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Paleodiet Studies Using Stable Carbon Isotopes From Bone Apatite and Collagen.

by

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ABSTRACT

Recently, carbon isotope studies using the mineral portion of bone have shown that this is a feasible area of research for providing paleodietary data. Stable carbon isotopes in collagen ($\delta^{13}C_{CO}$) reflect primarily the protein portion of diet, whereas stable carbon isotopes in carbonate from bone mineral ($\delta^{13}C_{CA}$) reflect the whole diet. While spacing between stable carbon isotopes in carbonate and diet is constant, spacing between stable carbon isotopes in collagen and diet may be variable depending on whether the carbon isotope value of protein equals that of the whole diet. Skeletal material from prehistoric human groups from southern Ontario and San Nicolas Island, Channel Islands, southern California were analyzed for carbon isotopes in both bone mineral and collagen. Results indicate that, in southern Ontario, maize was consumed at an earlier date than is indicated by the analysis of collagen alone, and that, on San Nicolas Island, marine food consumption did not decrease over time as occurred on nearby Channel Islands. The analysis of stable carbon isotopes from bone mineral provides additional paleodietary information to the analysis of stable isotopes from collagen. Low protein foods will be reflected in carbon isotopes in carbonate when consumed in

small amounts, whereas they will be reflected in collagen only when consumed in sizeable proportions. Spacing between carbon isotope values in carbonate and collagen permits determination of whether the carbon isotope value of dietary protein is greater or lesser than that of the whole diet.

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DEDICATION

I would like to dedicate this dissertation to my parents, Dr. Tony Harrison and Virginia Harrison, whose support throughout never wavered.

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CHAPTER 1 - INTRODUCTION

Paleodiet reconstruction is an important aspect of archaeology in that it is central to understanding past cultures and lifeways. Availability of food items and how they are obtained affects seasonal mobility, sedentary vs. nomadic lifestyles, and demographics. Analysis of paleodiet may be achieved through the study of floral and faunal remains, artifactual evidence, lithic microwear, chemical analysis of human bone, dental microwear, paleopathological lesions and functional anatomy. Prior to the 1980's, the primary method for reconstructing paleodiet was through the analysis of floral and faunal remains and through artifactual evidence. Although these types of evidence may provide a good picture of past diet, in some situations preservation problems may reduce the amount of data available for study. In addition, different types of floral and faunal remains may be differentially preserved and, as a result, may not be recovered in the proportions in which they were consumed. Only relatively broad inferences about diet composition can be made from functional anatomy, dental microwear, and paleopathological lesions. Stable isotope analysis is an additional technique which has been applied to problems of dietary reconstruction.

During the late 1970's, stable isotope analysis was first applied to problems of archaeological paleodiet reconstruction (Vogel and van der Merwe 1977, van der Merwe and Vogel 1978, DeNiro and Epstein 1978a). The use of stable isotopes for paleodiet reconstruction is predicated on the assumption that the isotopic composition of animal tissue is a direct and constant function of that of the animal's diet (DeNiro and Epstein 1978a). In simplified form this states that you are what you eat. Initially, the collagen component of bone was examined using stable isotopes of carbon and nitrogen, and trace elements from the mineral portion of bone including strontium, strontium/calcium ratios, and zinc. More recently, research has been directed toward the use of stable carbon isotopes in the carbonate component of bone mineral. The use of carbonate in bone mineral and carbon in bone collagen is the primary focus of this dissertation.

Stable isotope analysis using carbon isotopes in collagen was first used to describe maize consumption in eastern North America (Vogel and van der Merwe 1977, van der Merwe and Vogel 1978). Since these initial studies, there has been a large volume of work dedicated to quantifying the shift to maize horticulture in the various regions of eastern North America. One such region is southern Ontario, where Schwarcz and colleagues (1985) and Katzenberg and colleagues (1995) have shown, through the analysis of carbon

and nitrogen isotopes in collagen, that maize become a staple in the diet by approximately A.D. 1000.

The use of carbonate from the mineral phase of bone for human diet reconstruction was first demonstrated by Sullivan and Krueger (1981). Subsequent debate surrounding the validity of using the mineral phase of bone (based on questions concerning diagenetic effects on this tissue) appear to have been resolved through the use of suitable pretreatment procedures (Lee-Thorp 1989). Studies have indicated that while stable carbon isotope values in collagen primarily reflect those of the protein portion of diet (Chisholm et al. 1982), stable carbon isotope values of carbonate reflect those of the whole diet (Ambrose and Norr 1993, Tieszen and Fagre 1993). Questions surrounding the spacing of stable carbon isotope values in collagen and carbonate as reflected in human diet, and in particular in relation to early maize consumption in southern Ontario and marine resource consumption on San Nicolas Island, southern California, will be explored in this dissertation.

1.1 *Statement of Purpose*

In the present Ph.D. dissertation I have examined the relationship between data obtained from carbon isotope analysis of both collagen and carbonate using historic and prehistoric skeletal remains from selected sites in eastern North America, and from San Nicolas Island, southern

California. The dissertation will seek to: (1) compare and contrast the type of information provided by carbon isotope analysis from collagen and bone apatite, (2) provide an additional source of information surrounding early maize consumption in southern Ontario and marine resource use on San Nicolas Island using stable carbon isotopes from bone apatite, complementing the existing collagen and archaeological evidence, and (3) contribute to the database of information establishing the validity of bone apatite isotopic analysis for paleodiet reconstruction.

The dissertation will, through the analysis of prehistoric human bone, test whether stable carbon isotope analysis using bone mineral in addition to collagen can be used to provide dietary information above and beyond that which is provided by collagen analysis alone. By examining both collagen and biological apatite (bone mineral), information about the spacing of stable carbon isotope values between the two tissues is provided. In the first part of this study, human remains from prehistoric burials from southern Ontario are used to test whether temporal changes in the spacing of stable carbon isotope values between bone apatite and collagen suggests an earlier introduction of maize cultivation into the diet of prehistoric native populations in southern Ontario than does data provided by collagen alone. Ambrose and Norr (1993) hypothesize that because maize is a poor source of protein,

its dietary contribution is not reflected in stable carbon isotope ($\delta^{13}C$) values in collagen until it comprises a sizeable portion of diet (collagen reflects primarily the protein component of diet). However, because biological apatite reflects the whole diet, maize consumption will be reflected in the carbonate portion of bone mineral at or near its initial introduction. If maize is introduced slowly into the diet and gradually increases in proportion until it is a dietary staple, it will be reflected in the $\delta^{13}C$ signature in carbonate at an earlier date and in smaller proportions than in the $\delta^{13}C$ signature in collagen, and $\delta^{13}C$ spacing between carbonate and collagen ($\Delta^{13}C_{CA-CO}$) will change during this period of gradual increase because when maize is first introduced, $\delta^{13}C$ of carbonate will increase but $\delta^{13}C$ of collagen will not. However, if maize rapidly becomes a staple after initial introduction, $\Delta^{13}C_{CA-CO}$ will remain constant because when maize is first introduced, $\delta^{13}C$ of both collagen and carbonate will increase. In this dissertation I will test whether $\Delta^{13}C_{CA-CO}$ remains constant.

Based on controlled feeding experiments with rats, it has been suggested that the spacing between $\delta^{13}C$ between carbonate and collagen will equal 4.4‰ if the carbon isotope values of the protein portion of diet equals that of the whole diet (Ambrose et al. 1997). If $\delta^{13}C$ values of the protein portion of diet are more negative than those of the whole diet, spacing of $\delta^{13}C$ values between carbonate and

collagen will be greater than 4.4‰. Southern Ontario, where a C3 diet is supplemented by a C4 carbohydrate (maize), is an archaeological situation where this can be tested.

In addition, the dissertation will test whether stable carbon isotope analysis using biological apatite in addition to collagen provides additional information in the context of a coastal environment where marine resources are part of the diet. San Nicolas Island is one of the southern Channel Islands off the southern coast of California. A previous doctoral dissertation (Goldberg 1993) analysed stable carbon isotopes from collagen from skeletal remains from both the northern and southern Channel Islands and, among other results, concluded that marine resource use decreased relative to terrestrial resources through time in the southern Channel Islands. The addition of stable carbon isotope data from bone carbonate in this dissertation will be used to further evaluate marine resource use on San Nicolas Island.

Ambrose and colleagues (1997) suggest that when dietary protein has a less negative stable carbon isotope value than that of the whole diet, the spacing of $\delta^{13}C$ between carbonate and collagen will be less than 4.4‰. San Nicolas Island, where marine dietary protein is combined with terrestrial plant foods, is an archaeological situation where this can be tested.

Formal statements of hypotheses are presented at the end of section 2.5.2.

CHAPTER 2 - THEORETICAL BACKGROUND AND REVIEW OF LITERATURE

2.1 *Basic Chemical Concepts*

Isotopes are various forms of an element in which the number of neutrons in the nucleus of the atom varies. The different number of neutrons in the nucleus results in variation of the atomic weight between isotopes of the same element. For example, carbon (used in paleodietary studies) has seven different isotopes (^{10}C, ^{11}C, ^{12}C, ^{13}C, ^{14}C, ^{15}C, ^{16}C [Weast 1972]). Of these, all are radioactive except for ^{12}C and ^{13}C. All of the radioactive isotopes of carbon comprise less than 0.0001% by weight of all carbon isotopes in nature. ^{12}C and ^{13}C are stable isotopes, that is, they do not decay. The natural abundance of the stable isotope ^{13}C is small in relation to ^{12}C (1.11% and 98.89% natural relative abundance, respectively [Weast 1972]). Measurement of ^{13}C abundance involves measurement of the ratio of ^{13}C to ^{12}C using an isotope ratio mass spectrometer, with reference to the standard for carbon, the Peedee Formation *Belemnitella americana* marine fossil limestone from South Carolina (PDB) (Craig 1957). Isotope ratios are expressed using the δ (delta) notation in parts per thousand (permil or ‰). The carbon standard contains more ^{13}C than virtually

all dietary resources and most human tissues and therefore $\delta^{13}C$ values are usually negative.

Stable isotopes of nitrogen are also used in paleodietary studies. ^{14}N and ^{15}N are stable isotopes comprising 99.63% and 0.37% natural relative abundance, respectively (Weast 1972). The ratio of ^{15}N to ^{14}N is used for measurement of the abundance of ^{15}N. The standard for nitrogen is atmospheric air (AIR). Nitrogen isotopic values in plants and animals are usually positive in relation to atmospheric nitrogen.

δ values are calculated according to the following general equation (McKinney et al. 1950):

$$\delta\ (‰) = [\ (R_{sample}/R_{standard}) - 1\ ] \times 1000,$$

where R is the ratio of the heavier to lighter isotope.

2.2 *Bone Composition*

Bone includes two primary components, an organic phase which contains primarily the protein collagen, and an inorganic (mineral) phase which is predominantly hydroxyapatite. Collagen is the most abundant protein in the organic phase (approximately 30% by weight of bone), which contains both carbon and nitrogen. Collagen provides mechanical strength to bone, tendon and cartilage. It does not vary significantly in its composition and structure among vertebrates (Armstrong et al. 1983). In addition to

collagen, the organic phase of bone contains other proteins (approximately 2%) and water.

The other phase of bone is the mineral phase which is predominantly calcium and phosphate in the form of hydroxyapatite, comprising approximately 70 percent by weight of bone. Collagen is studded with crystals of hydroxyapatite. Hydroxyapatite has the approximate chemical formula $Ca_{10}(PO_4)_6(OH)_2$ and in bone contains carbonate (CO_3) substitutions in its crystal structure, usually substituting for phosphate. Other substitutions may include fluorine, chlorine, bromine, iodine, carbonate or oxygen in the hydroxyl position; strontium, barium, lead, radium, sodium, potassium or magnesium for calcium; and arsenate, biphosphate, carbonate or bicarbonate for phosphate (Hillson 1996). Water or a vacancy may also occur in either the calcium or hydroxyl sites. The source of carbonate is the reservoir of bicarbonate found in blood, and is introduced into bone mineral through blood-bone exchange via the Haversian system. Carbonate comprises 2-5 percent by weight of hydroxyapatite (Chickerur et al. 1980) and occurs as a defect in the form of either (1) structural carbonate substituting for PO_4 within the crystal, or (2) adsorbed carbonate occurring on the crystal surface or hydration layer.

In teeth, dentin contains approximately the same proportion of collagen to hydroxyapatite as does bone, while

tooth enamel contains approximately 98 percent hydroxyapatite and most of the remaining 2 percent is amelogenin, a protein unique to enamel (Hillson 1996).

Carbon isotope ratios can be determined in carbon in collagen and in the carbonate of hydroxyapatite. Nitrogen isotope ratios can be determined in collagen.

2.3 *How Carbon is Introduced into the Foodchain*

Carbon is introduced as atmospheric CO_2 through photosynthesis into terrestrial plants through one of three pathways, causing a difference in carbon isotope ratios between different plant groups based on the pathway utilized. C3 plants, which utilize the Calvin-Benson pathway, discriminate more against the heavier carbon isotope (^{13}C) during photosynthetic CO_2-fixation than do C4 plants, which utilize the Hatch-Slack pathway (Calvin and Bassham 1962, Hatch and Slack 1970). Therefore, C3 plants have lighter, or more negative, $\delta^{13}C$ values than C4 plants (Deines 1980). The terms C3 and C4 refer to the number of carbon atoms in a molecule formed during the first stage of photosynthesis. In C3 plants, carbon dioxide (from air or dissolved in seawater) is reduced and bound to RuDP (ribulose diphosphate) which then splits to form two molecules of PGA (phosphoglycerate), each of which contains three carbon atoms, hence the three-carbon, or C3, pathway. This is called the Calvin cycle. In C4 plants, carbon

dioxide is first bound to the four-carbon compound oxaloacetic acid, then bound to RuDP, and then enters the Calvin cycle, hence the four-carbon, or C4, pathway.

Nutritionally important C4 plants include maize, sorghum, millet, some chenopods and amaranth, and the tropical pasture grasses. Nutritionally important C3 plants include all root crops, legumes, vegetables, nuts, most fruits, forest, montane and wetland grasses, wheat, barley and rice. Cacti and succulents utilize a third pathway, Crassulacean acid metabolism (CAM).

While the $\delta^{13}C$ values of C3 and C4 plants are distinctive, there can be some overlap between that of CAM plants and C4 plants (Bender et al. 1973). However, as cacti and succulents (CAM plants) formed part of the diet in only a small number of human groups, this potential overlap is rarely problematic and can often be resolved through archaeological evidence. In general, plants which utilize different photosynthetic pathways can be distinguished using $\delta^{13}C$ values.

The differences in isotopic ratios of plants caused by the different photosynthetic pathways are maintained, with slight variation, up the foodchain. These slight variations are due to differences in the physical and chemical properties of different isotopes of the same element, called isotope effects (Hoefs 1987). The quantum theory of isotope effects explains why differences in physico-chemical

properties exist between different isotopes. Since the electronic, translational and rotational energies for isotopes of the same element are approximately equal, vibrational energy must be the source of isotope effects. Because vibrational energy of a molecule is dependent on the mass of the molecules involved, molecules containing different isotopes (which have different masses) will have different energies. These different energies cause the bonds formed by the light isotope to be broken more readily than the bonds of the heavy isotope. Molecules having the lighter isotope more readily enter into chemical reactions than molecules having the heavier isotope (Hoefs 1987). The process by which different isotopes are partitioned between the beginning and end products of a chemical reaction is called fractionation. Isotopic fractionation occurs because, generally, heavier isotopes (for example, ^{13}C) enter into chemical reactions at slower rates than lighter isotopes (^{12}C). The additional weight of extra neutrons in the nucleus of the atom causes differences in melting, freezing, crystallization, condensation and evaporation rates and temperatures (Urey 1947).

Differential fractionation of the stable isotopes of carbon in different plant groups and between different trophic levels forms the basis for inferring diet. Plants utilize carbon from atmospheric CO_2 which has a $\delta^{13}C$ value of -7.7‰ in the present day (Keeling 1961). Atmospheric CO_2

$\delta^{13}C$ values have decreased since the industrial revolution (since approximately A.D. 1800) due to the burning of fossil fuels which are isotopically lighter and as a result reconstruction of pre-1800 diets requires an upward adjustment of approximately 1.4‰ (van der Merwe 1989, Boutton 1991). During the conversion of atmospheric CO_2 to phosphoglyceric acid, the first product of photosynthesis, C3 plants fix carbon with $\delta^{13}C$ values in the range of -36‰ to -22‰ and an average of -26.5‰ (Bender 1971, Smith and Epstein 1971). C4 plants do not discriminate as effectively against the heavier carbon isotope and, as a result, fix carbon with $\delta^{13}C$ values in the range of -19‰ to -6‰ and an average of -12.5‰ (Smith and Epstein 1971, Smith 1972). This difference of $\delta^{13}C$ values between C3 and C4 plants is maintained up the foodchain depending on the mixture of C3 and C4 plants in the diet, and with further variation as trophic level increases, causing a trophic level effect. Spacing of $\delta^{13}C$ between plant diet and bone collagen caused by isotopic fractionation during collagen formation is in the order of +5‰ but lab experiments indicate that it varies according to the specific diet (Ambrose 1993, discussed in further detail below). Between herbivores and carnivores the trophic level effect causes approximately a +1‰ shift (Chisholm et al. 1982, van der Merwe 1982, Tieszen et al. 1983, Schoeninger 1985).

2.4 *Nitrogen Isotopes*

Nitrogen enters the food web when it is taken up by plants either from the soil or directly from the atmosphere. Some plants, for example, legumes, are able to fix atmospheric nitrogen. Nitrogen-fixing bacteria (the most common of which is *Rhizobium*) that grow on the roots of leguminous plants produce cellulose tubes which permit the bacteria to invade the cortical cells of the root. The bacteria multiply and produce ammonium (NH_4^+) which combines with carbon compounds producing amino acids (Delwiche and Steyn 1970). Non-nitrogen fixing plants derive their nitrogen from NH_4^+ and NO_3^- in soils and, therefore the $\delta^{15}N$ value for non-nitrogen fixing plants is dependent on that of the soil. The $\delta^{15}N$ value for atmospheric nitrogen is 0‰ and for soils averages about 10‰ depending on soil type (Mariotti 1983, Shearer and Kohl 1989). Therefore, nitrogen fixing plants have lower $\delta^{15}N$ values than non-nitrogen fixing plants. The spacing factor for $\delta^{15}N$ in collagen for herbivores over the plants they consume is approximately +3‰ (DeNiro and Epstein 1981, Minagawa and Wada 1984, Schoeninger and DeNiro 1984). Herbivores eating non-nitrogen fixing plants will have higher $\delta^{15}N$ values than herbivores which consume primarily nitrogen fixing plants. The spacing factor for $\delta^{15}N$ in collagen for carnivores over the herbivores they consume is approximately +3‰ (Minagawa and Wada 1984, Schoeninger and DeNiro 1984).

2.5 *Stable Isotope Analysis*

2.5.1 *History of Research*

The potential for diet reconstruction using isotopic analysis was first recognized in 1967 by Robert Hall who noted that maize and other grasses which have high $\delta^{13}C$ values, produced anomalously young ^{14}C dates, and therefore grass-eating and leaf-eating animals could theoretically be differentiated using stable carbon isotopes (Hall 1967). Practical application of this concept to human diet first occurred in the late 1970's when Vogel and van der Merwe (1977, van der Merwe and Vogel 1978) quantified long term consumption of maize in human groups from eastern North America using carbon isotopes from bone collagen. They suggested that maize consumption in Woodland societies was minimal prior to A.D. 1000. A large volume of work using bone collagen has accumulated over the past two decades quantifying the shift to maize cultivation in this part of the world. Southern Ontario provides an example of one particular part of eastern North America where studies have shown that the prehistoric population first began supplementing their diet with maize cultivation at about A.D. 700, but did not utilize it as a staple until approximately A.D. 1200 (Schwarcz et al. 1985, Katzenberg 1989, Katzenberg et al. 1995).

DeNiro and Epstein (1978a, 1981) demonstrated through the analysis of stable carbon and nitrogen isotope ratios in collagen and bone mineral from laboratory animals in controlled feeding experiments that the isotopic composition of animal tissue was determined by that of the diet. They also studied prehistoric human diet from the Tehuacan Valley of Mexico and demonstrated a dramatic shift in diet through time (DeNiro and Epstein 1981).

The characterization of marine resource consumption in relation to terrestrial foods was first demonstrated by Tauber (1981) in his study of Danish Mesolithic and Neolithic groups. Although both groups were coastal or near-coastal dwellers, the carbon isotope signature from collagen indicated that, while Mesolithic groups subsisted on a diet of primarily marine foods (less negative $\delta^{13}C$ values), Neolithic groups ate primarily terrestrial foods (more negative $\delta^{13}C$ values). Chisholm and colleagues (1982) examined this relationship between marine and terrestrial based diets in a study of prehistoric groups from the Canadian Northwest Coast and Ottawa Valley in Quebec.

The application of nitrogen isotope studies to the problem of marine resource consumption was demonstrated by Schoeninger and DeNiro (1984) in a study which examined stable isotope ratios of nitrogen and carbon for 66 species of birds, fish and mammals. They demonstrated that animals that fed exclusively in a marine environment had on average

9‰ more positive $\delta^{15}N$ values than those that fed in a terrestrial environment with overlap of less than 1‰. In addition, spacing of $\delta^{15}N$ values due to trophic level shifts in both a marine and terrestrial environment were shown to be +3‰.

The ability to use carbonate from the mineral phase of bone and tooth enamel for diet reconstruction was first demonstrated by DeNiro and Epstein (1978b) in a study of two sympatric Hyrax species in Tanzania. Application of the use of this mineral phase for human diet reconstruction was first demonstrated by Sullivan and Krueger (1981) in a study of modern and fossil bone, including humans. They examined 26 samples from a variety of different animals and environmental conditions and showed a high correlation between carbonate and collagen $\delta^{13}C$ values, with a consistent spacing between the two values of approximately 8‰. Although there was originally debate surrounding the validity of using the mineral phase due to problems of diagenesis (Schoeninger and DeNiro 1982, 1983, Krueger 1991), later studies have shown that it can be used profitably (Lee-Thorp and van der Merwe 1987, Lee-Thorp et al. 1989a, discussed in greater detail below). In Schoeninger and DeNiro's (1982) study of animals, including humans, from six sites in Peru and Mexico, their analysis of $\delta^{13}C$ values from both carbonate and collagen showed a correlation between the two values of $r^2 = 0.80$ (in contrast

to Sullivan and Krueger's [1981] correlation value of r^2 = 0.99), and concluded that the use of carbonate from the mineral portion of bone was an unreliable source of dietary data due to contamination by calcium carbonate from the burial environment. Schoeninger and DeNiro (1982) used stronger acids (50% glacial acetic acid) for removal of adsorbed and diagenetic carbonate. Sullivan and Krueger (1983) questioned the use of a strong acetic acid and, indeed, later studies have shown that stronger acids tend to recrystallize apatite and incorporate the diagenetic and adsorbed carbonate fractions into the crystal structure rather than remove them (Krueger 1991, Lee-Thorp and van der Merwe 1991). Thus a weaker acid which would remove diagenetic carbonates but not recrystallize structural apatite and incorporate diagenetic material, was required. This was demonstrated by Lee-Thorp (1989) who used 1M acetic acid (5.8% glacial acetic acid in double distilled water [ddH_2O]), which removed diagenetic and adsorbed carbonate without recrystallizing and incorporating these fractions into the crystal structure, thus leaving only the structural carbonate.

Carbonate from the mineral phase of tooth enamel has been shown to preserve the dietary isotopic signature for several million years and has been used to study Plio-Pleistocene hominids (Lee-Thorp and van der Merwe 1987, Lee-Thorp et al. 1989b). Lee-Thorp (1989, Lee-Thorp and van der

Merwe 1991) has demonstrated from the analysis of individuals from Plio-Pleistocene to Upper Pleistocene age (including those from hominid bearing limestone cave sites in South Africa) that the isotopic composition of enamel is only slightly susceptible to diagenetic alteration with the removal of post-mortem and adsorbed carbonates. Bone and tooth dentin produced unreliable results. The use of carbonate from enamel has the potential for dietary studies of pre-hominid and other paleo-species.

Whereas Sullivan and Krueger (1981) had reported a constant spacing between $\delta^{13}C$ of collagen and biological apatite, later studies by Schoeninger and DeNiro (1982, 1983) produced variable spacing between $\delta^{13}C$ of these two tissues. A study by Lee-Thorp and colleagues (1989a) demonstrated that trophic level affects the spacing between $\delta^{13}C$ of collagen and biological apatite. They suggested that different diets produced characteristic $\delta^{13}C$ spacing between collagen and biological apatite. They sampled a number of herbivores, carnivores and omnivores of known diet and trophic level from a variety of habitats in southern Africa with the goal of determining $\delta^{13}C$ spacing for different trophic levels. Mean differences between $\delta^{13}C$ for biological apatite and $\delta^{13}C$ for collagen were 6.8‰ for herbivores, 5.2‰ for omnivores and 4.3‰ for carnivores, and concluded that an all inclusive addition or subtraction of a spacing value should not be used, but rather the

spacing value of $\delta^{13}C$ between collagen and biological apatite should be determined by trophic level. Lee-Thorp and colleagues (1989a) showed that there is a trophic level effect on the spacing of $\delta^{13}C$ between collagen and biological apatite.

In a further study, Lee-Thorp and colleagues (1989b) studied two species of extinct baboons from Swartkrans cave, South Africa and distinguished between browsers (feeding on C3 plants) and grazers (with mixed C3 and C4 plants) using carbonate from biological apatite. The slight isotopic shift in the enamel of older specimens is of the order of approximately +3‰, and is likely due to small increases in the $\delta^{13}C$ value of Plio-Pleistocene atmospheric CO_2 and terrestrial plants, and lower atmospheric CO_2 concentrations (Lee-Thorp and van der Merwe 1987, Lee-Thorp et al. 1989a).

Potentially, carbon isotope ratios from tooth enamel carbonate can be used to infer diet of early hominids dating back to the beginnings of the hominid line. Lee-Thorp and colleagues (1994) studied carbon isotopes in biological apatite from fossilized enamel of 1.8 to 1.0 million year old robust Australopithecines and a number of other species from Swartkrans cave in South Africa. They found that $\delta^{13}C$ values were consistent with the proposed diets of various grazing and browsing herbivores and carnivores. However, carbonate analysis of the robust Australopithecine remains indicated that they were not herbivores (contrary to

Robinson's [1954] dietary hypothesis) but rather were consuming both C3 and C4 plants. As it was unlikely that they were directly consuming savanna grasses, a more likely explanation was that these robust Australopithecines were consuming the savanna animals who consumed those grasses. In a further study, Sponheimer and Lee-Thorp (1999) examined older *Australopithecus africanus* remains from Makapansgat Limeworks in South Africa. The analysis of carbonate from bone mineral suggested that 3.0 million year old *Australopithecus africanus* was not only eating fleshy fruit and leaves (as is the common consensus) but also C4 grasses or the animals that fed on C4 grasses. Sponheimer and Lee-Thorp suggest that *Australopithecus africanus* was omnivorous, providing evidence refuting the contention that encephalization in the *Homo* genus was the result of a shift in diet from a herbivorous diet to a more protein rich omnivorous diet. The isotopic evidence from bone mineral indicates an omnivorous diet in two earlier hominid groups, the robust and gracile Australopithecines.

Although carbon isotopes are the focus of this dissertation, some mention of nitrogen isotopes should be made. Briefly, trophic levels in terrestrial, marine and freshwater systems have been differentiated using nitrogen isotopes (Ambrose and DeNiro 1986b, Katzenberg 1989, Minagawa and Wada 1984, Schoeninger 1985, Schoeninger and DeNiro 1984). This has been extended to the evaluation of

the proportion of the consumption of meat using nitrogen isotope ratios from collagen (Ambrose and DeNiro 1986a), and has also been used to determine age at weaning (since nursing infants are effectively feeding on mother's tissues) (Katzenberg 1993).

