.7	9.6	3.3	43.2	15.4
Glubokoe Ozero II U	GLU 86	*Sus scrofa (dom)*	forest-steppe	-20.1	8.0	3.2	39.8	14.5
Kazach'ia Pristan'	KAZ 15	*Bos taurus*	steppe	-19.1	8.1	3.2	40.6	14.9
Kazach'ia Pristan'	KAZ 16	*Bos taurus*	steppe	-19.6	6.8	3.2	41.1	15.0
Kazach'ia Pristan'	KAZ 26	*Capra hircus*	steppe	-19.7	7.1	3.2	33.3	12.1
Kazach'ia Pristan'	KAZ 1	*Cervus elaphus*	steppe	-21.0	3.1	3.3	40.7	14.2
Kazach'ia Pristan'	KAZ 10	*Equus caballus*	steppe	-21.3	6.5	3.2	41.5	15.1
Kazach'ia Pristan'	KAZ 11	*Equus caballus*	steppe	-21.0	3.7	3.3	38.7	13.8
Kazach'ia Pristan'	KAZ 9	*Equus caballus*	steppe	-20.9	3.9	3.3	38.8	13.8
Kazach'ia Pristan'	KAZ 27	*ovicaprid*	steppe	-19.6	6.7	3.2	41.4	14.9
Kazach'ia Pristan'	KAZ 21	*Ovis aries*	steppe	-19.1	9.6	3.2	37.6	13.6
Kazach'ia Pristan'	KAZ 22	*Ovis aries*	steppe	-19.8	6.3	3.2	36.9	13.5
Kazach'ia Pristan'	KAZ 23	*Ovis aries*	steppe	-19.7	7.9	3.2	35.5	12.8
Kazach'ia Pristan'	KAZ 3	*Sus scrofa (dom)*	steppe	-19.9	6.7	3.3	34.2	12.2
Khamush-Oba	KHA 10	*Ovis aries*	steppe	-19.8	6.4	3.2	34.0	12.6
Khamush-Oba	KHA 11	*Ovis aries*	steppe	-20.3	6.3	3.2	32.0	11.7
Khamush-Oba	KHA 3	*Ovis aries*	steppe	-20.0	6.8	3.2	40.2	14.7
Khamush-Oba	KHA 4	*Ovis aries*	steppe	-19.7	6.8	3.2	35.6	13.2
Khamush-Oba	KHA 5	*Ovis aries*	steppe	-19.9	7.0	3.2	41.2	15.1
Khamush-Oba	KHA 6	*Ovis aries*	steppe	-20.0	6.9	3.2	36.7	13.5
Khamush-Oba	KHA 7	*Ovis aries*	steppe	-20.4	6.6	3.3	21.5	7.7
Khamush-Oba	KHA 8	*Ovis aries*	steppe	-20.1	6.8	3.2	37.4	13.6
Khamush-Oba	KHA 9	*Ovis aries*	steppe	-19.6	6.8	3.2	40.8	15.0
Khamush-Oba	KHA 12	*Saiga tatarica*	steppe	-20.3	6.6	3.2	34.4	12.7
Khamush-Oba	KHA 13	*Sus scrofa (dom)*	steppe	-20.6	7.9	3.2	36.1	13.2
Razdol'noe	RAZ 10	*Bos taurus*	steppe	-19.3	7.8	3.3	38.0	13.6
Razdol'noe	RAZ 8	*Bos taurus*	steppe	-19.5	7.1	3.3	37.0	13.3
Razdol'noe	RAZ 9	*Bos taurus*	steppe	-19.2	7.5	3.3	35.1	12.6
Razdol'noe	RAZ 3	*Equus caballus*	steppe	-20.9	4.1	3.3	39.7	14.1

Site	Sample	Species	Environment	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Razdol'noe	RAZ 4	*Equus caballus*	steppe	-21.1	4.1	3.3	40.7	14.6
Razdol'noe	RAZ 13	*Ovis aries*	steppe	-19.2	7.7	3.2	40.9	14.7
Razdol'noe	RAZ 7	*Ovis aries*	steppe	-19.1	7.1	3.3	40.9	14.7
Razdol'noe	RAZ 6	*Sus scrofa (ferus)*	steppe	-19.5	5.9	3.2	41.9	15.4
Vinogradyi Sad	VIN 6	*Asinus domesticus*	steppe	-20.9	5.0	3.2	39.7	14.3
Vinogradyi Sad	VIN 10	*Bos taurus*	steppe	-19.4	7.7	3.3	41.4	14.8
Vinogradyi Sad	VIN 11	*Bos taurus*	steppe	-20.3	6.4	3.2	42.0	15.2
Vinogradyi Sad	VIN 7	*Bos taurus*	steppe	-18.7	6.0	3.2	39.1	14.2
Vinogradyi Sad	VIN 13	*Canis lupus*	steppe	-19.4	10.3	3.3	42.6	15.0
Vinogradyi Sad	VIN 14	*Canis lupus*	steppe	-18.1	11.6	3.2	42.6	15.6
Vinogradyi Sad	VIN 15	*Canis lupus*	steppe	-18.3	11.7	3.2	43.8	15.9
Vinogradyi Sad	VIN 16	*Capra hircus*	steppe	-18.6	8.9	3.2	44.2	15.9
Vinogradyi Sad	VIN 19	*Castor fiber*	steppe	-21.0	9.7	3.2	43.6	15.7
Vinogradyi Sad	VIN 21	*Castor fiber*	steppe	-18.2	11.2	3.3	40.3	14.4
Vinogradyi Sad	VIN 22	*Cervus elaphus*	steppe	-19.7	7.2	3.2	43.4	15.7
Vinogradyi Sad	VIN 23	*Equus caballus*	steppe	-20.6	5.6	3.2	41.6	15.1
Vinogradyi Sad	VIN 24	*Equus caballus*	steppe	-20.7	5.1	3.2	44.0	16.0
Vinogradyi Sad	VIN 25	*Equus caballus*	steppe	-20.4	5.1	3.3	40.0	14.4
Vinogradyi Sad	VIN 27	*Equus caballus*	steppe	-20.3	5.9	3.3	39.8	14.3
Vinogradyi Sad	VIN 28	*Lepus europaeus*	steppe	-17.9	8.0	3.3	44.1	15.5
Vinogradyi Sad	VIN 29	*Ovis aries*	steppe	-17.4	10.1	3.2	42.0	15.1
Vinogradyi Sad	VIN 30	*Ovis aries*	steppe	-19.1	8.8	3.2	42.6	15.4
Vinogradyi Sad	VIN 31	*Saiga tatarica*	steppe	-19.1	8.9	3.2	42.9	15.6
Vinogradyi Sad	VIN 32	*Sus scrofa (dom)*	steppe	-20.6	9.6	3.3	43.4	15.5
Vinogradyi Sad	VIN 33	*Vulpes vulpes*	steppe	-18.7	8.8	3.2	42.7	15.5
Vinogradyi Sad	VIN 34	*Vulpes vulpes*	steppe	-17.8	10.5	3.2	42.5	15.3
Vinogradyi Sad	VIN 35	*Vulpes vulpes*	steppe	-18.5	11.0	3.3	43.1	15.5
Vozrozhdenie 2	VOZ 13	*Capra hircus*	steppe	-19.6	6.5	3.2	41.1	15.0
Vozrozhdenie 2	VOZ 1	*Ovis aries*	steppe	-19.8	5.4	3.5	11.6	3.9
Vozrozhdenie 2	VOZ 10	*Ovis aries*	steppe	-19.5	6.1	3.2	33.6	12.3
Vozrozhdenie 2	VOZ 11	*Ovis aries*	steppe	-19.4	6.9	3.3	40.5	14.4
Vozrozhdenie 2	VOZ 3	*Ovis aries*	steppe	-19.5	6.4	3.2	37.0	13.5
Vozrozhdenie 2	VOZ 4	*Ovis aries*	steppe	-20.3	6.5	3.2	41.8	15.2
Vozrozhdenie 2	VOZ 5	*Ovis aries*	steppe	-19.5	6.7	3.3	40.9	14.7
Vozrozhdenie 2	VOZ 6	*Ovis aries*	steppe	-19.6	6.7	3.4	40.5	13.9
Vozrozhdenie 2	VOZ 8	*Ovis aries*	steppe	-19.5	6.5	3.2	41.7	15.1
Vozrozhdenie 2	VOZ 9	*Ovis aries*	steppe	-20.1	6.3	3.4	17.5	6.1

Table A1.11. Human samples from Iron Age Ukrainian sites.

Site	Sample	Culture	$\delta^{13}C$	$\delta^{15}N$	C:N	%C	%N
Bazavluk	BAZA 1	Scythian	-17.0	11.4	3.2	42.4	15.4
Ordzhonikidze	ORD 11	Scythian	-17.4	12.1	3.2	45.7	16.6
Ordzhonikidze	ORD 3	Scythian	-17.3	11.4	3.2	44.5	16.3
Ordzhonikidze	ORD 4	Scythian	-16.7	10.5	3.2	32.2	11.8
Ordzhonikidze	ORD 6	Scythian	-16.1	10.6	3.2	42.2	15.4
Ordzhonikidze	ORD 7	Scythian	-14.4	11.7	3.2	42.9	15.7
Ordzhonikidze	ORD 8	Scythian	-14.1	11.1	3.2	42.6	15.6
Ordzhonikidze	ORD 9	Scythian	-15.3	11.4	3.3	33.8	12.1
Olvi'ia	OLV 1	Greek	-14.1	10.9	3.2	44.1	16.1
Olvi'ia	OLV 11	Greek	-13.9	11.1	3.2	40.5	14.8
Olvi'ia	OLV 12	Greek	-17.1	11.8	3.2	39.6	14.3
Olvi'ia	OLV 2	Greek	-14.0	9.9	3.2	40.1	14.5
Olvi'ia	OLV 3	Greek	-14.8	10.5	3.2	40.7	14.7

APPENDIX 2. *SITE LOCATION MAPS*

Figure A2.1. Total area from which samples were taken for this study, including the specific locations of Chicha, Kizil and Sebÿstei. Current national boundaries are shown for reference. Major lakes and rivers are labeled in italics. The broken line represents the approximate boundary between steppe and forest-steppe environments (Berg 1950; Masson and Taylor 1989; Marchenko and Vinogradov 1989; Dergachev 1989; Kremenetski 2000).

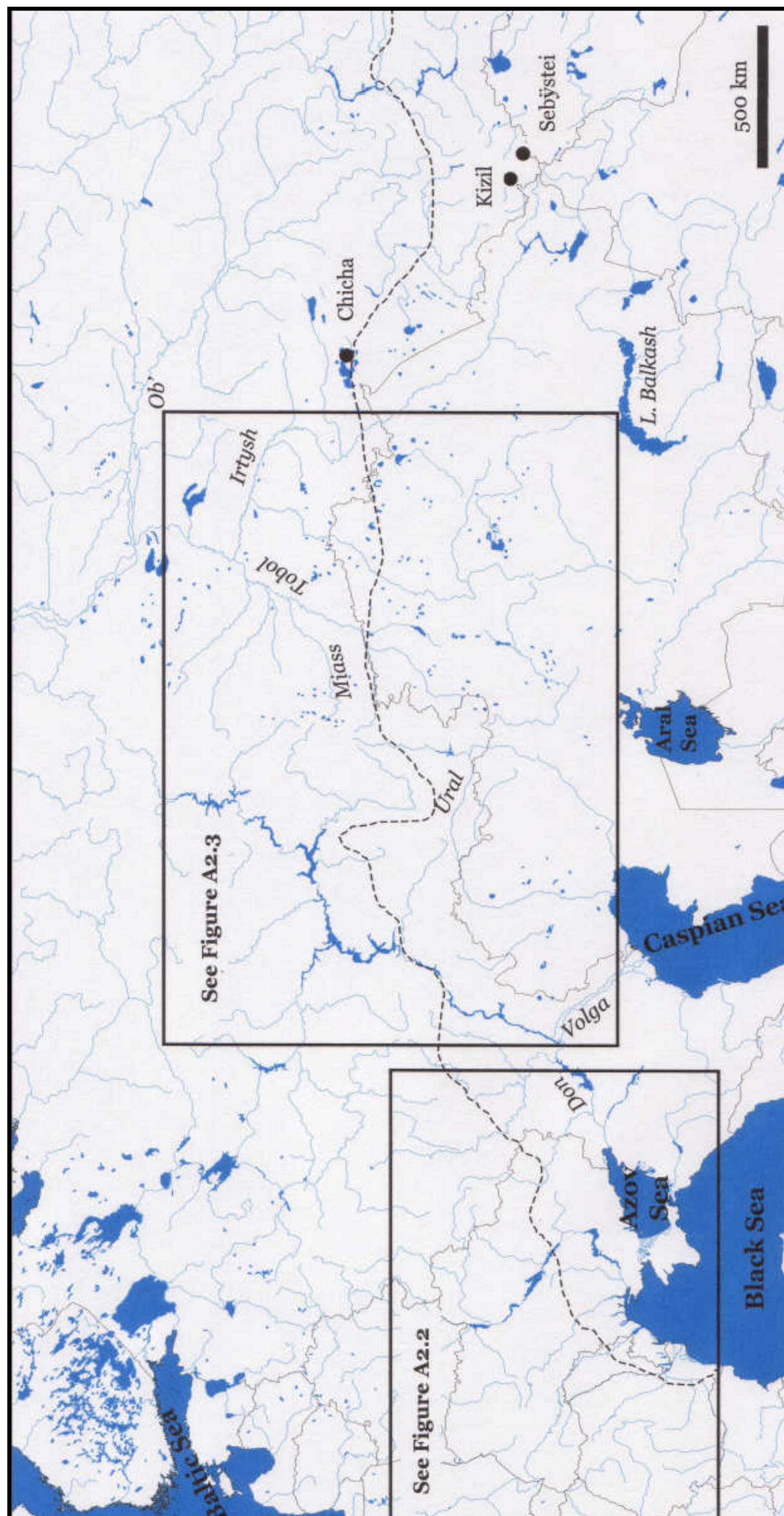


Figure A2.2. Ukrainian sites discussed in this study. Current national boundaries are shown for reference. Major lakes and rivers are labeled in italics. The broken line represents the approximate boundary between steppe and forest-steppe environments (Berg 1950; Masson and Taylor 1989; Marchenko and Vinogradov 1989; Dergachev 1989; Kremenetski 2000). Note: Notwithstanding the approximate borders given, Glubokoe Ozero II and Aleksandrovsk are situated in localized areas of forest-steppe. The exact locations of Novovasilievka and Novozhelannoe are not shown; these sites are located in a steppe environment in the Donetsk region (i.e., southeastern area) of Ukraine.

