

RECONSTRUCTION OF PREHISTORIC DIET IN BRITISH COLUMBIA
USING STABLE-CARBON ISOTOPIC ANALYSIS

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RECONSTRUCTION OF PREHISTORIC DIET IN
BRITISH COLUMBIA USING STABLE-CARBON
ISOTOPIC ANALYSIS

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Abstract

Information on prehistoric diet can be extremely useful for understanding human behaviour as it relates to food acquisition. In British Columbia prehistoric diet has been inferred from ethnographic, archaeological and zoo-archaeological evidence. However, lack of quantified data makes it difficult to determine the degree to which people relied on marine species for their protein, or to compare people from different sites and time periods.

This study uses a new approach, the application of stable carbon isotope analysis of human bone collagen, to obtain such quantified data. Following investigation of the applicability and variability of the analytical technique, ethnographic and archaeological evidence was used to identify dietary species representing marine and terrestrial alternatives. Samples of those species were taken for each of a number of different locales and environments, and representative isotope ratios determined for both marine- and terrestrial-based diets. Isotope ratios for prehistoric humans were then compared to those for the two alternative diets in order to determine the human's degree of reliance on marine species as a protein source.

Results indicate that prehistoric British Columbian coastal dwellers obtained about $90 \pm 10\%$ of their protein from marine species, while people along the Fraser and Thompson Rivers in Interior British Columbia obtained only about 40 to 60 (± 10)% of their protein from marine species, specifically salmon. People in areas away from major salmon streams obtained some of their protein from marine species although the amount was much less. These proportions do not appear to have changed significantly for the last 5000 years. However, some differences have been observed in results, between females and males, adults and children, and geographic areas.

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Table of Contents

Abstract	iii
Acknowledgements	iv
Table of Contents	v
List of Tables	ix
List of Figures	x
Introduction	1
Diet reconstructions	2
Problems with the standard approaches to paleodiet studies	4
New approaches	4
Scope of this study	5
Part I Carbon isotope analysis and its application in British Columbia	7
1 Overview of the carbon isotope technique	7
History of the technique	7
Definition of $\delta^{13}\text{C}$, fractionation and standards	9
Carbon reservoirs and plants	9
Herbivores, carnivores and food chains	11
2 Application of the technique and the importance of variability	14
Variations in reservoirs, food chains and dietary species	15
Materials analyzed: dietary species	20
Materials analyzed: collagen	20
Chemical makeup of collagen	24

The effects of diagenetic processes on collagen	25
The diet - consumer increment ' $\Delta\delta\text{C}$ '	27
Variability in consumer populations	32
Summary	33
3 Requirements of and problems with the laboratory procedures	35
Collagen extraction	35
Quality control of extractions	37
Sample measurement	40
Summary	41
4 Tests of the methods used	42
Sample analysis	42
Combustion of the samples to obtain CO_2	43
Collagen extraction	46
Determination of gelatin purity	50
Tests of lipid extraction	53
Variation in one individual's gelatin	55
Preparation of dietary samples	56
Discussion and summary	57
5 Determination of the diet - consumer increment, '$\Delta\delta\text{C}$'	59

6	The applicability of the technique, particularly in British Columbia	62
6.1	Dietary species	62
	Preliminary analyses	62
	Dietary sample requirements and sample selection	63
	Dietary sample results	65
6.2	Human population results in British Columbia	73
6.3	Interpreting the $\delta^{13}\text{C}$ results for humans	77
	Errors on the proportion determinations	78
	Summary	80
7	Conclusions about the technique and its application, particularly in British Columbia	81
	The technique	81
	Diet alternative values in British Columbia	83
	Results for human populations	83
	Proportion determinations	84
	Summary	85
	Part II Prehistoric diet in British Columbia	86
1	Archaeological evidence for paleodiet in British Columbia	86
	The coast	86
	The interior	94
	Human samples	97

2 Human results from British Columbia	99
2.1 The coastal results	99
The Namu - Central Coast people	99
The Prince Rupert area samples	103
The Queen Charlotte Island results	104
Hesquiat results	106
Gulf of Georgia sites	106
Summary and discussion of Coastal results	110
2.2 Interior results	115
Interior results: summary	122
Proportion determinations	123
British Columbia humans: summary and discussion	124
Conclusions	129
Appendix A Results for individual samples of dietary species	132
Appendix B Individual human results	138
References	148

List of Tables

1	Expected collagen values for modern consumers of specific diets	12
2	Values of ' Δdc ' that have been reported and used in the literature	28
3	Comparison of combustion methods	45
4	Comparison of gelatin extraction methods	49
5	C/N values, %C, and %N for extraction method test samples	50
6	Amino acid analysis of 5 representative samples	53
7	Effects of removing lipids from modern bone samples	55
8	Reproducibility of results for different extractions from the same individual	56
9	Results of the ' Δdc ' study	60
10	Results for the 46 paired samples used to test lipid extraction	67
11	North - south comparison for terrestrial species	69
12	Marine species results by regions	71
13	Summary of diet species results for British Columbia	72
14	Combined Central Coast results	102
15	Prince Rupert area individual results	103
16	Queen Charlotte Island results	104
17	Hesquiat results	106
18	Gulf of Georgia area results	107
19	Summary of coastal results	111
20	Interior results	117
21	Proportions of marine protein in diets: Coastal individual averages	123
22	Proportions of marine protein in diets: Interior individual averages	124

List of Figures

1	Proportions of modern C3 versus marine herbivore species in a diet	13
2	Locations of coastal sites sampled	100
3	Locations of interior sites sampled	116

1 Introduction

One of the major concerns of archaeologists is the study of human subsistence. Subsistence studies are concerned with the interactions of people, their technologies, and their local environments and biotas. Subsistence affects their day to day life, and to varying degrees, their social organization and cultural adaptations. Availability of food types can affect the location of sites and may lead to site specialization. Seasonal fluctuations in food availability may necessitate seasonal change in food procurement patterns and hence result in seasonal transhumance. Both of these factors could influence local and regional settlement patterns. The food types available will also influence the techniques of capture and/or harvest used, which will in turn influence the tools and site activities necessary for subsistence. Less visible aspects of subsistence techniques such as organization and division of labour, which may include specialization and sexual role differences, may also be influenced by the food types. Subsistence-related tool requirements may govern the materials selected and perhaps influence specialization in tool manufacture. Food types available or desired and tool material requirements may initiate or influence exchange patterns within a region.

Because of these influences, of foods on humans, we should be able to use data on diet to infer certain behaviour patterns for prehistoric people. For example, the differing proportions in which the foods available are used by a population may reflect personal or group preference as well as seasonal variations in availability and transhumance. Changes through time and space in the proportions of foods used by groups may reflect changes in food availability, in food preference, in subsistence technologies, or the introduction of new ideas, as well as changing trading patterns, political

affiliations and so on. Differences in foods used by various members of a group may reflect status differences. Obviously there are a number of questions that may be addressed, at least in part, through paleodiet reconstruction.

Diet reconstructions

The problem of reconstructing the diet of prehistoric people may be divided into three questions: 1) what foods were available to and used by the people, 2) in what relative proportions were the various foods present in the diets of the people, and 3) did these proportions vary through time and space or between and within populations? The first question is qualitative in nature and is usually dealt with through examination of preserved food materials or their remains, and of the tools used in their preparation. The other two questions are quantitative in nature and are more difficult to answer.

While there are many methods of obtaining data, the reconstruction of prehistoric diet has been carried out primarily through faunal analysis, with lists of recovered plant materials sometimes being included. In some cases paleo-diet has been inferred only from lists of modern animal and plant species that are presently found in the site areas. Of course there is no guarantee that any or all of these species were eaten, or that the same species were locally available in the past. Reports by early ethnographers, explorers, and traders provide useful information regarding diet, although many of them concentrate only on the techniques and technologies of harvest and capture. While some ethnographic reports mention which species are more important, most are unreliable in this matter. There is also the problem of how far back in time ethnographic and present day analogies may be taken.

Subsistence of local groups is also suggested by artifacts recovered during excavations. Bone, shell and wood points, hooks and barbs as well as cordage and basketry when preserved, and also lithic materials may be suggestive of harvesting techniques that are reported in the ethnographic literature. Obvious features such as fish weirs, pens and game fences, suggest harvesting techniques and hence species harvested. Lack of change in artifact types may indicate continuity in diets. While site locations and artifact types may be of some use in subsistence studies any interpretations based on such data will be highly inferential.

The above lines of evidence may indicate the species that were exploited for dietary purposes but it is often difficult, if not impossible, to estimate the relative importance of the various species in the local diets. To overcome this, analysts have devised various methods of quantifying faunal materials.