Nitrogen isotope values may also be affected by the local environment. Nitrogen fixation rates are higher in cool, moist forest soils and have lower $\delta^{15}N$ values, whereas hot, drier savanna and desert soils have higher $\delta^{15}N$ values (Delwiche and Steyn 1970, Shearer and Kohl 1986). The highest $\delta^{15}N$ values are found in saline soils and guano deposits (Shearer et al. 1983, Heaton 1987).

Recently, cholesterol has been suggested as having potential for providing a new source for paleodietary information using stable carbon isotopes. Stott and colleagues (1999) designed a study using data obtained from laboratory animal feeding experiments, and modern and archaeological humans. Results showed that cholesterol faithfully derived its isotopic signature from the diet, that turnover rates are faster than in collagen providing a means for analyzing carbon cycling over a shorter time frame than with collagen, that $\delta^{13}C$ values are biased toward dietary carbohydrates and fats, and that cholesterol indicated subtle differences between terrestrial and marine foods which were not apparent from collagen alone.

2.5.2 *Bone Apatite vs. Bone Collagen*

There has been debate surrounding what carbon isotope ratios from collagen actually represent in terms of dietary fraction (protein, carbohydrate or lipid). Chisholm and colleagues (1982) maintained that carbon isotope ratios obtained from collagen only reflect carbon isotope ratios of the protein portion of diet. If this is the case, then the carbohydrate and lipid fractions of diet are invisible in bone collagen based on carbon isotope data. However, diet experiments performed by Kennedy (1988) on rats fed low protein diets (manioc with a small supplement of higher protein lab chow) demonstrate that a significantly larger contribution of carbon in collagen came from manioc (41%) than could be accounted for by the proportion of protein provided by manioc in the controlled diet (23%). Therefore, some of the carbon in collagen must have come from a non-protein source - the starch in manioc. This indicates that carbon isotope ratios from collagen do indeed partially reflect non-protein fractions of diet. However, Ambrose (1993) noted that in this and other laboratory examples using small animals, the difference in $\delta^{13}C$ between that of diet and collagen is often smaller than that observed in natural observations of larger animals. This may be due to preparation procedures, proportions and quality of protein in the diet, or genetic factors between different animals (Ambrose 1993).

The biochemical basis for what portions of diet the different tissues in bone represent involves the source material for carbon. While carbonate in bone mineral is derived from dissolved bicarbonate in the blood which is derived from all dietary components, collagen, a protein, is composed of amino acids, in which carbon is contained. Some amino acids can be synthesized within the body (non-essential or dispensable amino acids), while others cannot be synthesized and must be derived intact as part of the diet (essential or indispensable amino acids). Table 2.1 provides a profile of the various amino acids contained in collagen, their relative weight percentages, and the percent of carbon and nitrogen in each amino acid.

In recent years a new tripartite division has been proposed to describe the various kinds of amino acids substituting for the more traditional terms essential and non-essential. This new system was proposed in order to take into account those amino acids which most organisms can synthesize within the body but are preferentially taken directly from the diet because they are metabolically expensive to synthesize (Young and El-Khoury 1995). These amino acids are termed conditionally indispensable. A lack of conditionally indispensable amino acids in the diet is not detrimental to the maintenance of body tissues (Young and El-Khoury 1995). However, growth and development and recovery from illness and injury may be retarded when

dietary resources lack conditionally indispensable amino acids. The newer tripartite system will be used in this dissertation.

Indispensable amino acids are contained in protein and, therefore, collagen reflects primarily the protein portion of diet. However, not all amino acids which form collagen are indispensable amino acids, some are dispensable. These can be synthesized within the body and, therefore, may be derived from non-protein sources. Clearly, some of the amino acids, and hence carbon, in collagen can be reflective of non-protein portions of diet. How much depends on how great a proportion of the diet is derived from protein (Kennedy 1988). Diets with a relatively higher protein component will provide more indispensable, and dispensable, amino acids and be more reflective of dietary protein than diets with a relatively lower protein proportion.

Krueger and Sullivan (1984) proposed that the $\delta^{13}C$ value of collagen is a function of the growth substrate (protein), whereas the $\delta^{13}C$ value of bone mineral is a function of the energy substrate (lipids, carbohydrates and protein not used for protein tissue synthesis). They proposed that, because the carbon in bone mineral is obtained from blood plasma CO_2 which is largely derived from the metabolism of carbohydrates and the carbon in collagen is obtained from amino acids, carbohydrates in the diet would be less strongly represented in collagen than in bone

mineral. If so, it should be possible to experimentally manipulate $\delta^{13}C$ values of the collagen and carbonate phases of bone based on the relative proportions of protein, lipid and carbohydrate in the diet, and on the $\delta^{13}C$ value of each of these components.

Ambrose and Norr (1993) designed a study to evaluate whether this was true in which they used rats as a test species. They analyzed $\delta^{13}C$ values for both collagen and carbonate in bone mineral for seven different diets, which varied according to whether the protein and energy portions of the diet were C3 or C4 derived, and in the proportion of protein within the whole diet. While the $\delta^{13}C$ value of rat collagen is poorly correlated to that of the whole diet when protein and non-protein $\delta^{13}C$ values differ significantly, collagen $\delta^{13}C$ values are well correlated to that of whole diet when protein and non-protein components have similar $\delta^{13}C$ values. If collagen $\delta^{13}C$ reflects dietary protein, then carbon atoms from the protein portion of diet must be preferentially routed to collagen. On the other hand, since the carbon atoms in bone mineral are derived from all dietary resources, $\delta^{13}C$ in bone mineral will be reflective of the whole diet (contra to Krueger and Sullivan's [1984] contention that bone mineral represents only the energy - carbohydrate, lipid and protein not used for protein tissue synthesis - portion of the diet). Ambrose and Norr (1993) found that the difference between $\delta^{13}C$ values of the diet

and the carbonate ($\delta^{13}C_{CA-D}$) in bone mineral was always 9.4‰ regardless of whether the $\delta^{13}C$ values of dietary protein and energy components were the same or different. On the other hand, they found that the difference between $\delta^{13}C$ values of diet and collagen ($\delta^{13}C_{CO-D}$) was 5‰ only if the $\delta^{13}C$ values of dietary protein and energy were the same. If the $\delta^{13}C$ values of dietary protein and energy were not the same, then $\delta^{13}C_{CO-D}$ were greater or lesser than 5‰. Therefore, the spacing between collagen and carbonate $\delta^{13}C$ values ($\Delta^{13}C_{CA-CO}$) will equal 4.4‰ (9.4‰ minus 5‰) only if the $\delta^{13}C$ value of protein is the same as that of the whole diet. The spacing between collagen and carbonate $\delta^{13}C$ values will vary from 4.4‰ depending on whether dietary protein is isotopically lighter or heavier as compared to the whole diet (Ambrose et al. 1997). In the case where the $\delta^{13}C$ value of dietary protein is more negative than that of the whole diet, $\Delta^{13}C_{CA-CO}$ is greater than 4.4‰, and a diet of C4 carbohydrates and C3 protein is suggested. This is consistent with a situation where a C4 cultigen, such as maize, is introduced into a C3 diet (for example, early maize cultivating groups in southern Ontario). On the other hand, if $\Delta^{13}C_{CA-CO}$ is less than 4.4‰, dietary protein is less negative than that of the whole diet, and a diet of C3 carbohydrates and marine protein is suggested (Ambrose et al. 1997). This is consistent with a situation where coastal groups obtain their protein from marine animals while utilizing C3

terrestrial plants (for example, prehistoric groups on San Nicolas Island, southern California).

Ambrose and Norr's (1993) experiment examined laboratory rats, not humans. Therefore, because spacings of $\delta^{13}C$ values between collagen and diet tend to be different between smaller and larger animals (Ambrose 1993), the human value for $\Delta^{13}C_{CA-CO}$ when $\delta^{13}C$ of the protein portion of diet is equal to that of the whole diet may not equal 4.4‰.

In another study, Tieszen and Fagre (1993) used laboratory mice as a test species in order to gain a better understanding of the dietary factors influencing $\delta^{13}C$ spacing between that of diet and collagen or carbonate in bone mineral. They analyzed eight experimental diets in which they varied the amount of C4 cellulose, C4 starch, C4 lipid and C4 protein added to a C3 diet. Diet 1 was all C3, and diet 8 was 92% C4, with diets 2 through 7 varying in these components. Results indicated that $\delta^{13}C$ values of collagen varied according to the specific dietary inputs in each diet but correlated strongly with protein rich tissues. They concluded that the carbon isotopic signal in collagen is largely determined by dietary protein, with some contribution by other major food components, but that their study did little to explain the differences in $\Delta^{13}C_{CA-CO}$, as has been documented in herbivores and carnivores (as discussed above, Lee-Thorp et al. 1989a). In sum, experiments using such diets show that collagen reflects

mostly the protein $\delta^{13}C$ value, while that of carbonate reflects the whole diet $\delta^{13}C$ value (protein, lipid and carbohydrate).

The experiments of Ambrose and Norr (1993), Tieszen and Fagre (1993), and Ambrose and colleagues (1997) suggest that collagen follows the 'routing' model (Chisholm et al. 1982, Chisholm 1989) whereby amino acids from the protein portion of diet preferentially go toward forming collagen. On the other hand, the laboratory diets suggested that carbonate in bone mineral reflects the whole diet adhering to the 'linear mixing' model (Schwarcz 1991). Through the analysis of the spacing between $\delta^{13}C$ values of collagen and carbonate, one can determine whether collagen reflects the whole diet in a particular situation, or whether it reflects primarily the protein portion of diet.

What is the theoretical logic behind the contention that dietary protein is routed to collagen? Ambrose and colleagues (Ambrose and Norr 1993, Ambrose et al. 1997) suggest that because 12.2% of the amino acids in collagen are indispensable amino acids they must therefore come from dietary protein. The indispensable amino acids in collagen have more carbon atoms than the dispensable amino acids and, as a result, contain a disproportionately high number of carbon atoms in relation to their percent composition of collagen (17.8% versus 12.2%). Some of the dispensable amino acids in collagen can only be synthesized from

indispensable amino acid precursors and therefore the carbon atoms in these dispensable amino acids must also be routed from dietary protein. The carbon atoms from these dispensable amino acids comprise 1.5% of the total carbon atoms in collagen. Therefore, at a minimum, a total of 19.3% (17.8% plus 1.5%) of carbon atoms in collagen must be derived from dietary protein, with the balance of carbon atoms being contained in the dispensable amino acids which can be formed from non-amino acid precursors, which can come from the carbohydrate and lipid portions of diet (Ambrose et al. 1997). This constitutes a minimum estimate of the amount of routing from dietary protein in the formation of collagen. Although the other dispensable amino acids can be synthesized from non-amino acid precursors, the energy required to do so is greater than if these dispensable amino acids were directly obtained from dietary sources. These dispensible amino acids which require greater energy for synthesis, and where their paucity in the diet may retard growth and development and recovery from illness and injury, are conditionally indispensable amino acids. Since it is reasonable to suggest that conservation of energy is adaptive in an evolutionary sense, it is likely that these conditionally indispensable amino acids will be obtained from dietary sources rather than independently synthesized when they are available in the diet. Therefore, in addition to the minimum estimate of 19.3% dietary routing of carbon

atoms in collagen, there would likely be a significantly greater amount of routing due to conservation of energy (Ambrose et al. 1997). If the diet provided an adequate source for all the amino acids in collagen, then the amount of routing of carbon atoms in collagen from dietary protein would be very large. Indeed, Ambrose and Norr (1993) demonstrated at least 60% routing of dietary protein to tissue protein rather than the theoretically expected minimum of 19.3% routing (Klepinger and Mintel 1986).

Because $\delta^{13}C$ values from both collagen and biological apatite are more similar in carnivores than in herbivores (Lee-Thorp et al. 1989a), data from the same individuals can provide information concerning trophic level. Bourque and Krueger (1994) examined five historical New England human populations and concluded that while the $\delta^{13}C$ values from collagen and biological apatite showed a high correlation, no additional dietary information was provided by the spacing of $\delta^{13}C$ values between collagen and biological apatite in these study groups. In another study, Ubelaker and colleagues (1995) compared the diet of low and high status individuals from highland Ecuador by comparing carbon and nitrogen from collagen and carbon from biological apatite. Although both collagen and carbonate $\delta^{13}C$ values differed between the two groups suggesting a greater consumption of maize in the higher status group, no difference in $\delta^{15}N$ values or in the spacing between the $\delta^{13}C$

values of collagen and carbonate was observed suggesting that there was no difference in the proportion of meat consumption between the two groups.

Stable carbon isotope ratios in collagen and biological apatite are different due to two factors. The first factor involves the different routes which carbon takes in the formation of these two tissues. The carbon in collagen is dependent on the specific amino acid profile of collagen, which is formed from a combination of dietary indispensable and dispensable amino acids and synthesized dispensable amino acids, which are preferentially derived from dietary protein. On the other hand, because carbonate is derived from dissolved CO_2 in blood plasma, it samples the total metabolic carbon pool of the body. This variable routing of carbon between the two tissues is one reason why $\delta^{13}C$ values are different between the two tissues.

The second factor involves different trophic level spacing values for collagen and bone mineral. Carnivores obtain the bulk of their energy from both herbivore lipid and protein. Lipids have $\delta^{13}C$ values 3‰ more negative than muscle (Vogel 1978, Deines 1980). Therefore, as compared to herbivores who obtain both protein and energy from plant tissue, a trophic level shift from herbivores to carnivores will cause an additional shift in the spacing between $\delta^{13}C$ of carbonate and collagen ($\Delta^{13}C_{CA-CO}$) of 3‰. This is because collagen primarily reflects dietary protein whereas

carbonate in bone mineral reflects the whole diet which, in carnivores, includes the isotopically lighter dietary herbivore lipid.

In herbivores, the difference in the values of $\delta^{13}C$ of plant diet and collagen ($\Delta^{13}C_{CO-D}$) will have an average value of +5‰ (Bender et al. 1981, DeNiro and Epstein 1978a, 1981, Kennedy 1988, Sullivan and Krueger 1981, Tiezsen and Boutton 1988, van der Merwe 1989, Vogel 1978, Vogel and van der Merwe 1977). Although it is not definitively known why enrichment occurs from diet to collagen, it has been suggested that it is largely due to fractionation during amino acid synthesis (Hare and Estep 1983, Macko et al. 1983) whereby collagen is enriched because it is synthesized using isotopically heavy amino acids (Tieszen and Boutton 1988). While indispensable amino acids are obtained from dietary proteins, dispensable amino acids may be resynthesized from any portion of the diet (Klepinger and Mintel 1986). Therefore, as has been suggested by the experiments of Kennedy (1988), Ambrose and Norr (1993) and Tieszen and Fagre (1993), it is entirely possible that some of the carbon in collagen is obtained from non-protein sources.

In a group of individuals with unchanging diet through time, $\delta^{13}C$ values obtained from collagen and biological apatite will be different between the two tissues but have high correlation through time. In other words, $\Delta^{13}C_{CA-CO}$

remains constant. However, if diet changes in the study group to incorporate, for example, a C4 plant such as maize, $\Delta^{13}C_{CA-CO}$ values may change at the point of maize introduction. Whether or not $\Delta^{13}C_{CA-CO}$ changes depends on how quickly maize becomes a dietary staple after initial introduction. If maize gradually increases in dietary proportion over a period of time, $\Delta^{13}C_{CA-CO}$ will change because, at initial introduction when maize forms a small proportion of diet, the $\delta^{13}C$ signature of maize will be reflected more so in the carbonate portion of bone mineral (representing all dietary components), but not in collagen (representing primarily the protein portion of diet). $\delta^{13}C$ in collagen alone will not reflect maize consumption until it comprises a sizeable portion of the diet (line A in Figure 2.1). If maize rapidly becomes a dietary staple soon after initial introduction, $\Delta^{13}C_{CA-CO}$ will not change because maize is consumed in a large enough proportion to be reflected in collagen from the earliest stages of maize cultivation (line B in Figure 2.1). In relation to the portion of this dissertation relating to early maize consumption in southern Ontario, an hypothesis can be formulated.

Hypothesis #1: *It is hypothesized that stable carbon isotope analysis of carbonate in bone mineral will permit detection of maize in smaller proportions and at an earlier*

date in the diet of southern Ontario prehistoric groups, than with analysis of stable carbon isotopes in collagen.

In addition, Ambrose and colleagues (1997) suggest that when the $\delta^{13}C$ value of dietary protein is more negative than that of the whole diet, $\Delta^{13}C_{CA-CO}$ is greater than 4.4‰, and a diet of C4 carbohydrates and C3 protein is suggested, can be tested.

Hypothesis #2: *It is hypothesized that in southern Ontario prehistoric groups, where maize (C4) supplements C3 protein and indigenous plants, $\Delta^{13}C_{CA-CO}$ will be greater than 4.4‰.*

In human groups where marine resources are part of the diet, the analysis of carbonate from biological apatite has the potential to provide additional dietary information than with the analysis of collagen alone. These diets often comprise a combination of protein derived from marine animals and carbohydrates derived from terrestrial plants. In this scenario, the $\delta^{13}C$ value for marine protein will be less negative than that of the whole diet if the source of carbohydrate is from C3 terrestrial plants. On the other hand, if the carbohydrate source is primarily C4 terrestrial plants, then the $\delta^{13}C$ value of the marine protein will be relatively more negative. This dichotomy can be addressed through the analysis of $\delta^{13}C$ from both collagen and biological apatite. As discussed above, when the $\delta^{13}C$ value of protein is not the same as that of the whole diet, $\Delta^{13}C_{CA-}$

$_{co}$ will be greater or lesser than 4.4‰ (Ambrose et al. 1997). In a situation where marine protein is combined with terrestrial plant carbohydrate, the value of $\Delta^{13}C_{CA-co}$ can be used to determine whether that terrestrial plant carbohydrate is C3 or C4 based. San Nicolas Island, where marine protein is combined with terrestrial plant carbohydrate, is a locale where this can be tested.

Hypothesis #3: *It is hypothesized that the spacing between stable carbon isotope values in collagen and biological apatite ($\Delta^{13}C_{CA-CO}$) will permit the identification of marine based foods and distinction between the consumption of C3 and C4 based terrestrial plants in the diet of San Nicolas Island prehistoric groups.*

2.6 *Diagenetic Processes*

Diagenetic changes in bone after death may alter isotope ratios. The burial environment can have a profound effect on the preservation of bone. Factors which can influence preservation include mechanical weathering (cracking and breaking of bones), the invasion of bone with plant microorganisms, and chemical alteration from contact with groundwater (Hare 1980). Of these, contact with groundwater can produce the most deleterious effects (Solomon and Hass 1967). Soil pH is also an important factor influencing bone preservation (Hanson and Buikstra 1987). Stable isotope ratios in bone may potentially be

affected through infiltration by organic material and chemical deposition of calcium carbonate via groundwater.

Diagenesis has little affect on collagen in relation to hydroxyapatite. Bone collagen can survive for thousands of years after burial, particularly in stable cool environments, and is resistant to diagenetic changes to isotopic ratios. Diagenetic processes causing poor collagen preservation can result in an absence of stable isotope data (due to too little CO_2 or N_2 for analysis) rather than an erroneous result (Nelson et al. 1986). DeNiro (1985) suggested that examining the atomic C/N ratio could be used as a guide for determining whether collagen is well preserved. Modern collagen C/N ratios are in the range of 3.2 to 3.3. DeNiro (1985) proposed that collagen with C/N ratios outside the range of 2.9 to 3.6 not be used in analysis due to diagenetic alteration. Well preserved bone or tooth with C/N ratios within the accepted range usually has more than 1 percent collagen by weight. Ambrose (1990) suggests that this collagen should have a minimum of 3 percent carbon and 1 percent nitrogen by weight in order for accurate isotope ratios to be determined. C/N ratios outside the 2.9 to 3.6 range may result from contamination with humic acids, lipids or carbonates (Kennedy 1988) and can be evaluated through the amino acid composition. Collagen is the only animal protein which contains the amino acid hydroxyproline and contains over 30% glycine (Hare

1980) and, therefore, through examination of the amino acid profile, an indication of potential contamination can be provided (Masters 1987).

The use of bone mineral requires additional controls to account for diagenetic processes (Lee-Thorp and van der Merwe 1987). The major contaminant of carbonate in bone mineral is groundwater calcium carbonate which is deposited in voids and on crystal surfaces. Bone apatite crystals have a large surface area on which adsorbed carbonate resides which favours rapid turnover in metabolic demands but also favours a high susceptibility to post-mortem groundwater contamination and diagenesis (Sillen 1989). Structural carbonate has a lower turnover rate and is much less susceptible to diagenesis. Therefore, carbon isotope ratios of adsorbed carbonate in hydroxyapatite may be affected by diagenetically deposited calcium carbonate, while structural carbonate is not affected. Appropriate pretreatment procedures have been developed which remove diagenetic and adsorbed carbonate without recrystallizing and incorporating these fractions into the crystal structure, thus leaving only the structural carbonate (as discussed in section 2.5.1, Lee-Thorp 1989). In a test of sample treatment procedures for carbonate, Koch and colleagues (1997) suggested that their results indicate that bone and tooth dentin (in relation to enamel) provide variable and unreliable results for $\delta^{13}C$ values. However,

whereas their acetic acid soak to remove non-structural carbonate was over a duration of 3 days, Lee-Thorp (1989) suggests a soak of up to 1 week for older samples, as was the case for the samples used in her study (of mid-Holocene and Pleistocene age).

There remain areas where further research is necessary to perfect dietary reconstruction. Diagenetic, nutritional, physiological (genetic differences between species) and environmental sources of variation in stable isotope ratios in human and other animal tissues must be understood and controlled for in order to increase the accuracy of diet reconstruction.

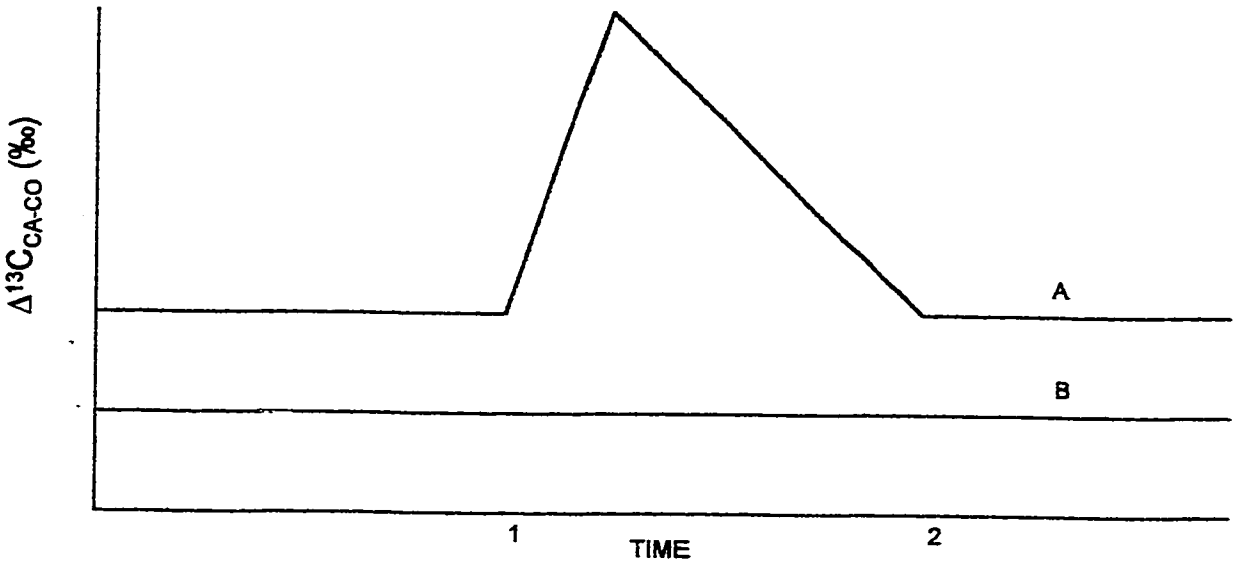
The use of carbonate from bone mineral has the additional advantage over the analysis of the collagen portion of bone in that samples of older provenience can potentially be analyzed where collagen has degraded. In addition, carbonate provides a clearer picture of the whole diet in relation to collagen and therefore can potentially be used to elucidate paleodiet in situations where specific dietary components may not be reflected in collagen. An example of this is the situation where C4 maize is not consumed in sufficient amounts to affect the carbon isotopic signature of collagen but does affect that of carbonate in bone mineral.

Table 2.1 - Amino acid, carbon and nitrogen contents of bone collagen. Adapted from Ambrose 1993, Hare and Estep 1983.

amino acid	% amino acid in collagen	% carbon in collagen	% nitrogen in collagen
Aspartic acid	4.4	4.61	3.69
Hydroxyproline	8.9	11.65	7.47
Threonine*	1.7	1.78	1.43
Serine	3.6	2.83	3.02
Glutamic acid**	7.4	9.69	6.21
Proline	13.0	17.02	10.91
Glycine	33.4	17.49	28.22
Alanine	11.2	8.80	9.40
Valine*	2.5	3.27	2.10
Methionine*	0.5	0.65	0.42
Isoleucine*	0.9	1.41	0.76
Leucine*	2.3	3.61	1.93
Tyrosine**	0.3	0.71	0.25
Phenylalanine*	1.2	2.83	1.01
Hydroxylysine	0.5	0.79	0.84
Lysine*	2.7	4.24	4.53
Histidine*	0.5	0.79	1.26
Arginine**	5.0	7.85	16.78

* Indispensable amino acid.
** Conditionally Indispensable amino acid.

Figure 2.1 - Change in $\Delta^{13}C_{CA-CO}$ values dependent on rapidity of C4 (maize) introduction. Scenario A occurs when maize gradually increases in dietary proportion over time. Scenario B occurs when maize rapidly becomes a staple soon after initial introduction. Note that between time 1 (at initial maize introduction) and time 2 (when maize becomes a staple) in scenario A, $\Delta^{13}C_{CA-CO}$ changes because $\delta^{13}C$ of maize is reflected in $\delta^{13}C$ of carbonate before collagen.



CHAPTER 3 - ARCHAEOLOGICAL BACKGROUND

3.1 *Southern Ontario*

One of the two examples analyzed in this dissertation, as outlined in Chapter 1, concerns prehistoric diet in southern Ontario, Canada. All archaeological remains utilized in this portion of the study are derived from sites in this region, bounded by Lake Huron to the west, Lake Erie to the south, Lake Ontario and the St. Lawrence River to the southeast, and the Ottawa River to the north extending westward to the northern end of Georgian Bay (Figure 4.1). The physiography of the region in the present day is largely a product of the glacial history of the Late Wisconsinan phase (25,000 - 10,000 years B.P.) of the final major glaciation of the North American continent. The following discussion of the physiography of southern Ontario is based on that of Karrow and Warner 1990. The bulk of southern Ontario, with the exception of the far northern reaches of the region in which the ground surface is largely comprised of bedrock, is characterized by features derived from this glacial history. These include sand plains, clay plains, beach ridges marking former shorelines and other features characteristic of glaciolacustrine deposits. Also present are end and lateral moraines deposited upon the retreat of

the glaciers, elongated drumlins formed parallel to ice movement, and glacial till. These features were formed through successive stadial and interstadial events as ice advanced and retreated. Underlying rock is a combination of limestone, sandstone and shale (sedimentary rocks) which produce slightly acidic podzolic soils. These are generally good for agriculture.

While maize is not indigenous to the area, the local climate does not preclude its cultivation. The area is characterized by cold winters, warm summers, with considerable snowfall in winter and moderate summer rainfall. The average number of frost free days varies somewhat throughout the area but has a minimum value of 135 days. These conditions are within the minimum parameters required for maize cultivation and, indeed, southern Ontario is located toward the more northerly limit for consistent maize cultivation.