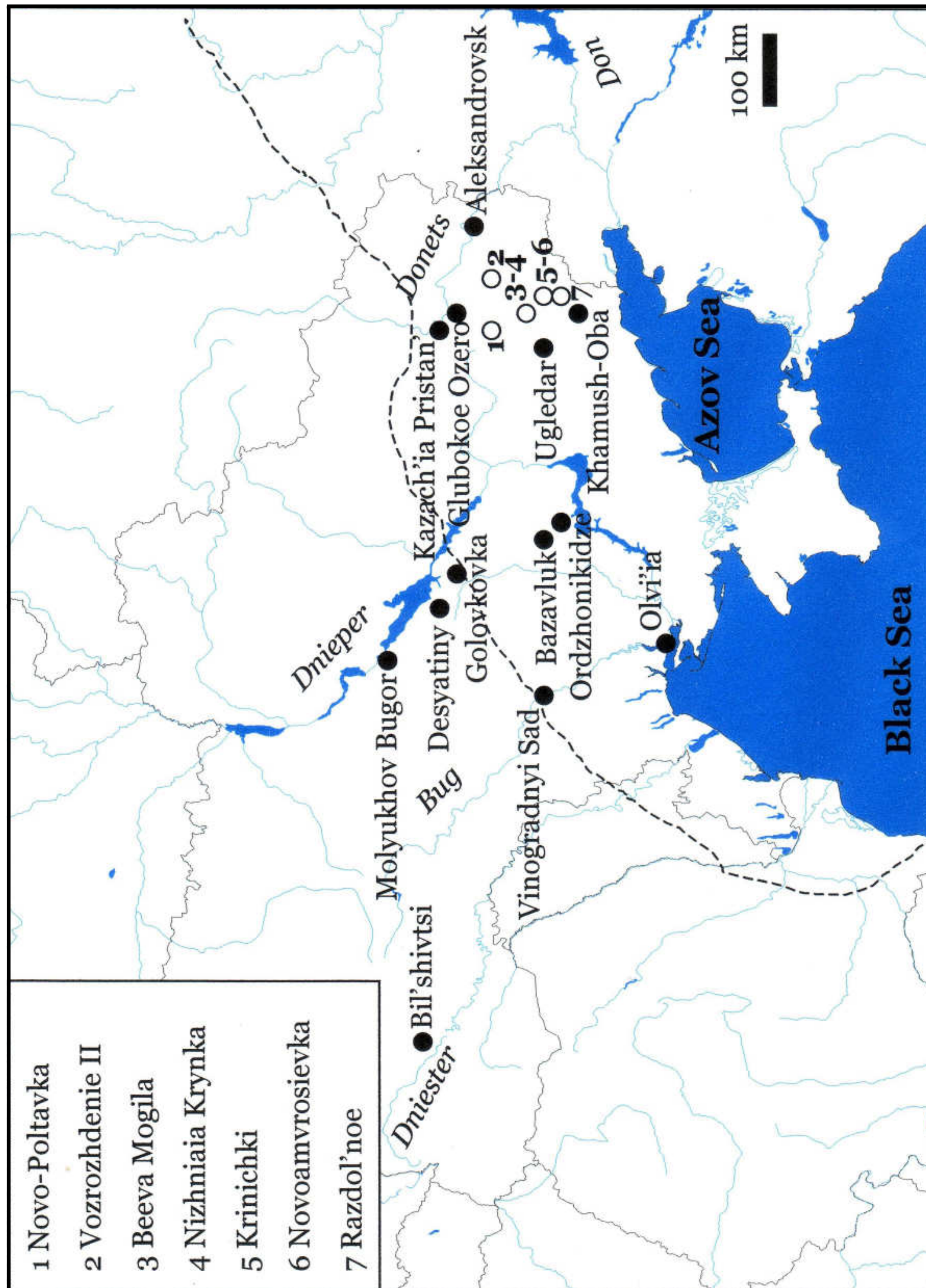
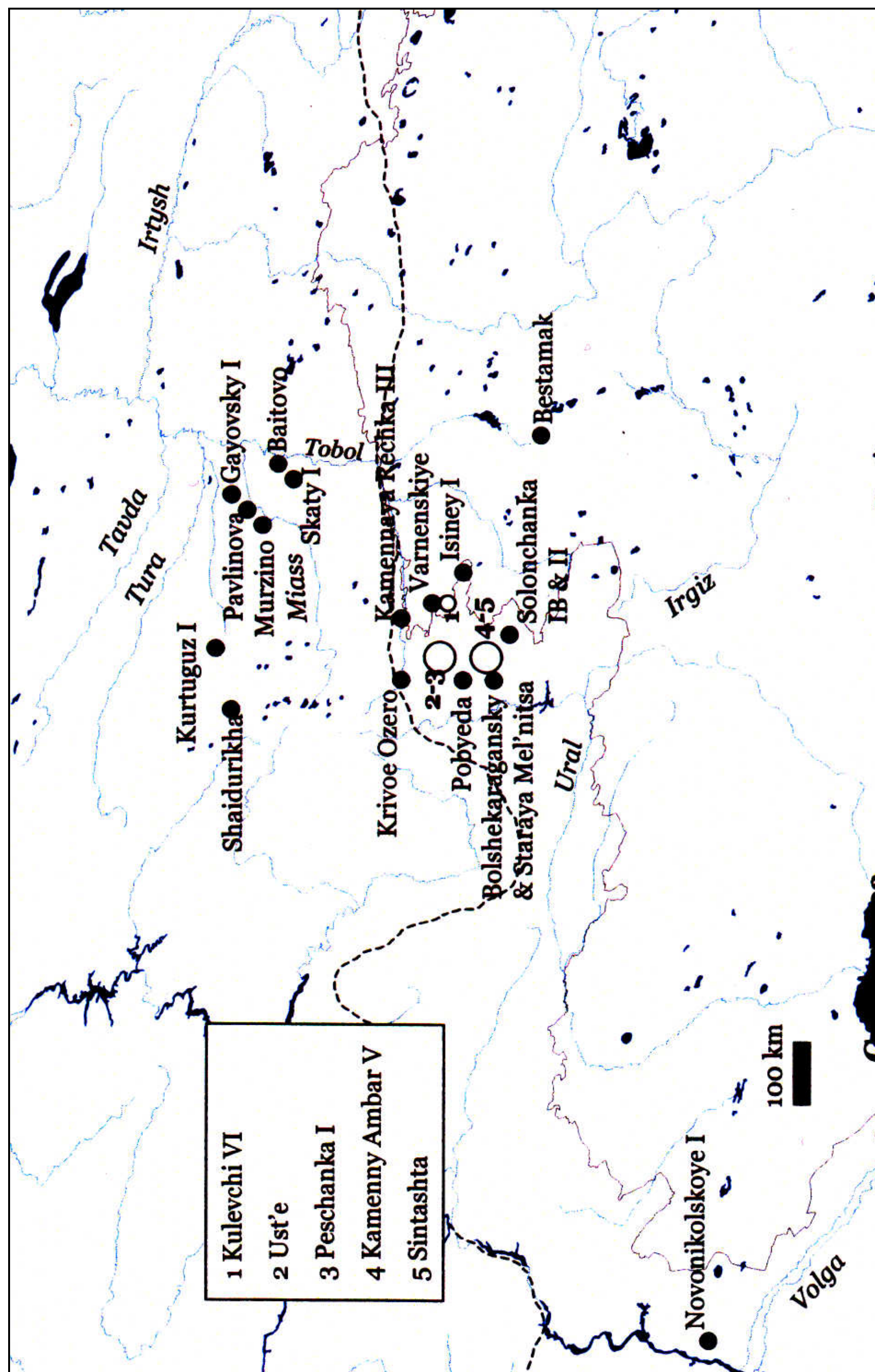


Figure A2.3. Sites included in this study, from the Urals and western Siberian region. Current national boundaries are shown for reference. Major lakes and rivers are labeled in italics. The broken line represents the approximate boundary between steppe and forest-steppe environments (Berg 1950; Masson and Taylor 1989; Marchenko and Vinogradov 1989; Dergachev 1989; Kremenetski 2000). The exact locations of Nikolaevka II and Berlik are not shown. Nikolaevka II is located in the South Urals steppe, and Berlik is located in the North Kazakhstan steppe.



APPENDIX 3. *CHAPTER 5 SAMPLE INFORMATION*

Table A3.1. $\delta^{34}S$, C:S, N:S and %S values for Chicha Samples. Values are averages of duplicate sample analyses, except for the samples marked with an asterisk (*), which were analysed once.

Sample	Species	$\delta^{34}S$	C:S	N:S	%S
CHA17	*Alces alces*	9.4	408	127	0.28
CHA6	*Alces alces*	12.3	467	142	0.25
CHA6 ult 2	*Alces alces*	9.1	444	135	0.26
CHA 43	*Bos taurus*	7.0	592	184	0.20
CHA10*	*Bos taurus*	10.8	461	141	0.23
CHA4	*Bos taurus*	10.2	412	126	0.30
CHA 44	*Canis familiaris*	29.5	457	145	0.25
CHA 45	*Canis familiaris*	6.2	378	120	0.28
CHA 46	*Canis familiaris*	15.8	508	154	0.24
CHA 47	*Canis familiaris*	16.0	490	155	0.24
CHA 48	*Canis familiaris*	18.4	466	143	0.25
CHA2*	*Canis familiaris*	10.9	359	106	0.32
CHA 59	*Carassius carassius*	21.3	258	80	0.48
CHA 60	*Carassius carassius*	20.0	286	89	0.41
CHA 61	*Carassius carassius*	19.7	282	88	0.41
CHA26	*Carassius carassius*	20.5	193	60	0.54
CHA28*	*Carassius carassius*	22.2	189	59	0.57
CHA29	*Carassius carassius*	20.9	197	61	0.58
CHA30*	*Carassius carassius*	19.8	201	61	0.57
CHA31	*Carassius carassius*	21.0	205	63	0.51
CHA 38*	*Equus caballus*	6.7	590	185	0.20
CHA 39	*Equus caballus*	5.4	488	153	0.23
CHA 40	*Equus caballus*	4.2	575	179	0.20
CHA 42*	*Equus caballus*	4.9	456	140	0.23
CHA3	*Equus caballus*	9.7	430	132	0.26
CHA 51	*Esox lucius*	21.8	256	81	0.43
CHA 52	*Esox lucius*	21.5	297	92	0.40
CHA 53	*Esox lucius*	15.3	260	80	0.48
CHA 55	*Esox lucius*	14.8	243	75	0.47
CHA22*	*Esox lucius*	11.9	157	50	0.63
CHA 32*	*Homo sapiens*	17.1	427	133	0.29
CHA 33*	*Homo sapiens*	18.6	420	131	0.29
CHA 34*	*Homo sapiens*	20.4	445	140	0.27
CHA 35*	*Homo sapiens*	19.2	471	147	0.26
CHA 36*	*Homo sapiens*	21.5	506	160	0.25
CHA 37	*Homo sapiens*	15.8	488	153	0.25
CHA1	*Homo sapiens*	11.3	491	150	0.23

Sample	Species	$\delta^{34}S$	C:S	N:S	%S
CHA13	*Homo sapiens*	13.8	461	146	0.24
CHA14	*Homo sapiens*	15.2	477	150	0.24
CHA15	*Homo sapiens*	9.5	444	139	0.25
CHA5	*Ovis aries*	9.0	391	118	0.23
CHA 56	*Perca fluviatilis*	21.0	298	93	0.41
CHA 57	*Perca fluviatilis*	20.9	287	90	0.40
CHA8b	*Perca fluviatilis*	15.0	223	69	0.47
CHA 49	*Sus scrofa*	11.2	516	158	0.23
CHA 50	*Sus scrofa*	10.9	550	167	0.23

Table A3.2. $\delta^{34}S$, C:S, N:S and %S values for Bil'shivtsi Samples. Values are averages of duplicate sample analyses, except for the samples marked with an asterisk (*), which were analysed once.

Sample	Species	$\delta^{34}S$	C:S	N:S	%S
BIL 26	*Bos taurus*	4.4	622	187	0.17
BIL 27	*Bos taurus*	6.9	498	154	0.18
BIL 39	*Capra hircus*	13.6	393	119	0.24
BIL 40*	*Capra hircus*	13.6	399	122	0.24
BIL 41*	*Capra hircus*	17.6	349	105	0.32
BIL 45*	*Cervus elaphus*	5.9	577	178	0.20
BIL 45 ult 2	*Cervus elaphus*	4.8	369	108	0.36
BIL 12	*Emys sp.*	17.3	480	149	0.20
BIL 13	*Emys sp.*	10.4	351	108	0.24
BIL 14	*Emys sp.*	10.0	257	76	0.46
BIL 15*	*Emys sp.*	11.4	351	107	0.22
BIL 46	*Equus caballus*	2.6	408	123	0.18
BIL 49*	*Equus caballus*	5.8	305	92	0.22
BIL 50	*Equus caballus*	2.6	254	78	0.26
BIL 23*	*fish*	3.2	258	78	0.40
BIL 25*	*fish*	6.9	184	55	0.36
BIL 1	*Homo sapiens*	3.0	390	121	0.22
BIL 32*	*Ovis aries*	14.9	423	130	0.22
BIL 33*	*Ovis aries*	11.3	328	101	0.32
BIL 34	*Ovis aries*	10.5	480	147	0.22
BIL 36*	*Ovis aries*	13.9	406	124	0.26
BIL 17*	*Sus scrofa dom.*	1.7	546	163	0.18
BIL 18	*Sus scrofa dom.*	9.1	345	107	0.32
BIL 19	*Sus scrofa dom.*	0.6	519	161	0.20

Table A3.3. %S, C:S and N:S data for all modern samples discussed in chapter 5.

Sample	Species	C:S	N:S	%S
CHA 62	*Carassius carassius*	366	116	0.31
CHA 63	*Carassius carassius*	221	66	0.53
SMBGd	*Bos taurus*	666	211	0.19
TOC NOC 1	*Homo sapiens*	434	134	0.28
TOC NOC 4	*Homo sapiens*	212	61	0.60
TOC NOC 11	*Homo sapiens*	106	27	1.07
Mullet1	*Liza argentea*	232	75	0.47
Luderick2	*Girella tricuspidata*	262	83	0.44
MangroveJack3	*Lutjanus argentimaculatus*	209	67	0.44
Morwong4	*Nemadactylus douglasii*	226	72	0.48
Flathead5	*Platycephalus fuscus*	253	82	0.45
Luderick7	*Girella tricuspidata*	254	82	0.43
Bream8	*Acanthopagrus australis*	246	80	0.46
Luderick9	*Girella tricuspidata*	247	79	0.47
Snapper10	*Chrysophrys auratus*	234	75	0.48
Bonito11	*Sarda australis*	272	86	0.43
Bonito12	*Sarda australis*	285	90	0.40
Mullet13	*Liza argentea*	280	90	0.40
Luderick14	*Girella tricuspidata*	254	82	0.45
Morwong15	*Nemadactylus douglasii*	225	72	0.49
Wrasse16	*Opthalmolepis lineolatus*	251	81	0.45
Goatfish17	*Parupeneus porphyreus*	252	81	0.40

APPENDIX 4. *PROVENANCE OF CHICHA SAMPLES FOR RESIDUE ANALYSIS*

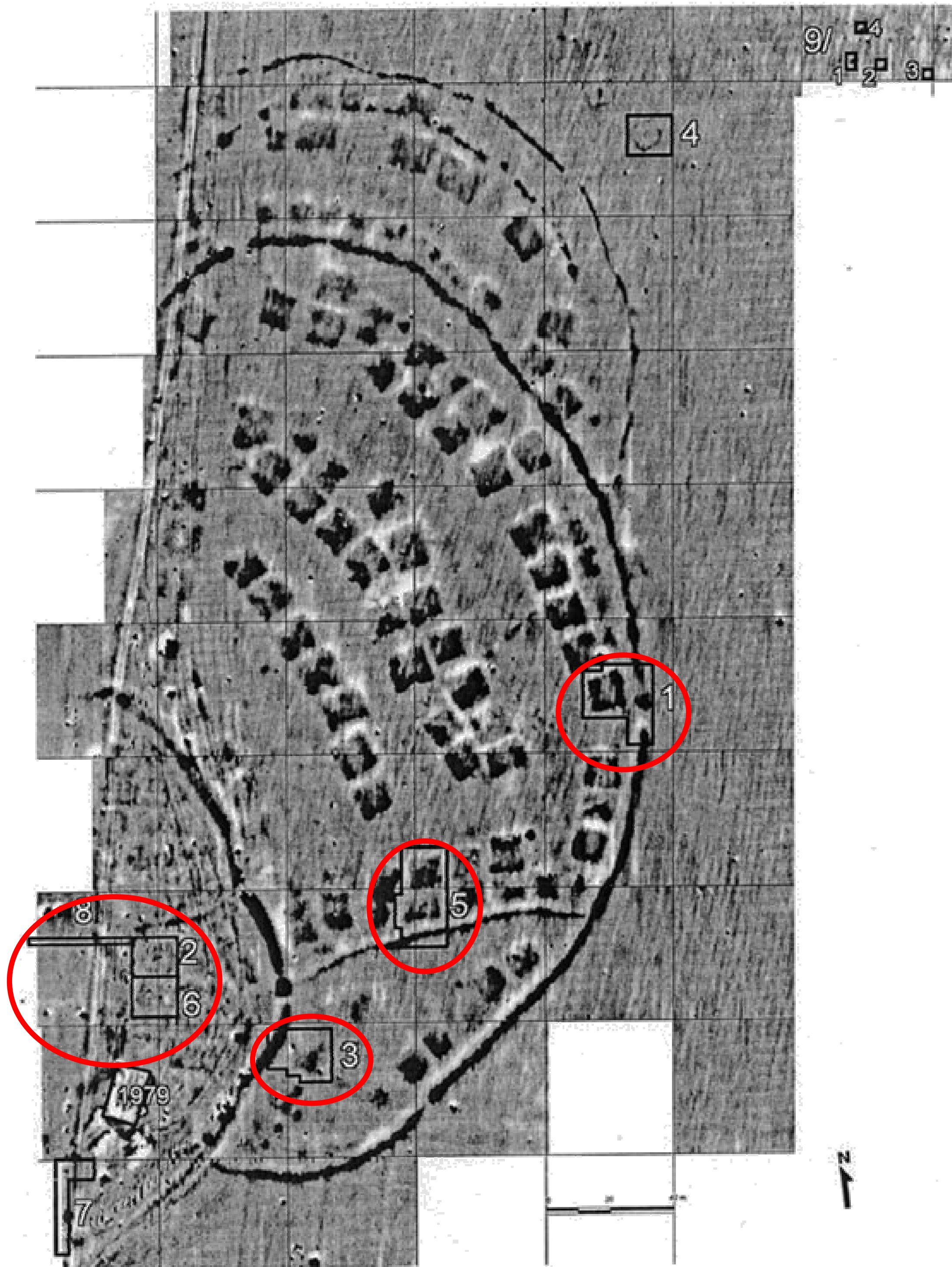


Figure A4.1. Magnetic prospection of Chicha (Molodin *et al.* 2001), displaying the location of excavation trenches. Circled trenches indicate areas from which potsherds were obtained for residue analysis.