The most commonly used approaches are to determine the minimum number of individuals (MNI) or to use the number of identified specimens (NISP) to arrive at a rank-order quantification of the importance of the various species (Casteel 1976-77; Chaplin 1971; Grayson 1984, 1979, 1973; and others). Another approach is to determine the weight of material recovered for each species, particularly for shell. This has been used in some instances to suggest the order of importance. A number of workers have determined the average amounts of edible meat available from modern individual animals of various species (Casteel 1974; Parmalee and Klippel 1974; Smith 1975; Thomas 1969; White 1953). These estimates are combined with the MNI values for a site to determine the amount of meat from each species that was eaten at the site and to rank the species in dietary importance. In some cases the meat weights or some derived value such as kCal of energy or man-days of food for a site have also been used to indicate

the number of people that might have inhabited that site (Shawcross 1967; Wheat 1972).

Problems with the standard approaches to paleodiet studies

A problem with these approaches lies in the necessary assumptions that recovered, or recoverable, material from a site is in fact representative of what was originally deposited in the site, and that this material is representative of diet. Taphonomic factors may have altered the makeup of the originally deposited assemblage which itself may have been initially biased by human selective behaviour or other cultural factors. The meat weight approach assumes that modern animals, often domesticated, are analogous to their prehistoric ancestors. It assumes knowledge of the butchering and consumption patterns of the prehistoric hunters.

The consequence of these problems is to limit the reliability of quantification and of any resulting interpretations. Many zooarchaeologists feel that useful absolute counts of remains are generally impossible to obtain. Further, NISP and MNI "can rarely provide unambiguous ratio scale measurements of taxonomic abundance" (Grayson 1984). Possibly the only type of quantified data that is reliable and useful is ordinal data, or rank ordering of abundances. While this is interesting and extremely useful information it will not give the fine resolution that we would like in order to distinguish between groups of people within a region, or perhaps in adjoining sites, or even within a site.

New approaches

These approaches to human diet reconstruction involve analogy and inference and the use of non-human data. If we can obtain data directly from

the humans then more accurate interpretations should be possible. Trace element analysis (Price and Kavanagh 1982; Schoeninger 1979; Sillen and Kavanagh 1982) is one method that has been used in attempts to determine diet proportions. Within the last ten years the analysis of stable isotopes, particularly of carbon, from preserved bone collagen has provided a new method for studying prehistoric diets. Isotope studies have provided useful information about the proportions of different plant groups in local diets, and of dietary change through time. While the method is still being developed it does have the advantage that the data are obtained directly from the individuals being examined and not by inference from associated faunal, floral and other archaeological materials. When combined with information obtained by other methods the isotopic data should provide a more complete picture of prehistoric subsistence.

Scope of this study

Since 1977 there have been a number of carbon-isotope ratio studies published dealing with the use of maize in prehistoric diets. However, in the coastal areas and along the interior rivers of British Columbia maize was not present and instead it is the marine food species that are of major interest. Before this study there had been only one indication of the applicability of stable-carbon isotopic data for determining the relative proportions of marine versus terrestrial species in paleodiets (Tauber 1979, 1981). Consequently, the first problem to be addressed here was whether such a study was possible and practical in coastal and riverine situations. Initial results indicated that indeed it was possible to distinguish between the two alternative sources of dietary protein, marine and terrestrial (Chisholm et al 1982, 1983). It also became clear that there are problems relating to the

technique that must be addressed before it can be applied satisfactorily to archaeological situations in British Columbia.

This thesis is therefore divided into two parts. Part I describes the technique and its requirements and discusses causes of variability that are known, or suspected, to affect the results or to limit their applicability to archaeological problems. Chapter 5 reports an investigation of the difference in isotope ratios between a consumer and its diet. Chapter 6 examines the food species available in British Columbia and attempts to determine the range of isotope values into which the human consumers should fit. It also considers the human population values for British Columbia. Chapter 7 summarizes what we know about the technique that allows its application in British Columbia. Part II deals with archaeological situations in British Columbia. Chapter 1 summarizes our knowledge of prehistoric subsistence and archaeological problems relating to subsistence in the region, and outlines an appropriate sample set for studying those problems. Chapter 2 and 3 present the study results, and relate them to the archaeological problems.

Because this project has been carried out over a number of years, and overlaps other projects, portions of it have already been published elsewhere (Chisholm et al 1982, 1983, 1983a, Lovell et al n.d.). Some of the published material is incorporated here, where necessary and if appropriate to the discussion.

Part I: Carbon isotope analysis and its application in British Columbia

1 Overview of the carbon isotope technique

History of the technique

Isotopic analysis of carbon has been known since the late 1930's when Urey and Grieff (1935) and Nier and Gulbranson (1939) began investigating fractionation of the stable isotopes of carbon. Later Craig (1953, 1954) and Wickman (1952) began measuring samples of carbon-containing materials in order to understand the isotopic behaviour of carbon in nature. Following Wickman's (1952) initial measurements, and from about 1960 onward, botanists have relied partly on isotopic evidence for the classification of plants according to photosynthetic cycle (Bender 1968, 1971; Park and Epstein 1960, 1961; Smith and Brown 1973; Smith and Epstein 1971; Troughton 1972; Troughton et al 1973, 1973a; and others). Analysis of food chains followed those studies (Degens et al 1968, 1968a; DeNiro 1977; DeNiro and Epstein 1978; Deuser et al 1968; Fry et al 1978; Haines 1976; Haines and Montague 1979; Minson et al 1975; Smith and Epstein 1970; Tieszen et al 1979; Vogel 1978).

Within the last decade the analysis of stable isotopes of carbon (and now nitrogen) in preserved bone has been used for studying both modern and past environments and diets. For example, this technique has been used in both laboratory and field studies to examine the relationship between a consumer and its diet (Bender et al 1981; DeNiro and Epstein 1978, 1981; Macko et al 1982; Schoeninger and DeNiro 1984; Teeri and Schoeller 1979; Tieszen et al 1979a, 1983; Vogel 1978). It has been used to examine herbivore foraging behaviour in both recent (DeNiro and Epstein 1978a; Tieszen et al 1979; Vogel

1978) and archaeological or palaeontological contexts (Bombin and Muehlenbachs 1985; Chisholm et al n.d.). The technique has also been used in archaeological studies to determine the presence of maize (or related C4 species of plants) in prehistoric diets (Bender et al 1981; Broida 1983; Bumsted 1984; Burleigh and Brothwell 1978; DeNiro and Epstein 1981; Katzenberg 1984; Lynott et al 1986; Norr 1981; Schwarcz et al 1985; van der Merwe and Vogel 1978; van der Merwe et al 1981). Previous to this study only Tauber (1979) had discussed marine consumer diet values, based on measurements of two Greenland Eskimo individuals. Since then, marine versus terrestrial comparison data have been reported for both archaeological and recent samples (Chisholm et al 1982, 1983; Hayden et al n.d.; Hobson and Collier 1984; Schoeninger and DeNiro 1984; Schoeninger et al 1983; Sealy and van der Merwe 1985, 1986; Tauber 1981).

The theory underlying the method of stable-carbon isotope analysis has been explained by DeNiro and Epstein (1978), van der Merwe and Vogel (1978) and others for dietary maize determinations, and by Chisholm et al (1982, 1983) for marine versus terrestrial comparisons. The use of stable-carbon isotope analysis in archaeology has been reviewed by van der Merwe (1982), and O'Leary (1981) has provided an overview of carbon isotope behaviour in plants. Galimov (1985) and Hoefs (1980) provide the theoretical background explanations of isotopic behaviour in natural systems. A short overview of the technique is presented here, based on the above literature, and is followed by a more detailed discussion of the requirements of the technique and of the sources of potential variation affecting the sample materials.

Definition of $\delta^{13}\text{C}$, fractionation, and standards

Isotopic fractionation is the selection for or against one or more isotopes of an element during the course of a chemical or physical reaction. As a result there is a change in the relative concentrations of the particular isotopes involved. In this case we are concerned with the ratio of Carbon 13 to Carbon 12. Ratios for samples are compared to the ratio for a standard and the results are expressed, in parts per mil (‰), as follows:

$$\delta^{13}\text{C} (\text{‰}) = [((^{13}\text{C}/^{12}\text{C})_{\text{sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{standard}}) - 1] \times 1000.$$

A negative value indicates a sample that is depleted in ^{13}C relative to the standard (i.e., it is isotopically 'lighter'). The standard may vary from lab to lab but the results are calibrated to the originally used PDB standard, a marine belemnite from South Carolina.

Sample preparation involves cleaning, isolation of the desired carbon bearing compounds, and combustion to obtain CO_2 . Measurements are taken on the CO_2 using a dual-beam isotope-ratio mass spectrometer. The sample and the laboratory standard are alternatively measured a number of times and an isotope ratio is determined for each relative to the PDB standard. Modern instruments are capable of providing measurement precisions of about ± 0.01 to 0.02‰ .