The climate of southern Ontario has been somewhat variable in the past. Since the recession of the Wisconsinan glaciation from the area approximately 15,000 years ago, the climate has undergone several warming and cooling periods but in general has followed a trend toward increasing temperature and humidity. The biotic environment has altered over time in tandem with climate change. The first post glacial vegetation pattern immediately following the recession of ice from southern Ontario was characterized

by a tundra-like environment including dwarf shrub and herbs. As the ice edge receded further north, a predominantly boreal forest environment (spruce and pine) existed giving way at approximately 7500 years B.P. to mixed hardwood (hemlock, birch, elm, maple and beech) with some interspersed spruce and pine. In the present day, southwestern Ontario (from the extreme southwestern margin of the province around Windsor extending along the northern shore of Lake Erie to the Niagara River) is encompassed within the Carolinian biotic province which is characterized by a predominance of mixed hardwood species, while areas to the north of the Carolinian biotic region are encompassed by the Canadian biotic province in which spruce and pine predominate (Karrow and Warner 1990).

Within the confines of these forests, prehistoric peoples were able to collect a wide variety of native foods including nuts, berries, fruits and grains, in addition to hunting the animals which lived in these environments (for example, deer and rabbit). Beginning at about A.D. 540, the first archeological evidence for maize cultivation in the form of radio carbon dated kernels and cupules occurs (Crawford and Smith 1996). Patterns of cultural change and food procurement will be explored more fully below.

3.1.1 *Theories of Cultural Change in Southern Ontario*

Archaeological research into the prehistory of southern Ontario dates to the early part of the twentieth century. Previously, thoughts on Iroquoian origins were based on meagre historical records and Native oral tradition (Smith 1990). These theories proposed that Iroquoian peoples had migrated into southern Ontario displacing the indigenous Algonquian inhabitants a few hundred years prior to European contact. Although archaeological excavation began to comprise a part of the data gathering process in the early part of the twentieth century, the migration hypothesis continued to prevail. Lloyd (1904) put forth his "southern hypothesis" which suggested that Iroquoian peoples had migrated into the Great Lakes area and displaced indigenous Algonquian groups and, therefore, there was little time depth to Iroquoian prehistory. Wintemberg (1931) was the first to seriously suggest that Iroquoian sites could be placed into a temporal framework (Archaic, Transitional and Pre-European stages). He did this through analysis of ceramic vessel shape and decoration. Wintemberg only applied his stages to Neutral development and did not recognize such changes in Huron sites. His was a functional rather than chronological classification and, as such, viewed the Iroquoian presence around the Great Lakes as having little time depth and subscribed to the prevailing migrationary hypothesis.

During the 1940s, the migrationary hypothesis began to be questioned as Iroquoian culture was considered to be an aspect of the Woodland pattern (following McKern's Midwestern Taxonomic System) rather than of the Mississippian (Griffin 1943, Kraus 1944). This chronological approach, the *in situ* hypothesis, permitted the recognition of greater time depth. Lee (1951), embracing the *in situ* hypothesis, proposed a more complex classification system which included the Glen Meyer, Uren, Middleport and Neutral stages, which he suggested could be applied to all of Ontario. Emerson (1954) suggested that the Midwestern Taxonomic System was too rigid for southern Ontario and introduced the concepts of the horizon and tradition (after Willey and Phillips [1958]) to the Iroquoian sequence.

MacNeish (1952), on the basis of a typological study of pottery rim sherds, using the same attributes as Wintemberg, applied chronometric dates to the Iroquoian sequence. He worked in conjunction with William Ritchie studying pre-Iroquoian pottery in New York state (Ritchie and MacNeish 1949) and formulated a chronology of Iroquoian and pre-Iroquoian prehistory for New York state and southern Ontario. His stages included Point Peninsula (400 B.C. - A.D. 600), Early and Late Owasco (A.D. 600 - 1100), Transitional Iroquois (A.D. 1100 - 1350), Prehistoric Iroquois (A.D. 1350 - 1500), Late Prehistoric Iroquois (A.D.

1500 - 1610), and Historic Iroquois (A.D. 1610 - 1687).

Wright (1966) rejected the Midwestern Taxonomic System and applied the idea of branches and stages within the horizon/tradition approach. Wright equated branches with distinct ethnic groups in his "Ontario Iroquois Tradition". In southern Ontario proper, the Iroquois tradition is separated into Early, Middle and Late periods (Wright 1966). Wright's Iroquoian scheme, with some subsequent debate and modification, remains in use to the present day.

During the Early Ontario Iroquois (A.D. 1000-1300), Wright suggests that two branches existed: the Pickering in the southeastern and the Glen Meyer in the southwestern portion of Ontario. Wright equates these two contemporary branches with ethnicity. Because certain ceramic attributes occur quite suddenly in southwestern Ontario and had previously only occurred in Pickering sites, Wright (1966) advanced a 'conquest theory' in which Pickering peoples moved into southwestern Ontario and absorbed Glen Meyer groups. More recently, research has tended to emphasize regional variation within a single cultural sphere rather than separating groups as Wright did (Sutherland 1980, Pearce 1984, Williamson 1985, 1990, Bursey 1997). Indeed, these studies have provided evidence indicating continuation of culture through Early Ontario Iroquois to later stages, and suggest that a more appropriate way of characterizing Early Ontario Iroquois culture is in terms of viewing them

as regional variants of the same cultural group as opposed to distinct groups. In a more general way, in past research an increase in a sedentary lifestyle accompanied by year-round village settlement and maize horticulture was seen as a relatively rapid occurrence at the beginning of the Early Ontario Iroquois (Noble 1975). However, as the recent dating of maize kernals from Princess Point sites (Crawford and Smith 1996) and human bone chemistry studies (Schwarcz et al. 1985, Katzenberg et al. 1995) suggest, it appears that maize introduction was a more gradual process.

The subsequent Middle Ontario Iroquois, as originally conceived by Wright (1966), is separated into chronological stages, the Uren (A.D. 1300-1350) and the Middleport (A.D. 1350-1400) based on their assemblages. Wright himself questions the validity of the Uren stage as it appears to be a mixture of Glen Meyer and Middleport assemblages. Subsequent research in the form of radiocarbon dates from a number of Uren and Middleport sites has suggested a realignment of Middle Iroquoian dating (Dodd et al. 1990). These dates place the Uren stage between A.D. 1280 and 1330 and the Middleport stage between A.D. 1330 and 1400. The Middle Ontario Iroquois period is characterized by an increasing intensification of maize cultivation and also the incorporation of squash, beans, sunflower and tobacco into the suite of horticultural items (Dodd et al. 1990).

Palisaded villages begin to incorporate longhouses with an increase in house length through the period.

The Late Ontario Iroquois (A.D. 1400-1650) is separated into geographical branches: the Huron-Petun in southcentral Ontario and the Neutral-Erie in southwestern Ontario. Wright (1966) equated these with precursor groups of local ethnographic populations which existed at European contact.

Stothers (1977) introduced the concept of the Princess Point Complex as transitional between non-cultivating Middle Woodland groups and agricultural Iroquoian groups. Stothers originally defined the Princess Point Complex and dated it to A.D. 600-900, with three phases: Early (A.D. 600-750), Middle (A.D. 750-850) and Late (A.D. 850-900), and consisting of three regional foci: Point Pelee, Ausable and Grand River. More recent reinterpretation by Fox (1990) has removed the Ausable designation as too poorly understood to classify, and integrated the Point Pelee focus into the Riviere au Vase phase of the Western Basin tradition in extreme southwestern Ontario, northern Ohio and southeastern Michigan (Fox 1990, Murphy and Ferris 1990) based on ceramic attributes. This has left the Grand River focus as the single remaining entity in the Princess Point Complex leading to debate concerning the validity of the use of the term "complex" for Princess Point (Fox 1990). At present, this has not been resolved and the literature continues to use the designation Princess Point Complex. Interpretation

of how Princess Point fits into the chronological scheme of southern Ontario continues to fuel the debate surrounding the origins of Iroquoian groups.

Although the *in situ* hypothesis remains a popular model for characterizing Iroquoian development, it is not universally accepted. In a series of recent articles, Snow (1994a, 1994b, 1995) re-evaluated the evidence and suggested that based on discontinuities of ceramic styles and settlement patterns, and linguistic history of the region, a case can be made for Iroquoian culture arising from an incursion from outside southern Ontario after A.D. 900 (suggesting that matrilineal, Iroquoian-speaking groups displaced earlier patrilineal, Algonquian-speaking groups). Indeed, based on these features, Snow suggested that a likely candidate for Iroquoian derivation was the Clemson's Island culture of central Pennsylvania which flourished after A.D. 775. However, Snow's recent hypothesis has been challenged from the standpoint that the Algonquian/Iroquoian-speaking and patrilineal/matrilineal dichotomies, indicating discontinuity, are not valid for this period (Crawford and Smith 1996, Smith and Crawford 1997). Indeed, Crawford and Smith suggest that Princess Point was not patrilineal, Algonkian-speaking and that the evidence suggests continuity between Princess Point and Glen Meyer. In addition, the basic construction of Princess Point ceramics has a closer affinity to later Early Ontario

Iroquois than earlier Point Peninsula pottery (Crawford and Smith 1996). They cite the Porteous site as an example of this continuity. Using another line of evidence, Molto's (1983) analysis of 21 discontinuous traits using the Mean Measure of Divergence indicated genetic continuity between Middle and Late Woodland groups in southern Ontario. Molto suggests that genetic admixture of Saugeen and Point Peninsula groups formed the Late Woodland Iroquois, in support of the *in situ* hypothesis.

In a response to Crawford and Smith's article, Snow (1996) evaluates the evidence provided by Crawford and Smith and concurs that there appears to be continuity between Princess Point and Glen Meyer. However, Snow does not abandon his essential theory of migration. He now suggests that Princess Point people are Iroquoian and that the migration of Iroquoian peoples occurred some three centuries previous to his initial assertion (Snow 1996), and that neither the Ontario Iroquois Tradition nor Princess Point derive from Clemson's Island in central Pennsylvania because Princess Point and Clemson's Island are contemporaneous. Snow (1996) now suggests that the discontinuity separates Princess Point and Point Peninsula at around A.D. 600, and that the presence of this discontinuity is highly suggestive of migration of Iroquoian peoples into southern Ontario around this time. This implies that Princess Point peoples

were Iroquoian and migrated to southern Ontario displacing previous Middle Woodland Point Peninsula and Saugeen groups.

3.1.2 *The Beginnings of Agriculture in Southern Ontario*

The earliest evidence for plant cultivation in southern Ontario occurs in the form of maize, a non-indigenous cultigen, during the Woodland period. Unlike regions to the south of southern Ontario, indigenous plants were not cultivated. There has been considerable debate surrounding when exactly this initial cultivation of maize occurred during the Woodland. Evidence in the form of archaeological maize kernels and cupules, as well as bone chemistry indicating maize cultivation, has a bearing on interpretations concerning the *in situ* versus migration hypotheses of cultural change in southern Ontario. In this section, I will explore the Woodland sequence of southern Ontario in terms of the various types of evidence which exist indicating initial maize cultivation, the dating for initial introduction as indicated by this evidence, how this evidence relates to the *in situ* and migration hypotheses, and how my research contributes to this body of knowledge.

The Early Woodland of southern Ontario is distinguished from the preceding Archaic by the appearance of pottery, and is divided into the Meadowood Complex (900-400 B.C.), and the Middlesex Complex (450 B.C. - A.D. 0) (Ritchie 1944, 1955, Ritchie and Dragoo 1960, Spence 1967). Interaction

with the Adena complex of the Ohio Valley is indicated by pottery styles during the Meadowood (Spence et al. 1990). Vinette 1 ware, coil constructed and relatively crude with cord-marked surface decoration, was followed by the more refined dentate-decorated Vinette 2 ware during the Middle Woodland (Ritchie and MacNeish 1949). Subsistence strategies changed little from the preceding Late Archaic. Hunting and gathering of game, fish, shellfish, nuts, berries and other plant foods continued with a seasonal round influenced by the availability of the various resources (Spence et al. 1990). Indigenous plants of southern Ontario are C3 and local animals which consumed these plants reflect a C3 isotopic signature. Band size would increase during the summer months as plant resources became more abundant, dispersing into smaller groups during the winter. Although cultivation of native plant species such as squash has been documented in areas to the south (Ford 1985), no evidence exists for cultivation of plants, native or imported, during the Early Woodland in southern Ontario.

The Middle Woodland (200 B.C. - A.D. 700) is characterized by the beginnings of dentate and wavy line pottery decoration (Vinette 2) (Ritchie and MacNeish 1949) and is divided into the Couture Complex (centred in southwestern Ontario, Michigan and Ohio), the Saugeen Complex (centred on the shores of Lake Huron), and the Point

Peninsula Complex (centred in southcentral and southeastern Ontario) (Spence et al. 1990). Beyond geographical distinction, these three complexes are distinguished by a variety of ceramic features including vessel form, coarseness of paste, surface finish, and zones of decoration (Wright 1963). Subsistence and settlement patterns during this period are generally characterized as a continuation of a seasonal round based on availability of local resources, hunted and gathered. Microband groups would aggregate into macrobands during the spring to take advantage of lake resources including fishing and terrestrial mammals which also aggregated in these areas, while in the fall and winter months microband groups would disperse to take advantage of hunting and trapping in forested areas away from lakes (Spence et al. 1990). Larger sites with structures and middens appear during this period indicating a prolonged period of macroband aggregation during the spring and summer as well as an increased consistency in terms of returning to the same site year after year (Spence et al. 1990).

The first archaeological evidence for maize cultivation in southern Ontario occurs in the Middle to Late Woodland transition. The earliest sites providing floral evidence for maize cultivation have been suggested to occur in the Princess Point Complex. Four sites excavated during the 1970's and 1980's have produced carbonized maize remains and were suggested to provide evidence for the presence of maize

cultivation in Princess Point times, including the Porteous Village site, Grand Banks site and Princess Point type site (Stothers 1977), and the Moyer Flats site (Fox 1986). Of these the Porteous Village site has been reinterpreted to be Early Ontario Iroquois (Noble and Kenyon 1972, Williamson 1990), and the second two are multi-component sites which render the exact cultural provenience of the carbonized maize remains unclear (Fox 1990). Although the Moyer Flats site produced Princess Point-like ceramics, it has been dated to A.D. 995 and, along with its close proximity to the Early Ontario Iroquois Blair Flats site, has been suggested to be associated with the Early Ontario Iroquois (Williamson 1990). Clearly the maize cultivation evidence from floral remains from these sites does not definitively suggest maize cultivation during Princess Point times. Another site in southern Ontario, the Dawson Creek site on the northwest shore of Rice Lake, has provided archaeological maize kernels (Jackson 1983). This site has been dated to 1405±60 B.P. or cal. A.D. 550(650)760 from charcoal from a hearth (sample submitted to the Saskatchewan Research Council Radiocarbon Laboratory, Jackson 1983). This evidence is a little more convincing than those from the previously mentioned sites, but still does not date the maize evidence itself.

However, more recent analysis in relation to the migration/*in situ* debate, has provided AMS radiocarbon dates

of Princess Point age from maize kernels and cupules from the Grand Banks, Lone Pine and Young 1 sites of the Lower Grand River Valley and the Bull's Point and Bull's Cove sites at the Cootes Paradise locality at the west end of Lake Ontario (Crawford and Smith 1996, Smith 1997, Smith and Crawford 1997, Crawford et al. 1997). These dates show that maize was present in southern Ontario as early as A.D. 540 at the Grand Banks site. Five dates were obtained, calibrated at 2 sigma with the program CALIB 3.0 (non-bracketed numbers indicate the 2 sigma range, bracketed numbers indicate the intercept): A.D. 260(540)660, A.D. 240(570, 600)870, A.D. 650(789)980, A.D. 880(1000)1150, A.D. 990(1030)1210, (Crawford et al. 1997). Crawford and colleagues (1998) go on to suggest that occupation of the lower Grand River flood plain during Princess Point times was not necessarily seasonal as had been previously supposed (Stothers 1977). Rather, through examination of the geomorphological history of the lateral bar upon which the Grand Banks site sits, they determined that because the bar surface was relatively stable and flooding was minimal during the period A.D. 500-900, year-round occupation of the site was possible (Crawford et al. 1998).

The *in situ* versus migration debate centres around whether or not the Iroquois are indigenous to southern Ontario and New York state, or migrated from some other locality, usually suggested to be from an area to the south.

Additionally, the debate questions, if migration is accepted, when such migration occurred. In terms of southern Ontario, the *in situ* versus migration hypothesis potentially generates three possible scenarios. The first is that people (proto-Iroquoians) migrated into southern Ontario without bringing maize with them and independently began to cultivate maize once settled in southern Ontario. This scenario suffers from the fact that maize is not indigenous to southern Ontario and, therefore, maize would have had to have been introduced to the region through some mechanism involving either trade between groups resulting in movement of maize from some other region (presumably to the south) into southern Ontario, or through migration of groups of people from an area previously cultivating maize (to the south) to southern Ontario. The rejection of this first scenario leads to the other two possible scenarios: (1) groups of people migrated into southern Ontario bringing maize with them - the 'migration hypothesis', and (2) maize was transported to southern Ontario through trade networks without an actual movement of people - the '*in situ* hypothesis'.

As was stated at the end of section 2.5.2, it is hypothesized in this dissertation that the analysis of bone apatite for stable isotopes of carbon will demonstrate the consumption of maize at an earlier date when it is consumed in relatively small proportions in southern Ontario. The

demonstration of the presence of maize during Princess Point times through the use of the analysis of bone apatite would add credence to the hypothesis of *in situ* Iroquoian development from Princess Point to Early Ontario Iroquois. The more recent studies carried out by Crawford and Smith (1996) since the commencement of this dissertation provide an additional check on the results contained within the present work. Analysis of maize consumption from skeletal samples from sites of Princess Point provenience in southern Ontario provides an additional avenue of evidence supporting the *in situ* hypothesis of Iroquoian development from Princess Point to Early Ontario Iroquois.

3.2 *San Nicolas Island*

Archaeological remains from San Nicolas Island provide an opportunity to study the interplay between marine and terrestrial dietary resources. San Nicolas Island is one of the Channel Islands off the southern coast of California between Santa Barbara and San Diego. These islands are separated into two groups, the Southern Channel Islands and the Northern Channel Islands. San Nicolas Island is part of the Southern Channel Island group and is the most distant from the mainland coast. As with the other Channel Islands, sea levels have risen since the recession of the ice sheets associated with the Wisconsinan glaciation beginning approximately 10,000 years ago. There is no evidence to

indicate that San Nicolas Island was ever connected to the mainland via a land bridge (Schwartz and Martz 1992).

The southern California coast, including the Channel Islands, is characterized by a Mediterranean climate having mild, wet winters and dry, warm summers (Moratto 1984). San Nicolas Island is one of the smaller of the Channel Islands comprising 22 square miles. The bulk of the land area of the island consists of a central plateau which is surrounded by canyons leading down to a series of terraces to the shoreline. The following environmental information is taken from an article by Schwartz and Martz 1995. In the northwest portion of the island are large sand dunes. Its environment is semi-arid with sparse vegetation and limited terrestrial animals including island fox, mouse and lizard. The paucity of terrestrial life is counter balanced by an abundant sea life. Marine mammals include sea lions, elephant seals and harbour seals. Sea otters were present prehistorically but are now extirpated. Shell fish are abundant in numerous tide pools surrounding the island. Fish are abundant and a wide variety of sea birds make the island home. While prehistoric peoples would have been faced with a lack of terrestrial resources, the abundant marine life afforded a substantial subsistence base.

3.2.1 *Prehistory of the Southern California Bight*

Initial archaeological research in the area of the California Bight in southern California (including the Channel Islands) was primarily concerned with the construction of local chronologies. David Banks Rogers in 1930 (Rogers 1993) presented the first manuscript which addressed the problem of chronology in the southern California Bight region. This analysis was not originally published but the manuscript resides in the Santa Barbara Museum of Natural History and is summarized in Rogers (1993). Rogers presented three cultural divisions for the southern California Bight. The earliest Oak Grove People are so called because of the frequency of sites near oak trees or groves. Dietary indicators suggest that large mammals and fish comprised a relatively small proportion of the diet, while mollusk and urchin remains are moderately prevalent. Although no plant remains were recorded, Rogers suggests that the presence of a large number of grinding implements including manos and mortars are indicative of a large portion of the diet being obtained from acorns and other plant material. The subsequent Hunting Culture of Rogers' chronology is characterized by an increase in the number of larger mammal bones (including deer, elk, puma, grizzly bear, black bear, seal, sea elephant, various small mammals, mollusks and some fish) as well as in the number and quality of workmanship of projectile points suggesting

an increased emphasis on hunting and reduced emphasis on acorns and other plant foods. Of the evidence for the processing of plant material, mortars and pestles become more prevalent than manos and metates. Rogers calls his final division the Canaliño, characterized by an increase in tools used for intensive procurement of marine resources including fishhooks, net sinkers and plank canoes. Rogers considered the Canaliño to be the direct ancestors of the historic Chumash who occupied the mainland area around Santa Barbara and the northern Channel Islands. Although Rogers does not mention the historic Gabrieliño of the Los Angeles area and southern Channel Islands, it is presumed that in this scheme the Canaliño would also be ancestral to the Gabrieliño (Goldberg 1993).

Subsequent to Rogers analysis, several other revisions or reformulations of southern California Bight chronology have been proposed. By and large these have had a tendency to become excessively complex with extensive splitting of cultures (Goldberg 1993). This complexity arose, in part, due to the different subsistence strategies used in the interior, mainland coastal and island subregions of southern California. These differences are due to the different floral and faunal resources available in these subregions. While larger mammals (such as deer) are available on the mainland, only small and relatively few terrestrial mammals are available on the islands. Additionally, terrestrial

abundance is reduced on the islands as compared with the mainland coast, and therefore, marine resources are utilized in greater proportion. An example of a complex chronology is that proposed by King (1990) in which three major periods are each split into three to five phases with further subdivision within each phase. While these divisions may be characteristic of cultural change within the various subregions, Rogers three main divisions essentially characterized the three main temporal changes in dietary resource procurement in coastal and island areas (as discussed above).

Rogers (1993) Oak Grove People are equivalent to what are now commonly known as Millingstone cultures subsisting primarily on terrestrial plant food items, particularly seeds, with a relatively lower proportion of terrestrial mammal items (Moratto 1984). Radiocarbon dating of Millingstone sites in southern California have shown that they occur as late as 2000 B.P. and overlap with Rogers later Hunting and Canaliño populations (Johnson 1966, in Goldberg 1993). Other problems with Rogers' formulation include whether the Hunting culture is present in southern Chumash and Gabrieliño regions, and suggestions that Millingstone and Hunting sites might be representative of seasonal habitation (Leonard 1971, Landberg 1965, in Goldberg 1993). What is clear, however, is that mainland coastal and island populations share dietary similarities.

Both regions display a mixed diet including terrestrial plants and animals and marine foods, but with a reduced component of terrestrial mammals on the islands. In addition, the islands display a reduced component of terrestrial plant material as a result of the low terrestrial abundance on the islands (Goldberg 1993). Essentially, both mainland coastal and island populations display a mixed diet, while island groups are heavily biased toward marine resources.

Salls (1988) proposed a chronology which, while simplifying the previously mentioned complex chronologies, takes into account the differences between mainland interior and coastal (including island) groups. The interior was characterized by an early Archaic horizon from 10,000 B.P. to 1000 B.P., equivalent to the earlier proposed Millingstone groups, with an emphasis on terrestrial plant foods. The subsequent Late Prehistoric from 1000 B.P. to contact, included trade items with coastal groups including some marine resources.

Coastal and island groups are divided into three phases (Salls 1988). The earliest division is the Early Mariners from 10,000 B.P. to 5,000 B.P., temporally equivalent to Rogers' Oak Grove People. The Archaic Canaliño, spanning from 5,000 B.P. to 2,000 B.P., are equivalent to Rogers' Hunting and earlier Canaliño groups, while Salls' Formative

Canaliño (2,000 B.P. to contact) equates with Rogers' later Canaliño.

3.2.2 *Stable Isotope Studies*

In one of the earlier studies examining relative dependence on marine versus terrestrial resources, Walker and DeNiro (1986) used stable isotopes of carbon and nitrogen in collagen to characterize diet in the Santa Barbara Channel region of southern California during the late prehistoric period. They analyzed samples from forty archaeological human burials and concluded that groups living in the interior of the mainland consumed a diet composed mainly of terrestrial resources, that groups living on the mainland coast consumed a mixed diet containing substantial quantities of both marine and terrestrial resources, while northern Channel Island groups subsisted on a diet heavily dependent on marine resources. Walker and DeNiro did not examine sites from the southern Channel Islands. These results were consistent with the natural habitat of these regions. Marine resources were least accessible for inland mainland groups, whereas the availability of marine resources was greatest and the availability of terrestrial resources the least for island groups. Because a diet containing marine foods results in increased $\delta^{15}N$ and $\delta^{13}C$ values, an increased use of marine foods may potentially be confused with a C4 based

terrestrial diet, since the isotopic signatures of marine and C4 based diets overlap. However, in southern California there is no evidence for the use of C4 plants in the diet, hence suggesting that the consumption of marine foods accounted for the increased $\delta^{15}N$ and $\delta^{13}C$ values found in Walker and DeNiro's study. These data are also consistent with dental data which did not show increased caries and attrition rates as would be expected with increased plant food consumption, and ethnohistoric reports (Walker and Erlandson 1986). Walker and DeNiro (1986) suggest that there was little trade in terrestrial food resources between the mainland and northern Channel Islands as this would lower $\delta^{15}N$ and $\delta^{13}C$ values.

Goldberg (1993) in her Ph.D. dissertation, expanded upon Walker and DeNiro's work by evaluating carbon and nitrogen isotopes from collagen in southern California for both spatial and temporal variation in marine resource consumption utilized by prehistoric groups. Spatial divisions used were inland, mainland coastal, northern Channel Island and, as a supplement to Walker and DeNiro's study, southern Channel Island groups. Temporal divisions used were an early period pre-dating 3,000 years B.P. and a late period post-dating 3,000 years B.P. Goldberg concluded that in a general sense marine resource consumption increased spatially from inland to mainland coastal to northern Channel Island and finally to southern Channel

Island groups; and temporally from early to late periods in all geographic contexts with the exception of the southern Channel Island group. Here carbon and nitrogen isotope ratios from collagen indicated a decrease in marine resource use from early to late periods. Of the southern Channel Island group, Goldberg analyzed samples from San Clemente and San Nicolas Islands. However, while samples for both the early and late periods were available for San Clemente Island, she was only able to analyze early period samples from San Nicolas Island. For this reason, her determination that marine resource consumption decreased from the early to late period in the southern Channel Islands is based only on data emanating from San Clemente Island. When Goldberg examined the data from San Clemente Island only, the data also indicated a decrease in marine resource consumption from early to late periods. She attributes this to increased trade of terrestrial food items with the mainland during the late period relative to the early period (when the islanders would have been extremely limited in terrestrial food sources due to the arid nature of the Channel Islands, and relied more heavily on marine foods). Increased trade of terrestrial resources from the mainland during the late period permitted a proportionately greater consumption of terrestrial based foods relative to marine based foods on the southern Channel Islands.

While these data (Goldberg 1993) for the southern Channel Islands are suggestive of decreased marine resource use from early to late periods, the data set is incomplete because there were no available late period sites for San Nicolas Island in Goldberg's study. In the present dissertation, both early and late period sites from San Nicolas Island were available for analysis. Therefore, part of this dissertation will seek to fill in this gap by making a comparison between early and late sites on San Nicolas Island in relation to marine resource consumption. Both carbon and nitrogen isotopes from collagen and carbon isotopes from bone mineral will be analyzed with the object of determining whether $\delta^{13}C$ from carbonate provides additional information beyond that provided by collagen.