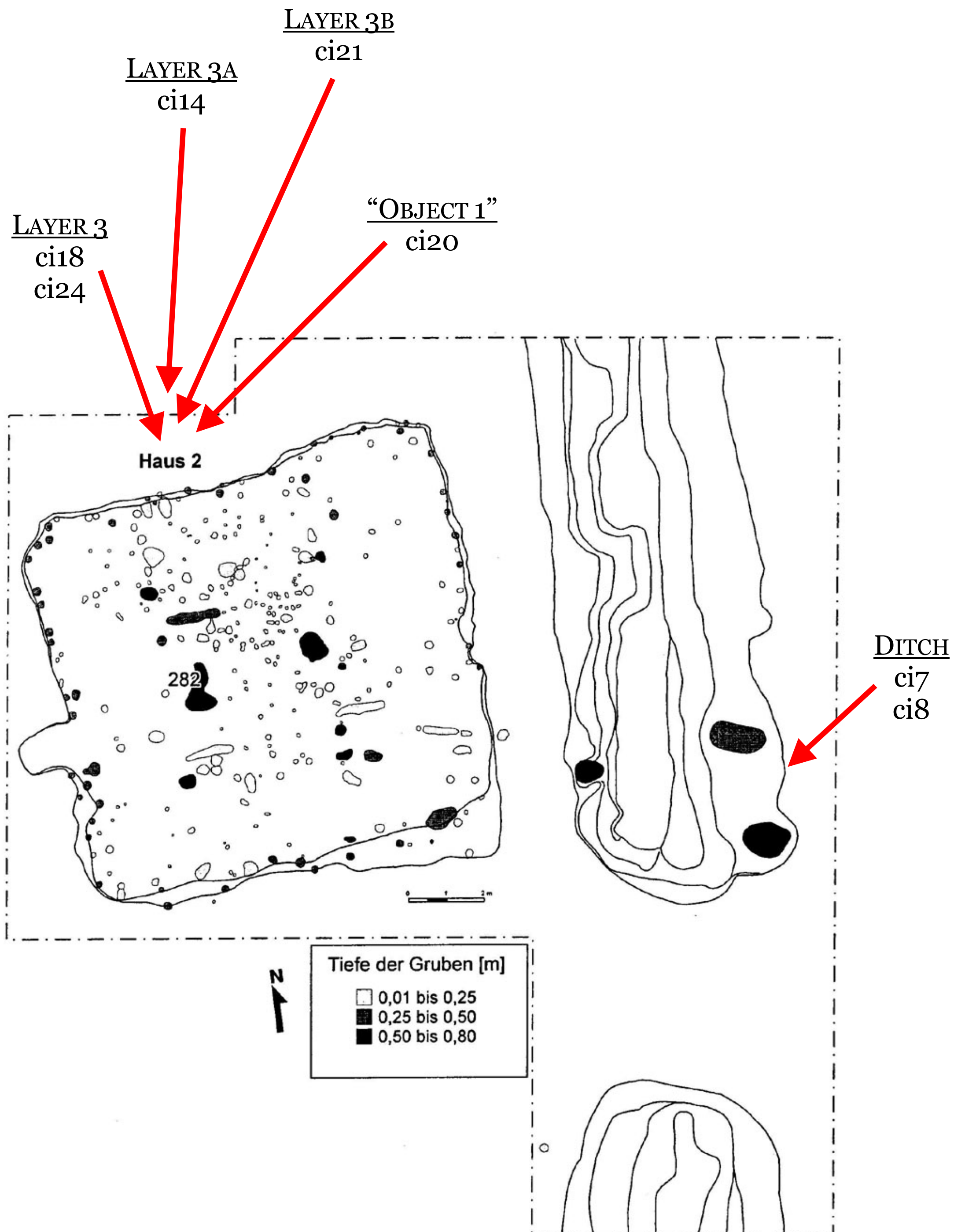


Figure A4.2. A drawing of Chicha trench 1, excavated in 2000 (provided by Jens Schneeweiβ, Deutsches Archäologisches Institut, Berlin, Germany). Areas from which potsherds were obtained are indicated.

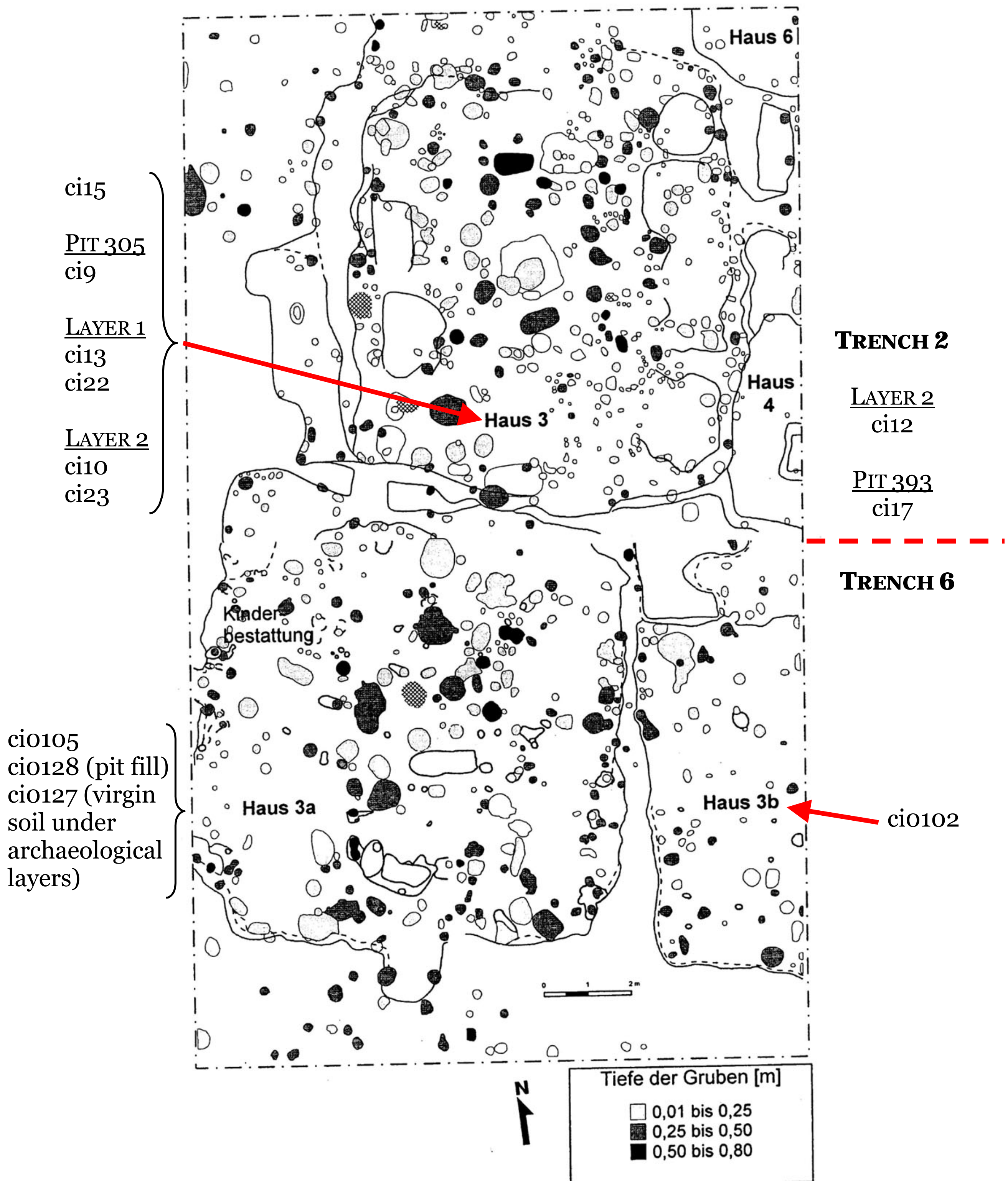


Figure A4.3. A drawing of Chicha trenches 2 and 6, excavated in 2000 and 2001, respectively (provided by Jens Schneeweiβ, Deutsches Archäologisches Institut). Areas from which potsherds were obtained are indicated.

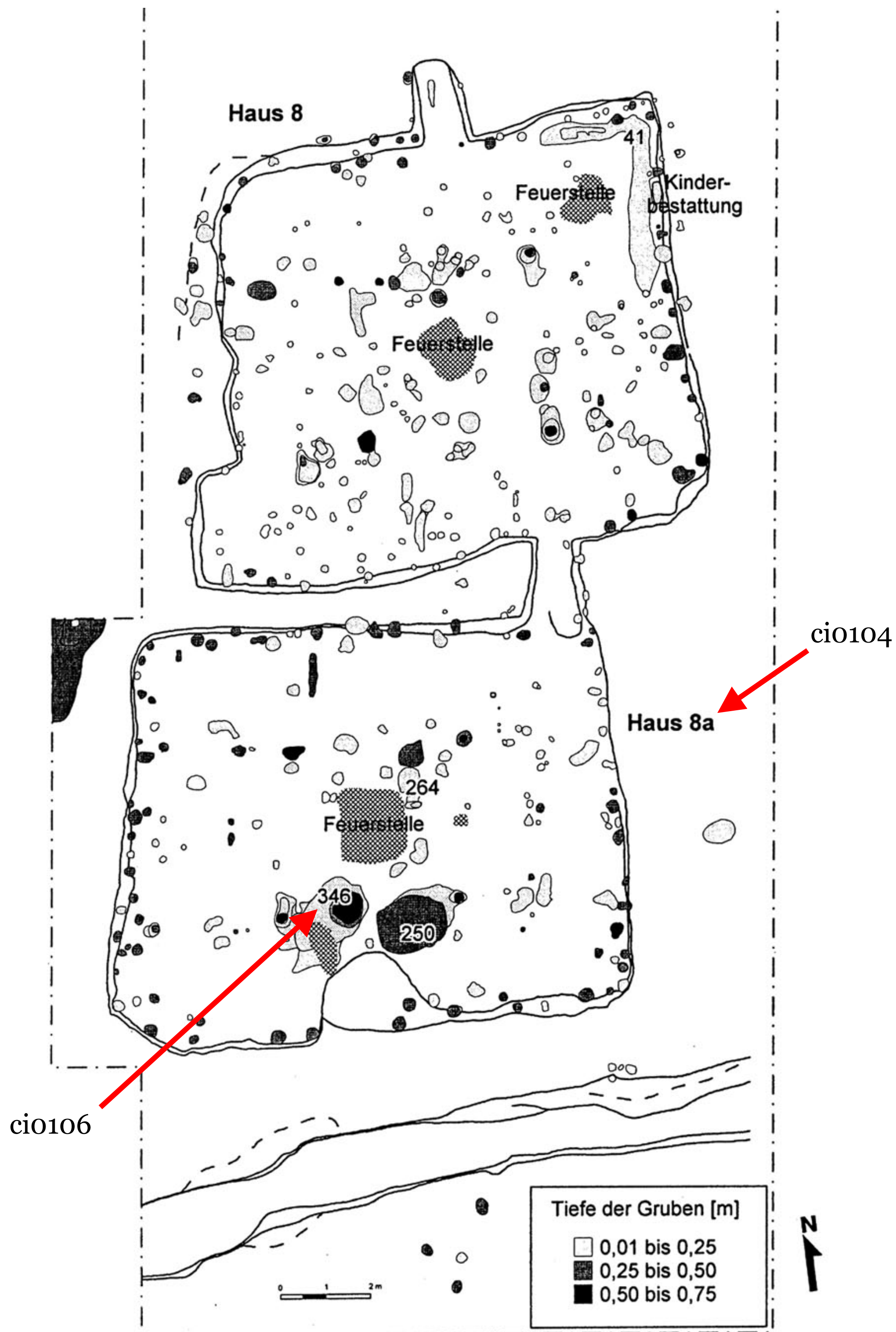


Figure A4.4. A drawing of Chicha trench 5, excavated in 2001 (provided by Jens Schneeweiβ, Deutsches Archäologisches Institut). Areas from which potsherds were obtained are indicated.