Carbon reservoirs and plants

Carbon is found in the atmosphere as CO_2 with a virtually constant $^{13}\text{C}/^{12}\text{C}$ ratio of about 1:99, corresponding to a modern $\delta^{13}\text{C}$ value of -7‰ . This CO_2 is incorporated into plant tissues via photosynthesis, at which time the $^{13}\text{C}/^{12}\text{C}$ ratio is altered. Most plants use the Calvin-Benson, or C_3 ,

photosynthetic pathway, which generates a molecule containing 3 carbon atoms (glyceraldehyde 3-phosphate) as one of its first intermediate products. Most of the flowering plants, trees and shrubs, and temperate zone grasses use this process. C₄, or Hatch-Slack, plants follow a pathway that incorporates the CO₂ into a 4 carbon molecule (oxaloacetate) as the first photosynthesis intermediate. The C₄ metabolism is present in about 10 plant families and the majority of species are xeric environment grasses. C₄ plants of particular interest to archaeologists are maize, some millets, some sorghums, cane sugar, some amaranths and some chenopods. A third group, the Crassulacean Acid Metabolism (CAM) plants, is made up of tropical succulents, such as pineapple and various cacti, few of which are found in any quantity in the diet of herbivores or humans. Although their photosynthetic process is not clearly understood yet, phytoplankton at the base of marine food chains fractionate carbon to the same degree as terrestrial C₃ plants. They obtain their carbon from dissolved upper-ocean bicarbonates, which presently have $\delta^{13}\text{C}$ values of about 1 to 2‰, and thus have isotope ratio values differing from C₃ species.

Because the photosynthetic pathways differ chemically they produce different degrees of isotopic fractionation. $\delta^{13}\text{C}$ values averaging about -26.5‰ characterize modern terrestrial C₃ plants, while modern C₄ species values average about -12.5‰ (O'Leary 1981; Smith and Epstein 1971; van der Merwe 1982; Vogel et al 1978). The separation of 14‰ between the two group averages allows for discrimination between them. CAM plant values vary with their environment. In xeric conditions they resemble C₄ plants while in less xeric conditions they tend towards C₃ values. Modern marine plankton values average about -19.5‰ (Brown et al 1972; Degens et al 1968; Deuser et al 1968; Sackett et al 1965).

Herbivores, carnivores and food chains

When herbivores (terrestrial or marine) eat plants their metabolism requires selection and recombination of plant chemicals and respiration of CO₂, which results in further fractionation of the carbon isotopes. While the whole body average value of a consumer is displaced from its diet by only 0.8 (±1.1)‰ (DeNiro and Epstein 1978), the increment between the diet and bone collagen of the consumers, called the 'collagen enrichment' or 'Δdc', is not well known but appears to be about 5‰ (Chisholm et al. 1982, 1983; van der Merwe 1982; van der Merwe and Vogel 1978). This means that the bone collagen from a modern herbivore subsisting solely on C₃ grasses should give a δ¹³C value of about (-26.5 + 5)‰ or -21.5‰. If the diet were based solely on C₄ grasses then the value should be about -7.5‰. Thus the 14‰ spread observed between C₃ and C₄ plants would be maintained at the consumer's level. In the case of the carnivores or omnivores, in the next trophic level, including humans, a further fractionation of about 1‰ from the diet average has been reported (Bender et al 1981, DeNiro and Epstein 1978, McConnaughey and McRoy 1979, Schoeninger 1985, Tieszen et al 1983), provided the same tissue is examined, (e.g., herbivore and carnivore muscle, or herbivore and carnivore collagen). However, due to the low numbers of samples examined and the uncertainties which, when given, are larger than the increment itself, the inter-trophic level difference cannot be considered to be adequately demonstrated yet. But if the value of about 1‰ is correct then carnivores subsisting upon either of the above two herbivore types would give values for bone collagen of about -20.5‰ and -6.5‰ for the C₃ and C₄ herbivore alternatives respectively. For marine plankton based diets the expected modern herbivore value would be about -18.5‰ for muscle tissues while the value for carnivore collagen would be about -13.5‰. Using these values we

may construct a table of expected values for the trophic levels of various modern food chains (Table 1) in order to illustrate their general relationship. Gelatin values from the literature tend to support these results in a general sense but the uncertainties, when given, are usually too large to support any strong conclusions about trophic levels. It is thus still necessary to examine a large number of individual animals from different trophic levels before these values can be accepted as correct.

Table 1: Expected collagen values for modern consumers of specific diets.*

<u>Diets and their $\delta^{13}\text{C}$ averages (‰)</u>	<u>Consumer collagen (‰)</u>	
C3 plants only	-26.5	-21.5
Meat from herbivores on C3 diets	-25.5	-20.5
C4 plants only	-12.5	-7.5
Meat from herbivores on C4 diets	-11.5	-6.5
Marine plankton only	-19.5	-14.5
Meat from marine herbivores	-18.5	-13.5
Meat from marine carnivores	-17.5	-12.5

* based on the literature data summarized above.

Based on these values, if a modern carnivore samples both C3 based and marine based herbivore species then the $\delta^{13}\text{C}$ value for its collagen will lie between the extremes, or end-points, of -20.5‰ and -13.5‰. A simple linear interpolation locating the sample value between the two extremes, results in a proportion estimate for the relative amounts of C3 herbivore and marine herbivore species in that particular consumer's diet (Fig. 1). For example, a value of $\delta^{13}\text{C} = -14.9\%$ will indicate a diet that is 80 percent marine for that

particular consumer. A similar approach is taken for any other pairs of dietary alternatives.

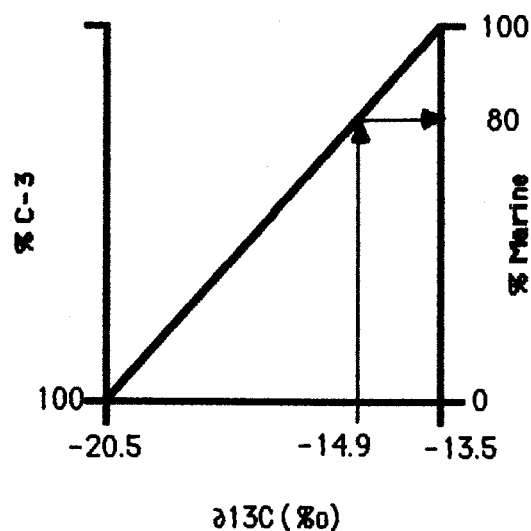


Figure 1: Proportions of modern C3 versus marine herbivore species in a diet.

The uncertainty on this proportion estimate is determined by combination of the uncertainties associated with the sample measurement and the two end-points between which the consumer value is interpolated. Most studies have not stated the uncertainties associated with their results, but for preliminary marine versus terrestrial comparisons on the British Columbia Coast, the reported uncertainty on proportion estimates was about $\pm 8\%$ (Chisholm et al 1983). Understanding the sources and magnitudes of uncertainties associated with the results is a major consideration in any of these studies because until we know them we will not know how well and in what situations the method works. This is discussed in the following chapters.

2 Application of the technique and the importance of variability

The easiest way of relating human $\delta^{13}\text{C}$ values to either C₃ -, C₄ -, or marine-based diet alternatives would be by comparing the values for unknown consumers to those of humans that subsisted exclusively on the alternative diets, as shown in Figure 1. However, the absence of consumers known to have subsisted exclusively on one or the other of the diet alternatives available would mean that the human end-point values cannot be determined directly. Other approaches are to determine the average values for the local diet alternatives and then either use them to derive values for the human end-points or relate the value of the unknown consumer(s) to them. In order to follow any of these approaches we need to know that:

- 1) the diet alternatives of interest are isotopically different,
- 2) there is a known and constant relationship between consumers and their diets,
- 3) any metabolic differences in consumers are small enough that they do not obscure any comparisons we wish to make, and
- 4) the material analyzed retains the isotopic information we want.

It is necessary to know the variability in dietary species values in order to determine whether the local alternatives are distinct enough for comparison. It will also be beneficial to understand any potential sources of variation affecting dietary species in order to determine the most appropriate samples to analyze. The variability associated with human populations will tell us if comparisons of the human groups are permissible and will also give us information on the amount of variability that may be attributable to inter-individual differences in metabolism and to variation in the local diets. Before examining the results it is also necessary to know

whether the observations are normally distributed and hence random and allow statistical comparison.

This chapter is concerned with what we already know about diet and consumers, and with known and potential causes of naturally occurring variability in sample isotope ratios, and their probable effects on the technique. Methodological details of the technique and related errors are discussed in Chapters 3 and 4 while results for British Columbian dietary species and humans are discussed in Chapters 6 and Part II.

Variations in reservoirs, food chains, and dietary species

Variations may arise from a number of natural causes, such as temporal shifts in isotope ratios for the carbon reservoirs (Stuiver et al 1984). Such fluctuations would be reflected in food species and their consumers and could, if large enough, obscure temporal shifts in diet proportions. Data presented by Broecker and Peng (1982: Figure 10-19), Craig (1954a), Jansen (1962), and Leavitt and Long (1986: Figure 4), suggest that prior to about 1860 the mean curve for tree ring $\delta^{13}\text{C}$ values was effectively flat, but that it fluctuated by up to 1‰. The burning of fossil fuels since about A.D. 1860 has added carbon to the atmospheric and oceanic reservoirs and resulted in a gradual non-linear shift in $\delta^{13}\text{C}$ values (Stuiver 1978: Figure 3). Since isotopic paleodiet studies are based in part on comparisons of modern diet alternatives with prehistoric consumers it is essential that this fossil fuel induced shift in $\delta^{13}\text{C}$ must be considered when data is being interpreted. This has not been done in previous isotopic studies of paleodiet. At the moment the best estimate for the magnitude of that shift is that both atmospheric and surface ocean average $\delta^{13}\text{C}$ values are about 0.7‰ lighter for modern samples (Broecker and Peng 1982, Stuiver 1978). While there is no stated

uncertainty for this shift, visual inspection of Stuiver's (1978) graph suggests that it should be about $\pm 0.2\%$.