On San Nicolas Island, where marine protein is supplemented by terrestrial resources (whether indigenous to the island or imported through trade with the mainland), terrestrial plant resources may be under represented in collagen. Because collagen reflects primarily the protein portion of diet, marine animal foods are likely to be over represented in collagen. This contrasts with southern Ontario, where C4 maize is introduced into a C3 based diet, in that maize is unlikely to be represented in collagen until it is consumed in sizeable proportions. Whereas on San Nicolas Island terrestrial foods are likely to be under represented in collagen in relation to marine protein, in

southern Ontario C4 plants are likely to be under represented in relation to indigenous C3 foods in collagen. This dissertation, through the analysis of carbonate and collagen from prehistoric bone samples from both southern Ontario and San Nicolas Island, seeks to demonstrate these relationships in archaeological samples.

CHAPTER 4 - SAMPLE SET AND METHOD OF ANALYSIS

4.1 *Sample Set*

4.1.1 *Southern Ontario*

Human skeletal remains from the following sites from southern Ontario were used for stable carbon isotope analysis of biological apatite and collagen and for stable nitrogen isotope analysis of collagen. Results of collagen analysis of remains from some of these sites have been previously published (Schwarcz et al. 1985, Katzenberg et al. 1995). Skeletal rib fragments from all but two sites resided in the laboratory of Dr. M. A. Katzenberg at the University of Calgary, Alberta and were utilized in this study. These rib fragments were originally obtained by Schwarcz and colleagues and Katzenberg and colleagues who received permission for stable isotope analysis. Further samples were not obtained due to recent changes in professional practice. In recent years native North American groups have become vocal in their desire for the scientific community and general public to respect the historical and spiritual aspects of native burials. This has resulted in legislation such as the Native American Graves Protection and Repatriation Act (NAGPRA) in the United States. Additional bone samples were obtained

through Dr. Susan Pfeiffer of the University of Guelph, Ontario (Moatfield and Uxbridge sites).

Samples used in this study from sites in southern Ontario range in date from the Laurentian Archaic (Morrison's Island, 2300 B.C.) to the historic Huron (Ossossane Ossuary, A.D. 1636). Sites were chosen based on the availability of material for analysis and with the object of having a temporal range of sites spanning from hunter/gatherer, pre-maize horticultural times, through the introduction and initial cultivation of maize, to the historical period. A site location map for sites used in the southern Ontario portion of the study is provided in Figure 4.1. Discussion of the cultural affiliation of the various sites is provided in Chapter 3.

Samples from a single site of Archaic age were analyzed. Morrison's Island, while technically part of Quebec, is located in the lower reaches of the Ottawa River which forms the border between Ontario and Quebec. The site was initially excavated by Kennedy in 1953 (1953, 1966), and provided two radiocarbon dates of calibrated (cal.) 2750±85 B.C. and 1845±85 B.C., giving a Laurentian Archaic provenience. Eighteen burials were excavated by Kennedy and analyzed by Pfeiffer (1977).

The Donaldson Site, located on the east coast of Lake Huron, encompasses two cemeteries, both of which are of Middle Woodland, Saugeen provenience. The burials of

Cemetery I, initially excavated by Wright (1963), were analyzed by Anderson (1963), and contain 12 individuals. Cemetery II, containing 11 individuals, was initially excavated by Finlayson (1977), while the burials were analyzed by Molto (1979). The radiocarbon dates obtained from wood and charcoal fragments for Cemetery I span a wide range from an early date of 585 B.C. to a late date of A.D. 1225 (Wright 1963). Wright rejects the later dates and dates Cemetery I to uncalibrated (uncal.) 555±25 B.C. (Wright 1963). Cemetery II has provided a single radiocarbon date of uncal. A.D. 5±75 (Finlayson 1977). Levesconte Mound, also of Middle Woodland provenience is located approximately 100 km northeast of Toronto. The site was excavated by W. A. Kenyon of the Royal Ontario Museum (Kenyon 1986). Two radiocarbon samples from grave goods provided dates of uncal. A.D. 230±55 and A.D. 120±50 (Spence et al. 1979).

Serpent Mounds, located approximately 100 km northeast of Toronto, is a multicomponent site which produced 159 skeletons. The site was excavated by Johnston (1968), and the burials analysed by Anderson (1968). Of the various components, samples from burials from Mounds E, G and I were analyzed in the present study. Mound E, the earliest of the mound complexes, provided two radiocarbon dates obtained from wood and charcoal fragments of uncal. A.D. 120±200 and A.D. 290±150 (Johnston 1968). Mounds G & I represent

secondary burials and are thought to be later than Mound E. Johnston (1968) states that they date to between A.D. 300 and 500. All three mounds are of Middle Woodland, Point Peninsula provenience.

The Surma site, located at the northeastern end of Lake Erie in Fort Erie, Ontario, has been dated to A.D. 700 through the archaeological analysis of grave goods (Emerson and Noble 1966). The site was excavated by Emerson in 1965 (Emerson and Noble 1966) and the 22 burials were analysed by Cybulski (1968). The Middle to Late Woodland transition, Princess Point provenience of this site (and the subsequent Varden site) is of particular interest to this study because this is when initial archaeological evidence for maize consumption occurs, as discussed in Chapter 3. The Varden site, located at the eastern end of Long Point which extends into Lake Erie, has provided an AMS date of cal. A.D. 918±164 (MacDonald 1986), indicating a provenience of Middle to Late Woodland transition Princess Point or Early Ontario Iroquois, Glen Meyer Branch. Nine individuals were excavated by MacDonald (1986).

The Miller site is of Early Ontario Iroquois, Pickering Branch provenience and has been radiocarbon dated to uncal. A.D. 1152±118 (Wright 1966), cal. A.D. 1115±70 (Katzenberg et al. 1995). This village site with a palisade is located just east of Toronto. Thirty-two individuals were excavated from seven pits at the site by Kenyon (1968), and the

burials were analyzed by Ossenberg (1969). The Force site is also of Early Ontario Iroquois provenience but of the Glen Meyer Branch and is located in the Grand River drainage in central southern Ontario. It was excavated by Fox in 1978 and radiocarbon dates range between cal. A.D. 1235±75 and A.D. 1325±75 (Fox 1980).

Moatfield, located in northern Greater Toronto, and radiocarbon dated to cal. A.D. 1330±60 (Susan Pfeiffer, personal communication) is of Middle Ontario Iroquois provenience. Under Wright's (1966) original formulation, this site would be classified as belonging to the Uren stage. However, as discussed in Chapter 3, more recent thinking suggests that Uren is a mixture of Glen Meyer and Middleport assemblages, and therefore it is more appropriate to classify Moatfield as Middleport. Four femora samples were provided by Pfeiffer for the present study. These were of subadults ranging in age from 12 to 16 years. Samples from Moatfield are the only ones utilized in this study that were not adults. While there may be differences in stable carbon isotope values between infants and adults due to nursing infants effectively feeding off mother's tissues causing a trophic level effect (Katzenberg 1993), there is no evidence to suggest that dietary, and hence isotope, differences exist between adolescents and adults in prehistoric southern Ontario groups. Moatfield and Uxbridge (see below) were the only two sites where rib samples were

not utilized. While isotope turnover rates have been suggested to be shorter in ribs than other long bones because of reduced cortical thickness, femora were the only bones from Moatfield and Uxbridge available in the present study.

The second Middle Ontario Iroquois, Middleport site used in the present study is Fairty Ossuary, located on the northwestern outskirts of Toronto, and dated to A.D. 1350±50 through association with the nearby village sites of Robb and Faraday (Donaldson 1962, Wright 1966). The burials were analyzed by Anderson (1964) and the ossuary provided a minimum number of 512 skeletons.

Uxbridge Ossuary, located just west of Rice Lake, dating to cal. A.D. 1490±80, is of Late Ontario Iroquois, Huron-Petun Branch provenience (Pfeiffer 1983). Nine femora samples were provided by Pfeiffer. Kleinberg Ossuary is also of Late Ontario Iroquois, Huron-Petun Branch provenience and is located just west of Toronto. The site was excavated by Melbye (1974) and contains a minimum number of 561 individuals. Artifacts including beads and axes were used to date the site to approximately A.D. 1600 (Melbye 1974). Ossossane Ossuary, located at the southeastern end of Georgian Bay is a historic Huron site and has been dated to A.D. 1636 using ethnohistoric and archaeological data (Kidd 1953).

Unless otherwise noted, bone samples were taken from ribs of up to a maximum of five individuals from each site, where possible. With the exception of Moatfield (as noted above), only skeletal material from adult individuals was utilized so as to remove the variable of the possibility of different diet for infants and subadults.

4.1.2 *San Nicolas Island*

Bone samples from three sites from San Nicolas Island were analyzed for stable carbon isotopes from collagen and biological apatite and for stable nitrogen isotopes from collagen. Of these sites, two date from Goldberg's (1993) early period and one from her late period, as described in Chapter 3. All samples analyzed were from adult individuals. The samples were obtained through the laboratory of Dr. M. A. Katzenberg at the University of Calgary, Alberta. They were originally supplied by Dr. J. Ezzo for collagen and carbonate analysis as part of a project exploring San Nicolas Island diet and lifeways. Permission was granted by Dr. Ezzo for inclusion of carbon isotope data from collagen and carbonate in the present dissertation.

SNI-16, located on the northwest coast of San Nicolas Island, was excavated by the UCLA Archaeological Survey in 1958 (Reinman and Townsend 1960), with subsequent work performed by Reinman (1964). Radiocarbon analysis provided

two dates: cal. 3300±100 B.P. from *Haliotis* fish remains recovered from the burial area, and cal. 3682±149 B.P. from charcoal from a hearth (Schwartz and Martz 1992). This places the site in Goldberg's (1993) early period. The site is a dune site and consists of patches of shell midden capping a large dune. The burials were located at the landward end of the dune.

SNI-40 is another midden site located near the west coast of the island overlooking a rocky intertidal area, excavated by the UCLA Archaeological Survey in 1959 (Reinman and Townsend 1960). Twenty burials were recovered from the inland slope of the midden and analyzed by Rootenberg (1960). Radiocarbon analysis from Olivella beads from two of the burials provided a date of cal. 3980±100 B.P. (Schwartz and Martz 1992). SNI-40 can also be placed in Goldberg's (1993) early period.

SNI-18 is located on the northwest side of the island and consists of a large dune with shell midden scattered over the top and sides of the dune. The site was excavated by the UCLA Archaeological Survey in 1959 (Reinman and Townsend 1960), and the five burials were analyzed by Rootenberg (1960). Radiocarbon analysis from *Haliotis* fishhook blanks from the burial area provided a date of cal. 300±60 B.P. (Schwartz and Martz 1992). SNI-18 is associated with Goldberg's (1993) late period.

4.2 *Sample Preparation*

The purpose of sample preparation for isotope analysis is to transform the raw bone into a form in which only the pertinent tissues are present. This entails the removal of impurities and phases of bone not used in the analysis. Sample preparation includes cleaning the sample, removing contaminants due to post-mortem alteration and diagenesis, and conversion of the sample to a gas. The sample is then prepared for the quantification of stable isotope ratios using an isotope ratio mass spectrometer.

4.2.1 *Isolation of Collagen*

There exists a large body of research characterizing bone collagen preparation techniques (Hare 1980, Armstrong et al. 1983, Chisholm et al. 1983, DeNiro 1985, Garfinkel 1987, Stafford et al. 1987, DeNiro and Weiner 1988a, Tuross et al. 1988, Schoeninger et al. 1989, Ambrose 1990). Compact lamellar bone and unburned tooth dentin are preferred for collagen analysis as there is less surface area for chemical weathering and physical contamination. The major contaminants in bone, for collagen analysis, include lipids, biological carbonate from bone apatite, post-depositional carbonates, carbon and nitrogen in adhering sediments, and organic matter (Hassan and Ortner 1977, Hassan et al. 1977, Kyle 1986, Piepenbrink 1986, Hanson and Buikstra 1987). In the present study, these

contaminants were largely removed through, firstly, mechanical procedures including scrubbing in distilled water and scraping off discoloured surfaces, adhering contaminants, connective tissues and all cancellous bone. This was followed by cleaning with distilled water in an ultrasonic cleaner. The cleaned bone was broken into chunks no larger than approximately two cubic centimetres for the purpose of chemical purification so that the inorganic portion of bone and organic materials from the burial environment are removed, isolating the protein collagen.

Once the bones were cleaned and allowed to dry, they were weighed. One to two grams of sample was retained for analysis, and any remaining sample was put in a sample bag for possible future use.

In the present study, isolation of collagen followed the method of Sealy (1986) whereby 1% HCl was used to demineralize the bone and then was followed by treatment with NaOH which removed humic acids and any remaining lipids. In some cases, the reaction with HCl was extremely slow and, therefore, 2% HCl was used for part of the process. HCl was changed every two days, until the reaction ceased (CO_3^{-2} has all been converted to CO_2 and H_2O). A 0.125M solution of NaOH was used and left to soak for 20 hours. If left to soak too long, collagen may begin to degrade, while if not left to soak long enough, not all contaminants will be removed, in either case potentially

skewing results (Chisholm 1989). Between the HCl and NaOH treatments, samples were rinsed to neutrality using double distilled water, and again after NaOH treatment. Samples were then freeze dried, and then weighed a second time in order to facilitate calculation of percent collagen yield.

4.2.2 *Isolation of Bone Apatite*

As with collagen analysis, compact lamellar bone is preferred for bone apatite analysis as there is less surface area for chemical weathering and physical contamination. Isolation of bone apatite was refined by Lee-Thorp (1989). This technique was followed in the present study. Bones were initially cleaned using the procedure as outlined above for collagen. Each sample was then ground to a 0.375 mm particle size and 100 - 500 mg of bone powder was placed in 15 ml centrifuge tubes. Centrifuge tubes were weighed both before and after bone powder was placed into them.

Non-structural carbonate is subject to post-mortem diagenetic alteration. Therefore, adsorbed and diagenetic carbonate in bone mineral must be removed prior to isotopic analysis. Sample preparation for the analysis of bone mineral involves the removal of organic matter and adsorbed and diagenetic carbonate, leaving only the structural carbonate for analysis. Organic matter (i.e. collagen and humic contaminants) was removed through treatment with Clorox (sodium hypochlorite - NaOCl). Clorox treatment was

repeated until effervescence ceased, usually 3 to 5 repeats. Samples were then rinsed to neutrality and then treated for 24 hours with 1M acetic acid for removal of adsorbed and diagenetic carbonate. Samples were then rinsed a second time until neutrality was reached. Samples were then freeze dried and weighed a second time in order to facilitate calculation of percent yield. Clorox does not remove all organic material but the remaining organic component does not affect the carbonate isotope results because it does not react with phosphoric acid (DeNiro and Weiner 1988b).

4.3 *Gas Preparation*

It is necessary to convert the purified collagen or bone apatite to a gas for the purpose of analysis with an isotope ratio mass spectrometer. For collagen, in the lab of Dr. H. R. Krouse at the University of Calgary, the Carla Erba gas analyser converts the sample to CO_2 and N_2 gas and the Finnegan Tracer-mat mass spectrometer analyzes the resulting gas by first oxidizing a tin wrapped 1 mg sample at 1030°C, and then reducing with copper at 640°C. N_2 gas and CO_2 gas is then automatically fed through to the mass spectrometer part of the unit.

For bone mineral the procedure follows that of Lee-Thorp (1989) whereby carbon in carbonate is converted to CO_2 gas by reacting 50 to 100 mg of purified carbonate with 100% phosphoric acid under vacuum at constant temperature for 24

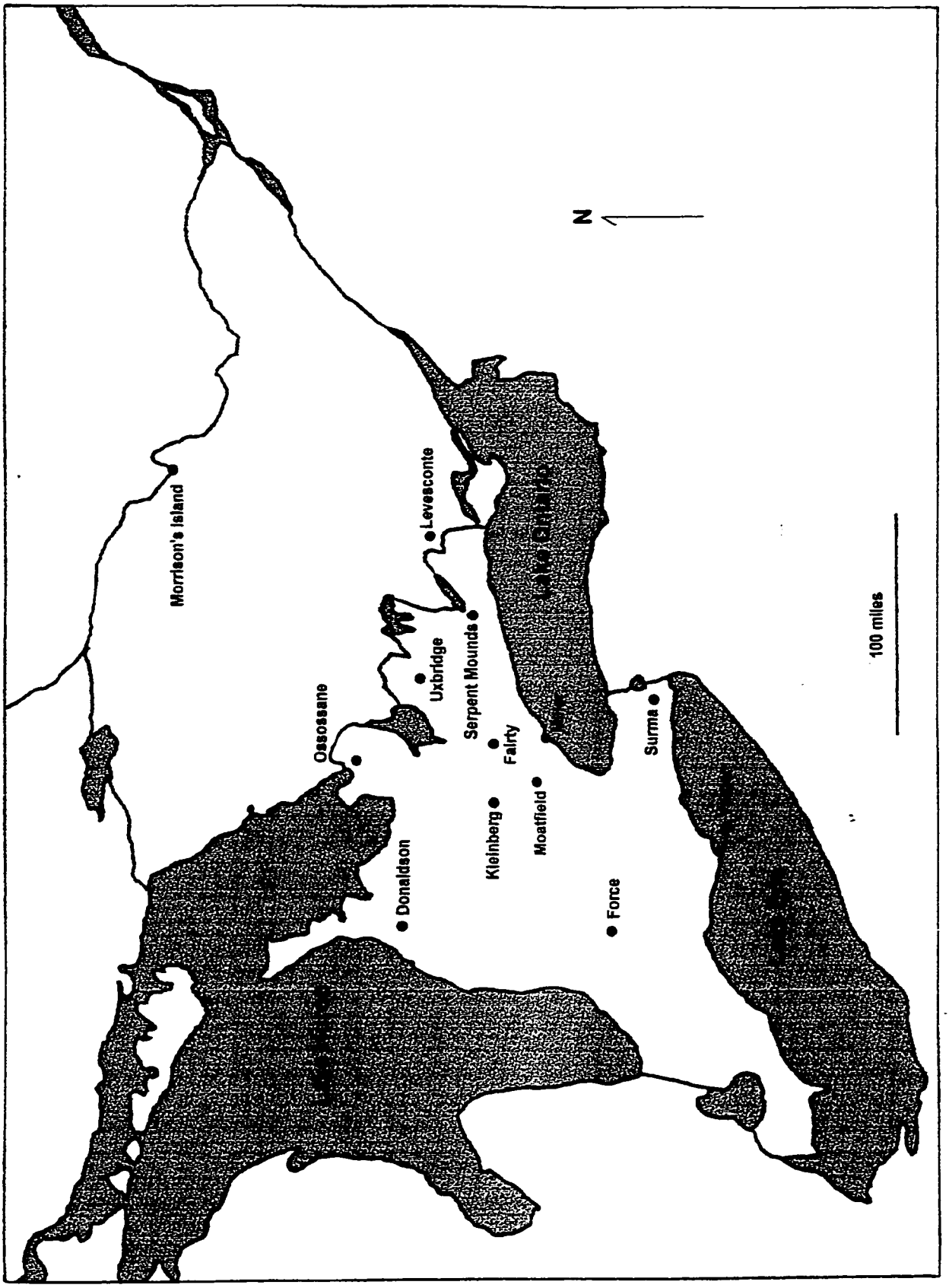
hours. CO_2 gas was then collected in a glass tube and flame sealed. The sample is then manually entered into the Sira mass spectrometer.

4.4 *Isotope Ratio Mass Spectrometry*

An isotope ratio mass spectrometer measures the abundances and masses of stable isotopes in gases and as N_2 and CO_2 in the form of ions. The three sections of the mass spectrometer include: (1) the source, in which the sample gas is ionized and accelerated, (2) the analyzer, where the ions are separated according to their mass by a magnetic field or electrostatic analyzer, and (3) the detector, where the separated ions of different mass are counted (Bauer et al. 1978). Sample and standard gases are alternately let into the mass spectrometer. The ratio of sample stable isotopes and ratio of standard stable isotopes is printed out using a computer program which also provides a δ value. Precision can be measured through calculating the standard deviation for each standard used. For carbon in collagen, the internal KNOX standard averaged -20.75±0.38‰ (n=16), while the international standards Graphite averaged -26.30±0.53‰ (n=7), and NBS-21 averaged -28.09±0.55‰ (n=6). For nitrogen in collagen, the internal KNOX standard averaged 7.76±0.35‰ (n=15), and the international standards IAEA-N1 averaged 0.40±0.09‰ (n=7), and IAEA-N2 averaged 20.34±0.25‰ (n=7). For carbon in

carbonate, the internal Lublin standard averaged 2.86±0.17‰ (n=8). At the University of Calgary, we are fortunate to have the use and services of the equipment and research associates of the Stable Isotope Laboratory of Dr. H. R. Krouse in the Department of Physics and Astronomy.

Figure 4.1 - Site locations for southern Ontario.



CHAPTER 5 - RESULTS

5.1 *Southern Ontario*

Data were obtained for samples for carbon isotopes from carbonate ($\delta^{13}C_{CA}$) and collagen ($\delta^{13}C_{CO}$), and nitrogen isotopes from collagen ($\delta^{15}N$). A total of 56 samples were analyzed from the 13 sites as outlined in Chapter 4: Morrison's Island, Donaldson Cemeteries 1 and 2, Levesconte, Serpent Mounds E, G and I, Surma, Varden, Miller, Force, Moatfield, Fairty Ossuary, Uxbridge, Kleinberg Ossuary, and Ossossane Ossuary. Data are presented in Table 5.1. Dates for each site are indicated. All $\delta^{13}C_{CA}$ data were obtained through preparation and analysis in the present study. Some $\delta^{13}C_{CO}$ data were obtained through previous work by Schwarcz and colleagues (1985) and Katzenberg and colleagues (1995) because there was an insufficient amount of remaining bone from these earlier studies for collagen analysis in addition to carbonate analysis, of which no previous data from earlier studies existed. For those samples with sufficient remaining bone, $\delta^{13}C_{CO}$ data were obtained through preparation and analysis in the present study. This distinction is noted on Table 5.1 (values with an asterisk next to them). Where possible, a minimum of five samples per site were

included in the study for each of collagen and carbonate analysis (see Table 5.1).

Table 5.1 also provides values for the spacing between carbonate and collagen $\delta^{13}C$ ($\Delta^{13}C_{CA-CO}$) for each sample, with the exception of RLE 1 and RLE 2 (Levesconte) where $\delta^{13}C_{CO}$ values were not available in the previous studies and the remaining bone for each of these did not provide a sufficient quantity after preparation for mass spectrometer analysis. Also provided in Table 5.1 are the average values of $\delta^{13}C_{CA}$, $\delta^{13}C_{CO}$ and $\Delta^{13}C_{CA-CO}$ for each site (bracketed values). Nitrogen isotope values ($\delta^{15}N$) from collagen analysis are also provided in Table 5.1.

The obtained isotope data were plotted in a series of graphs (Figures 5.1 through 5.10). Each sample datum was plotted against time in order to observe both inter- and intra-site variability. Figure 5.1 shows this variability for $\delta^{13}C_{CA}$ values, Figure 5.2 for $\delta^{13}C_{CO}$ values, and Figure 5.3 for $\Delta^{13}C_{CA-CO}$ values. (Note that while there are five values for Levesconte for $\delta^{13}C_{CA}$, there are only three values for $\delta^{13}C_{CO}$ and $\Delta^{13}C_{CA-CO}$ as discussed above.) The data can be broken into two discrete groups when $\delta^{13}C_{CA}$ and $\delta^{13}C_{CO}$ are observed individually (see Figures 5.1 and 5.2). On Figure 5.1 ($\delta^{13}C_{CA}$ by sample) samples plotted on the left hand side of the figure have more negative $\delta^{13}C_{CA}$ values than samples plotted on the right hand side. The same distinction can be observed on the plot of $\delta^{13}C_{CO}$ by sample (Figure 5.2). The

date at which this distinction occurs is at about A.D. 1000 (temporally between Varden [A.D. 918] and Miller [A.D. 1152]). The more negative $\delta^{13}C_{CA}$ and $\delta^{13}C_{CO}$ values in the left hand group of data points on both Figures 5.1 and 5.2 are indicative of a C3 based diet, whereas the more positive $\delta^{13}C_{CA}$ and $\delta^{13}C_{CO}$ values in the right hand group of data points are indicative of the inclusion of C4 (maize) plant consumption. This is not to suggest that pre-existing foods native to southern Ontario ceased to be consumed at the time of maize introduction but rather that maize was supplemental to these native foods.

Figure 5.3 shows the spacing between $\delta^{13}C_{CA}$ and $\delta^{13}C_{CO}$ ($\Delta^{13}C_{CA-CO}$) for each sample from each site. No discernible temporal pattern can be observed in this particular plot.

Mean values from samples from each site were plotted for $\delta^{13}C_{CA}$, $\delta^{13}C_{CO}$ and $\Delta^{13}C_{CA-CO}$. Figure 5.4 shows mean $\delta^{13}C_{CA}$ values for each site plotted versus date, Figure 5.5 shows mean $\delta^{13}C_{CO}$ values for each site plotted versus date and Figure 5.6 shows mean $\Delta^{13}C_{CA-CO}$ values for each site plotted versus date. Both figures 5.4 and 5.5 show the same distinction between more negative values for the group of sites on the left hand side of the plot and more positive values for the group of sites on the right hand side of the plot, as was shown in the corresponding plots by sample (Figures 5.1 and 5.2). No pattern is readily discernible when all points on the plot of $\Delta^{13}C_{CA-CO}$ by site means (Figure

5.6) are considered. However, if certain sites (points) are removed from the graph (with justification) a pattern does emerge. Only a single sample (showing an elevated $\Delta^{13}C_{CA-CO}$ value) was available for study from Donaldson Cemetery 1 (dated at 555 B.C.). This sample may not be representative of the population as a whole. An alternate explanation for a high $\Delta^{13}C_{CA-CO}$ value for Donaldson Cemetery 1 involves the accuracy of the dating of the site. Serpent Mound E (A.D. 205) also shows a high $\Delta^{13}C_{CA-CO}$ value and may be the result of dating problems. These concerns will be addressed in Chapter 6.

The large value for $\Delta^{13}C_{CA-CO}$ on Figure 5.6 for Moatfield is the result of a less negative value for $\delta^{13}C_{CA}$ (-1.4‰, Figure 5.4) as compared to $\delta^{13}C_{CA}$ values for other temporally surrounding sites, rather than a more negative $\delta^{13}C_{CO}$ value (-11.4‰, Figure 5.5) as compared to $\delta^{13}C_{CO}$ values for other temporally surrounding sites. This suggests that the specific nature of the diet at Moatfield is affecting $\delta^{13}C_{CA}$ values but not $\delta^{13}C_{CO}$ values. The reason for this is explored in Chapter 6.

If the data points representative of these sites are removed from the analysis on Figure 5.6, we are left with data points from Donaldson Cemetery 2, Levesconte, Serpent Mounds G and I, Surma, Varden, Miller, Force, Fairty Ossuary, and Uxbridge. These nine points form a curve with a minimum $\Delta^{13}C_{CA-CO}$ value among the earliest sites, increasing

to a maximum value for Varden, and decreasing towards the later sites. This pattern is consistent with the pattern postulated in Chapter 2 as being representative of a gradual increase in maize consumption from initial introduction until it becomes a staple food item, rather than maize quickly becoming a staple food soon after introduction (see Figure 2.1, Chapter 2). This will be elaborated more fully in Chapter 6.