APPENDIX 5. *GAS CHROMATOGRAPHS OF SAMPLES WITH LOW LIPID YIELD*

Figure A5.1. ci8a

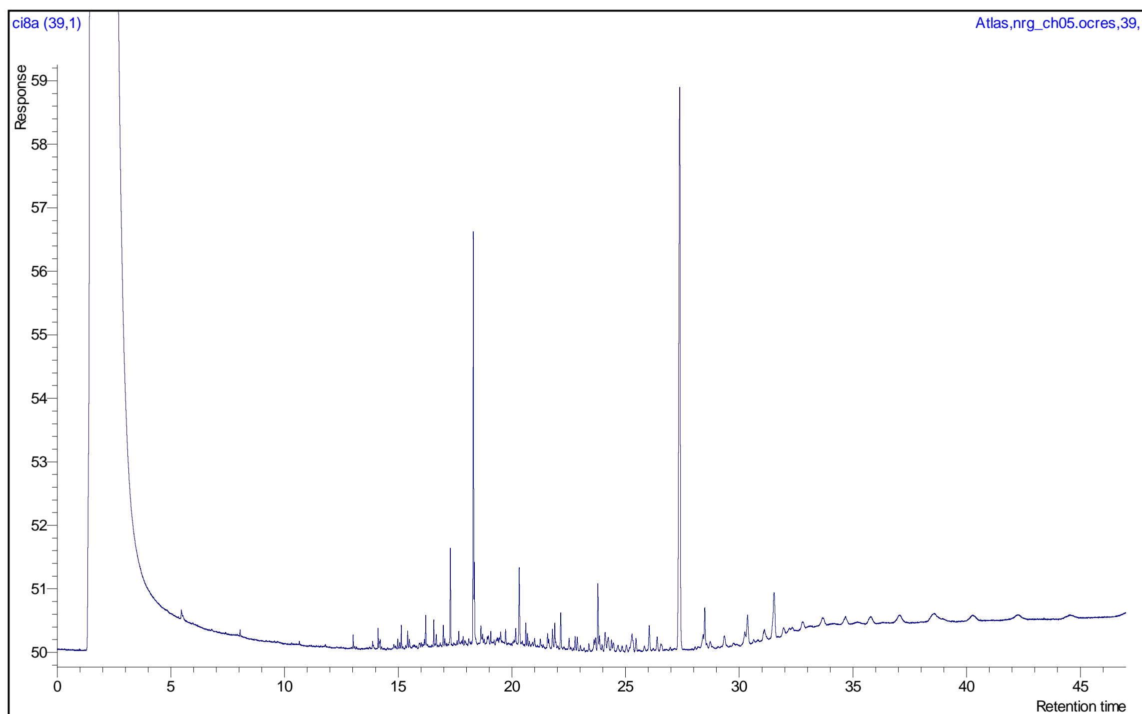


Figure A5.2. ci9a

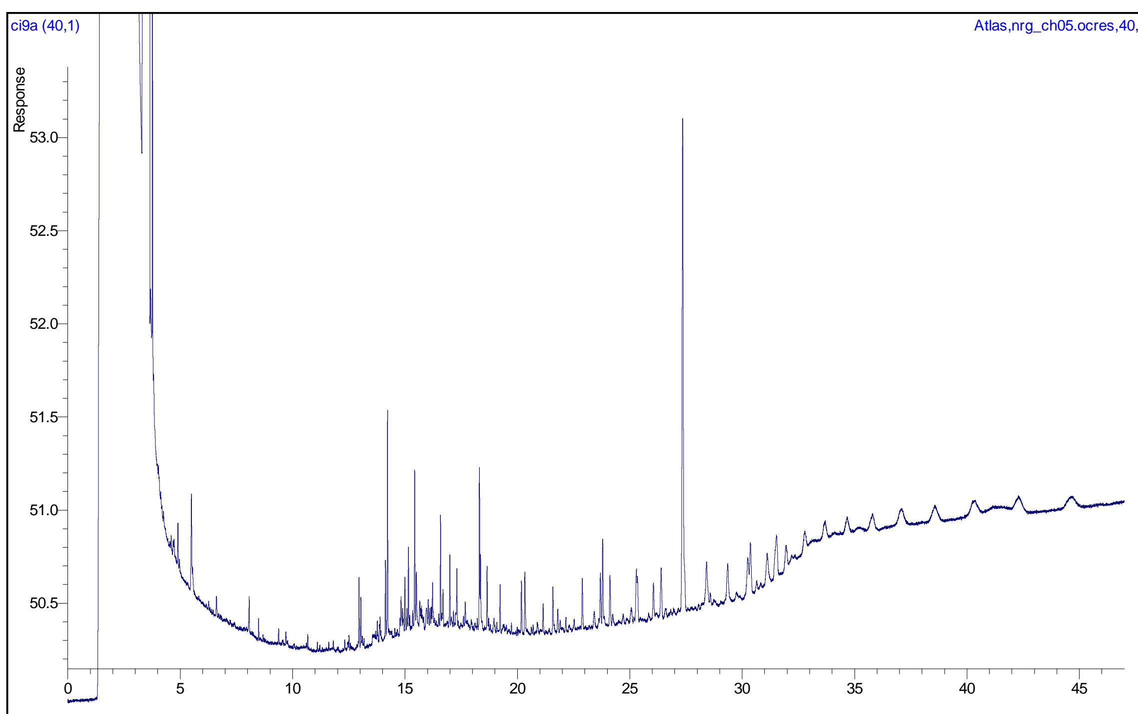


Figure A5.3. ci17a

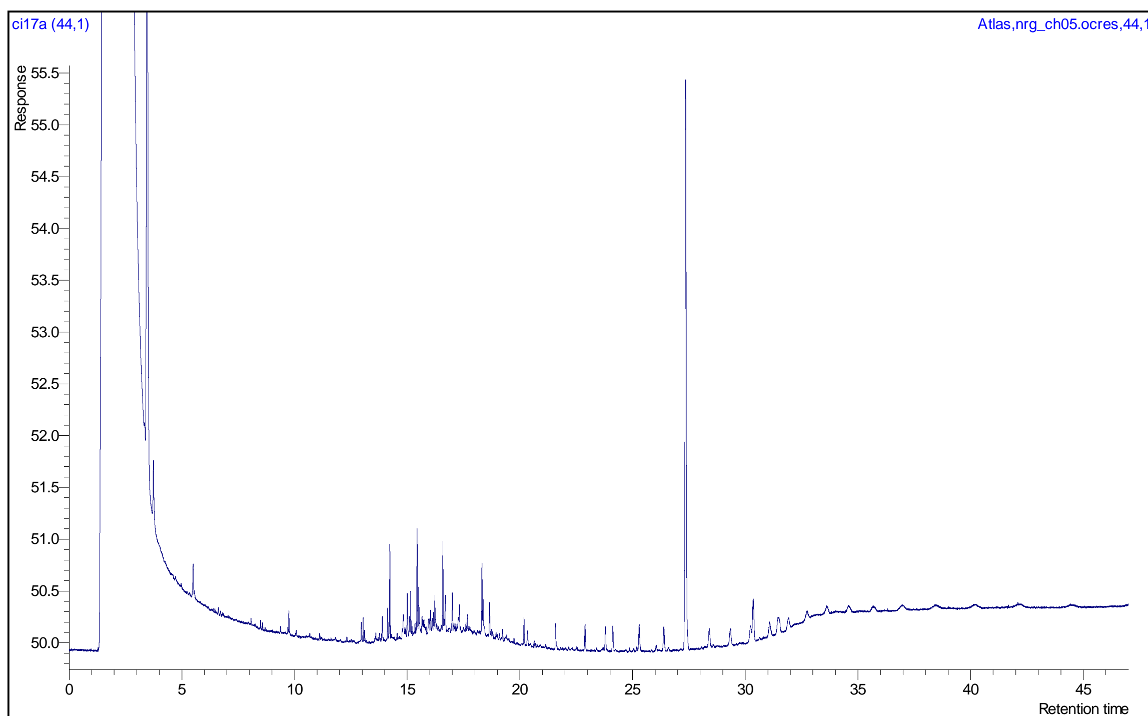


Figure A5.4. ci19a

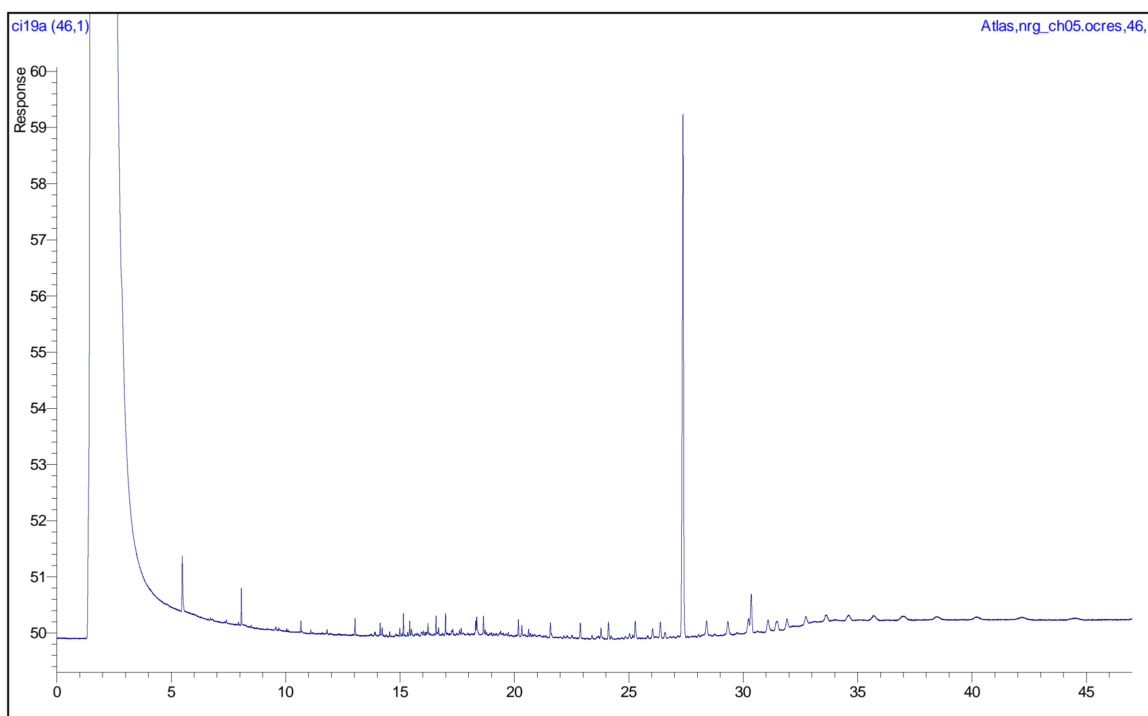


Figure A5.5. ci0103a

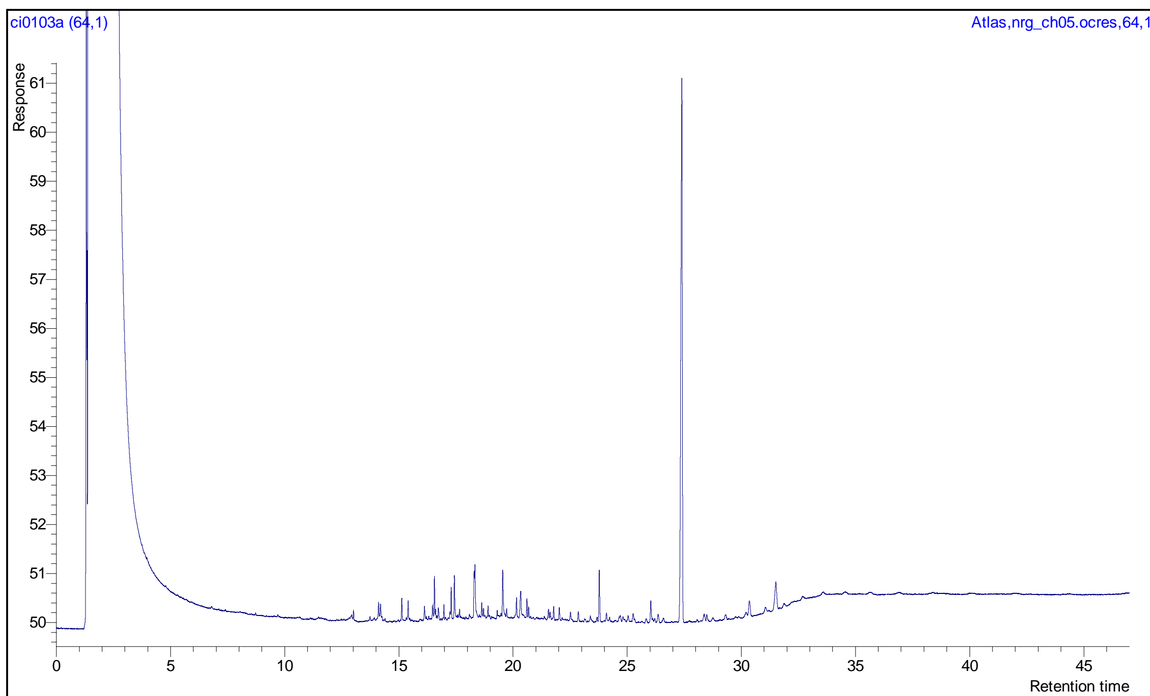


Figure A5.6. ci0107a

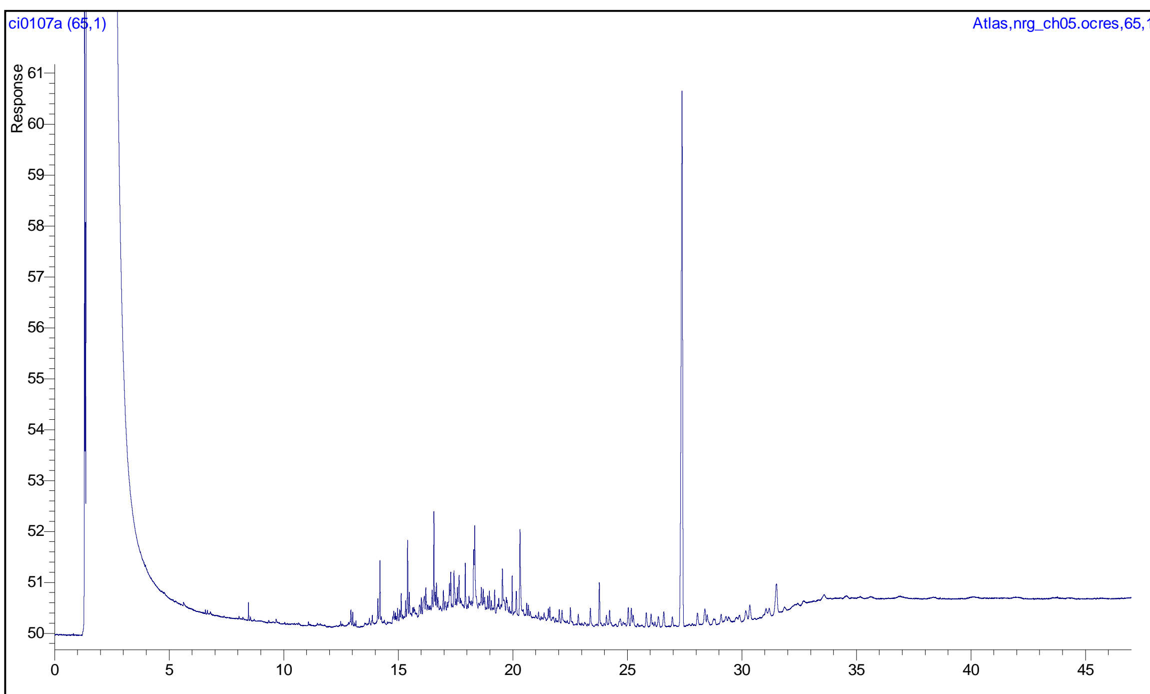


Figure A5.7. ci0112c

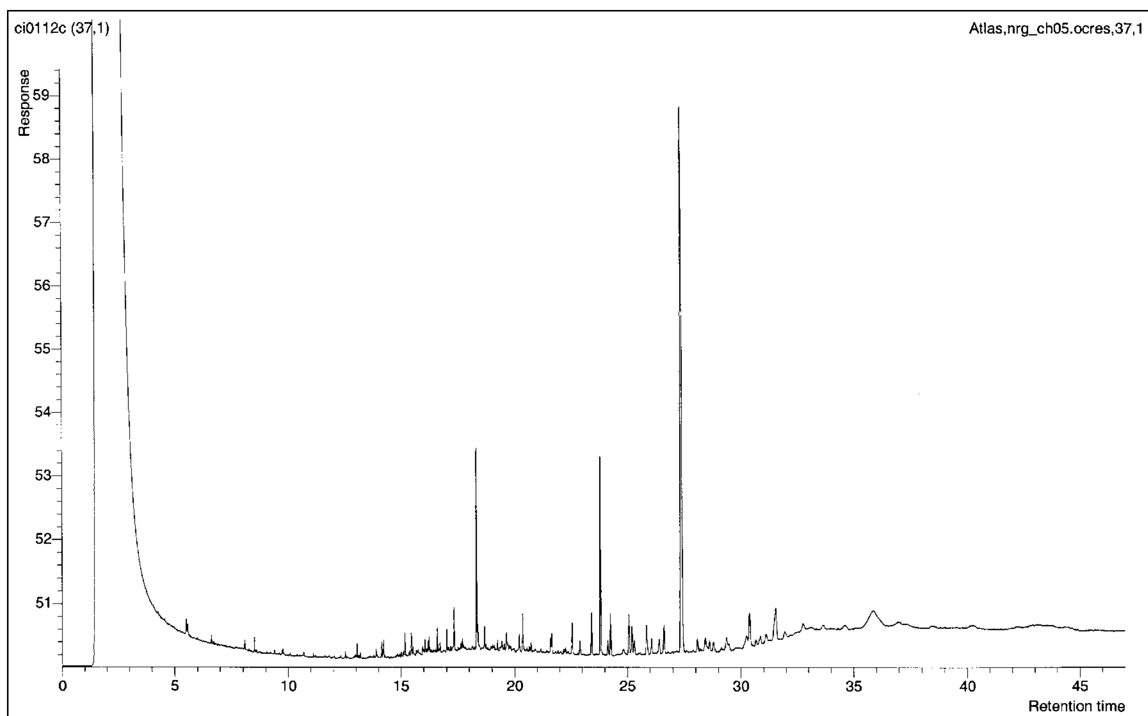


Figure A5.8. ci0119a

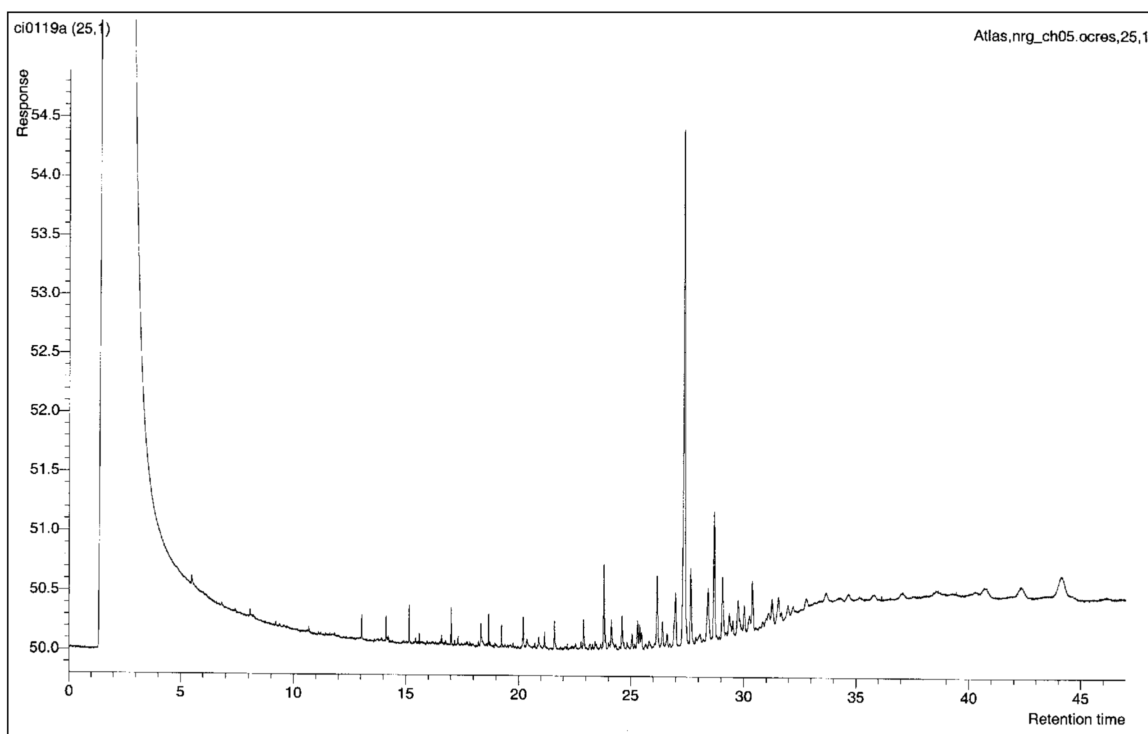
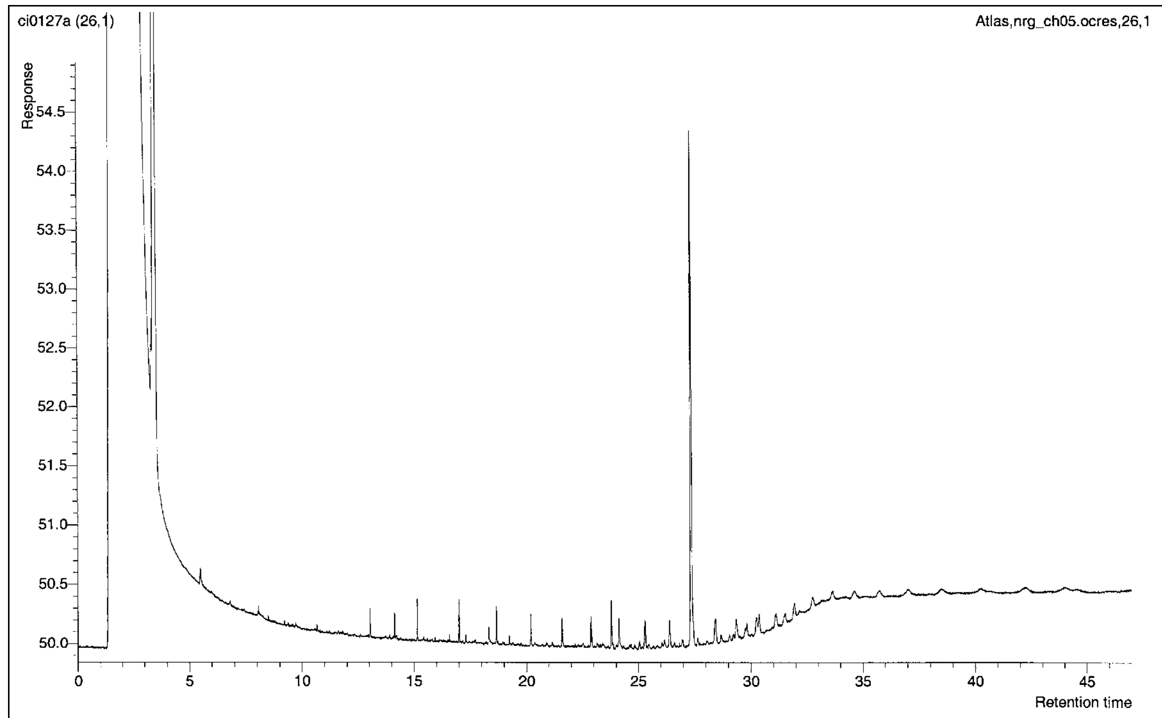