The present atmospheric $\delta^{13}\text{C}$ value is about -7% and is generally uniform over large areas due to the rapid mixing of the atmosphere. However, Lowdon and Dyck (1974) and van der Merwe (1986, personal communication) have seen localized differences in air CO_2 values related to the respiration of tree species. In species growing below heavy tree canopies there is often a difference in the isotopic ratios of the CO_2 . This variation occurs on a seasonal basis, related to the growth cycle of the trees in the area and on their respiratory rate as it changes through the annual growth cycle. Decay and oxidation of organic remains affect the layers of air near the the ground (Keeling 1961; Munnich and Vogel 1959; Vogel 1978a) and therefore affect plants that live close to the ground, particularly if tree canopies shelter them from air movement. In areas where air circulation is greater these differences are not noticed (Keeling 1961). Such differences in the local plants should be reflected in the herbivores and so on up the food chains.

Geographic and seasonal variations in the proportions of C3 and C4 grass species in an area (Teeri and Stowe 1976; Tieszen et al 1979a) could affect $\delta^{13}\text{C}$ results for grazing herbivores (Chisholm et al n.d.) and hence the food chains into which they fit. The grazers may also migrate to new ranges, which might have different proportions and species of C3 and C4 plants, and this may be reflected in the herbivore's isotopic ratio averages. Different species of grazers may change their ranges at different times due to dissimilar foraging behaviours and plant species preferences, therefore showing different average isotopic ratios. This would contribute to variation in the overall averages for diet alternatives and hence to variation in the results for local humans that rely on those grazers. Data from Bumsted

(1984) and Schwarcz et al (1985) also indicate geographic differences in $\delta^{13}\text{C}$ values for maize, a human food in many areas.

The major difference between terrestrial and marine species arises from differences in the atmospheric and oceanic carbon reservoirs. The reservoir for the marine biosphere is the upper mixed layer of the ocean for which the modern average $\delta^{13}\text{C}$ value is about 0‰ (Craig 1953). The modern atmospheric value averages about -7‰ (Craig 1953, Keeling 1961). The difference between the two reservoirs arises from fractionation as CO_2 (gas) hydrates while entering solution in the sea water. Molecular CO_2 that may be dissolved in sea water will have the same value as atmospheric CO_2 , while the hydrated form, HCO_3^- , has a value that is about 7‰ lighter than that (Degens 1969:308). Since normal sea water (at pH 8 - 8.1) contains about 90% of its dissolved inorganic carbon as HCO_3^- it should have a value of about 0‰ but the two carbon species are in an equilibrium which varies with temperature and pH (Hoefs 1980) so we may expect to find regional variations in oceanic $\delta^{13}\text{C}$ values. Stuiver (1983) has stated that the correct modern value for North Pacific surface water is $\delta^{13}\text{C} = +1.0\text{‰}$ while Deuser and Hunt (1969) and Kroopnick (1974) have found that many ocean basin surface waters have modern values around +2‰.

Degens et al (1968a), Eadie and Sackett (1971), Fontugne and Duplessy (1981), Sackett et al (1965) and Wong and Sackett (1978) have observed a linear relationship between water temperature and $\delta^{13}\text{C}$ values for marine plankton, and presumably for food chains that rely on them, wherein the more negative values are found in colder water. This is no doubt one of the mechanisms that generates differences in values between areas as the water temperature does vary, often widely, between different geographic regions.

This difference may result from variation in the $\text{CO}_2 - \text{HCO}_3^-$ equilibrium, or from the plankton's CO_2 use changing with temperature (Galimov 1985:25).

One possible source of variation in local temperatures would be seasonal upwellings of colder water along coastal zones. In addition to causing temperature changes the upwelling currents could also carry carbon that is isotopically different than that of the local surface waters. Another possible factor in temperature-induced ratio differences is the lipid content of the specimens examined. Lipids (fats and oils) are isotopically lighter than protein and carbohydrates (DeNiro 1977:90-98; Galimov 1985:27; Jacobson et al 1970; Parker 1964; Smith and Epstein 1971; Vogel 1978), so if they are in greater abundance in an organism that organism will show a lighter isotopic average than one with a lower lipid content. In colder temperatures an organism has higher energy requirements than in warm conditions. Much of the energy for an organism is obtained through dietary and stored lipids so that in colder regimes the lipid content of the organism may be higher, resulting in an isotopically lighter average (McConnaughey and McRoy 1979). Fontugne and Duplessy's (1981) results indicate that samples (from which lipids were not removed) from colder surface temperature areas in the ocean were isotopically lighter, as might be expected. Unfortunately we have no data on the relative lipid content of the various samples analysed. We do however know that the proportions of lipids versus proteins is highly variable in fish species (Geiger and Borgstrom 1962). Also, lipids differ from each other in isotopic ratios (Galimov 1985:103-9) so that if species have different mixtures of lipids their average $\delta^{13}\text{C}$ values may differ.

Yet another factor that may influence the isotopic carbon in marine species is the terrestrial runoff of biogenic carbon. This would be a problem particularly for intertidal and estuarine species (Galimov 1985:25; Haines and

Montague 1979; Rau et al 1981; and others), but should not noticeably affect pelagic and migratory marine species. If the intertidal species are part of the local diet, as they commonly are, then we can expect greater variation in dietary average values for the coastal areas.

Marine species that migrate over long distances, through potentially varied conditions, will reflect the average values of the reservoirs through which they travel. In the North Pacific, salmon are the most obvious examples. Species such as tuna which also migrate widely have their northern limits in the coastal region of British Columbia and as a result should exhibit values reflecting a mixture of the local and a more southerly reservoir, which may differ somewhat from the local one in isotope ratio.

Fresh water (of pH 5 to 7) contains primarily molecular CO₂ so its modern average value should be close to the modern atmospheric value of -7‰ (Degens 1969), or slightly lighter (Oana and Deevey 1960; Vogel 1959). Thus, freshwater plants should have $\delta^{13}\text{C}$ values similar to C₃ plants, which would be reflected by their dependent consumer species. Influx of lighter biogenic CO₂ causes fresh water plant and plankton values to be in the range of C₃ terrestrial plants (Galimov 1985:25). Large bodies of fresh water with good circulation and low sediment loads will be closer to oceanic values (Galimov 1985:25).

The food choices of the individual consumers will also be reflected in the variability of consumer populations. Each individual may select different foods from the local menu that may have been affected by any or all of the above sources of variation. The possible trophic level difference mentioned earlier may introduce further variation because individuals that are eating a mixture of species of the same basic type, but from different trophic levels, may show slightly different results if their diets included different

proportions from the trophic levels. This could be complicated further in marine food chains because they usually include more trophic levels than terrestrial chains. For example, marine chains may include species such as salmon and tuna which are second or third level carnivores, i.e., they eat other carnivorous fish. An additional complication could arise due to terrestrial animals that may have been eating some marine foods, such as bear that were eating salmon or coastal deer that may have been eating seaweeds.

While many food species values may be available from the literature the potential existence of these regional differences in reservoir and food species values means that it is necessary to sample dietary alternative species within each study area. We cannot assume literature values to be correct from one area to another. However, we need not sample species that are not contributing to the local diets. If the difference between the diet alternatives is the same as that between the air and ocean reservoirs, of about 7 to 8‰, then this method should be applicable. If for some reason the difference between diet alternatives should decrease to the point where the two alternatives cannot be clearly distinguished then the method would not be applicable in that situation. For studies in British Columbia it is necessary to obtain dietary species values for both the terrestrial and the marine environments in order to confirm the difference between the terrestrial and marine diet alternatives. This is discussed in Chapter 6.

Materials analyzed: dietary species

Since foods are generally either plants, animal muscle, or animal organ tissues, and not bone, we should use those materials to determine average values for dietary alternatives. DeNiro and Epstein (1978, 1981) and Tieszen et al (1983) have shown that animal tissues may differ significantly in $\delta^{13}\text{C}$

values. Similarly, Troughton (1972) has observed differences between plant tissues, such as seeds and leaves. Therefore we must use samples from the tissues that were eaten. As animal soft tissues and vegetal materials do not usually preserve well, in nearly all cases we are limited to the use of modern analogs of identified prehistoric food species. Bone samples from modern individuals may be compared to preserved bone from individuals of the same species to establish temporal control over the $\delta^{13}\text{C}$ values for diet species.

Materials analyzed: collagen

In archaeological situations bone collagen extract (commonly called gelatin) from the consumer is analyzed because bone collagen is usually the only organic tissue reliably preserved in sufficient quantity, and because the inorganic portion of the bone is known to be unreliable for this purpose.