$\delta^{13}C_{CA}$ (X-axis) was plotted against $\delta^{13}C_{CO}$ (Y-axis) for each sample datum from each site (Figure 5.7) and for mean values from each site (Figure 5.8). The plot on Figure 5.8 shows three groupings of data. The distinction between these groups is due mainly to a difference in $\delta^{13}C_{CO}$ (Y-axis values) in relation to $\delta^{13}C_{CA}$ (X-axis values). On Figure 5.8, the data points are separated into three groups, labeled I, II, III. In group I, $\delta^{13}C_{CA}$ values are in the range of -14‰ to -15‰, and $\delta^{13}C_{CO}$ values cluster in the -19‰ to -21‰ range. These values for both $\delta^{13}C_{CA}$ and $\delta^{13}C_{CO}$ are indicative of C3 based diets. Group II again have $\delta^{13}C_{CO}$ values in the -17‰ to -20‰ range, but $\delta^{13}C_{CA}$ values have a low of approximately -14‰ but increase to -9‰. In other words, in group II, carbonate values are increasing while collagen values are remaining constant. This is suggestive of relatively small amounts of maize in the diet which are reflected in the isotopic signature of carbonate but not in the isotopic signature of collagen. In group III, both

collagen and carbonate show elevated $\delta^{13}C$ values ($\delta^{13}C_{CO}$ in the range of -10‰ to -12‰, and $\delta^{13}C_{CA}$ in the range of -1‰ to -6‰) indicating that C4 plants (maize) form a significant proportion of the diet.

These data suggest that the C4 isotopic signature of maize is reflected in collagen at around A.D. 1000 (temporally between Varden and Miller), but not earlier. However, if we look at the distribution of $\delta^{13}C_{CA}$ values (along the X-axis on Figure 5.8), we observe a greater degree of continuity with no sudden jump. Indeed, the pre-Miller site data points show a gradual increase in $\delta^{13}C_{CA}$ along the X-axis, suggesting that the C4 isotopic signature of maize is reflected in bone carbonate at an earlier date than in collagen.

Figures 5.9 and 5.10 show stable nitrogen isotope values for the data set. $\delta^{15}N$ for individual samples was plotted against time (Figure 5.9) and $\delta^{15}N$ for site means was plotted agaenst time (Figure 5.10). The range of values for site means is from a low of 11.3‰ to a high of 14.8‰, and do not increase nor decrease through time. These values are suggestive of dietary protein being obtained from fish resources (Katzenberg 1989). Serpent Mounds E and Serpent Mounds G & I show elevated $\delta^{15}N$ values in relation to temporally surrounding sites. These values may be indicative of an increased reliance on fish protein relative

to terrestrial animal protein in the diet of Serpent Mounds groups.

5.2 *San Nicolas Island*

As with the southern Ontario data set, data were obtained for all samples for $\delta^{13}C_{CA}$, $\delta^{13}C_{CO}$ and $\delta^{15}N$. A total of 33 samples were analyzed from three dated sites on San Nicolas Island: SNI-40, SNI-16 and SNI-18, dating to respectively, 4000 B.P., 3300 B.P. and 300 B.P. Sites SNI-40 and SNI-16 date to Goldberg's early period and SNI-18 dates to the late period (Goldberg 1993). All samples provided the aforementioned data except for samples SNI 06 and SNI 07 from site SNI-16 (see Table 5.2). In the case of these samples, too little sample material remained after preparation for mass spectrometer collagen analysis, providing no $\delta^{13}C_{CO}$ and $\delta^{15}N$ data. Carbonate preparation provided enough material for carbonate analysis. In addition to the 33 dated samples, data ($\delta^{13}C_{CA}$, $\delta^{13}C_{CO}$ and $\delta^{15}N$) were obtained for nine other samples from six undated sites on San Nicolas Island (see Table 5.2). Table 5.2 also provides the spacing between carbonate and collagen $\delta^{13}C$ values for each sample. Also present on Table 5.2 are mean values for $\delta^{15}N$, $\delta^{13}C_{CA}$, $\delta^{13}C_{CO}$ and $\Delta^{13}C_{CA-CO}$ for each of the three dated sites.

The data were then plotted on scatter diagrams (see Figures 5.11 to 5.16). The data were plotted by sample for

each of the three sites for $\delta^{15}N$ (Figure 5.11), $\delta^{13}C_{CO}$ (Figure 5.12) and $\delta^{13}C_{CA}$ (Figure 5.13). In each of these, no trend of isotope ratios with time was detected. Figure 5.14 shows $\Delta^{13}C_{CA-CO}$ by sample for each site. Again, no trend can be seen from the early to late period.

Mean values for each of the three sites for each of $\delta^{15}N$ (Figure 5.15), $\delta^{13}C_{CO}$ (Figure 5.16) and $\delta^{13}C_{CA}$ (Figure 5.16) were plotted. Nitrogen isotope values (Figure 5.15) are high in relation to terrestrial environments for all three sites indicating a high proportion of marine foods in the diet of San Nicolas Island people. There is little variation in $\delta^{15}N$ values between the early sites (the two points on the left hand side of the diagram) and the late site (the point on the right hand side of the diagram). Indeed, total variation for mean $\delta^{15}N$ is 1.1‰ (see Table 5.2). This minimal variation with no trend indicates that, through time, there was no increase nor decrease in marine food consumption on San Nicolas Island. The relatively high values for $\delta^{15}N$ for the three sites (ranging between 17.3‰ and 18.4‰) may be also interpreted as indicative of an arid environment (Delwiche and Steyn 1970, Shearer and Kohl 1986). However, because archaeological evidence suggests a diet heavily reliant on marine foods (Goldberg 1993), it is more likely that the high $\delta^{15}N$ values are due moreso to marine food consumption rather than the arid environment.

Figure 5.16 shows mean values for $\delta^{13}C_{CA}$ and $\delta^{13}C_{CO}$ for each of the three sites. In each of these there is a very slight decrease in $\delta^{13}C$ value from early to late period sites, though only of the magnitude of 0.8‰ and 0.4‰, respectively. In essence, we can conclude from the $\delta^{13}C_{CA}$ and $\delta^{13}C_{CO}$ data that these values remain constant through time from the early to late period. This agrees with the nitrogen data and, in combination, suggests little change in the proportion of marine resource use from the early to late period on San Nicolas Island.

$\Delta^{13}C_{CA-CO}$ values between the three sites remain constant, varying only slightly from the earliest (SNI-40) to latest (SNI-18) sites, that is from 2.8‰ to 2.5‰, respectively (see Table 5.2). The absolute value of $\Delta^{13}C_{CA-CO}$ values is of some significance and will be discussed in Chapter 6.

The absolute values of $\delta^{15}N$ and $\delta^{13}C_{CO}$ can be compared between Goldberg's dissertation (1993) and the present study. For nitrogen, both studies' collagen data display a range between approximately 15‰ and 21‰. For carbon, both studies' range between -15‰ and -9‰, as would be expected given that both sets of data emanated from collagen.

Table 5.1: Carbon and nitrogen isotope data for bone collagen and apatite for sites from southern Ontario.

date	sample	$\delta^{15}N$	$\delta^{13}C_{CA}$	$\delta^{13}C_{CO}$	$\Delta^{13}C_{CA-CO}$
2300±400BC	Morrison's Island	(11.9)	(-13.6)	(-19.2)	(5.6)
	RMO 1	11.9*	-13.6	-19.2*	5.6
555±25BC	Donaldson, Cem. 1		(-9.5)	(-19.5)	(10.0)
	RDO 1		-9.5	-19.5*	10.0
AD5±75	Donaldson, Cem. 2	(12.3)	(-12.2)	(-19.3)	(7.1)
	RDO 2	12.5*	-12.4	-19.1*	6.7
	RDO 3	11.6*	-11.9	-19.9*	8.0
	RDO 4	12.5*	-12.3	-19.1*	6.8
	RDO 4R	12.5*	-12.0	-19.1*	7.1
	RDO 5	12.5*	-12.3	-19.1*	6.8
	RDO 5R	12.5*	-12.4	-19.1*	6.7
AD175±55	Levesconte	(14.6)	(-15.1)	(-21.8)	(6.7)
	RLE 1		-14.6		
	RLE 1R		-15.2		
	RLE 2		-15.4		
	RLE 3	14.6	-15.1	-22.7	7.8
	RLE 4	13.9	-14.8	-20.4	5.6
	RLE 5	15.3	-15.1	-22.2	7.1
AD205±90	Serpent Mounds E	(14.8)	(-9.2)	(-17.9)	(8.7)
	RSM 6	15.1	-9.2	-17.8	8.6
	RSM 7	14.5	-9.1	-18.0	8.9
AD400±100	Serpent Mounds G & I	(12.4)	(-14.6)	(-19.9)	(5.3)
	RSM 1 (G)	12.4*	-14.4	-21.1*	6.7
	RSM 2 (G)	11.8*	-14.9	-20.6*	5.7
	RSM 3 (I)	12.3	-14.2	-19.8	5.6
	RSM 4 (I)	13.6	-14.9	-19.1	4.2
	RSM 5 (I)	12.0	-14.8	-19.1	4.3
AD700	Surma	(12.7)	(-12.0)	(-18.3)	(6.4)
	RSU 1	13.0*	-12.5	-18.3*	5.8
	RSU 2	13.4*	-12.1	-18.4*	6.3
	RSU 3	11.8*	-11.4	-18.5*	7.1
AD918±164	Varden	(11.3)	(-12.0)	(-19.4)	(7.4)
	RVA 1	11.2*	-11.3	-19.2*	7.9
	RVA 2	10.8*	-10.5	-19.5*	9.0
	RVA 3	12.3*	-12.9	-19.0*	6.1
	RVA 4	11.2*	-11.0	-19.5*	8.5
	RVA 5	11.2*	-14.4	-19.6*	5.2
AD1115±70	Miller	(13.5)	(-6.6)	(-13.3)	(6.7)
	RMI 1	13.5*	-6.6	-13.3*	6.7
AD1268±75	Force	(11.6)	(-5.2)	(-12.4)	(7.2)
	RFO 1	11.6*	-4.4	-11.7*	7.3
	RFO 2	11.1*	-5.8	-12.8*	7.0
	RFO 3	11.8*	-4.6	-12.1*	7.5
	RFO 4	11.9*	-5.8	-13.1*	7.3
AD1330±60	Moatfield	(11.8)	(-1.4)	(-11.4)	(10.0)
	RMF 1	11.4	-0.7	-11.8	11.1
	RMF 2	12.4	-1.6	-10.7	9.1
	RMF 3	11.7	-0.5	-11.1	10.6
	RMF 4	11.7	-2.8	-11.8	9.0
AD1350±140	Fairty Ossuary	(11.7)	(-4.3)	(-11.2)	(6.9)
	RFA 1	11.6*	-3.9	-10.1*	6.2
	RFA 2	11.8*	-4.7	-12.2*	7.5

Table 5.1 (continued):

date	sample	$\delta^{15}N$	$\delta^{13}C_{CA}$	$\delta^{13}C_{CO}$	$\Delta^{13}C_{CA-CO}$
AD1490±80	Uxbridge	(11.1)	(-4.9)	(-10.8)	(5.9)
	RUX 1	10.2	-4.1	-10.1	6.0
	RUX 2	11.0	-5.0	-11.3	6.3
	RUX 3	12.0	-5.1	-11.2	6.1
	RUX 4	10.1	-5.3	-11.0	5.7
	RUX 5	11.0	-4.3	-10.2	5.9
	RUX 6	11.8	-5.4	-10.8	5.4
	RUX 7	10.9	-4.5	-11.2	6.7
	RUX 8	11.4	-4.8	-10.3	5.5
	RUX 9	11.6	-5.2	-11.3	6.1
AD1600	Kleinberg Ossuary	(12.2)	(-5.4)	(-12.0)	(6.6)
	RKL 1	12.0*	-5.4	-11.7*	6.3
	RKL 2	12.3*	-5.4	-12.2*	6.8
	RKL 3	12.4*	-5.5	-12.2*	6.7
AD1636	Ossossane Ossuary	(13.4)	(-5.1)	(-12.7)	(7.6)
	ROS 2	12.4	-4.9	-12.1	7.2
	ROS 3	13.9	-5.9	-11.1	5.2
	ROS 4	11.6	-5.4	-11.1	5.7
	ROS 5	16.7	-5.1	-15.9	10.8
	ROS 6	12.3	-4.1	-13.5	9.4

Notes: * data from Schwarcz et al. 1985, Katzenberg et al. 1995.
() mean value for site.

Table 5.2: Carbon and nitrogen isotope data for bone collagen and apatite for sites from San Nicolas Island.

sample	Ezzo #	$\delta^{15}N$	$\delta^{13}C_{CA}$	$\delta^{13}C_{CO}$	$\Delta^{13}C_{CA-CO}$
4000 B.P., 'early' period		(18.0)	(-7.1)	(-10.0)	(2.8)
SNI 23	SNI-40, BURIAL 1	19.0	-6.9	-9.9	3.0
SNI 24	SNI-40, BURIAL 2	18.6	-6.7	-9.1	2.4
SNI 25	SNI-40, BURIAL 3	18.2	-9.2	-11.3	2.1
SNI 26	SNI-40, BURIAL 6	19.0	-8.2	-11.1	2.9
SNI 27	SNI-40, BURIAL 9	17.2	-6.8	-9.2	2.4
SNI 28	SNI-40, BURIAL 10	16.5	-8.1	-10.6	2.5
SNI 29	SNI-40, BURIAL 12	19.5	-7.2	-9.7	2.5
SNI 30	SNI-40, BURIAL 16	19.2	-7.0	-9.7	2.7
SNI 31	SNI-40, BURIAL 17	16.4	-6.9	-10.5	3.6
SNI 32	SNI-40, BURIAL 18 (257-83)	16.9	-5.4	-9.7	4.3
SNI 33	SNI-40, BURIAL 19	16.4	-6.2	-9.5	2.3
SNI 34	SNI-40, BURIAL 20	18.5	-7.5	-11.1	3.6
SNI 35	SNI-40, 257-72	18.9	-6.7	-9.1	2.4
3300 B.P., 'early' period		(17.3)	(-7.7)	(-10.4)	(2.7)
SNI 03	SNI-16, BURIAL A	17.9	-6.9	-10.3	3.4
SNI 04	SNI-16, BURIAL B	17.0	-9.0	-11.6	2.6
SNI 05	SNI-16, BURIAL C	20.6	-8.4	-9.9	1.5
SNI 06	SNI-16, BURIAL 1	-	-5.9	-	-
SNI 07	SNI-16, BURIAL 2	-	-7.3	-	-
SNI 08	SNI-16, 256-91	15.6	-8.2	-11.2	3.0
SNI 09	SNI-16, 256-93	16.5	-8.2	-11.6	3.4
SNI 10	SNI-16, 256-98	17.8	-8.0	-10.9	2.9
SNI 11	SNI-16, 256-99	16.4	-5.1	-8.8	3.7
SNI 12	SNI-16, 256-101	17.6	-7.6	-11.1	3.5
SNI 13	SNI-16, 256-104	17.8	-7.8	-10.5	2.7
SNI 14	SNI-16, 256-105	16.7	-5.6	-8.5	2.9
SNI 15	SNI-16, 256-107.1	17.5	-8.6	-9.7	1.1
SNI 16	SNI-16, 256-107.0	18.2	-8.0	-10.5	2.5
SNI 17	SNI-16, 28-F-152	14.9	-8.8	-10.7	1.9
300 B.P., 'late' period		(18.4)	(-7.9)	(-10.4)	(2.5)
SNI 18	SNI-18, ACC.5-A16	16.4	-7.8	-10.6	2.8
SNI 19	SNI-18, ACC.16	17.9	-8.9	-11.3	2.4
SNI 20	SNI-18, BURIAL 2	19.1	-7.8	-10.2	2.4
SNI 21	SNI-18, BURIAL 3	17.8	-8.0	-11.2	3.2
SNI 22	SNI-18, 221-65	20.8	-6.9	-8.8	1.9
no date					
SNI 01	A1664-829	16.7	-6.7	-9.4	2.7
SNI 02	A1664-846	19.9	-8.0	-10.2	2.2
SNI 36	SNI-56, BURIAL 2 (UCLA)	20.0	-9.3	-11.8	2.5
SNI 37	SNI-56, BURIAL 2.1	21.5	-8.1	-10.8	2.7
SNI 38	SNI-56, BURIAL 2.3	20.4	-8.5	-11.2	2.7
SNI 39	SNI-56, 17-137	18.3	-8.2	-10.9	2.7
SNI 40	SNI-117, BURIAL 2	17.4	-5.8	-9.2	3.4
SNI 41	SNI-146, BURIAL 1	20.4	-8.4	-11.8	3.4
SNI 42	SNI-214, FEATURE 1	21.3	-8.5	-9.9	1.4

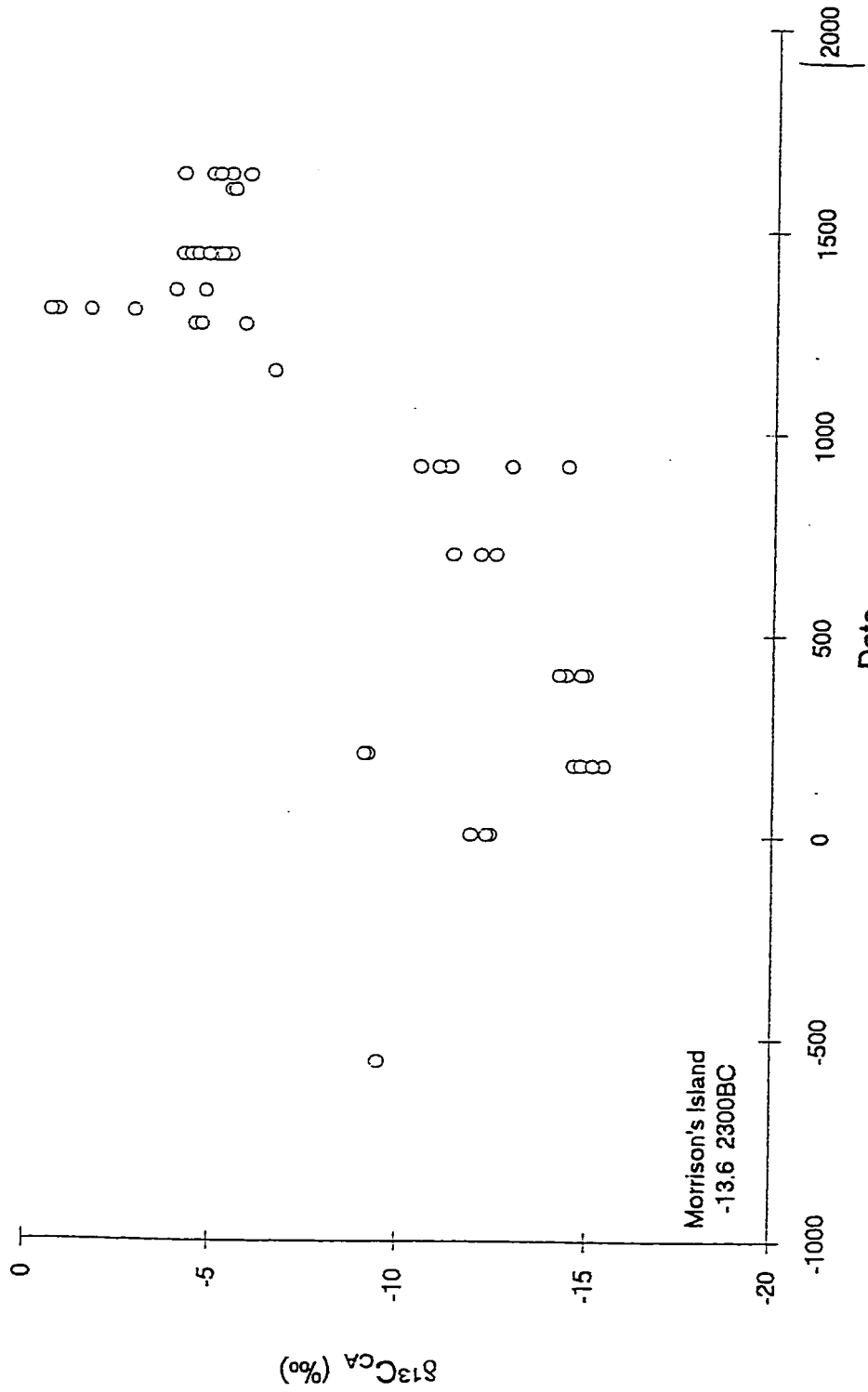


Figure 5.1: Carbon isotope ratios of bone carbonate versus time for samples from Ontario.

Figure 5.2: Carbon isotope ratios of bone collagen versus time for samples from Ontario.

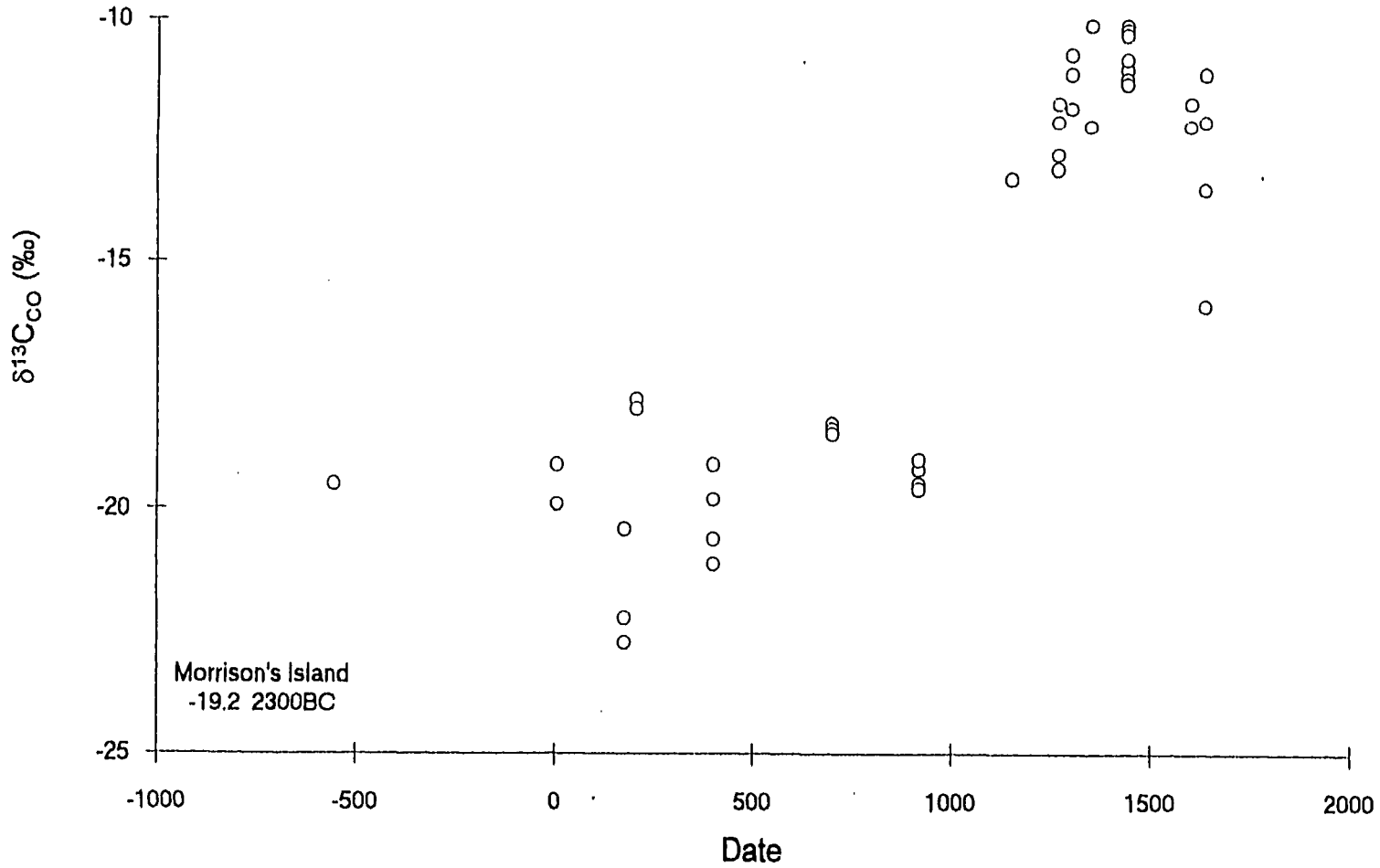


Figure 5.3: Spacing of carbon isotope ratios of bone carbonate and collagen versus time for samples from Ontario.

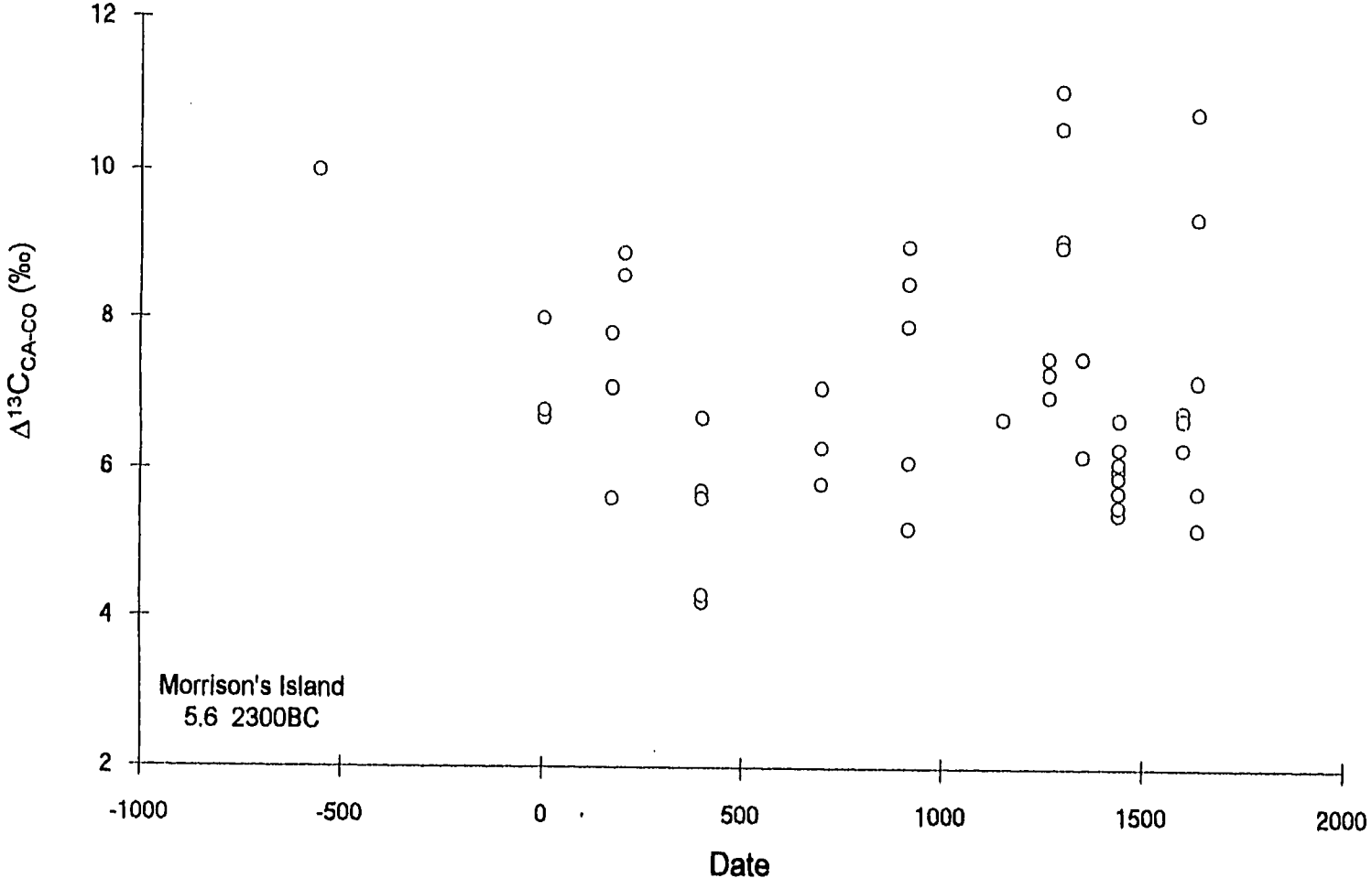
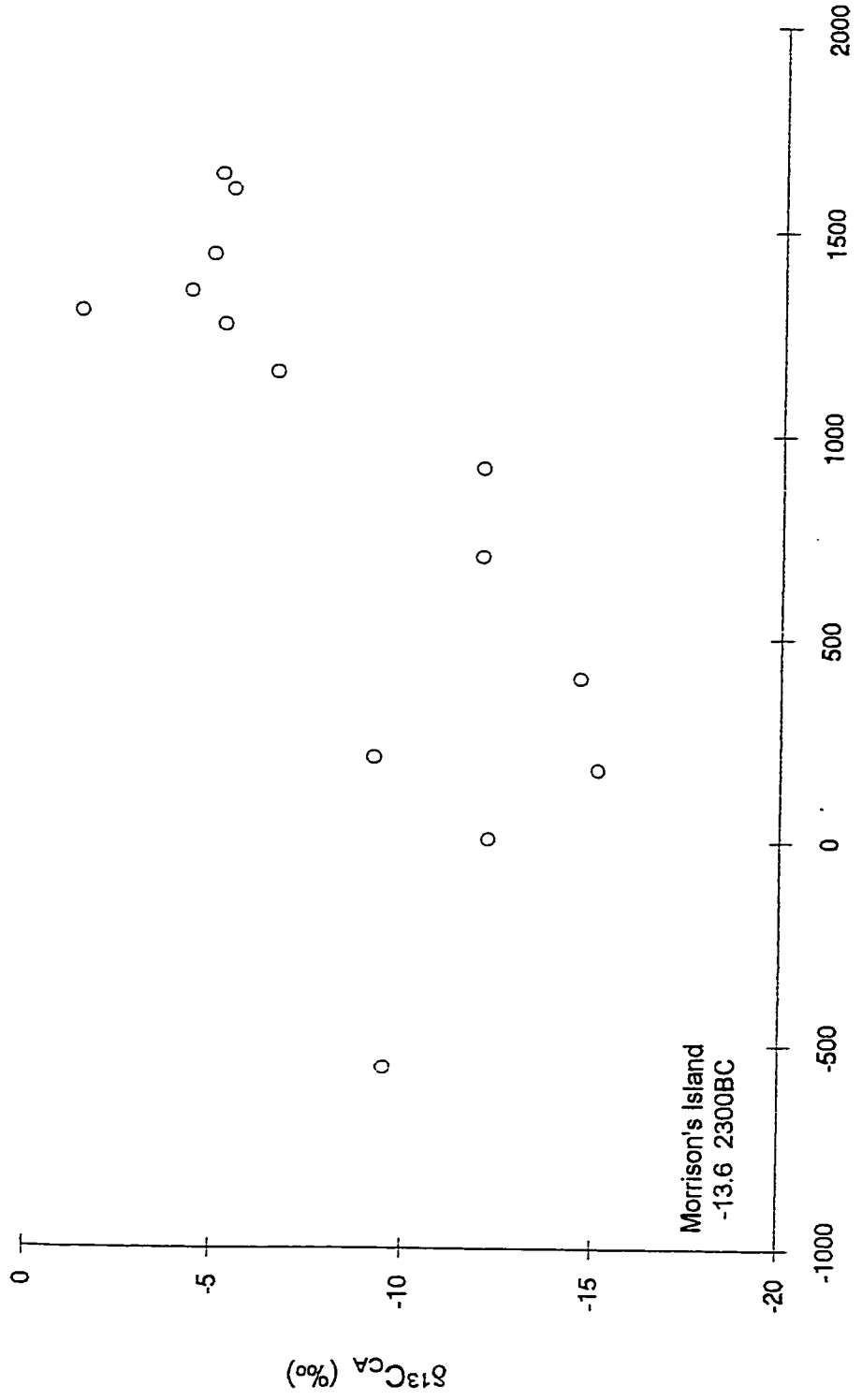


Figure 5.4: Carbon isotope ratios of bone carbonate versus time for site means from Ontario.