Figure A5.9. ci0127a



APPENDIX 6. *TRACES FOR SAMPLES RUN BY GC-MS*

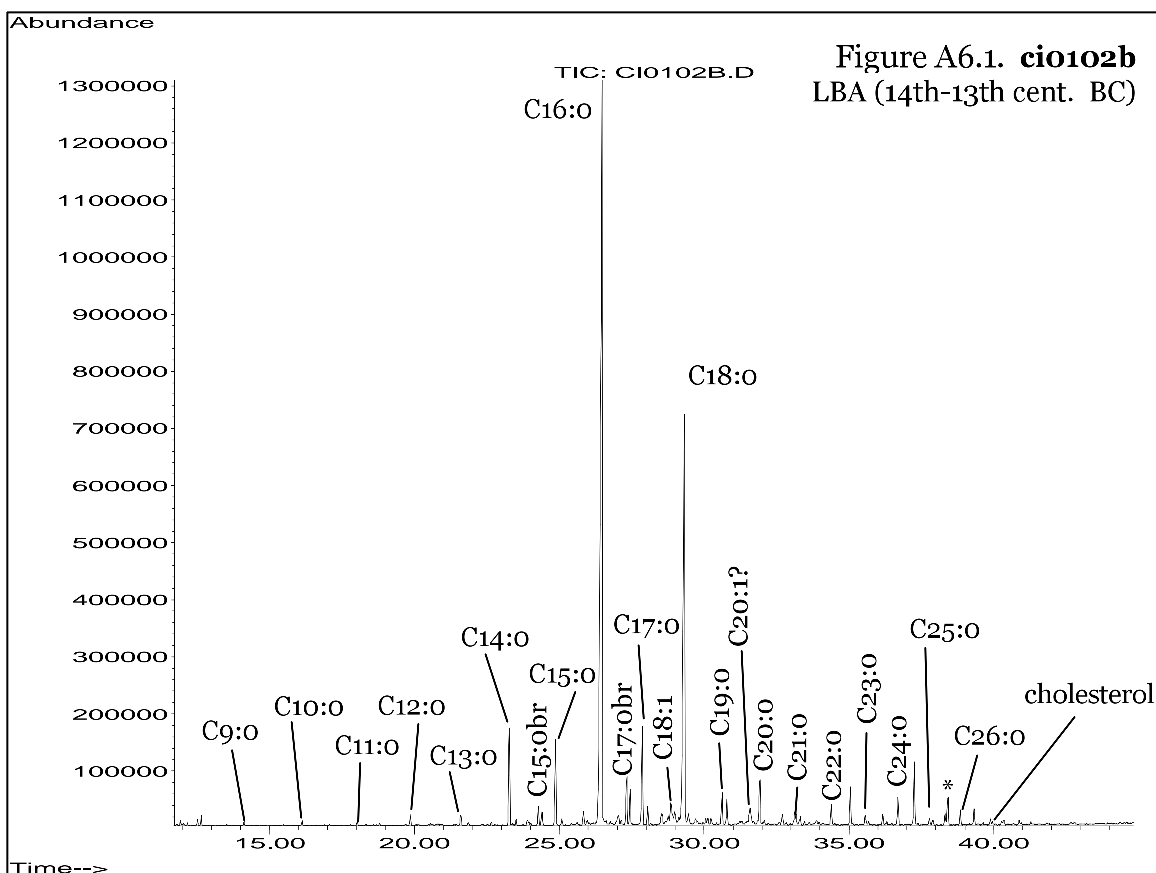


Figure A6.1. **ci0102b**
LBA (14th-13th cent. BC)

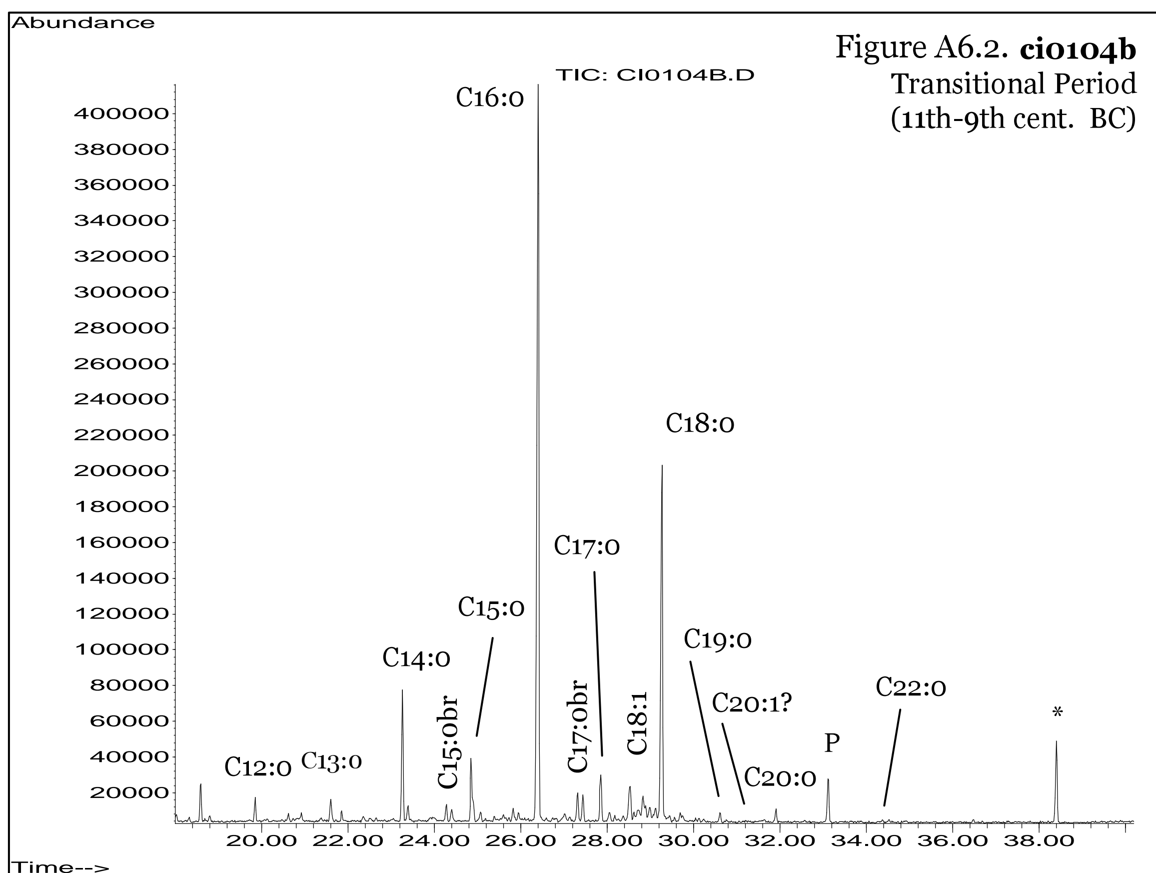


Figure A6.2. **ci0104b**
Transitional Period
(11th-9th cent. BC)

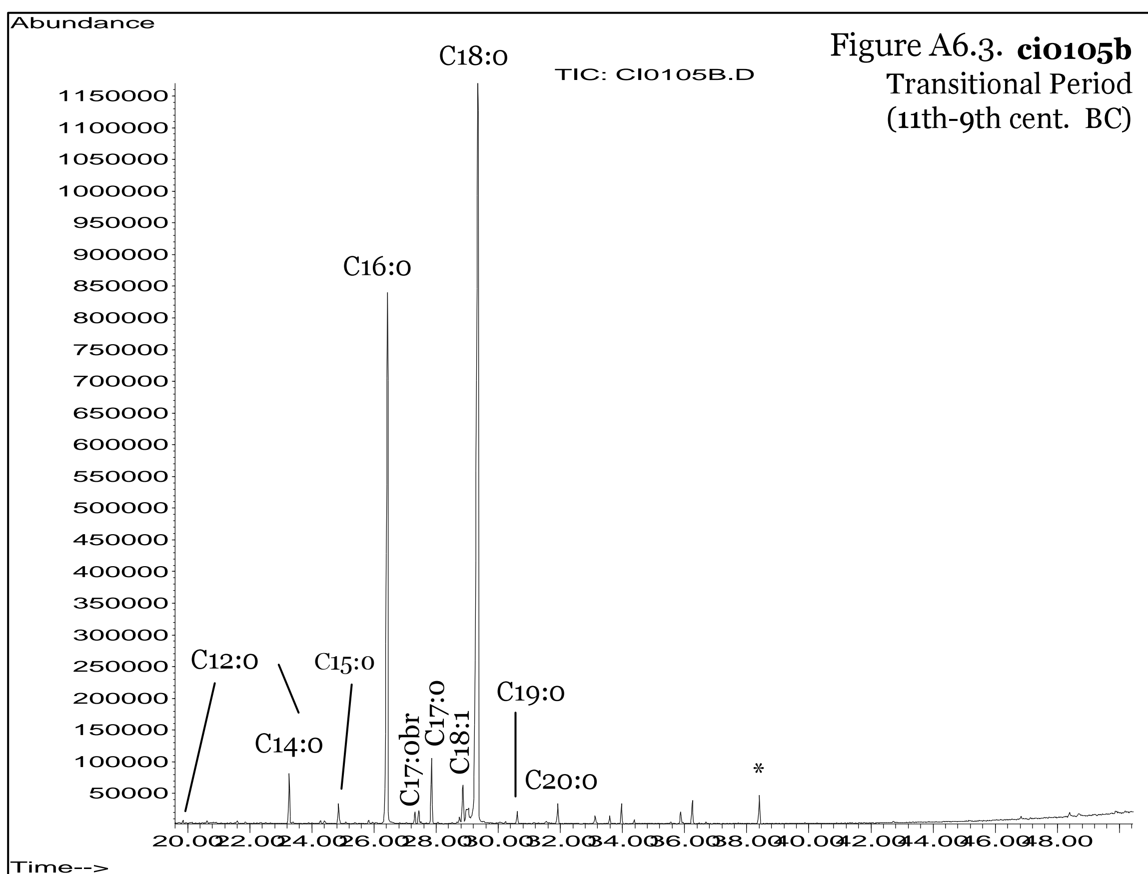


Figure A6.3. **ci0105b**
Transitional Period
(11th-9th cent. BC)

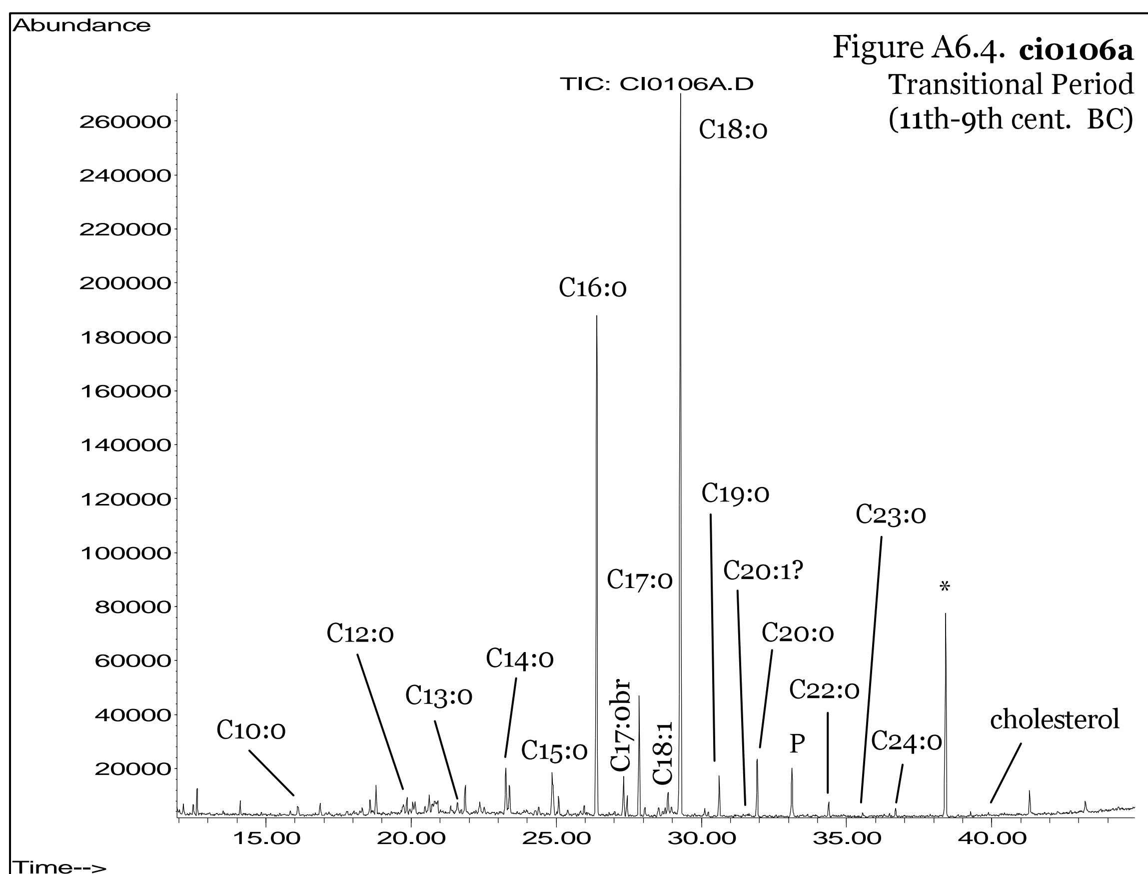


Figure A6.4. **ci0106a**
Transitional Period
(11th-9th cent. BC)

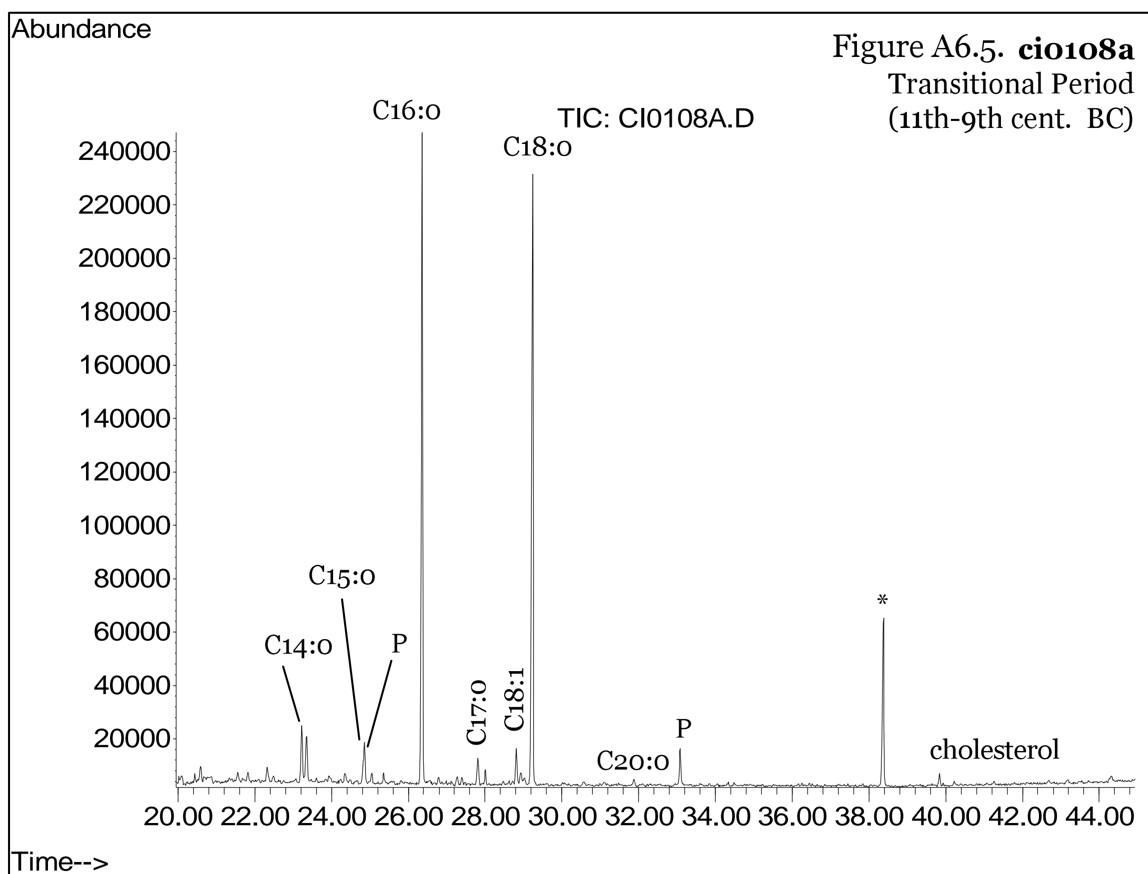


Figure A6.5. **ci0108a**
Transitional Period
(11th-9th cent. BC)