Because the formation of bone collagen is governed by RNA, as is the formation of all proteins, all bone collagen for a single individual, and in fact for all members of a single species should be structurally very similar (Glimcher and Kranz 1968, Mahler and Cordes 1966, Shubert and Hammerman 1968, White et al 1978). In addition, collagens for mammalian species are sufficiently similar that they may be considered the same. If the sequencing of amino acid residues in collagen molecules is not correct then the collagen may not be able to assume its stable structure (Yutani et al 1985) and therefore may not stabilize in the intracellular bone matrix. While some variation in the amino acid sequence may occur at the terminal ends of the collagen fibrils (Kuhn 1982) the number of variant residues should be very small (i.e., 1 or 2 residues in a thousand, Mahler and Cordes 1966), or the molecule will not function correctly. In addition, these procollagen non-helical end units are removed as the collagen fibrils form (Kuhn 1982;

Tanzer 1982). Any variant amino acid residues will have come from the same dietary sources as all of the others in the collagen and therefore should not introduce any isotopic variation. Therefore, if minor structural irregularities do occur they should not affect the isotopic character of mature collagen samples. In addition, when we analyze gelatin we are looking at many molecules so that minor differences in one molecule should be masked out.

Bumsted (1985) argues that sex, age and metabolism affect $\delta^{13}\text{C}$ values. Evidence presented by Lovell et al (1986) suggests otherwise. For a large sample of 50 prehistoric humans subsisting on one food reservoir the average $\delta^{13}\text{C}$ value was $-17.5 \pm 0.3\text{‰}$. Between males (n=9) and females (n=9) the difference was $\leq 0.1\text{‰}$, about the same as the measurement uncertainty, while specific identified age groups were within 0.3‰ of the population average (Lovell et al 1986). These results suggest that variation among humans of different age, or sex, that is greater than 0.3‰ and 0.1‰ respectively must be based on dietary differences, and is not metabolic in origin (Lovell et al 1986). In addition DeNiro and Schoeninger (1983) showed that there was less than 0.3‰ variation in results for bone samples taken from a group of 3 rabbits and 15 mink fed on monotonous diets.

Since all bone collagen in an individual is structurally identical and since the amino acids for forming the collagen come from one pool, different bones and different areas of the same bone should not differ isotopically for an individual on a constant diet. However, the two studies just mentioned did not report results for bones from single individuals so we are not certain how little variation we may expect within a single individual on a constant diet. Multiple samples from one individual were examined in this study and are discussed in chapter 4.

It is possible that the isotopic composition of collagen could differ from one area of bone to another because of change in an individual's diet as he/she grows and accumulates or replaces collagen. Different bones in different regions of the body mature at different times in an individuals' development. If the diet of an individual changes during periods of active growth then we may expect to see differences between collagen formed before and collagen formed after the change in diet. This potential for variation has not yet been tested but could be examined if samples, including active bone growth zones, from a group of immature individuals should become available.

The turnover rate of bone collagen is not adequately documented. Stenhouse and Baxter (1979) suggest a 30 year period for complete replacement, Libby et al (1964) suggest a period of about 10 years. While turnover studies have been carried out for various other forms of collagen, few, if any, have been done for bone collagen. We do know that bone collagen turns over slowly, likely requiring at least 10 years for complete replacement. While this period allows for change in the isotopic composition of bone collagen, any sample of collagen molecules will include a mixture of old and newly formed collagen, thereby averaging and obscuring short term changes. This phenomenon would preclude the use of bone collagen for determinations of short term seasonal or annual events. However, non-bone collagen or other stable proteins from tissues such as skin, hair, and nails may be useful for such studies since they are replaced much more rapidly.

A possible cause of inaccuracy would be the effects of diet stress or starvation on a group of consumers. During such periods the consumers may use exotic species that have different $\delta^{13}\text{C}$ values than their normal foods, although this was not too likely in British Columbia. If dietary protein was not being used to form bone collagen at those times then the isotopic

signature of the exotic foods would not be recorded in the consumer's collagen. This problem would become evident if the exotic species were present in some abundance in the zooarchaeological record.

Chemical make up of collagen

The amino acids from which collagen is assembled are obtained from dietary materials. Seven of these amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine) are considered essential in the human diet. This means that they must be ingested as either the specific amino acids or as their non-aminated precursors (Falconer 1969; Mahler and Cordes 1966; Meister 1965; White et al 1978: 329ff., 678ff.) since humans cannot synthesize the carbon skeletons for these particular acids. Arginine may be essential in some species, such as the rat, but is only essential in growing humans, not adults (Falconer 1969). The remaining amino acids are considered non-essential because the human body can synthesize them from metabolic materials if necessary. Some of the non-essential amino acid precursors are derivable from the carbohydrate metabolism (Mahler and Cordes 1966; Meister 1965; White et al 1978: 329ff., 678ff.). However, in normal circumstances protein intake is sufficient to provide non-essential amino acids as well as the essential ones and little or no synthesis is necessary (White et al 1978: 678). In areas such as the British Columbia coast where mammals, fish and shellfish are the major dietary items and where carbohydrate is not a major dietary component (Drucker 1951; Suttles 1968) it is likely that virtually all collagen-forming amino acids had their origin in dietary protein. Therefore, in these areas our diet reconstructions are concerned with alternative protein sources and not with carbohydrates or lipids. We are not looking at a complete food chain but instead are examining

what may be termed a protein chain. In other areas where carbohydrate intake is higher both dietary protein and carbohydrates must be considered as potential sources of carbon for the consumer's bone collagen.

Lipids in the diet are a source of energy but do not contribute to amino acid synthesis (White et al 1978: 329ff., 678ff.), except perhaps in the case of serine, a minor constituent of collagen (Mahler and Cordes 1966), so they can be safely excluded from the samples to be analysed. Data assembled by DeNiro (1977: 90-98) show that $\delta^{13}\text{C}$ values for the protein and carbohydrate components in 12 different plants and animals of different species differ by $0.8 \pm 1.3\%$. Further, for 6 animal species the lipids were isotopically lighter than protein by $4.8 \pm 1.8\%$. Vogel (1978) observed that "fat" was $7.9 \pm 1.6\%$ lighter isotopically than gelatin for 8 South African ungulates. Since the lipid content of different individuals and of different species may vary greatly (Geiger and Borgstrom 1962; McConnaughey and McRoy 1979) lipids may differentially affect the $\delta^{13}\text{C}$ values for dietary flesh, and possibly for bone samples. Therefore, unless the lipids are removed before measurement they may partially obscure the relationship between diet and collagen. This fact, in addition to the previously discussed problems with lipids in diet species, indicates that we must extract lipids from both bone and dietary samples before analysis.

The effects of diagenetic processes on collagen

One of the advantages of working with collagen as a material for analysis is that it is an extremely stable molecule, increasingly so in older individuals (Eyre 1980; Hedges and Wallace 1978). Samples of collagen have been found in bone as old as 100,000 years (Sinex and Faris 1959) and perhaps up to 380 million years (Abelson 1956; Isaacs et al 1963). While the stability of

collagen makes it a reliable source of carbon for dating and stable-isotopic analysis, we must still consider the possibility of diagenetic change in the collagen molecules. Such changes could be in the form of 1) the addition of contaminant carbon materials to the collagen, or 2) the replacement of some of the collagen carbon by contaminating carbon atoms.

After death and deposition, bone collagen breaks down into large peptide units (chains of amino acids, but not complete proteins) which themselves eventually break into individual amino acids. At each step the degradation products become more soluble and easily removed by ground water (Ortner et al 1972). Selective removal of particular collagen parts should not be a problem because it could only occur after denaturing and partial hydrolysis of the collagen, which should also render the remaining products soluble, permitting their removal from the bone. In any case, peptides and amino acid breakdown products that may remain after diagenesis should be removed during sample pretreatment, leaving the remaining intact collagen for analysis.

The addition of carbon contaminants to deposited bone, possibly including collagen from other bones as well as other proteins and organic compounds, requires a transport mechanism such as ground water. If the contaminants are sufficiently soluble to be transported then they should be sufficiently soluble to be removed by either more ground water or by laboratory pretreatment. Any other mechanism would likely involve alteration of the collagen. Addition of contaminants to the collagen itself would have to occur at a position on the molecule where a projecting group (NH_2 , COOH , OH or R) was accessible and where the carbon skeleton would not be altered. Proper sample pretreatment should reverse such reactions and remove the attached contaminants as easily as they replaced the original projecting groups,

leaving the carbon skeleton intact. Interstitially deposited contaminants, such as humic and fulvic acids, should be removed by proper treatment. Replacement of main skeleton carbon atoms would require cleaving of the molecule leading to its denaturing, increasing solubility, and subsequent removal, either by ground water or during pretreatment.