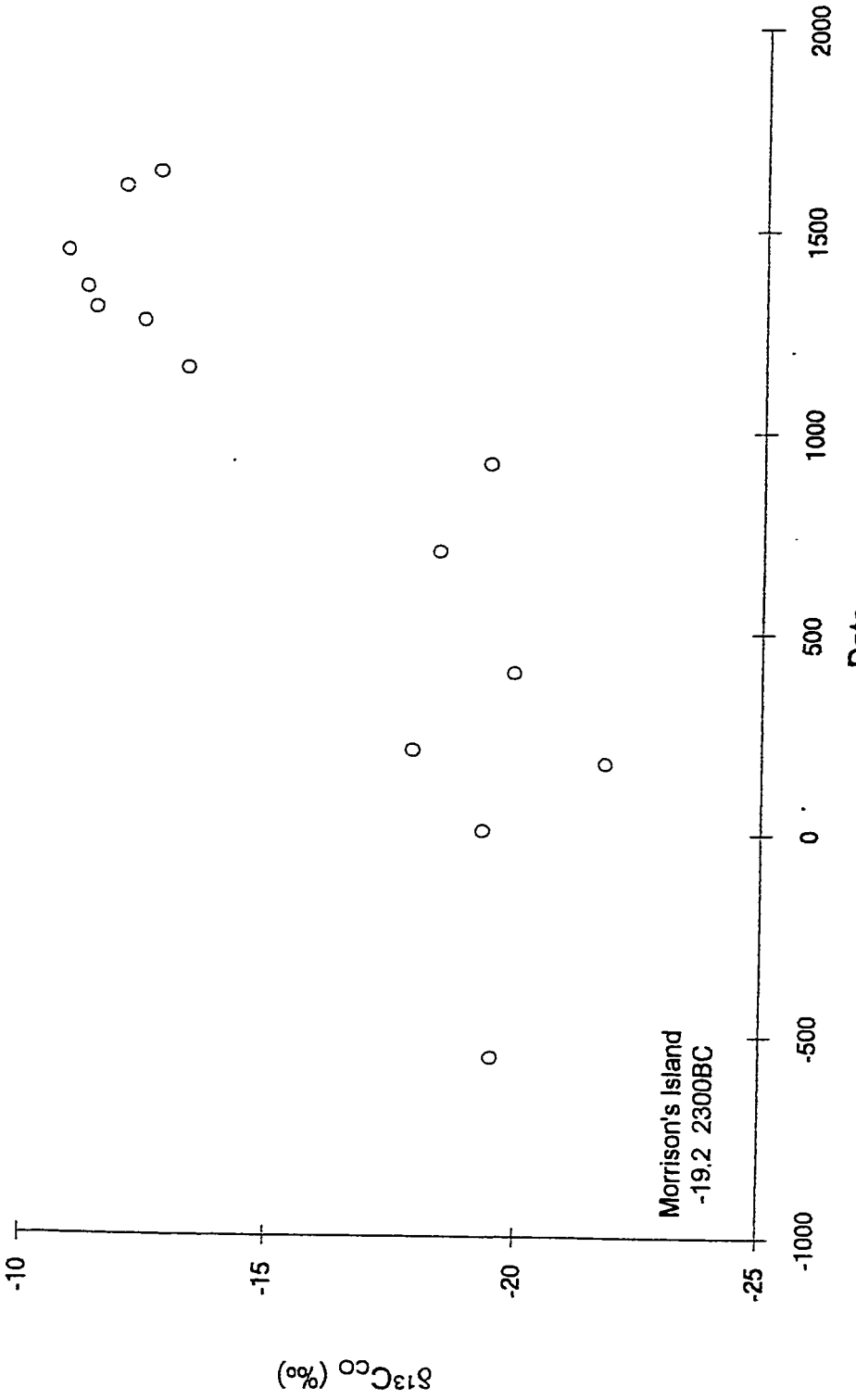


Figure 5.5: Carbon isotope ratios of bone collagen versus time for site means from Ontario.

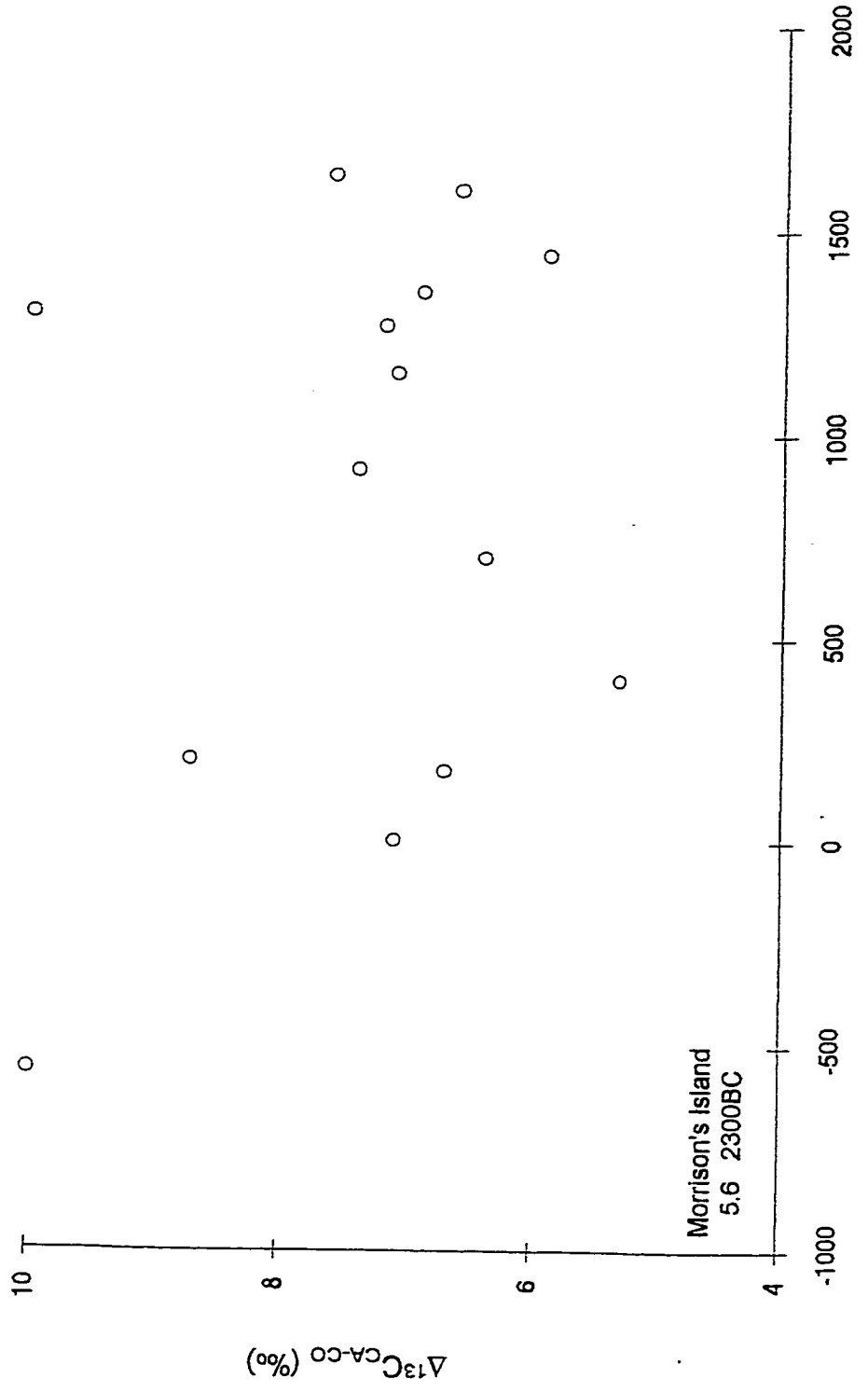


Figure 5.6: Spacing of carbon isotope ratios of bone carbonate and collagen versus time for site means from Ontario.

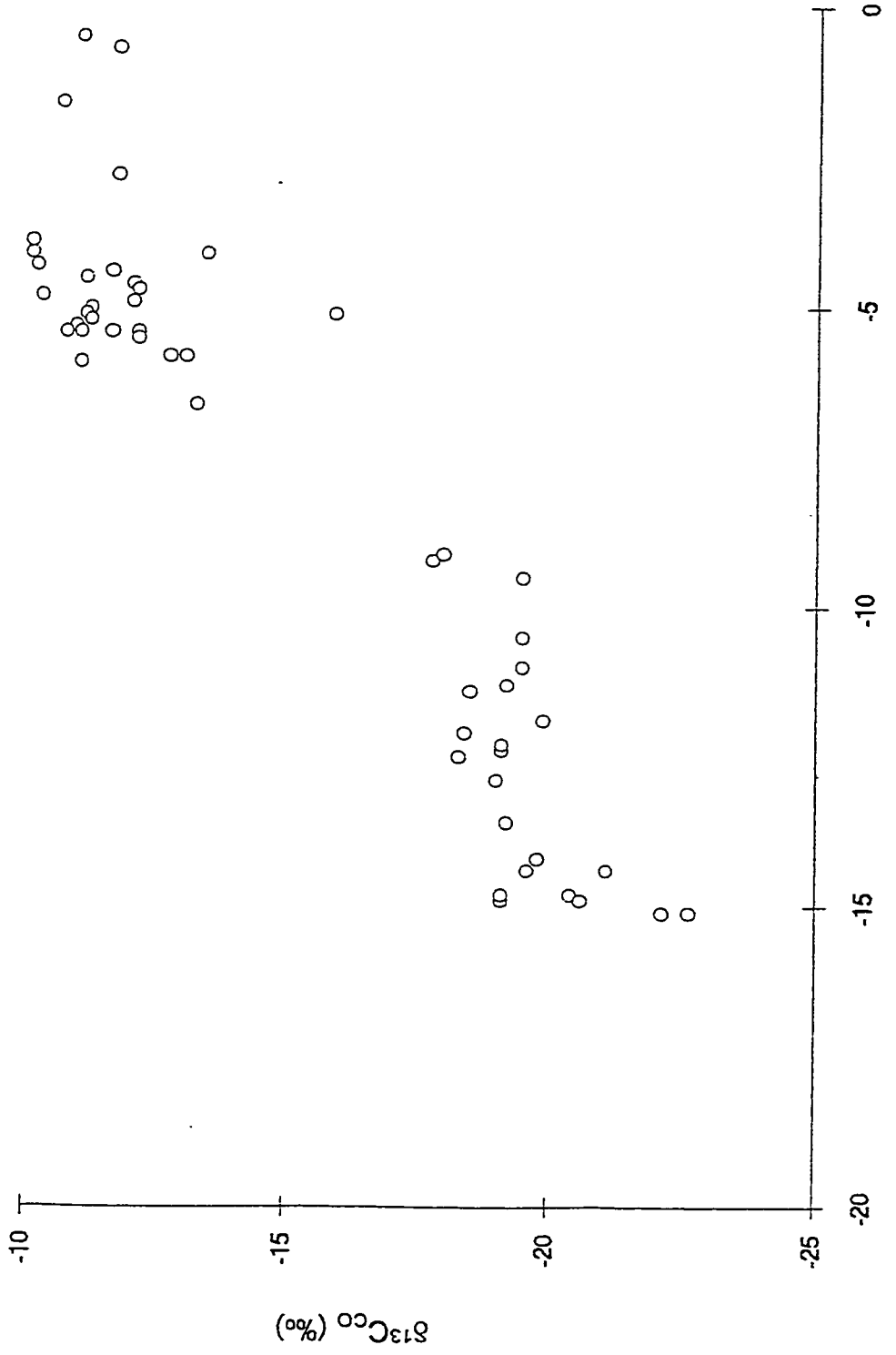


Figure 5.7: Carbon isotope ratios of bone carbonate versus bone collagen for samples from Ontario.

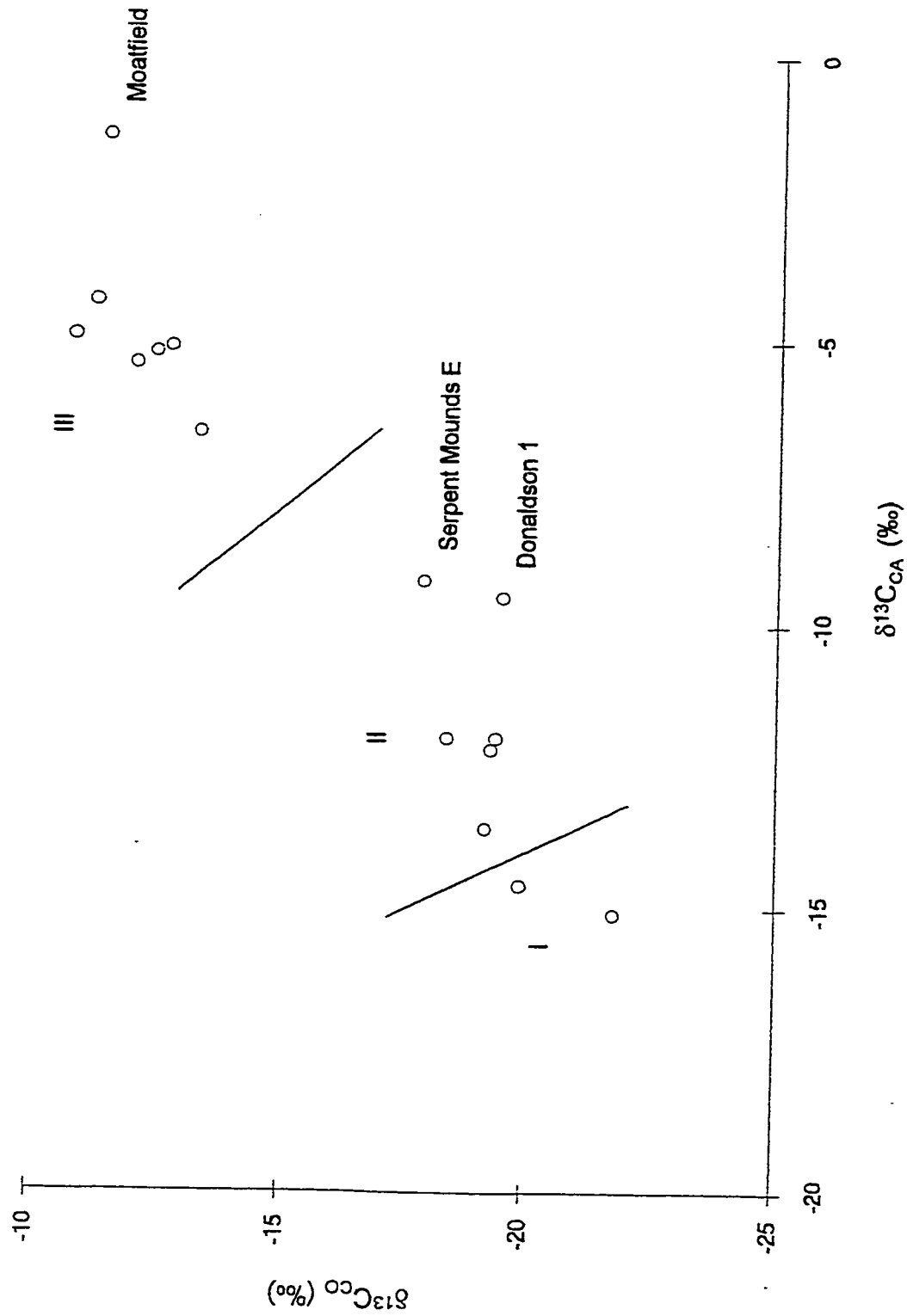


Figure 5.8: Carbon isotope ratios of bone carbonate versus bone collagen for site means from Ontario.

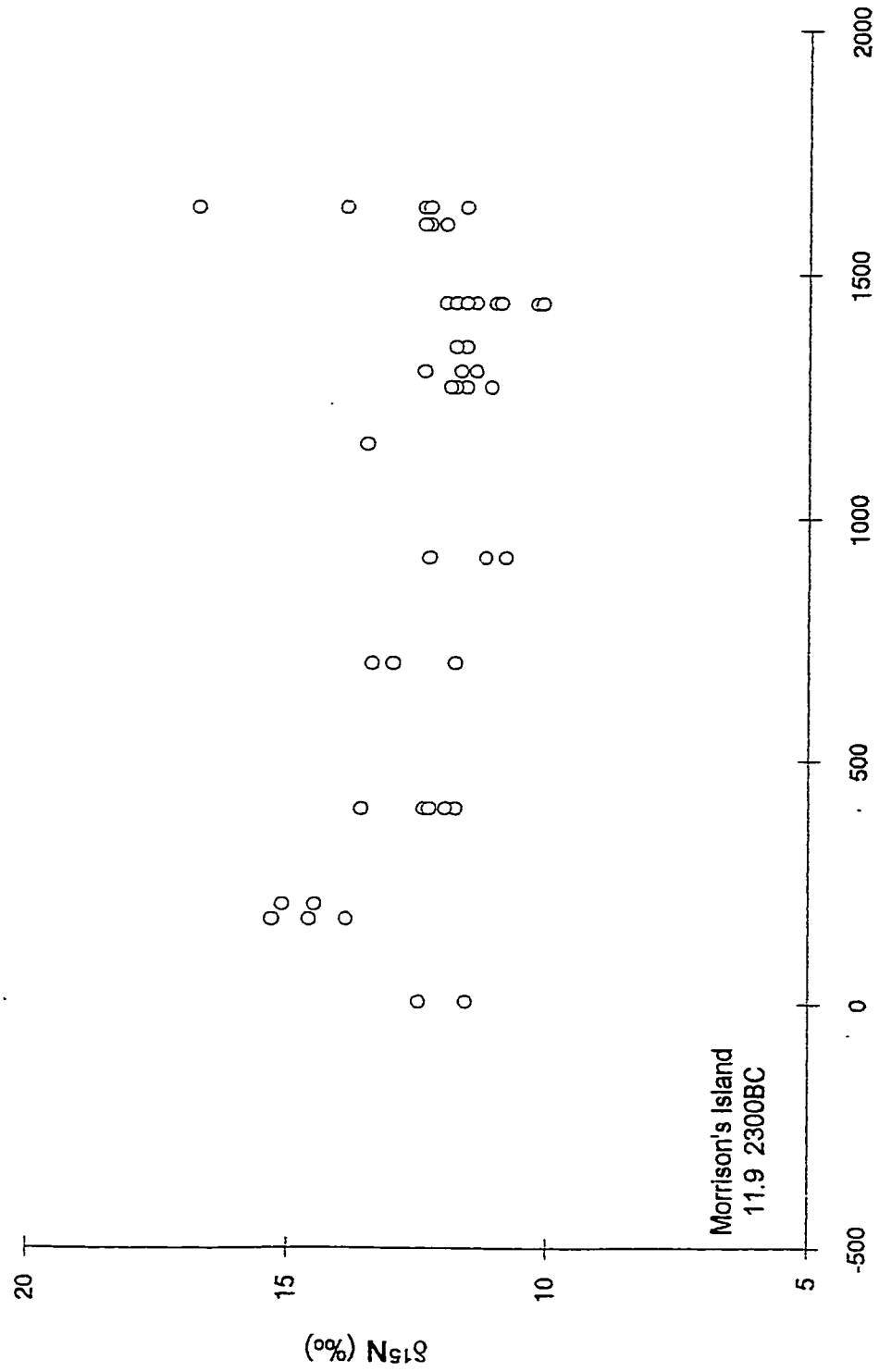


Figure 5.9: Nitrogen isotope ratios versus time for samples from Ontario.

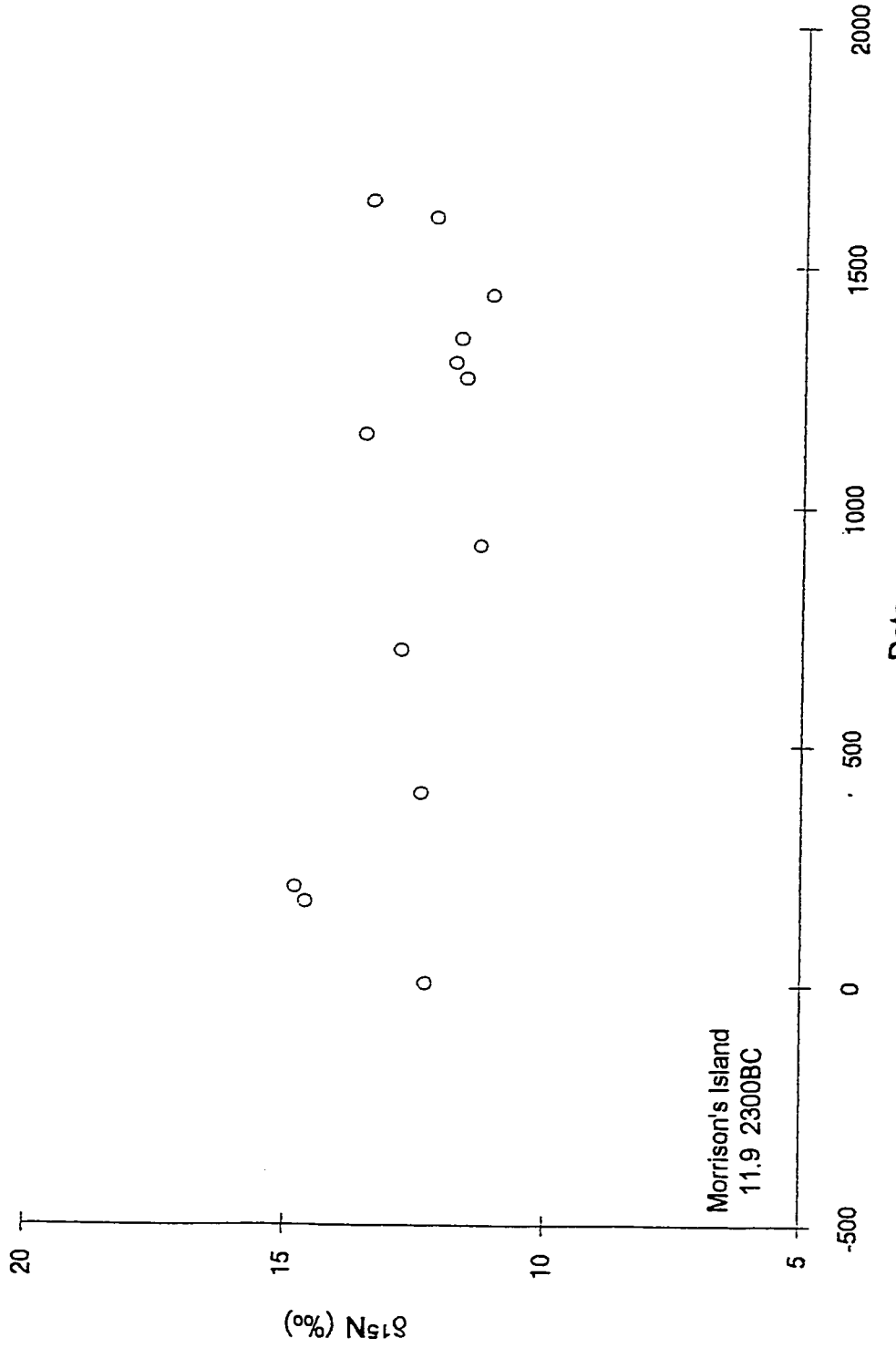


Figure 5.10: Nitrogen isotope ratios versus time for site means from Ontario.