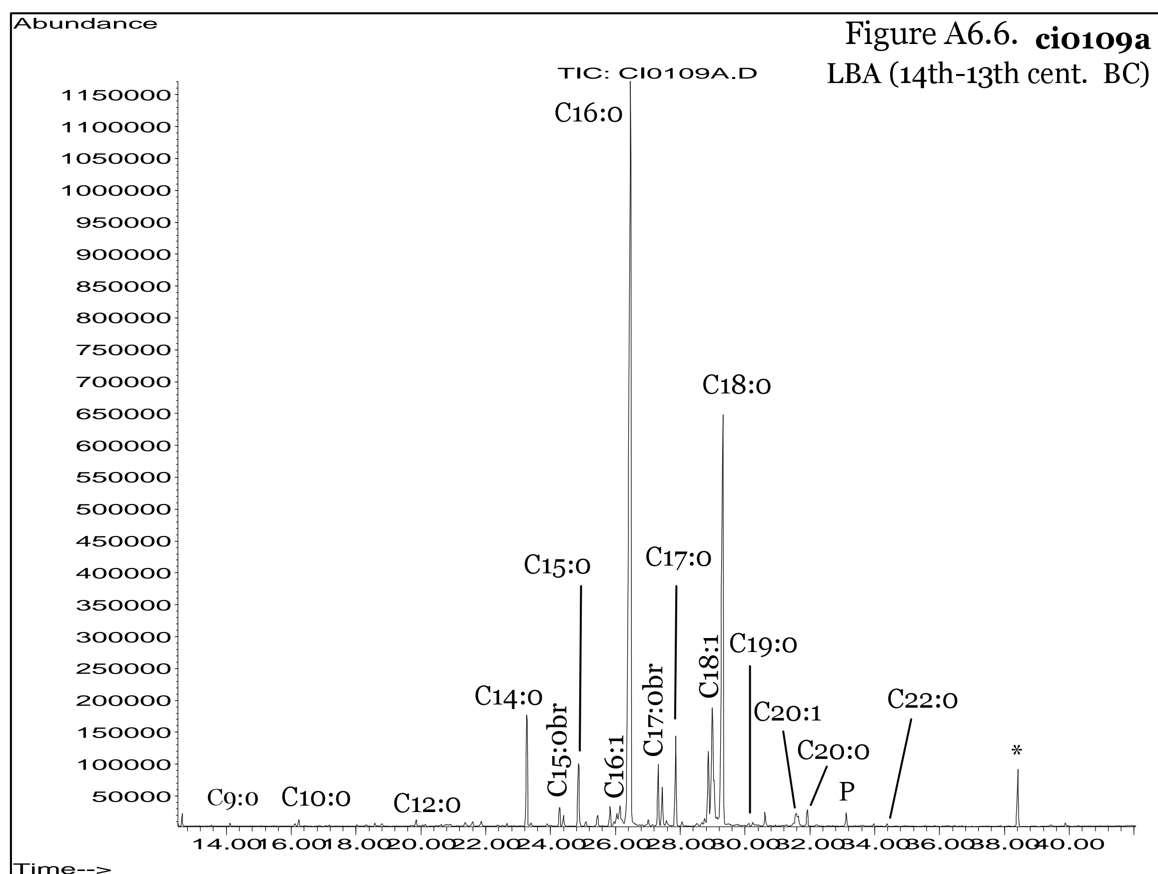


Figure A6.6. **ci0109a**
LBA (14th-13th cent. BC)

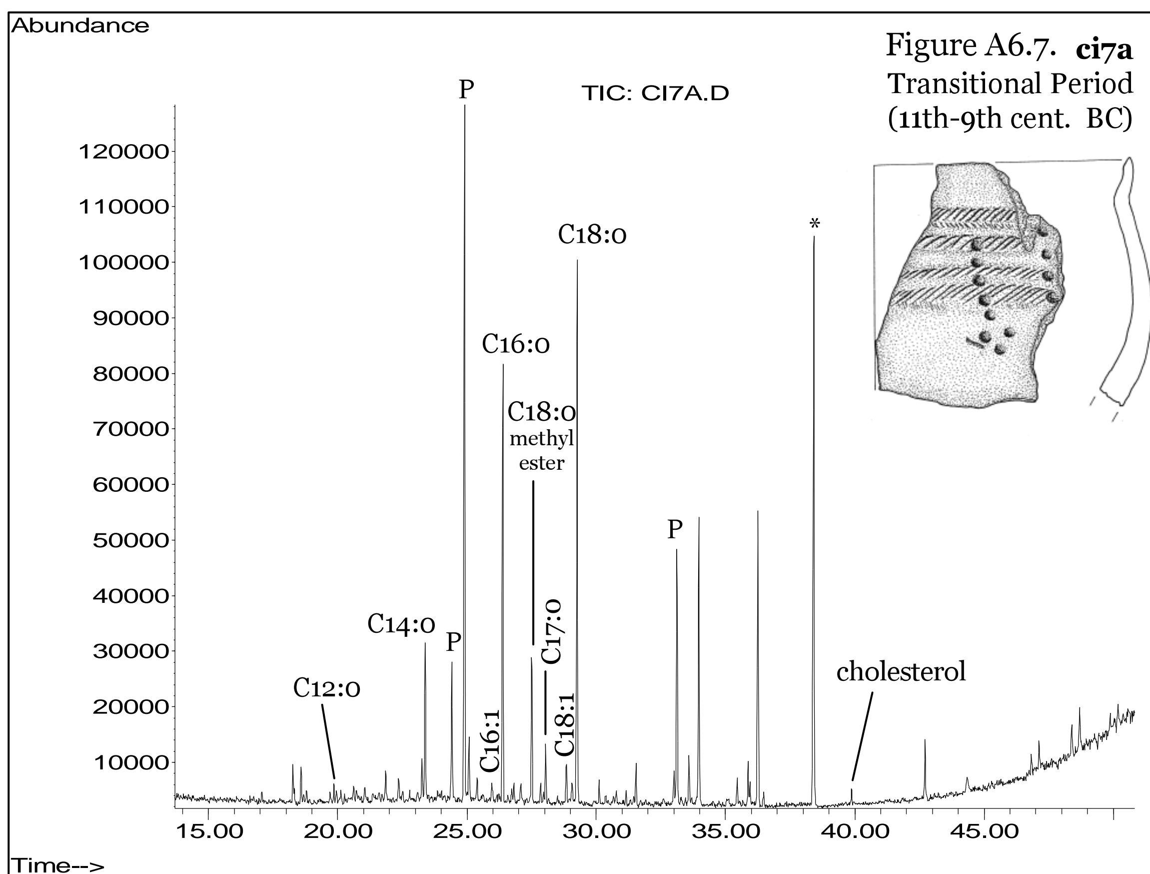


Figure A6.7. **ci7a**
Transitional Period
(11th-9th cent. BC)

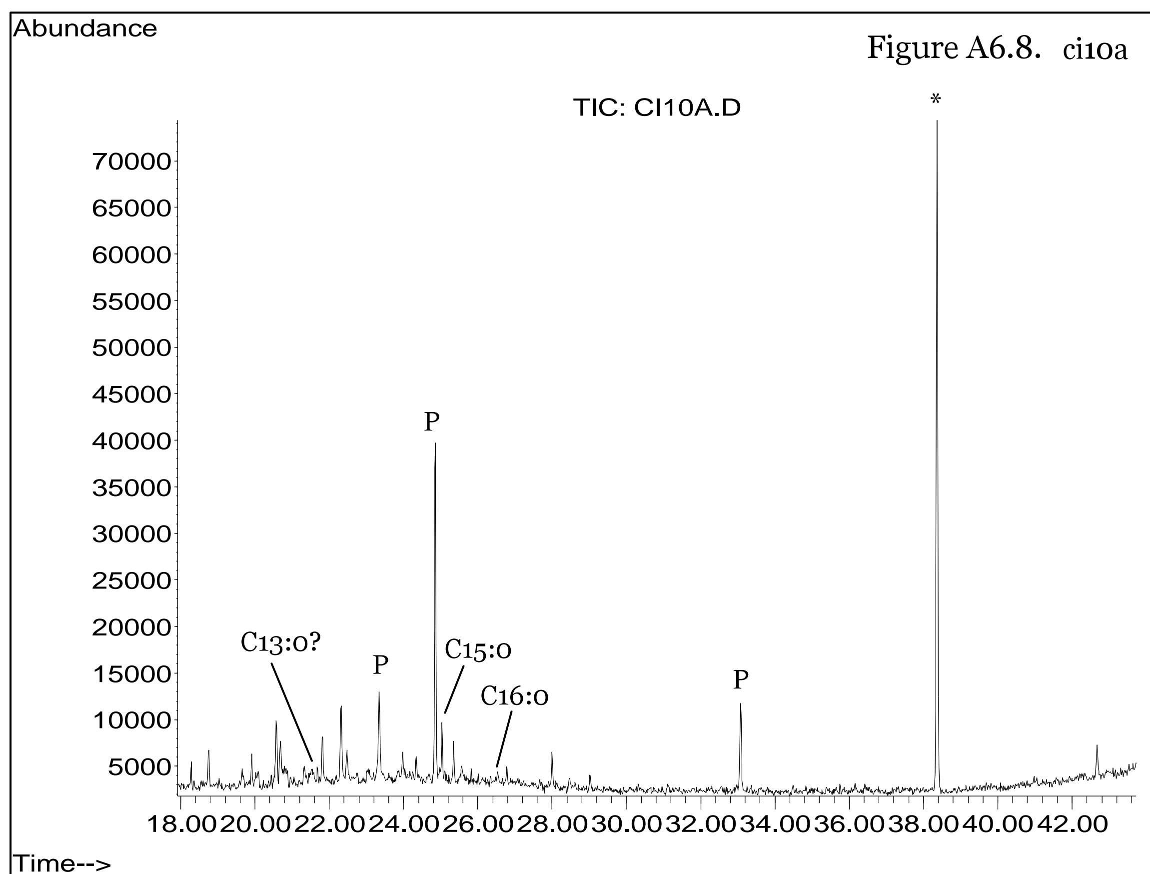


Figure A6.8. ci10a

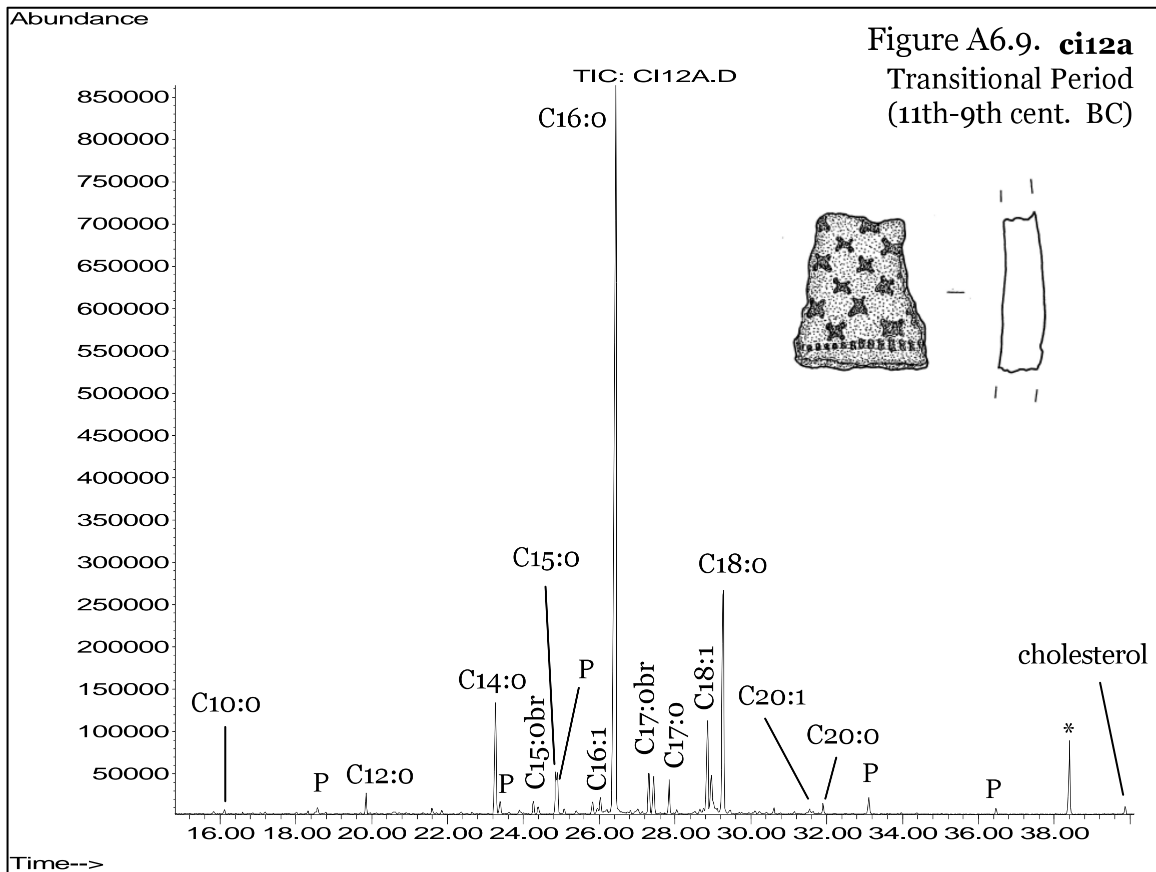


Figure A6.9. **ci12a**
Transitional Period
(11th-9th cent. BC)

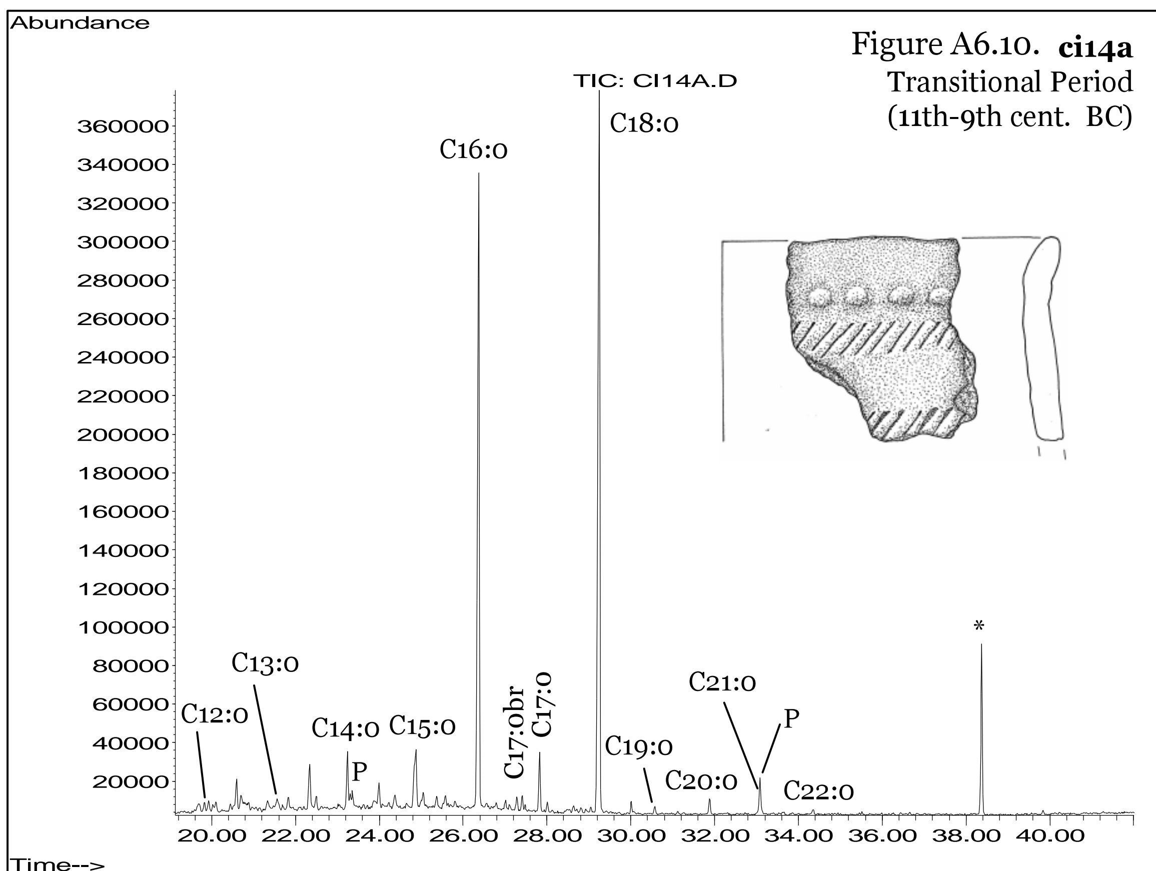


Figure A6.10. **ci14a**
Transitional Period
(11th-9th cent. BC)

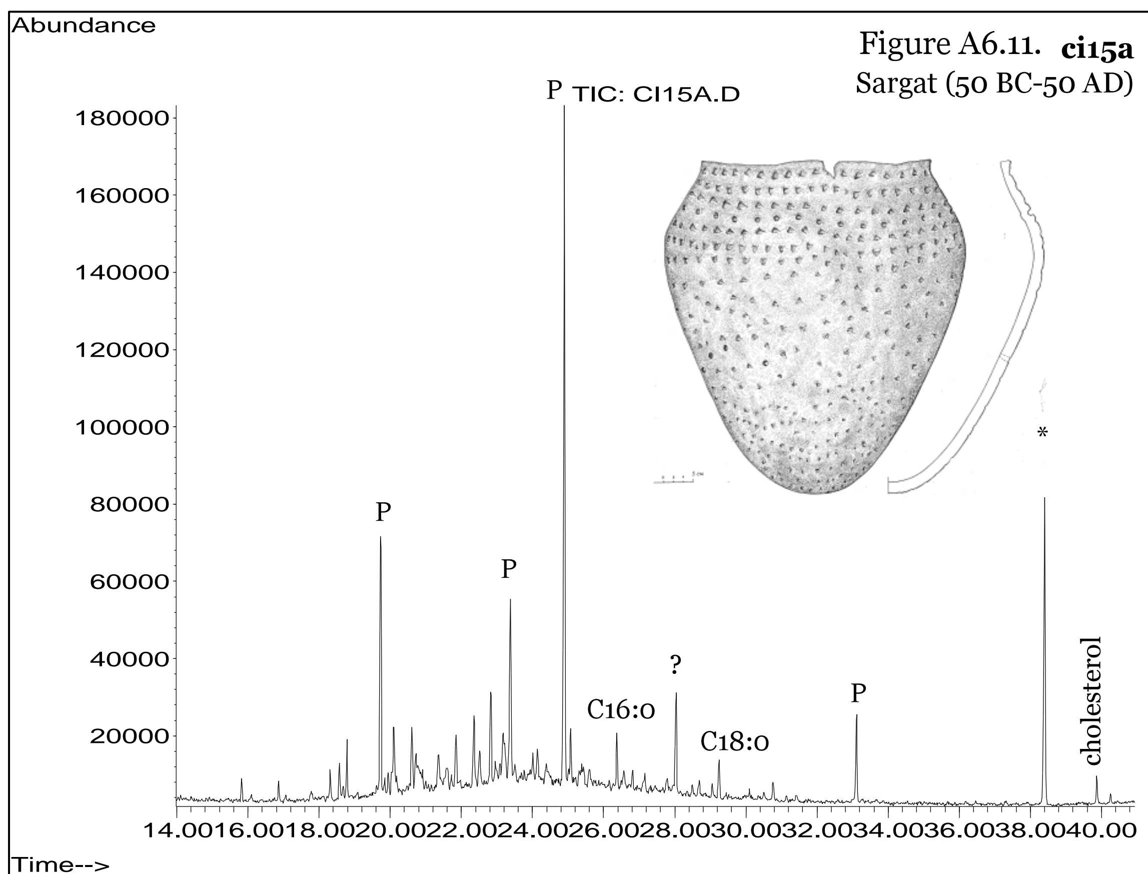


Figure A6.11. **ci15a**
Sargat (50 BC-50 AD)