After the carbon contaminants are removed, either in the ground or the laboratory, the only other organic materials that may be present in bone samples should be other bone proteins, carbohydrates, and lipids from the same individual. They are less stable than collagen and should be removed more readily. The presence of other proteins in any significant quantity should be revealed by amino acid analysis, since they will have different relative proportions for amino acid residues and will lack the distinctive glycine, hydroxyproline and proline characteristics of collagen (see Table 6). Carbohydrates and lipids do not contain nitrogen so that variations in the ratio of carbon to nitrogen may possibly be used to detect their presence. As their origin will have been the same as the collagen carbon, these other proteins and carbohydrates should differ very little isotopically from the collagen, if at all.

The diet - consumer increment, ' Δ_{dc} '

To relate the $\delta^{13}\text{C}$ values for consumers to those of their diet it is essential that we know the correct value for the increment between them, called ' Δ_{dc} ', as well as the variability associated with it. The variability will give us information on whether ' Δ_{dc} ' differs between individuals. Examination of the published isotopic studies of paleodiet reveals that of those papers using ' Δ_{dc} ' only 7 rely on primary data (Table 2); the remainder refer back to those 7 papers. Of the 7 papers, 2 use data for consumers that were reported

Table 2 Values of ΔDC that have been reported and used in the literature.

Consumer Species (n=)	Average ΔDC (%)	Location	Diet Species (n=)	Average ΔDC (%)	Location	ΔDC
Vogel and van der Merwe (1977)						
Humans	-20.	N. Europe	C3 Plants	-26.	various	+6
van der Merwe and Vogel (1978)						
Browsers	-21.2	S. Africa	C3 Plants	-26.5	S. Africa	+5.3
Humans (31)	-21.4 \pm 0.78	S. Africa	C3 Plants	-26.5	S. Africa	+5.1
DeNiro and Epstein (1978/1981)						
Mice (3)	-18.6 / ? -17.9	Laboratory	JAX 911A (10)	-22.3 \pm 0.5		+3.7 / 4.4
Mice (3)	-16.5 / ? -15.5	Laboratory	Lab Blox F6 (4)	-19.3 \pm 0.4		+2.8 / 3.8
Mice (4)	?	Laboratory	Rat Chow	?		+3.5
Bender et al (1981)						
Mice	?	Laboratory	Mixed grains	?		+1
Mice	?	Laboratory	Maize	?		-1
Chicks	?	Laboratory	Mixed grains	?		+2
Chicks	?	Laboratory	Maize	?		-1
Sullivan and Krueger (1981)						
Browsers	-21.5	N. America	C3 Plants	-26.5	S. Africa	+5
Grazers	-6.5	and Kenya	95% C4	-12.5	S. Africa	+6
Bombin and Muhlenbacks (1985)						
Bison	-23.5	N. Canada	C3 grasses	-26.6	N. Canada	+2.8

by Vogel and Waterbolk (1972, 1967). The other 5 obtained their own consumer samples. In four cases dietary species were analysed. The remaining three studies assumed dietary species values from the literature, generally from regions well removed from the study areas. Sample preparation methods were described in only a few cases, but it appears that lipids were not removed from any of the dietary samples, or from any of the bones, modern or archaeological.

Vogel and van der Merwe (1977) used Vogel and Waterbolk's (1972, 1967) data on prehistoric northern European humans to derive an average of about -20‰ for consumers living on a C₃ based diet. The problem with this value is that marine species may have been present in the diet of the Europeans sampled. A modern C₃ plant average value, of about -26‰ , was derived from Troughton (1972) and Vogel's own work. Vogel's data would have been from South African samples, somewhat removed geographically and temporally from the northern Europe humans. ' Δdc ' in this case was determined to be about 6‰ . In a subsequent paper (van der Merwe and Vogel 1978) modern South African browsers, with an average value of -21.2‰ but who may have eaten a small amount of C₄ species, were compared with modern South African C₃ plants, with an average of -26.5‰ . Since both sets of samples were modern and were obtained from the same general area this study (van der Merwe and Vogel 1978) remains as the best field study thus far conducted. The potential flaws are 1) the possibility that browsers were eating C₄ species, 2) the failure to remove lipids from plant samples, and 3) the possibility that plant tissues sampled were not those being eaten by the browsers. This study also includes thirty one human samples from pre-maize contexts in the northeastern United States, with an average $\delta^{13}\text{C}$ value of $-21.4 \pm 0.78\text{‰}$, only 5.1‰ away from the modern C₃ plant average of -26.5‰ .

Further field studies include that of Sullivan and Krueger (1981) in which prehistoric North American and Kenyan browsers (average $\delta^{13}\text{C} = -21.5\text{‰}$; $n=3$) were compared with modern South African C3 plants (Vogel et al 1978), and prehistoric North American and Kenyan grazers (average $\delta^{13}\text{C} = -6.5\text{‰}$, $n=2$) were compared with modern South African C4 plants (Vogel et al 1978). Aside from the small sample size, there is a problem with the C4 grazers because the 2 samples are from distinctly different archaeological time periods, and are alleged to have eaten a diet that was only 95% C4 (Sullivan and Krueger 1981: Table 1). No account is taken of possible geographic and temporal differences. In spite of these difficulties the paper concludes that for C3 consumers ' Δdc ' is $+5\text{‰}$, while for C4 consumers it is $+6\text{‰}$. These two differing ' Δdc ' values have been assumed correct in subsequent work (van der Merwe 1982) resulting in discussion of metabolic differences between consumers eating either C3 or C4 species.

Bombin and Muehlenbachs (1985) compared modern bison's tissues with their diet for field samples obtained in Northern Canada. They do not mention which tissues specifically were examined, although it appears that bone was not one. Apparently they have assumed that all of the bison tissues were equivalent, which is known for other species to be untrue. Further, Bombin and Muehlenbachs (1985) do not explain their sample preparation techniques, so it is not clear which tissue components are being examined, i.e., protein only, protein plus carbohydrates, or other combinations. For these reason their study results relating to ' Δdc ' are not reliable.

The remaining ' Δdc ' determinations are based on laboratory studies. DeNiro and Epstein (1978) analysed samples of two groups of 3 mice each, with each group on a different diet, and reported a different ' Δdc ' value for each group, averaging about 3.5‰ . Unfortunately they did not remove lipids

from the bone samples, which were modern and hence likely to be high in lipid content. This failure would likely have given collagen results that were lighter in ^{13}C , and hence closer to the dietary values than would be found with lipid free samples, thus reducing ' Δdc '. More recent results, including one more group of 4 mice and re-analysis of the first two groups, produced different average values for each of the groups, with an overall average of about 3.9‰ (DeNiro and Epstein 1981: figure 3). In the second investigation the samples were treated to an NaOH wash stage and gave values that differed from the earlier results for the same, but not NaOH treated, samples. Whether the NaOH acted on the lipids or proteins, or both is not known. One point not addressed by DeNiro and Epstein is the fact that the three different diet - consumer pairings each produced different ' Δdc ' estimates. A possible explanation for this is the failure to remove lipids from the dietary samples. If diet samples had measureably different lipid contents then we would expect differences in diet average values, and hence in ' Δdc '.

Bender et al (1981) report results for 7 mice and 7 chicks fed on either mixed grains or maize diets. Bone from these animals was first dried at 80°C (a temperature at which we might expect collagen to begin denaturing) and then treated with 4% HCl for an unspecified length of time. It was not treated with hot acidic water to separate the collagen. It is not known how successful this process was in isolating pure gelatin, although experience at Simon Fraser University suggests that it is not a particularly reliable approach. For that reason, and also because lipids were not considered, Bender et al's (1981) data must be considered unreliable for ' Δdc ' estimates.

We are thus left with van der Merwe and Vogel's (1978) result of ' Δdc ' = 5.3‰ as the most reliable field result. For laboratory studies the work of DeNiro and Epstein (1978, 1981) is the best data available, although both of

these studies are flawed. The other studies, including Sullivan and Krueger's (1981) determination that C4 consumers had a different ' $\Delta\delta C$ ' of +6‰ must be considered unreliable and hence not useable.

Further investigation of this problem is required and must include analysis of lipid-free samples, obtained from laboratory populations, and possibly from localized, well controlled field studies, or preferably both. Such a study is discussed in Chapter 5.

Variability in consumer populations

The variation in a set of consumer collagen samples will be made up of 4 components: 1) that introduced by the foods that the consumers ate, 2) any intra-individual variation due to the consumer's metabolism, 3) inter-individual variation in the increment between the consumers and their diets, and 4) that due to sample preparation and measurement. Until now little evidence has been presented in the literature regarding the variability associated with large populations of consumers, human or otherwise. Van der Merwe and Vogel (1978) measured 31 humans, not from one but from 6 Archaic Period sites in Illinois and Ohio, obtaining an average value of $-21.4 \pm 0.78\%$ (at 1σ). Lovell et al (1986) have examined a prehistoric human population of 50 individuals from one site, who lived on an essentially homogenous diet, and observed an average value of $-17.5 \pm 0.3\%$ (at 1σ) for the whole population. Hobson and Schwarcz (1985) have examined populations of 55 mule deer and 22 voles, observing variabilities of $\pm 0.5\%$ and 0.6% respectively. DeNiro and Schoeninger (1983) observed an average value of $-12.8 \pm 0.2\%$ for both humeri and femora from 15 mink fed on the same monotonous diet. In all but the last of these cases the diets were not well known but were thought to have been similar for each individual in the

sampled populations. These results will include variability from all four of the components mentioned, and give an idea of the variability that may be expected for other populations living on homogenous diets. The low variability reported by Lovell et al (1986) suggests that any human population variability in excess of $\pm 0.3\%$ will result from inhomogeneity in diets within that population. Data on isotopic variability in other human populations are desired to support these observations. British Columbia human populations are discussed in Chapter 6.