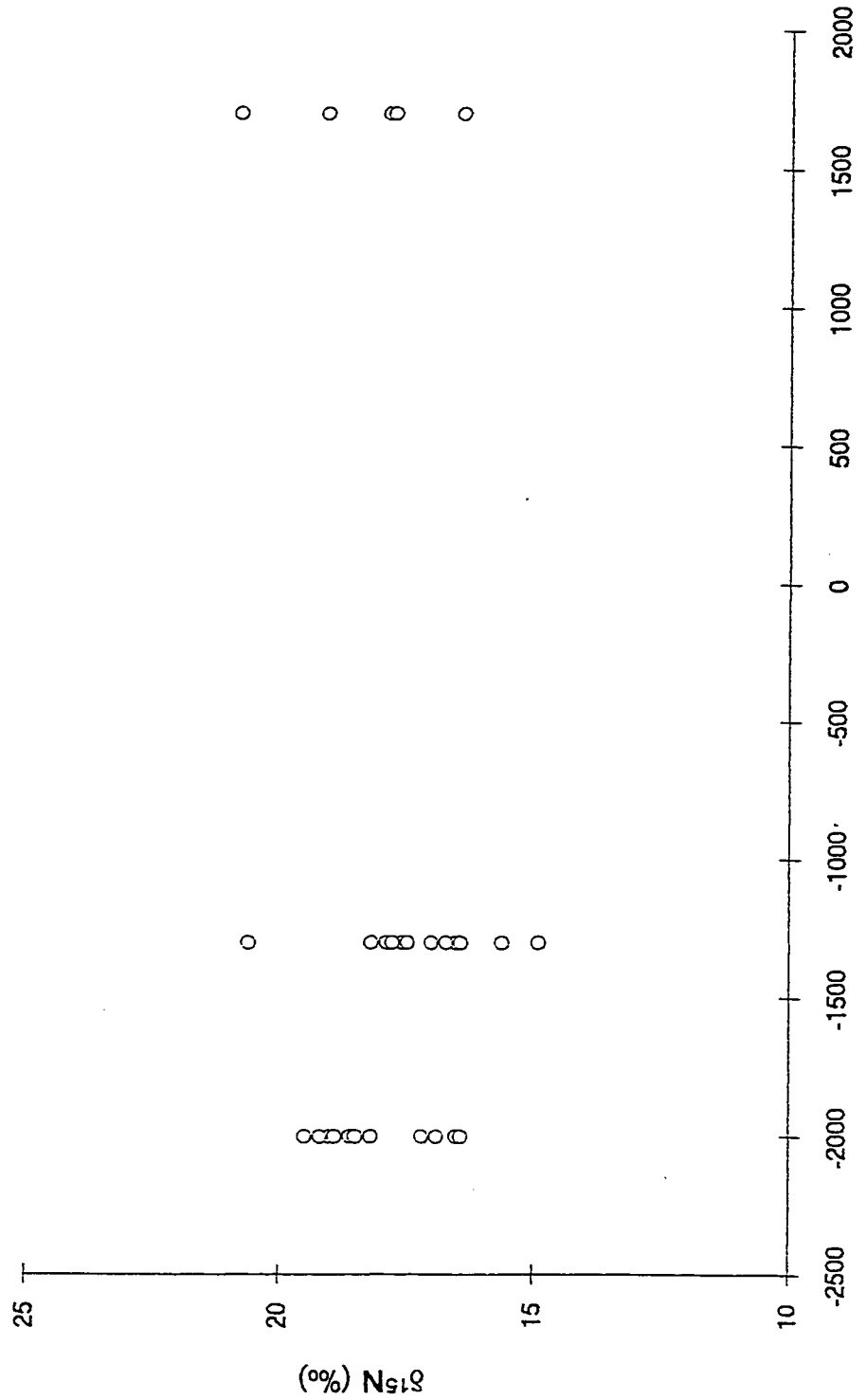


Figure 5.11: Nitrogen isotope ratios versus time for samples from San Nicolas Island.

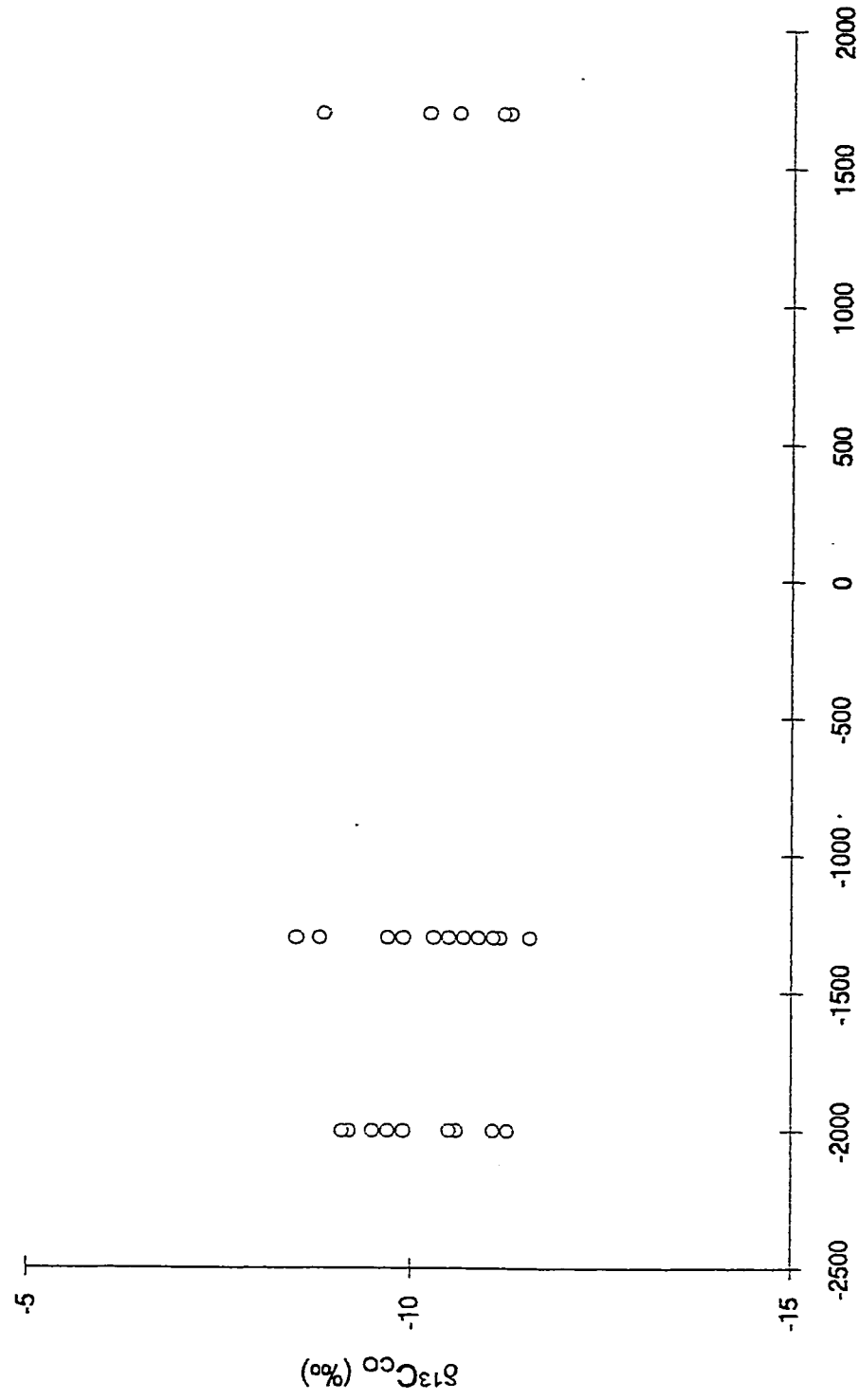


Figure 5.12: Carbon isotope ratios of bone collagen versus time for samples from San Nicolas Island.

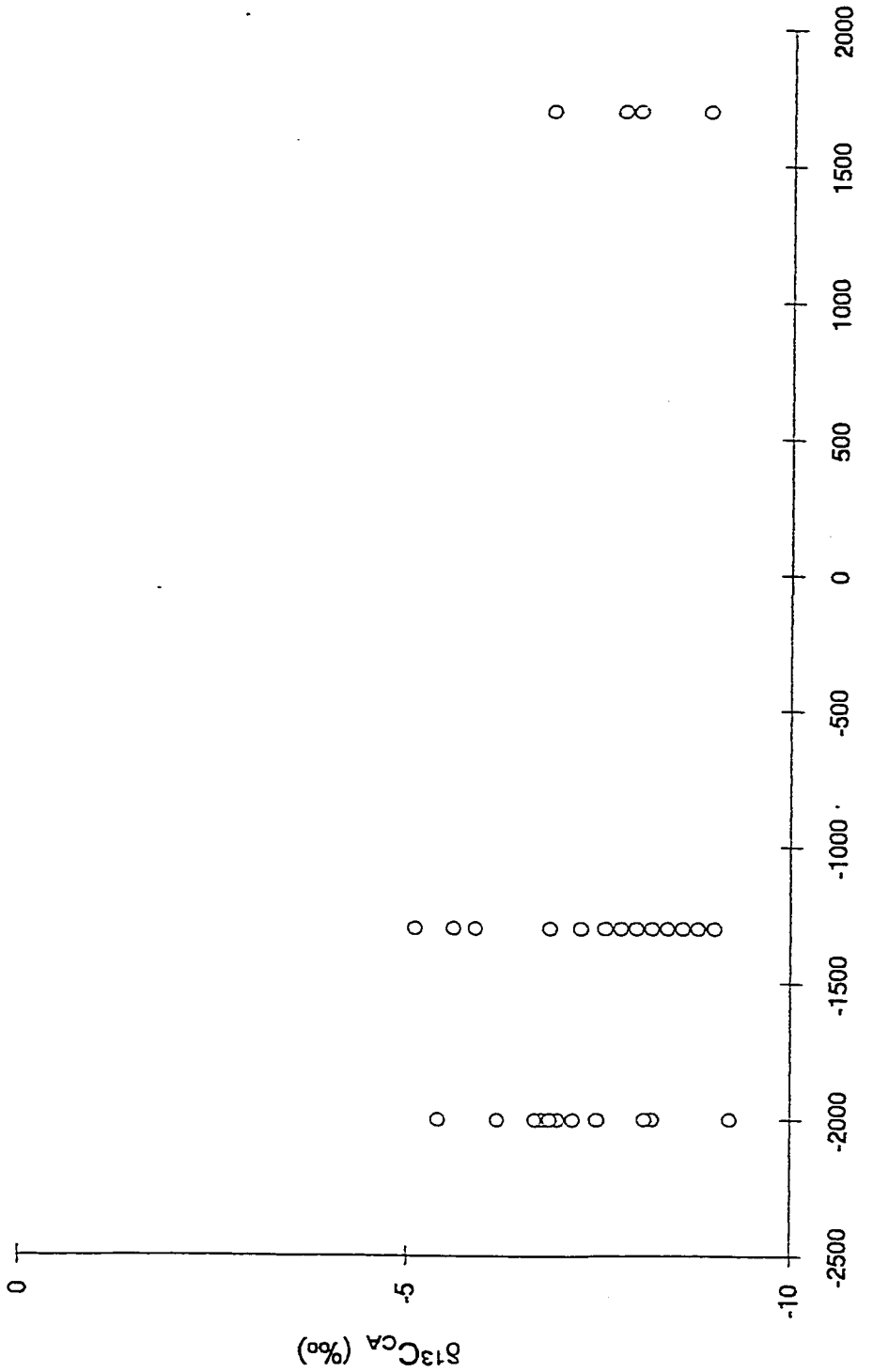


Figure 5.13: Carbon isotope ratios of bone carbonate versus time for samples from San Nicolas Island.

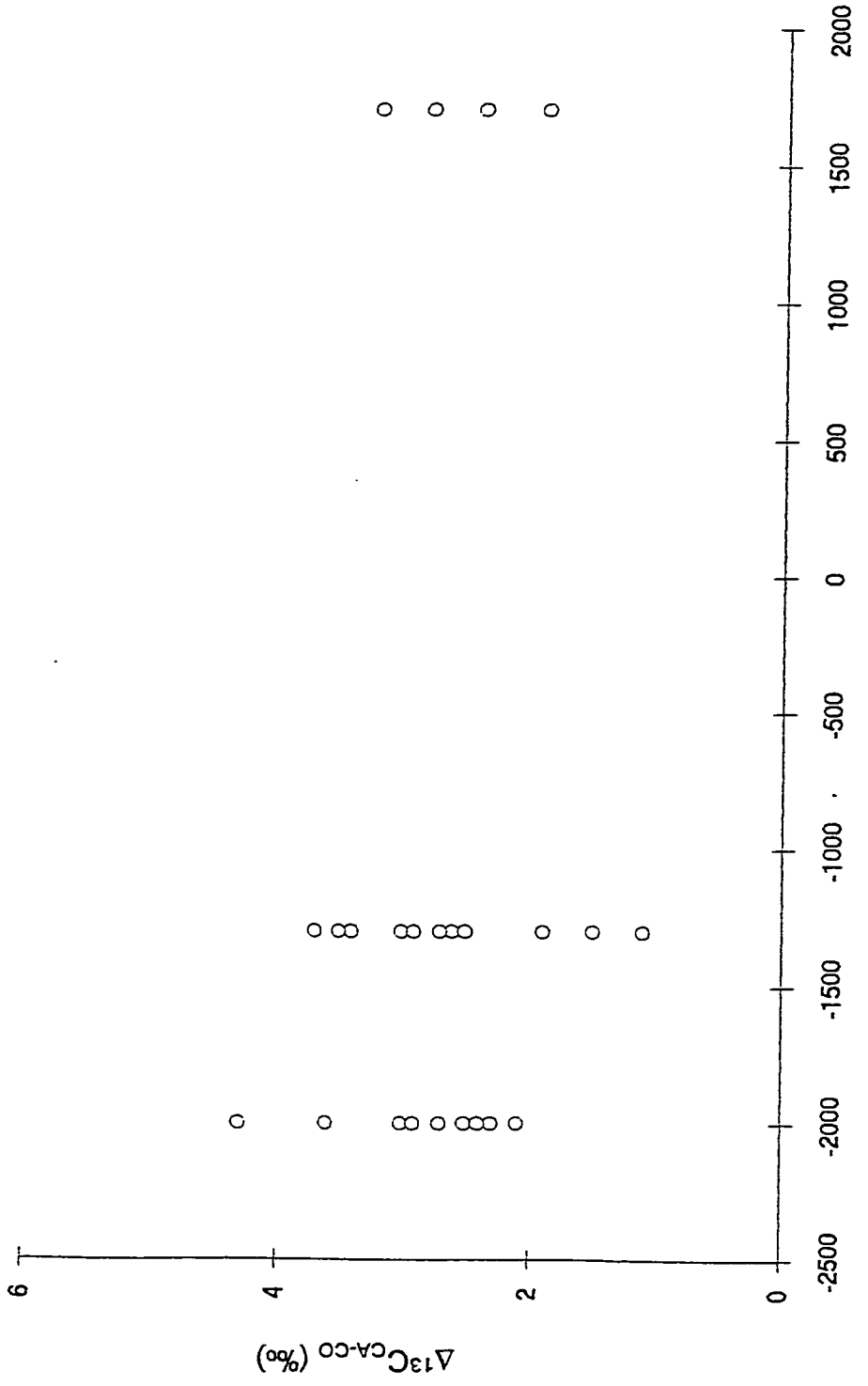


Figure 5.14: Spacing of carbon isotope ratios of bone carbonate and collagen versus time for samples from San Nicolas Island.

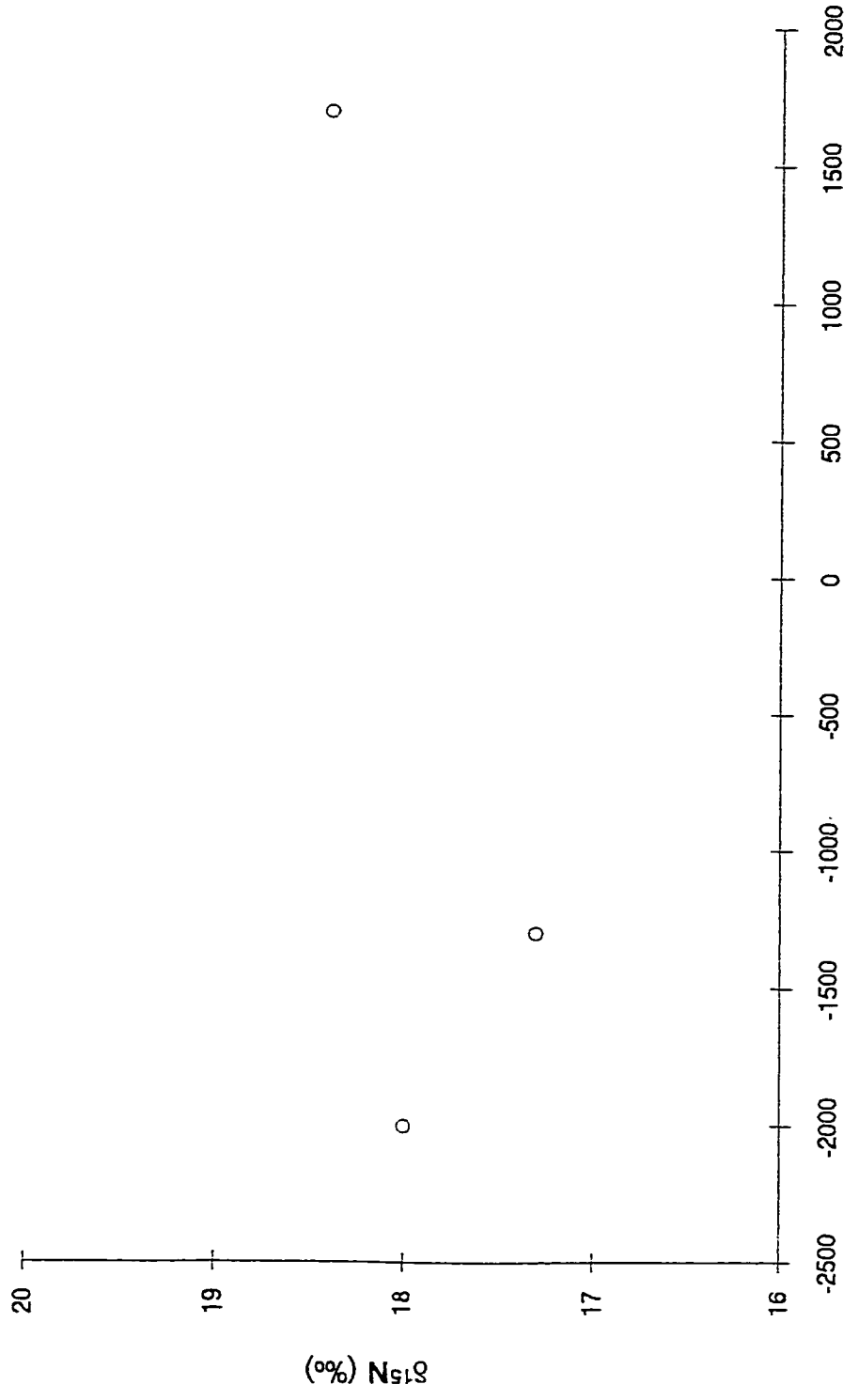


Figure 5.15: Nitrogen isotope ratios versus time for site means from San Nicolas Island.

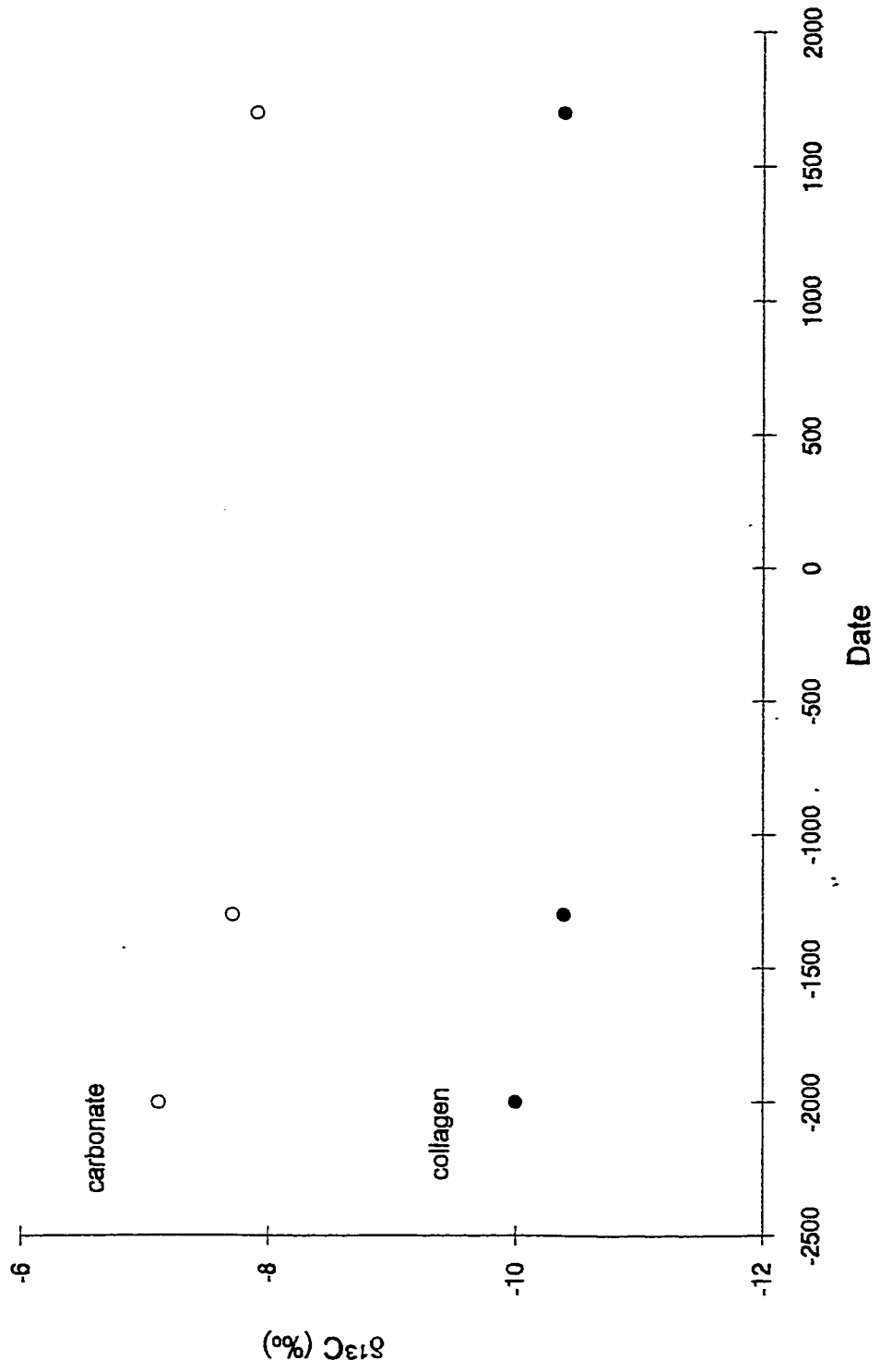


Figure 5.16: Carbon isotope ratios of bone carbonate and bone collagen versus time for site means from San Nicolas Island.

CHAPTER 6 - DISCUSSION AND CONCLUSIONS

6.1 *Southern Ontario*

Recent studies dating maize remains in sites of Princess Point provenience have established the presence of the cultigen maize as early as A.D. 540 in southern Ontario (Crawford and Smith 1996, Smith and Crawford 1997). Schwarcz and colleagues (1985) and Katzenberg and colleagues (1995) established through the analysis of carbon isotopes from human bone collagen that maize comprised a dietary staple by A.D. 1000, and suggested that maize was introduced into the diet over a period of two to four centuries. Indeed, although the recently obtained dating by Crawford and Smith was not available to Schwarcz and colleagues and Katzenberg and colleagues at the time of their studies, these recently obtained dates agree with their contention. The present study sought to add to this body of knowledge through the addition of carbon isotope analysis from bone mineral.

As described in Chapter 2, because maize is a poor source of protein, it is hypothesized that its dietary signature will not be reflected in collagen (representing mostly the protein portion of diet) until it comprises a significant proportion of the diet, whereas the dietary

signature of maize will be reflected in bone mineral at an earlier date when it is consumed in smaller proportions because bone mineral represents the whole diet (Ambrose and Norr 1993, Tieszen and Fagre 1993). Given that we now know that maize was present in Princess Point sites as early as A.D. 540 (Crawford and Smith 1996, Smith and Crawford 1997), we should be able to use isotope studies to observe dietary maize in earlier Princess Point material in bone mineral but not bone collagen. Is this the case in the present study? As discussed in the previous chapter, the pattern illustrated in Figures 5.7 and 5.8 is indicative of maize being introduced into the diet of southern Ontario native groups and gradually increasing in dietary proportion until it becomes a staple food item at about A.D. 1000.

Ambrose and Norr (1993) suggest, through laboratory studies using experimental diets based on Krueger and Sullivan's (1984) model for metabolic pathways which suggested that collagen represents the protein portion of diet and bone mineral represents the energy portion (carbohydrate, lipid and protein not used for protein tissue synthesis), that in a hypothetical situation where diet is comprised of a C3 carbohydrate and C4 protein $\Delta^{13}C_{CA-CO}$ should have a value of -7‰. In the reverse situation where diet is comprised of a C4 carbohydrate and C3 protein $\Delta^{13}C_{CA-CO}$ should have a value of +21‰. The dietary composition in the present study most closely reflects Ambrose' second

scenario above. Maize is the C4 carbohydrate (but also contains some protein) and native plant and animal foods provide the C3 protein. The data obtained in the present study do not agree with Ambrose' suggested $\Delta^{13}C_{CA-CO}$ value of +21‰ as based on the Krueger and Sullivan model. Indeed, as can be viewed on Figure 5.6, $\Delta^{13}C_{CA-CO}$ values are in the range of +5-10‰. What this suggests is that bone mineral reflects protein components of diet that are used for protein tissue synthesis, as well as carbohydrate, lipid and protein not used for protein tissue synthesis components. Controlled laboratory experiments have suggested that this is the case (Ambrose and Norr 1993, Tieszen and Fagre 1993).

Ambrose and Norr (1993) suggest that as diet varies, so should the spacing between collagen and diet $\delta^{13}C$ values ($\delta^{13}C_{CO-D}$), carbonate and diet $\delta^{13}C$ values ($\delta^{13}C_{CA-D}$), and collagen and carbonate $\delta^{13}C$ values ($\Delta^{13}C_{CA-CO}$). In their study, they present hypothetical spacings for these values for seven experimental diets. In theory, if the $\delta^{13}C_{CO-D}$ or $\delta^{13}C_{CA-D}$ value is subtracted from the $\delta^{13}C_{CO}$ or $\delta^{13}C_{CA}$ value, the $\delta^{13}C$ value for the diet should be obtained because the $\delta^{13}C_{CO}$ or $\delta^{13}C_{CA}$ value will equal the diet $\delta^{13}C$ value plus the $\delta^{13}C_{CO-D}$ or $\delta^{13}C_{CA-D}$ value for a particular diet. Since the spacing values between diet and tissue are different for collagen and carbonate, in theory the $\delta^{13}C_{CO}$ value minus the $\delta^{13}C_{CO-D}$ value should equal the $\delta^{13}C_{CA}$ value minus the $\delta^{13}C_{CA-D}$ value (M. A. Katzenberg, personal communication). Both should

equal the diet $\delta^{13}C$ value for a particular diet. Does this work with the data set used in this dissertation?

Figure 6.1 shows both $\delta^{13}C_{CA}$ and $\delta^{13}C_{CO}$ mean values for each site plotted by date. The two sets of data roughly parallel each other. Where carbonate $\delta^{13}C$ values increase, so do those of collagen, and vice versa. The spacing between carbonate $\delta^{13}C$ values and collagen $\delta^{13}C$ values is roughly equal across time. Can we subtract a collagen-diet spacing value from the collagen tissue values for each site, and subtract a carbonate-diet spacing value from the carbonate tissue values for each site, and arrive at the same 'diet' value for both? Using a $\delta^{13}C_{CA-D}$ value of +9.4‰ and a $\delta^{13}C_{CO-D}$ value of +5.1‰, we obtain the configuration observed in Figure 6.2. The two sets of data do indeed come closer together, but not completely. Clearly, these values for $\delta^{13}C_{CA-D}$ and $\delta^{13}C_{CO-D}$ do not reflect the true diet. If a $\delta^{13}C_{CA-D}$ value of +12.0‰ and a $\delta^{13}C_{CO-D}$ value of +5.1‰ are used, the two sets of data coincide, with the notable exceptions of Donaldson Cemetery 1, Serpent Mounds E and Moatfield (Figure 6.3), thus indicating that these tissue-diet spacings are reflective of the true diet. The data used in the present study are from human remains whereas Ambrose and Norr's (1993) study analysed rats. As explained in Chapter 2, spacing values may be variable between large and small mammals depending on genetic factors.