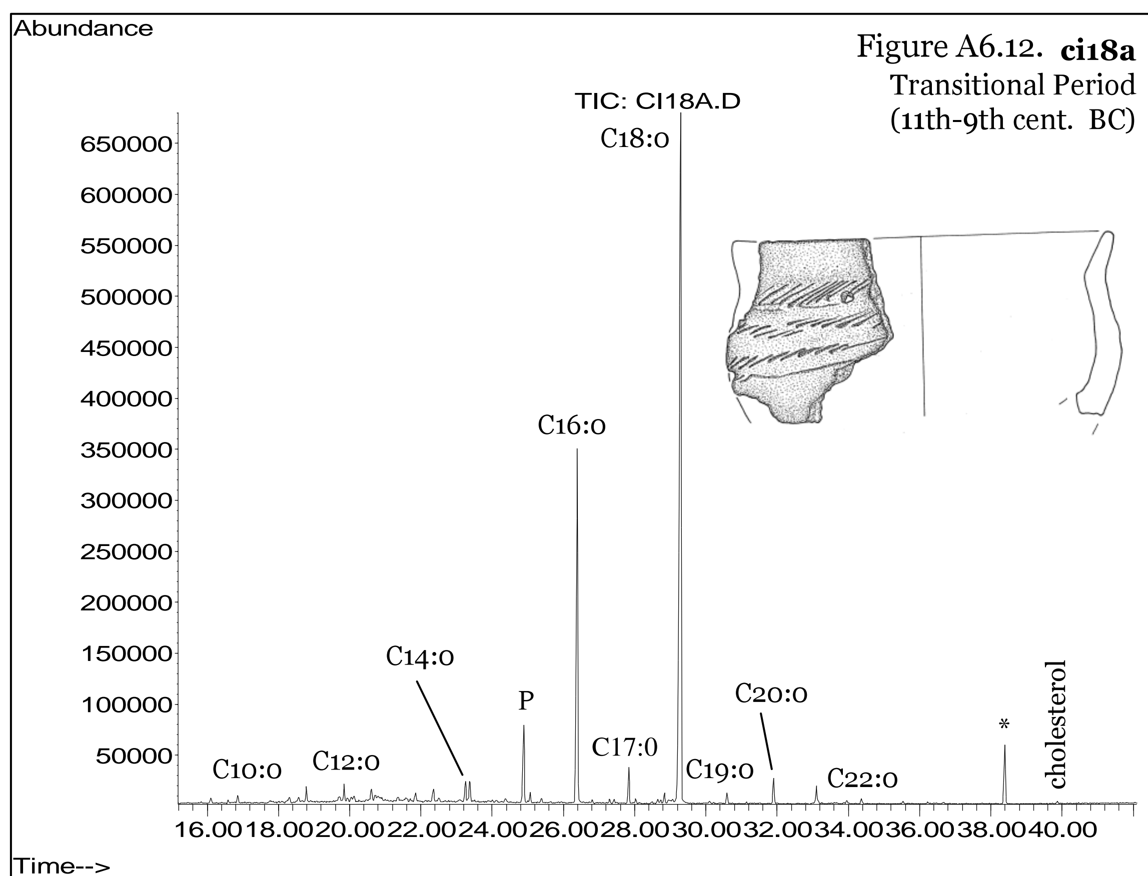


Figure A6.12. **ci18a**
Transitional Period
(11th-9th cent. BC)

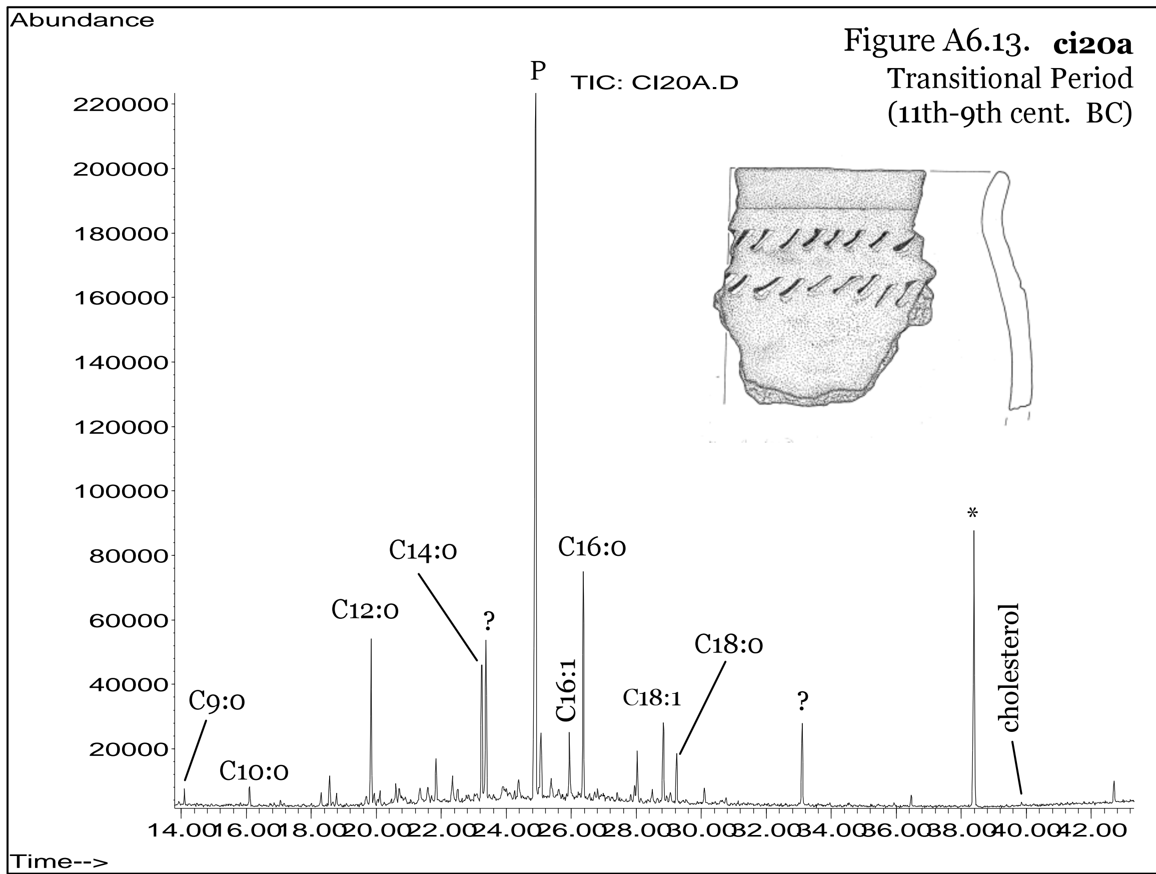


Figure A6.13. **ci20a**
Transitional Period
(11th-9th cent. BC)

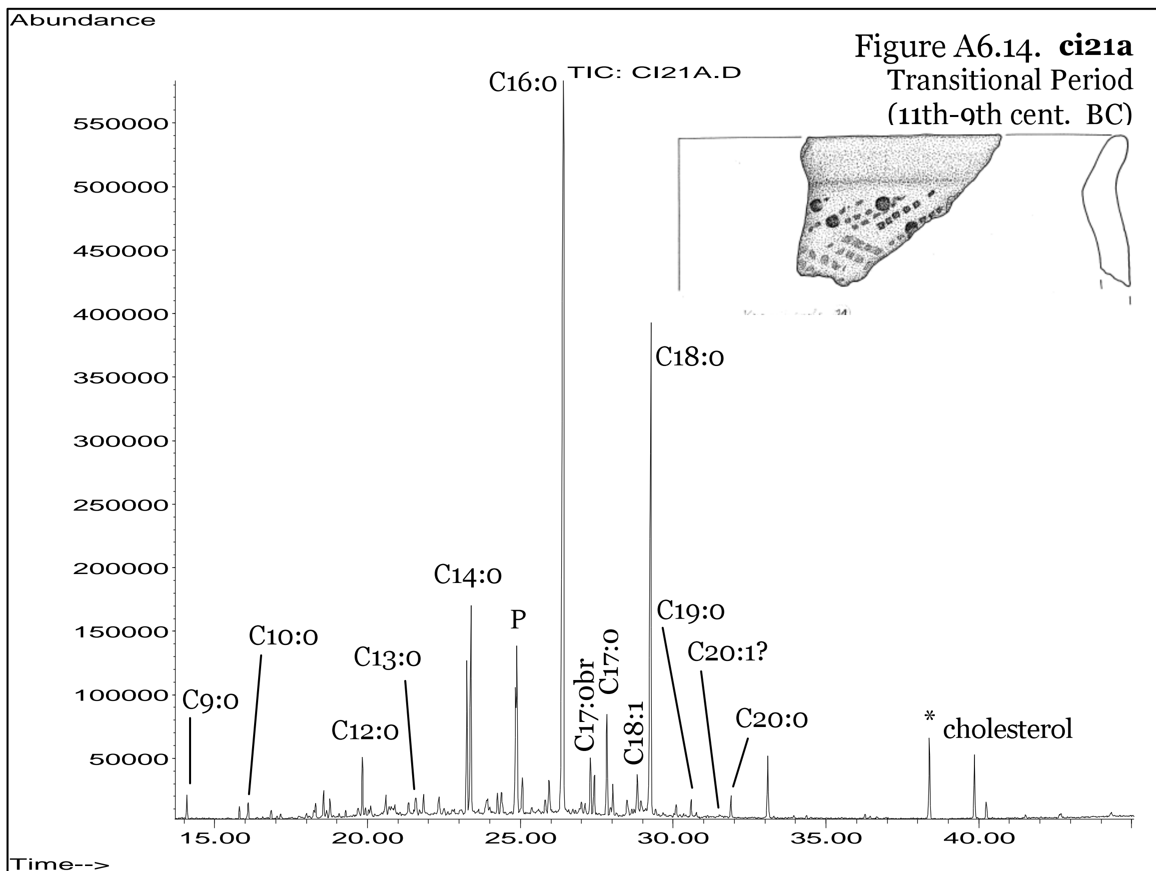


Figure A6.14. **ci21a**
Transitional Period
(11th-9th cent. BC)

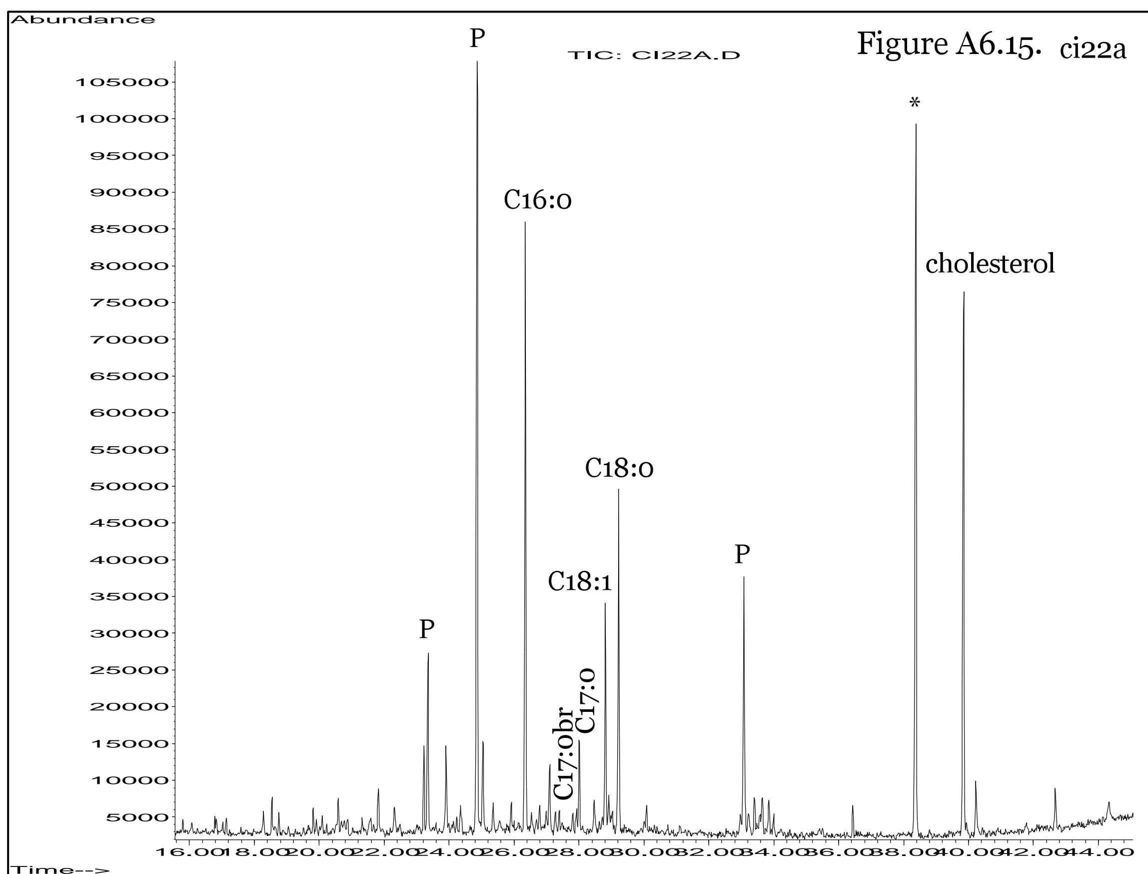
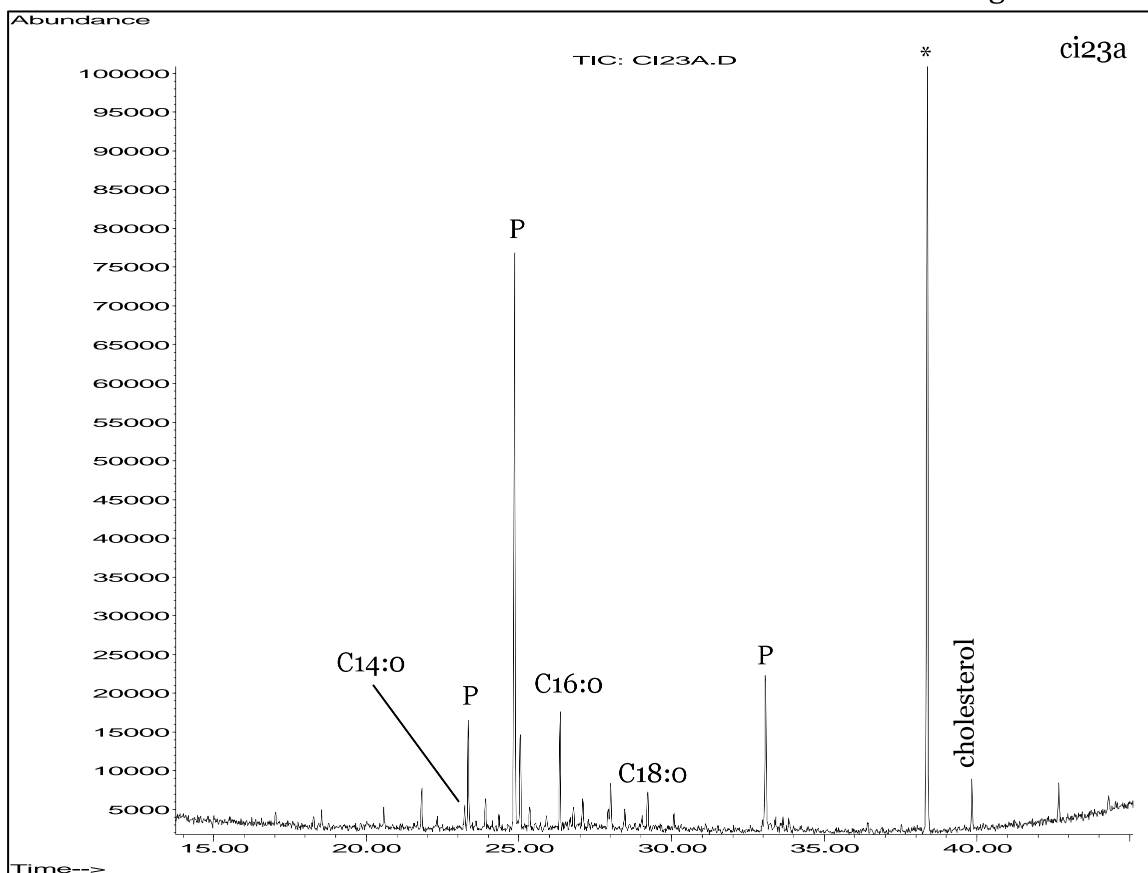
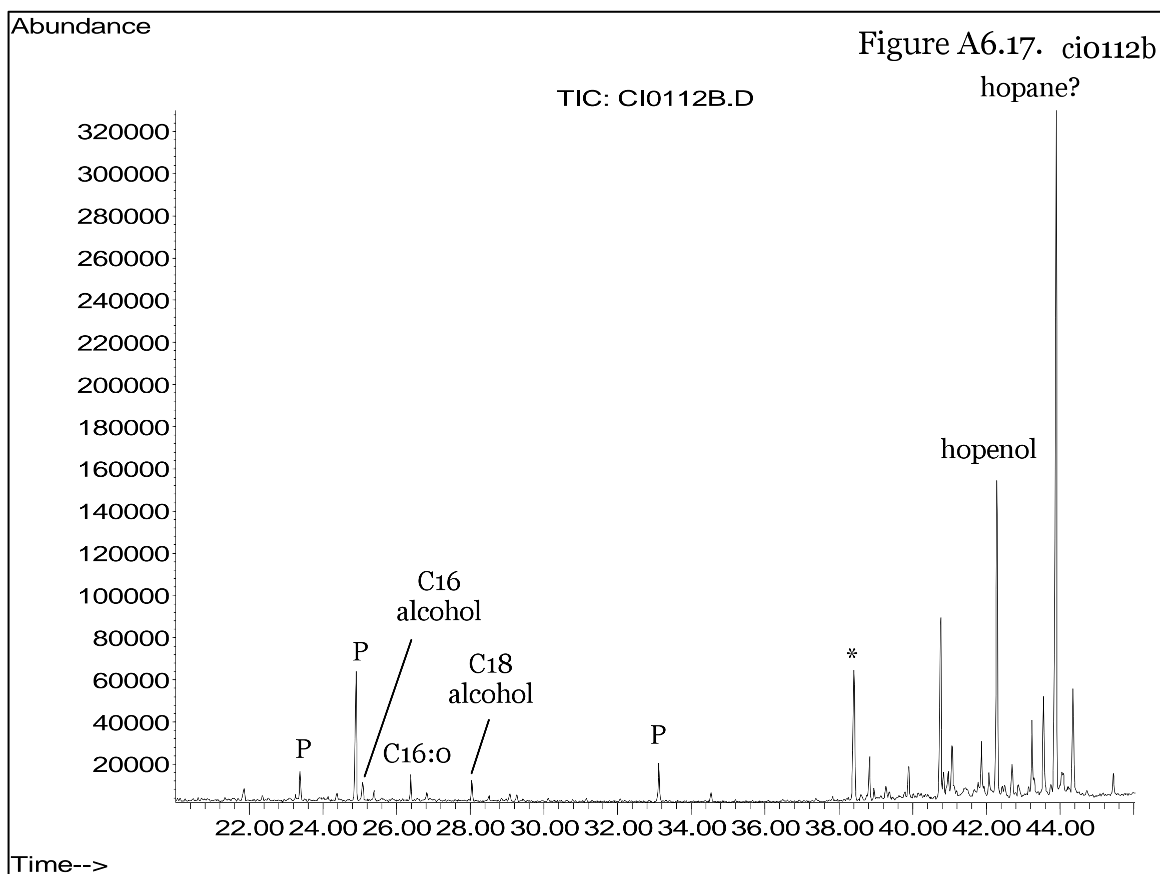