Summary

To carry out isotopic studies of paleodiets it would be ideal if we knew the end-point values for human consumers in the study area. Since this information is not usually available we need to know the average values and variabilities for 1) the diet alternatives of interest in the study; 2) the correct value for ' $\Delta\delta c$ '; and 3) metabolic differences in and between consumers, which are indicated in part by the variability for human population results. We also need to know that collagen retains the isotopic information we want and can be recovered without contaminants.

Investigation of the literature reveals that none of this information is well known yet. Work mentioned in the introduction suggests that the marine and C_3 based terrestrial diet averages are separated by about 7 to 8%. However, variability in dietary species averages may arise from a number of sources; consequently, it is necessary to sample the dietary species in each region before addressing Question 1.

Neither the value for ' $\Delta\delta c$ ' or the variability associated with it are well known. The value appears to be about 5% but this is not based on any well controlled study and therefore must be investigated further.

Because the structure of collagen is well known and is constant for humans and other mammals its metabolic variability should be low. This is supported by Lovell et al's (1986) measurements which show no age or sex related differences in human values for a population living on a homogenous, or nearly homogenous diet. They suggest that variabilities exceeding $\pm 0.3\%$ should result from differences in dietary species values and are not metabolic in origin. Further supporting evidence on this point is desirable.

Present knowledge about collagen indicates that if pure gelatin can be extracted from bone samples then it should retain the isotopic information that was originally laid down in the bone. Appropriate quality control methods must be applied to check for sample purity and the absence of diagenesis. These points are discussed further in the following chapters.

Evidence has been cited for a fossil fuel induced temporal shift in reservoir $\delta^{13}\text{C}$ values since about A.D. 1860, of about $+0.7 \pm 0.2\%$. Since studies of this type rely on comparisons of prehistoric human results with those for modern diet alternative samples it is essential that this shift be considered when the data are interpreted.

3 Requirements of and problems with the laboratory procedures

This chapter discusses laboratory procedures and errors associated with sample handling and measurement.

Collagen extraction

Since there is no way of avoiding the gradual breakdown of collagen or the addition of contaminants to bone samples it is clear that we must focus on sample purification processes. The most obvious problems leading to variation in results are: 1) the failure to remove contaminants from bone samples and 2) the alteration or degradation of the extracted collagen due to improper procedures. Sample extraction procedures therefore must meet a number of requirements: 1) they must remove all contaminating carbon-bearing materials, 2) they should not selectively remove or alter any of the collagen, but if they do then they should remove all fragments of collagen and any amino acids hydrolysed from the collagen, leaving only intact molecules or representative large peptide fragments for analysis, 3) they should not add any carbon-containing materials to the sample, and 4) for the purposes of archaeological interpretation and economy, it is desirable that the process should be simple.

A process that is commonly used in isotopic paleodiet studies is that of Longin (1971), or some variant of it. In this process bone samples are first mechanically cleaned to remove any obvious contamination such as rootlets, glue residues, inked and laquered numbers etc., and then are ground in a Wiley Mill or similar device. Demineralization and removal of acid and water soluble materials is achieved by treatment with HCl. Collagen is extracted from the bone matrix by using hot water at about pH 3. This procedure is intended to remove all acid and water soluble materials, including free amino

acids and soluble peptides. While gelatin produced by this method appears to be pure and reliable there may be problems if an NaOH wash is included to remove humic and fulvic acids, as is sometimes the case (DeNiro 1985, DeNiro and Epstein 1981, Land et al 1980, Schoeninger 1985).

Reasons for avoiding the NaOH wash are that it may noticeably decrease the gelatin yield (Grootes 1980, personal communication), and that it may affect the isotopic character of the gelatin. Experience in the SFU radiocarbon laboratory indicates that this commonly used method gives somewhat lower yields than does Longin's (1971) method. This may be due in part to the solubility and loss of some amino acids (e.g., serine and threonine) and smaller peptides to the NaOH (Meister 1965). DeNiro and Epstein (1981) found a shift of about 0.7‰ to 1.0‰ between NaOH treated and untreated fresh bone samples. While the reasons for this shift are not clearly understood there are a few factors that may explain it. Different amino acids have different $\delta^{13}\text{C}$ values (Abelson and Hoering 1961; Hare and Estep 1982; Macko et al 1982). If amino acids are selectively removed then the overall gelatin value could be biased. We also know that NaOH affects some amino acids more strongly than others (Meister 1965; Veis 1964), particularly arginine and tyrosine (Eastoe and Leach 1977), and serine and threonine which have $\delta^{13}\text{C}$ values as much as 10 to 12‰ heavier than the other collagen amino acids (Hare and Estep 1982; Macko et al 1982). Eastoe and Leach (1977) indicate that gelatins are slightly poorer in the rarer amino acids and richer in the common ones than the original corresponding collagens. It is likely that alkali treatment selectively removes peptides poorer in hydroxyproline and richer in tyrosine (Eastoe and Leach 1977). Thus, the use of an NaOH treatment may selectively bias the collagen $\delta^{13}\text{C}$ results, although this needs further investigation. If this is the case, it may explain the postulated

diagenetic alteration of the isotopic character of buried collagen noted by Land et al (1980) and DeNiro (1985). In both cases an NaOH wash was included in the extraction process. Of interest is the fact that all the altered $\delta^{13}\text{C}$ values reported by DeNiro (1985) appear to be isotopically lighter than those for other individuals of the same species.

Bumsted (1984) has also determined that NaOH washes are detrimental to collagen extraction and has used an Amberlite XAD-2 resin column to remove contaminants from the gelatin. However, the $\delta^{13}\text{C}$ values were altered by as much as 1.5‰ with this approach (Bumsted 1984: 82-83), possibly due, at least in part, to contamination from column degradation products. Many resins gradually degenerate and can contribute carbon to samples being analysed (D. Eckert, Beckman Instruments 1982, personal communication). Alterations of this nature are unfortunately not acceptable if we wish to reduce errors to minimal levels. Extractions based on enzymes and EDTA also hold the possibility of introducing contaminant carbon. Dialysis techniques are slow and unwieldy. Comparisons of three different extraction methods are discussed in Chapter 4.

Quality control of extractions

Ensuring that the samples to be analyzed contain no contaminant carbon requires some method(s) for confirming sample purity. One approach that has been suggested and that is being actively promoted at conferences and in the literature (Ambrose, 1985, personal communication; Bumsted, 1986, personal communication; DeNiro 1985; Schoeninger, 1986, personal communication) is the use of elemental analysis to determine C/N values.

DeNiro (1985) has suggested that carbon - nitrogen ratios within the range 2.9 to 3.6 are an indication of collagen sample purity. Values falling

outside that range will indicate contamination. He found that for 212 modern bone gelatin samples the C/N values fell within the range 2.9 to 3.6. The percent by weight of nitrogen in gelatin is about 18.6% (Berger et al 1964, Eastoe 1961, Edsall and Wyman 1958, Sellstedt et al 1966). Since proteins, including collagen, are about 50% carbon (Berger et al 1964; Regenstein and Regenstein 1984; Sellstedt et al 1966) the theoretical C/N value will be about 2.7. Values higher than this would suggest either low nitrogen levels or higher carbon levels, resulting from either the removal of nitrogen or the addition of carbon contaminants. If the added contaminants were other proteins, with similar C/N values, then their presence would not be detected by this method. Similarly, removal of collagen breakdown products, i.e., peptides and free amino acids, with similar C/N values would also not be detected. Since DeNiro's (1985) 212 samples were modern, diagenesis is not a factor. The difference between his observed range of values and the theoretical one may result from sample preparation technique effects, particularly the failure to remove lipids specifically, which contain carbon but not nitrogen.

DeNiro (1985) also reports on about 100 archaeological samples with C/N values falling mostly around 3.2 to 3.5. Twenty-three of 25 aberrant carbon - nitrogen ratios reported by (DeNiro 1985) deviate in such a direction as to indicate either the loss of nitrogen or the addition of contaminant carbon. As it is known that alkali treatment in the preparation of gelatins will remove amide nitrogen (Balian and Bowes 1977), it is possible that DeNiro was not observing evidence for diagenetic change, but for bias introduced by sample pretreatment. This must be investigated further, using splits from the same sample extracted by different methods. However, it is reasonable to conclude

that C/N values may be useful in detecting gross contamination or alteration of gelatin, but values falling near 2.7 will not necessarily indicate purity.

A second check on sample purity is to carry out amino acid analysis to determine if any non-gelatin proteins are present, or if the gelatin has been altered (Bumsted 1984, Lynott 1985). This approach will be discussed further in chapter 4.