There are three sites which do not follow this pattern: Donaldson Cemetery 1, Serpent Mounds E and Moatfield. At Moatfield the $\delta^{13}C_{CO}$ value is in the same range as $\delta^{13}C_{CO}$ values at temporally surrounding sites, while the $\delta^{13}C_{CA}$ value is elevated above those of temporally surrounding sites (Figure 6.3). One possible explanation for this situation involves the proportion of C4 foodstuffs in the diet at Moatfield. In a study of African herbivores, Lee-Thorp and van der Merwe (1987) show that C4 grazers had $\delta^{13}C_{CA}$ values in the range of -2.0 to +1.6‰. These values are similar to the $\delta^{13}C_{CA}$ values for Moatfield. It is not here suggested that the Moatfield people were C4 grazers. However, maize is a C4 grass, and one possible explanation for the high Moatfield $\delta^{13}C_{CA}$ values may be that they were consuming a higher proportion of maize in their diet than other contemporaneous groups of south-central Ontario. Indeed, this type of situation is borne out in a study by Schober and Ambrose (1995), in which they found that one out of four females analyzed from Cahokia had a $\delta^{13}C_{CA}$ value of -1.5‰, within the same range as the Moatfield group. Schober and Ambrose (1995) attribute this to an increased proportion of maize in the diet. This relates to Ambrose and Norr's (1993) test of experimental diets using rats in a laboratory situation (as described in Chapter 2). They showed that when a 95% C3 non-protein/5% C4 protein diet was compared with a 95% C4 non-protein/5% C3 protein diet, the

$\delta^{13}C_{CO}$ difference between the two diets was 1‰, but the $\delta^{13}C_{CA}$ difference between the two diets was 12‰. Diets with percentages between these extremes produced $\delta^{13}C_{CO}$ and $\delta^{13}C_{CA}$ values which varied according to the dietary percentages. The point here is that the higher the percentage of C4 non-protein (maize) in relation to C3 protein (meat, nuts), the greater the difference between $\delta^{13}C_{CO}$ and $\delta^{13}C_{CA}$ ($\Delta^{13}C_{CA-CO}$ increases). If at Moatfield the proportion of maize consumption is elevated in relation to C3 protein, $\delta^{13}C_{CA}$ will be more positive relative to $\delta^{13}C_{CO}$, which is what is observed in the present data set. In addition, if the Moatfield people were consuming a greater proportion of maize and lower proportion of animal protein in relation to other groups, lipid intake would be reduced. Because lipids have more negative $\delta^{13}C$ values than the protein and carbohydrate portions of diet, and because lipids form a small portion of plant tissue in relation to animal tissue, the effect of lipid content will be more pronounced in diets with greater proportions of animal foods. The greater lipid content in diets with higher proportions of animal foods will decrease the $\delta^{13}C$ value of those diets. The collagen $\delta^{13}C$ value will be depressed in relation to the carbonate $\delta^{13}C$ value because collagen is more reflective of protein (animal foods with greater lipid content), while carbonate reflects all dietary components. Where diet has a reduced amount of animal protein, collagen $\delta^{13}C$ values will be

depressed in relation to carbonate $\delta^{13}C$ values, and the value of $\Delta^{13}C_{CA-CO}$ will increase. This is what is observed at Moatfield. Therefore, it is here suggested that Moatfield diet had an increased proportion of maize and decreased proportion of animal protein in relation to temporally surrounding sites.

Both Serpent Mounds E and Donaldson Cemetery 1 have $\delta^{13}C_{CA}$ values more positive than what would be suggested from the dating of these sites. The $\delta^{13}C_{CA}$ values for these two sites (in the -9‰ range, Figure 5.8), are suggestive of maize consumption. However, lower $\delta^{13}C_{CO}$ values (in the -20‰ range, Figure 5.8) do not suggest substantial maize consumption. Even so, even minor maize consumption (as suggested by the carbonate data) at the dates suggested for Donaldson Cemetery 1 (555 B.C.) and Serpent Mounds E (A.D. 205) is questionable. However, there has been discussion concerning the dating of these two sites. Spence and colleagues (1990) question the original dating analysis for Donaldson Cemetery 1. Original radiocarbon dates spanned from 550 B.C. to A.D. 1225 (Wright 1963, Anderson 1963), with the later dates being rejected due to their improbability. However, in a reanalysis by Spence and colleagues (1990), it is suggested that a date of A.D. 550 is more likely. If this is the case, the presence of maize at Donaldson Cemetery 1 becomes more likely. A further complication arises because none of the original radiocarbon

dates were taken from human bone. Instead, associated wood and charcoal fragments were dated, leading to the possibility that the burials may have been intrusive or other commingling within the site occurred.

Similarly at Serpent Mounds E, Spence and colleagues (1990) suggest that because associated wood and charcoal fragments and not human bone were dated, similar problems of commingling and intrusion may be occurring at this site. In addition, the accepted date of A.D. 205 for Serpent Mounds E does not remove it from the time range for maize consumption in northeastern North America in general. At the Edwin Harness (Ohio) and Icehouse Bottom (Tennessee) sites, maize is present in the first century A.D. (Ford 1987, Chapman and Crites 1987), and at the Holding site (Illinois), maize is present in the first century B.C. (Riley et al. 1994). Although no other southern Ontario sites appear to have maize evidence at A.D. 205, the presence of a C4 (maize) isotopic signature in Serpent Mounds E carbonate may indicate an initial importation of maize to southern Ontario.

Excepting these problematic sites, the data contained in the present study provide evidence for consumption of C4 plants in southern Ontario as early as A.D. 500. Archaeological evidence suggests that maize was the C4 plant. The carbonate data from bone mineral provides evidence that maize was consumed in smaller proportions and

at an earlier date than does the evidence derived from collagen. The two lines of evidence taken together (carbonate and collagen) suggest that maize was first introduced into the diet at around A.D. 500, but did not become a dietary staple, when it comprised a sizeable portion of diet, until approximately A.D. 1000. $\Delta^{13}C_{CA-CO}$ increases to a maximum and then decreases (Figure 5.6) as maize is introduced into the diet, gradually increases in proportion, and becomes a dietary staple, as was postulated in Chapter 2, Figure 2.1. During the transitional period when maize is consumed but is not a dietary staple, its isotopic signature can be observed in the $\delta^{13}C$ value of carbonate but not collagen (Figure 5.8).

The data presented in this dissertation add credence to the suggestion that carbon atoms used to form the carbonate in bone mineral follow the linear mixing model whereas the carbon atoms used to form collagen follow the routing model. Ambrose and colleagues (1997) suggest that $\delta^{13}C_{CA-D}$ should always equal 9.4‰ because bone mineral follows the linear mixing model. However, $\delta^{13}C_{CO-D}$ will equal 5‰ only if the $\delta^{13}C$ value of the protein portion of the diet and the whole diet are the same, because collagen follows the routing model. Therefore, $\Delta^{13}C_{CA-CO}$ will equal 4.4‰ only if the $\delta^{13}C$ value for the protein portion of diet equals that of the whole diet. Is this the case in the present study? Table 5.1 provides $\Delta^{13}C_{CA-CO}$ values for both individual samples and

site averages. These $\Delta^{13}C_{CA-CO}$ values are consistently greater than 4.4‰ indicating that $\delta^{13}C$ values of carbohydrates are less negative than that of protein in the diet. In the sites where maize is consumed, this is a result of the consumption of a C4 plant (maize). In southern Ontario, maize constitutes the C4 carbohydrate and animals feeding off indigenous foods constitutes the C3 protein according to Ambrose and colleagues' (1997) theory. In the sites which pre-date maize consumption this is likely a result of the bulk of the diet being comprised of gathered plant material as opposed to protein from meat, but may also be the result of even earlier consumption of maize in southern Ontario as may be suggested from the problematic sites of Donaldson Cemetery 1 and Serpent Mounds E. AMS dating of human bone samples from these two sites is the subject of ongoing research.

Snow (1994b, 1995) originally suggested that Iroquoian-speaking peoples migrated into southern Ontario at about A.D. 900-1000. Crawford and Smith (1996, Smith and Crawford 1997) presented radiocarbon dates from maize kernels and cupules indicating the presence of maize in southern Ontario as early as A.D. 540, and also suggested that Princess Point peoples were matrilineal and Iroquoian-speaking, thus suggesting that maize cultivating Iroquoian-speaking peoples were present in southern Ontario at this early date. Snow (1996) later concurred with Crawford and Smith and now

suggests that any Iroquoian migration into southern Ontario occurred at or prior to approximately A.D. 500. The data presented in this dissertation show, through the analysis of carbon isotopes from bone mineral, that maize was being consumed as early as A.D. 500, adding further evidence in addition to the archaeological evidence to suggest that Iroquoian development from Princess Point to Early Ontario Iroquois Glen Meyer times followed the *in situ* model.

The present data set does not conclusively answer the question of whether Iroquoian peoples were present in southern Ontario prior to Princess Point times. Although Serpent Mounds E and Donaldson Cemetery 1 show $\delta^{13}C_{CA}$ values which may be indicative of maize consumption, there is some discussion concerning the precise dating of these sites. Therefore, no firm conclusion concerning maize consumption prior to Princess Point times can be made from the present data set. If Serpent Mounds E and Donaldson Cemetery 1 do provide evidence for maize consumption prior to Princess Point times, it can not be definitively concluded that these people were of Iroquoian ethnicity. On the carbon isotopic evidence alone, it is not possible to equate maize cultivation with Iroquoian ethnicity. It is possible that other antecedent groups not linked by ethnicity to later Iroquoian groups were present in southern Ontario during the pre-Princess Point period.

6.2 *San Nicolas Island*

In the present study, $\Delta^{13}C_{CA-CO}$ values for the three San Nicolas Island sites are 2.8‰, 2.7‰ and 2.5‰ from early to late period. Ambrose and colleagues (1997) suggest that for a diet where protein and whole diet carbon isotopic values are equal, $\Delta^{13}C_{CA-CO}$ will equal 4.4‰. In the present San Nicolas Island data set $\Delta^{13}C_{CA-CO}$ is less than 4.4‰ for all three sites. Ambrose and colleagues (1997) suggest that this is the result of dietary protein being less negative than that of the whole diet. In relation to San Nicolas Island, the $\Delta^{13}C_{CA-CO}$ values are consistent with a scenario whereby the isotopically less negative dietary protein constitutes marine protein combined with C3 carbohydrate. Goldberg (1993) using carbon isotopes from collagen alone, suggested that this may be the case. The addition of the analysis carbon isotopes from bone mineral add evidence confirming Goldberg's suggestion. If the plant material being consumed prehistorically on San Nicolas Island had been C4, there would be the potential for the $\delta^{13}C$ values of the marine protein and C4 plants being approximately equal and, therefore, $\Delta^{13}C_{CA-CO}$ equaling approximately 4.4‰. As this is not the case, C3 rather than C4 plants supplementing marine protein in the diet of San Nicolas Island prehistoric peoples is suggested from the carbonate data.

The results obtained in the present study can be compared with collagen data results obtained by Goldberg (1993). As described in Chapter 3, Goldberg's dissertation examined differences in diet between coastal and inland mainland groups, and northern and southern Channel Island groups of southern California, for her early and late periods. San Nicolas Island is one of four southern Channel Islands, the others being Santa Barbara Island, Santa Catalina Island and San Clemente Island. Goldberg used data from San Nicolas Island and San Clemente Island only. Amongst other findings, Goldberg's data suggested that collagen $\delta^{13}C$ values indicate that marine resource use decreased through time in the southern Channel Islands (Goldberg 1993). On the other hand, $\delta^{15}N$ values showed no increase nor decrease through time. If San Clemente Island is examined by itself, both $\delta^{13}C$ and $\delta^{15}N$ indicate a decrease in marine foods through time. Unfortunately, Goldberg did not have late period samples from San Nicolas Island and, as a result, a comparison can only be made between Goldberg's generalized data set for the southern Channel Islands as a whole, and the present data set from both early and late period San Nicolas Island.

What then does this comparison show? The collagen data in the present study from San Nicolas Island only, indicate no increase nor decrease from early to late periods, whereas Goldberg's data for the southern Channel

Islands in general indicate a decrease in marine food consumption. The discrepancy between the two data sets is likely a result of a difference in dietary change patterns between San Clemente Island (decreasing marine resource use through time) and San Nicolas Island (constant marine resource use through time).

The present data set adds late period sites from San Nicolas Island which show a different pattern to that of Goldberg's San Clemente Island data. Why might this be so? San Nicolas Island is the furthest island from the mainland in the Channel Islands. Goldberg suggests that marine resource use decreased through time on San Clemente Island as trade with the mainland increased, allowing increased supplementation of mainland plant foods to the sparse island terrestrial plant foods. The carbon isotope data from San Nicolas Island, suggesting no change in the proportion of marine resource use through time, may be explained by the greater distance of San Nicolas Island from the mainland in relation to San Clemente Island (whereas San Nicolas Island is 120 km from the coast with no intermediate islands, San Clemente Island is 80 km from the coast and Santa Catalina Island is situated approximately half-way between San Clemente and the coast [Goldberg 1993]), thus reducing the amount of potential trade of plant foods with the mainland.

6.3 *Conclusions*

The use of stable carbon isotopes from bone mineral in addition to collagen provides the potential for increased information to be derived from bone chemistry studies. $\delta^{13}C$ values of collagen reflect primarily the carbon isotope ratios of the protein portion of diet because in its formation collagen follows the routing model. $\delta^{13}C$ values of carbonate from bone mineral reflect those of the whole diet because carbonate follows the linear mixing model. When a C4 plant is introduced into a C3 based diet, it will be reflected in the carbon isotope signature of carbonate from bone mineral when it forms only a small portion of the diet. On the other hand, the C4 signature will not be reflected in collagen until it forms a significant portion of the diet.

In southern Ontario, maize is a C4 plant which was introduced into a C3 based indigenous diet. Bone chemistry studies in this dissertation have shown that the carbon isotopic signature of maize was detectable in carbonate from bone mineral at an earlier date, when maize was consumed in a smaller amount, than it can be detected in collagen. The data supports Hypothesis #1: *Stable carbon isotope analysis of carbonate in bone mineral permits detection of maize in smaller proportions and at an earlier date in the diet of southern Ontario prehistoric groups, than with analysis of stable carbon isotopes in collagen.*

The spacing between $\delta^{13}C$ from collagen and carbonate, $\Delta^{13}C_{CA-CO}$, can be used to determine when the value of dietary protein is more negative than that of the whole diet. In southern Ontario, in sites containing maize, $\Delta^{13}C_{CA-CO}$ is greater than 4.4‰, indicating that C4 maize supplemented C3 based protein. The data supports Hypothesis #2: *In southern Ontario, where maize (C4) supplemented C3 protein and indigenous plants, $\Delta^{13}C_{CA-CO}$ is greater than 4.4‰.*

The spacing between $\delta^{13}C$ from collagen and carbonate, $\Delta^{13}C_{CA-CO}$, can be used to determine the type of carbohydrates which may potentially supplement a marine based diet. On San Nicolas Island, $\Delta^{13}C_{CA-CO}$ is less than 4.4‰, indicating that marine protein was supplemented with C3 carbohydrate. The data supports Hypothesis #3: *$\delta^{13}C$ values from collagen and carbonate suggest a marine based diet and spacing between $\delta^{13}C$ from collagen and carbonate, $\Delta^{13}C_{CA-CO}$, suggests that C3 based plants supplemented a marine based diet.*

These conclusions provide two archaeological situations in which the theoretical proposals concerning $\Delta^{13}C_{CA-CO}$ values presented by Ambrose and colleagues (1997) based on laboratory rat and mouse studies (Ambrose and Norr 1993, Tieszen and Fagre 1993) are supported.

Isotope analysis of carbonate as well as collagen in human bone provides an important and potentially more accurate means of extracting dietary information from the archaeological record than other methods, thus increasing

our knowledge of past human subsistence patterns and adaptive strategies. The benefit of the use of stable isotope analysis of bone is that it allows for the direct measurement of the components of past diet, and allows the researcher to relate human diet to the local environment. This aids in the construction of subsistence models which can be problematic when based only on indirect evidence such as artifactual and floral and faunal remains, which may not be preserved in the proportions in which they were consumed.

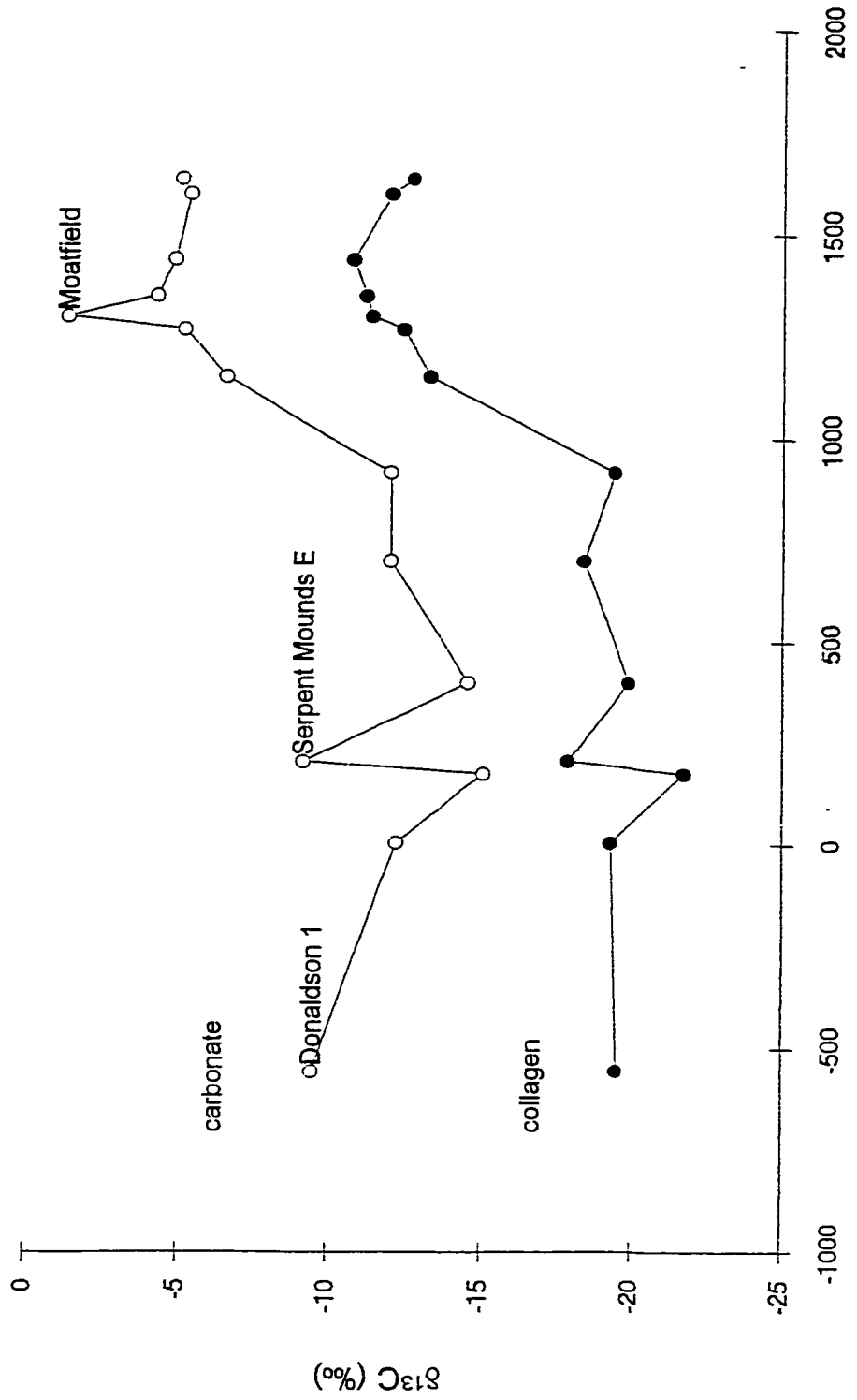
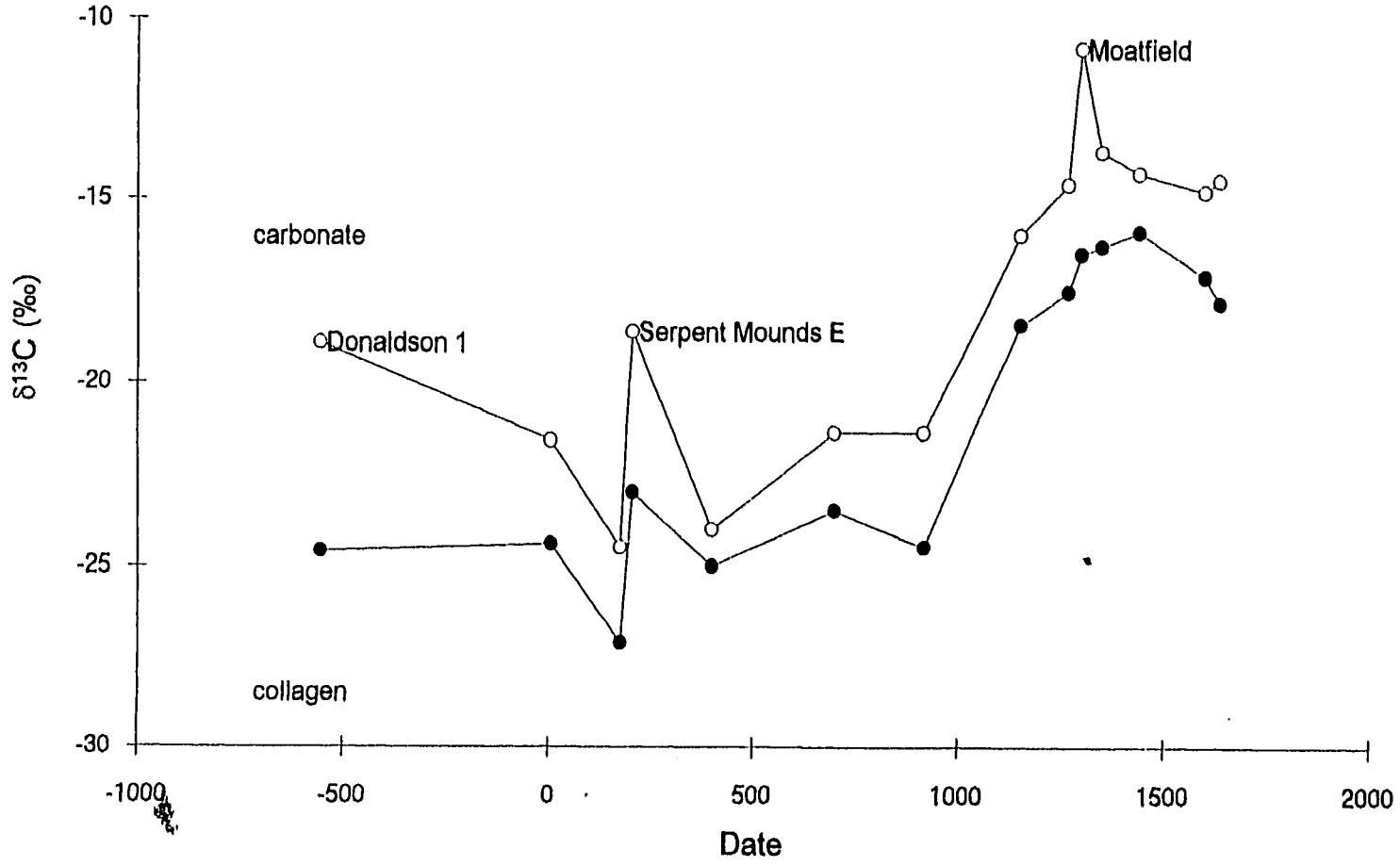


Figure 6.1: Carbon isotope ratios of bone carbonate and bone collagen versus time for site means from Ontario.

Figure 6.2: Carbon isotope ratios of bone carbonate minus a carbonate-diet spacing value of 9.4, and bone collagen minus a collagen-diet spacing value of 5.1, versus time for site means from Ontario.



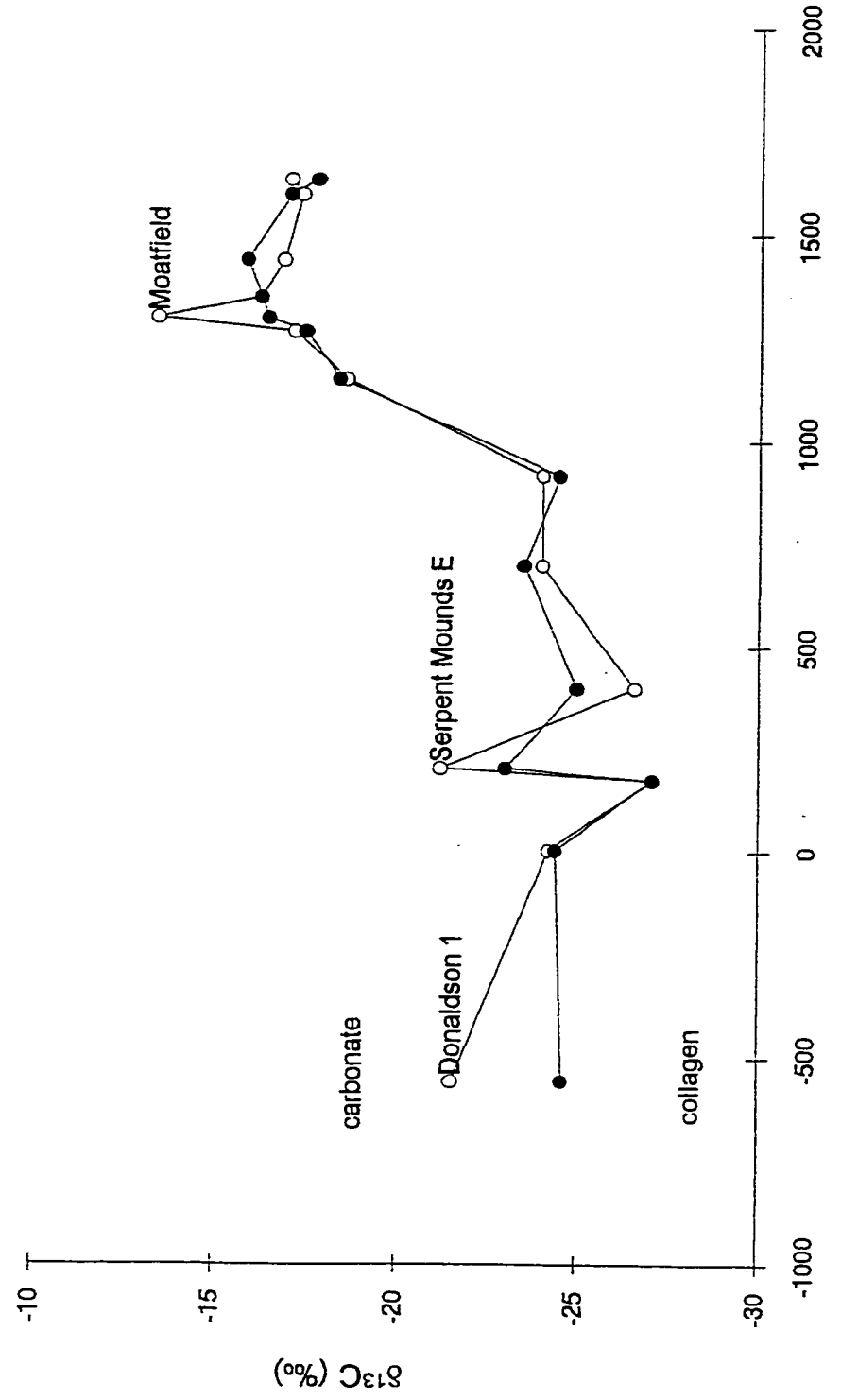


Figure 6.3: Carbon isotope ratios of bone carbonate minus a carbonate-diet spacing value of 12, and bone collagen minus a collagen-diet spacing value of 5.1, versus time for site means from Ontario.

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