Figure A6.16.





Abundance
Figure A6.17. ci0112b
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hopane?
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260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
hopenol
C16
alcohol
P
C18
alcohol
*
P
C16:0
P
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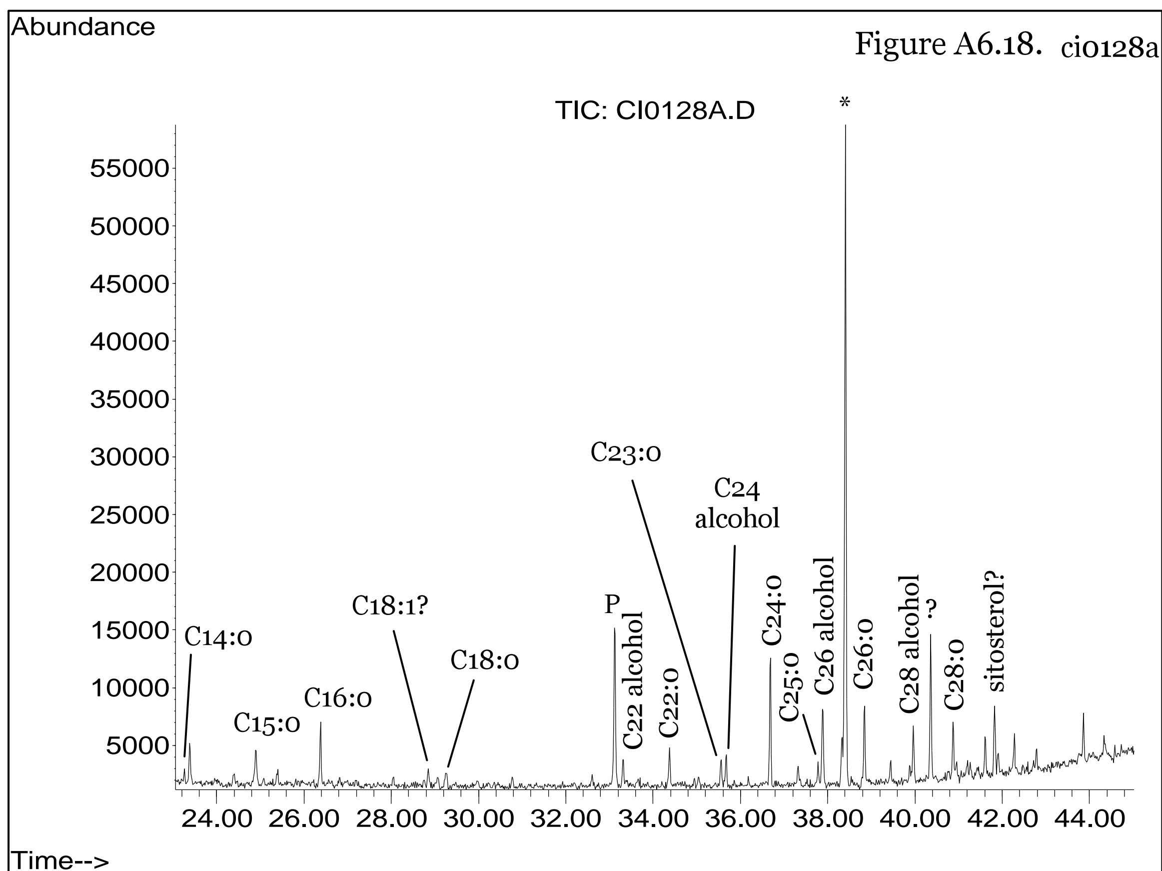
Time-->



Abundance
Figure A6.18. ci0128a
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*
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45000
40000
35000
30000
25000
20000
15000
10000
5000
C23:0
C24
alcohol
C14:0
C15:0
C16:0
C18:1?
C18:0
P
C22 alcohol
C22:0
C24:0
C25:0
C26 alcohol
C26:0
C28 alcohol
?
C28:0
sitosterol?
24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00
Time-->

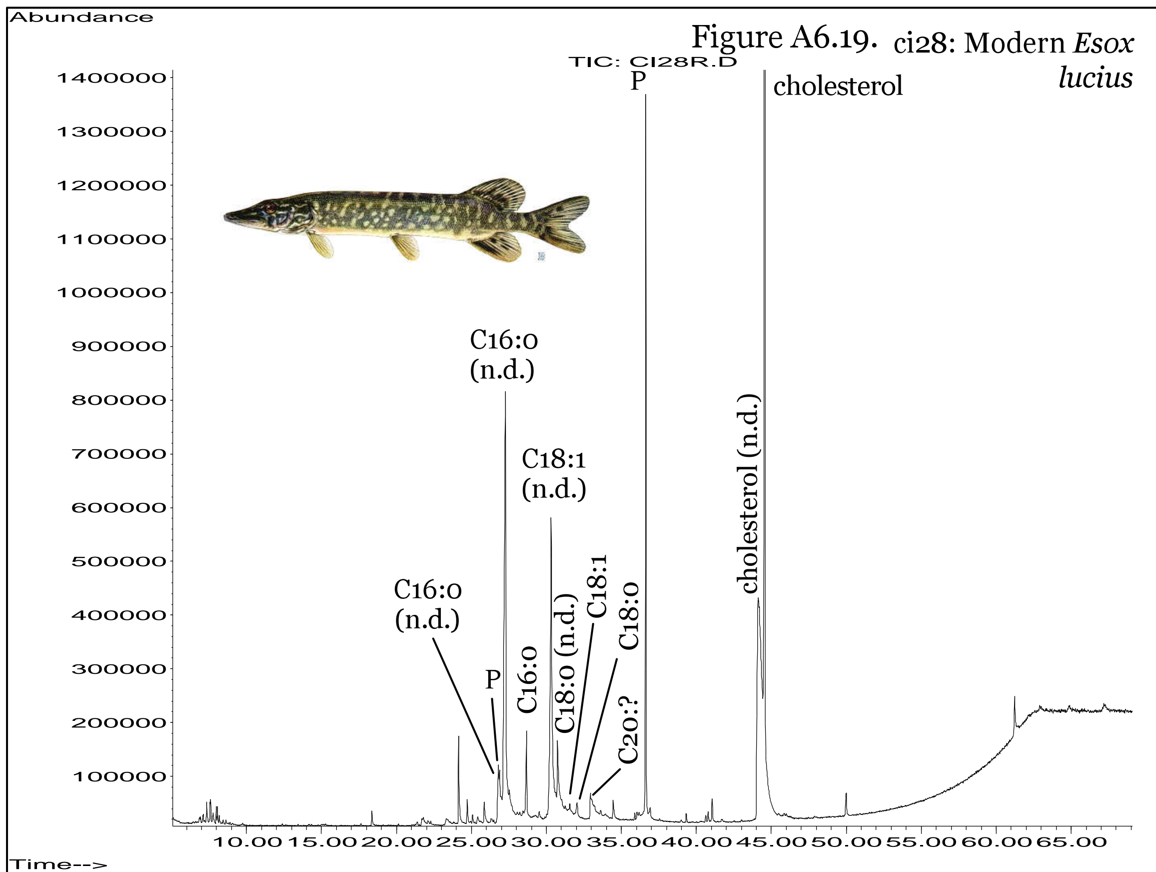

Figure A6.19. ci28: Modern *Esox lucius*

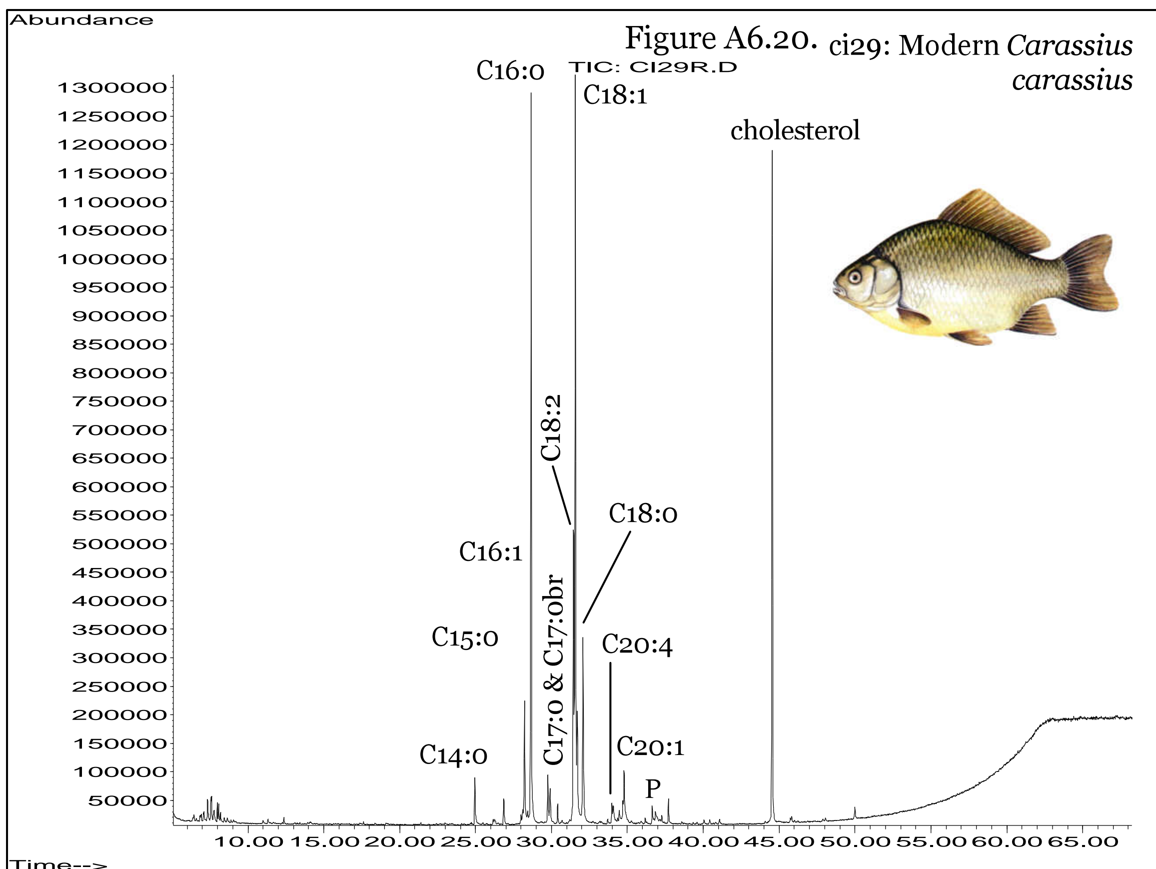

Figure A6.20. ci29: Modern *Carassius carassius*

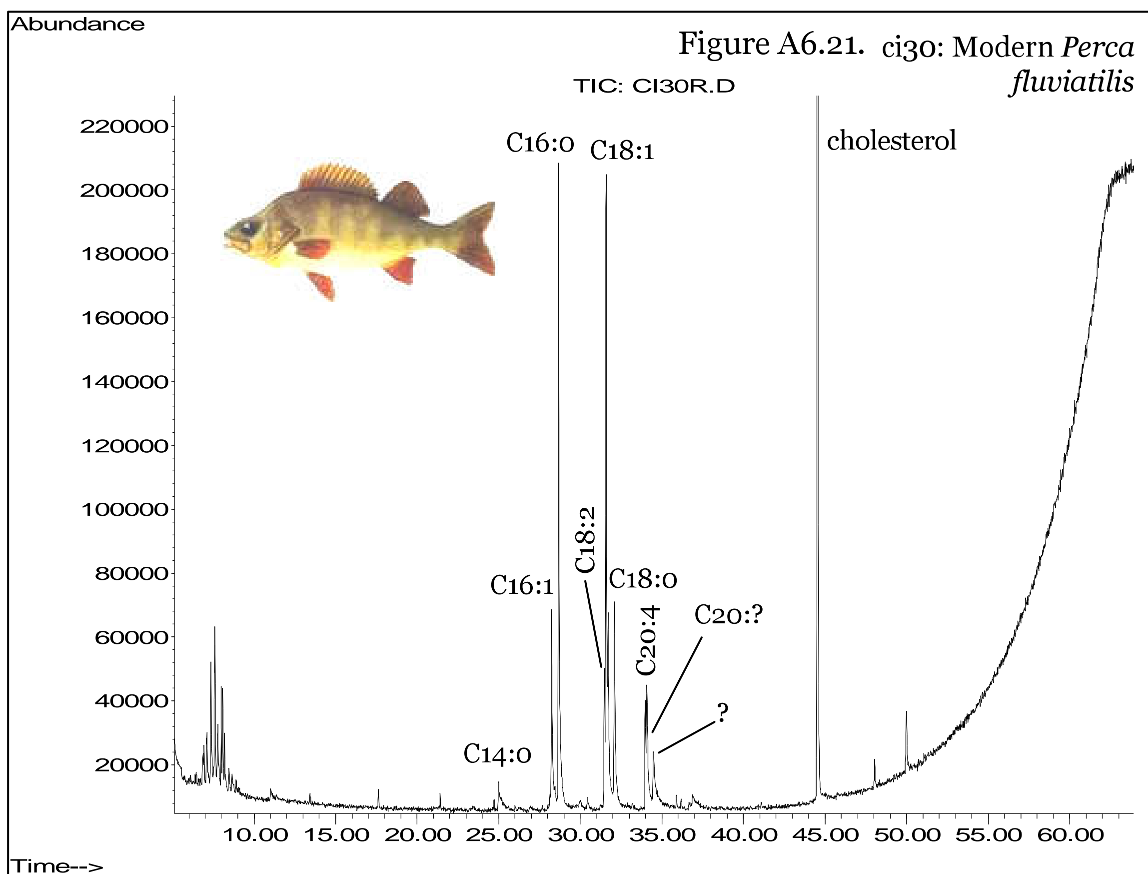

Figure A6.21. ci30: Modern *Perca fluviatilis*