It is also desirable to have standard bone and collagen samples with compositions similar to those analysed in paleodiet studies. They would be useful in accounting for any variations due to sample preparation and combustion differences. This problem was discussed at the 11th International Congress of Anthropological and Ethnological Sciences in Vancouver, B.C. in August 1983, but to date no new standard has been forthcoming.

Such a standard should probably include a group of samples, i.e., material from a large C₃-plant-consuming herbivore mammal, from a large C₄-plant-consuming herbivore mammal, and from a large marine mammal. These three types would provide examples for each of the three major dietary alternative groups. In addition to carefully prepared, well mixed, collagen extracts (gelatin), it is necessary to include some powdered, but unextracted, material from the same bone sample in order to compare each laboratory's extraction procedures. At the moment the only bone standard available appears to be one developed for trace elements by the International Atomic Energy Agency in Vienna. This may suffice until more appropriate standards are made up.

Sample measurement

The most straight forward and easily controlled variation relating to this analytical approach is associated with sample combustion and measurement.

As mentioned previously, modern instrumentation can provide measurement precisions on CO₂ samples of about ± 0.01 to $\pm 0.02\%$ under ideal conditions. Although not all instruments provide this level of precision the contribution of this error should not normally be a problem. However, a potential problem lies in the possibility of interlaboratory calibration errors in isotopic analysis. This was illustrated by Mann (1982) who arranged to have a number of laboratories carry out isotopic measurements of NBS radiocarbon standards. The reported results have variations of 0.5 - 0.6‰ (at 1σ) with the range of values being about 1 to 1.5‰ (Mann 1982). A further indication of this problem comes from initial analyses for this study (Chisholm et al 1983). In that case 48 human samples from 15 different sites on the British Columbia coast were measured at three different laboratories yielding a variation of 1.0‰ (1σ). When only the 37 samples, from 11 of the sites, that were prepared at Simon Fraser University and measured at McMaster University were examined the variation was reduced to 0.4‰, indicating the presence of inter-lab differences in absolute values.

Control of this variation can only be obtained when laboratories doing isotope analysis adhere closely to some conventional standard, as they generally do. The above results indicate, however, that such is not always the case. Such carbon standards do exist for radiocarbon dating and mass spectrometry and must be used regularly and carefully to prevent errors.

Summary

Sample extraction procedures must meet a number of requirements: 1) they must remove all contaminating carbon, 2) they should leave only intact and unaltered collagen molecules or representative large peptide fragments for analysis, and 3) they should not add any contaminant carbon. Sample extractions have generally been done following Longin's (1971) approach, often including a NaOH wash which may adversely affect the gelatin. Tests for sample purity have been generally lacking although the use of C/N values and of amino acid analysis results have been suggested as a check on purity. As a result we still do not know the most appropriate methods of sample extraction and quality control. In addition, there is a lack of comparative standards for gelatin extraction and measurement with which to calibrate different laboratories' procedures and measurements. We do know that instrumental errors in measurement should be small, about ± 0.1 to $\pm 0.2\%$, and thus should not contribute significant uncertainty to the results. These points are discussed further in the next chapter.

4 Tests of the methods used

This chapter describes and discusses the specific methods used in this study. It includes the results of tests of the techniques used, some of which have already been published (Chisholm et al 1983a).

Sample analysis

The particular mass spectrometer used for most of this study, a VG Micromass 602D in the laboratory of Prof. Henry P. Schwarcz, Geology Department, McMaster University, gives analyses of replicate samples of the same CO₂ gas with a precision of $\pm 0.08\%$ (1σ) or better (Schwarcz, 1982, personal communication). The absolute $\delta^{13}\text{C}$ values are determined with respect to the National Bureau of Standards Solenhofen limestone standard (NBS 20) and reported in the normal fashion relative to the PDB standard. Measurements of the NBS Graphite standard (NBS 21) gave the result of $-28.25 \pm 0.1\%$ which differs from the accepted value (-28.1%) by 0.15% . The NBS Oxalic acid Radiocarbon standards SRM-4990 and RM-49 were also used to check the absolute accuracy. The average values obtained were -19.11% and -17.42% , respectively. These are about 0.1% and 0.2% different from the average values obtained by a number of laboratories for these materials (Mann 1982). A second instrument, a VG Micromass 903 in the Quaternary Isotopes Laboratory, University of Washington, Seattle, was used for measuring samples for the ' Δdc ' study, discussed later in this chapter. Its precision is 0.04% for replicates of the laboratory standard, and 0.03% on averages for paired samples (T. Saling, University of Washington, 1986, personal communication). Since the ' Δdc ' study measurements constitute a complete study in themselves and are not affected by the remainder of this study, cross calibration of the two instruments was not

considered necessary. Human samples that had been measured in other laboratories were redone on the McMaster instrument so as to avoid calibration problems.

Combustion of the samples to obtain CO₂

The major considerations in sample combustion are that: 1) the combustion products include those that are desired for analysis, 2) carbon from the samples is not distributed through a number of combustion products, but that it all goes into only the desired product (in this case CO₂), and 3) the combustion is complete so that there is no chance for fractionation due to incomplete conversion of the sample. For archaeological studies it is usually necessary to handle large numbers of samples, therefore a combustion system that is simple, inexpensive and fast is desirable. For this reason dynamic combustion systems (e.g., Craig 1953; DeNiro 1977) are to be avoided if possible as they are relatively complex and time consuming. However, the technique proposed by Stump and Frazer (1973) and modified by Sofer (1980) provides a simple and satisfactory alternative. This procedure has been adopted here and is as follows:

- 1) tubes (6mm o.d. x ca. 20 cm) of Vycor are prepared and baked at 900°C for 2 hours to remove any organic contaminants that may adhere to them;
- 2) CuO (wire form), which provides the oxygen for combustion, is similarly treated at 900°C to remove contaminants, (to avoid recontamination of the tubes and the CuO it is advisable to store them in a sealed container, perhaps under vacuum and over a dessicant, until use);
- 3) appropriate quantities of the desired sample (typically about 5 mg) are loaded into the Vycor tubes along with about 1 gm of the prepared CuO;
- 4) sample tubes are evacuated (ca. 2×10^{-3} Torr) and sealed; and

5) loaded tubes are baked at 900°C for 2 hours in a preheated oven and then allowed to cool slowly in the oven before removal (usually overnight), after which the samples are ready for analysis.

For measurement the tubes were scored with a file, placed in a metal bellows connected to the mass spectrometer, and broken under vacuum by flexing the bellows (Des Marais and Hayes 1976). The tube gases were passed through a dry ice slush trap to remove water vapour and the CO₂ was frozen on a cold finger while non-condensable gases were pumped away. The CO₂ was then expanded into the mass spectrometer inlet for measurement.

Analysis of the gases resulting from the combustion has shown that the only carbon-containing gas present is CO₂ (T. Brown, SFU, 1986, personal communication). While SO₂ was not observed there were indications of sulfur and/or CuSO₄ remaining in some of the sample tubes. Although there have not been any problems, it may be beneficial to use a small amount of pure silver wire to catalyse N₂ formation and aid in removal of sulfur from the gases (Bombin and Muehlenbachs 1985). In some cases, particularly where water vapour is present, it is conceivable that some of the carbon could be diverted to form carbonates or carbonic acid if sample gases are left in the combustion tubes for an extended period (Stuiver et al 1984). Loss of CO₂ to carbonates etc., could result in fractionation of the CO₂ carbon. To avoid this potential problem it is only necessary to ensure that the samples are dry before the tubes are sealed and combusted, and the combustion and analysis occur within a short period of each other (i.e., the same day). Since most amino acids and many peptides are hygroscopic to some degree it will be necessary to use the vacuum system to evaporate any residual water before sealing the tubes. The simplest method of detecting problems in the combustion stage is to measure the yield of CO₂. If there is conversion and

loss of carbon to other compounds then the yields will be lower. Yields for samples prepared in conjunction with this study were within estimated measurement errors of 100%, i.e., 97 - 100% (M. Knyf, 1981, personal communication).

In the early stages of this project sample combustion was carried out at 550°C in borosilicate glass (Pyrex) tubing, as suggested by Sofer (1980). DeNiro (1981, personal communication) was concerned that at this temperature combustion would be incomplete and therefore might lead to fractionation. This possibility was tested by burning replicate samples of collagen and of meat in both Pyrex tubing at 550°C and in Vycor tubing at 900°C. In addition, two replicates of collagen were burned in Vycor at 550°C. The relative CO₂ yields and values are shown in Table 3.

Table 3 Comparison of combustion methods

<u>Sample and No. of replicates</u>	<u>Method</u>	<u>Relative CO₂ yield* ($\pm 1\sigma$)</u>	<u>$\delta^{13}\text{C}_{\text{PDB}}$ (‰) ($\pm 1\sigma$)</u>
Collagen (6)	Pyrex 550°C	1.6 \pm 0.2	-12.7 \pm 0.1
Collagen (2)	Vycor 550°C	1.7 \pm 0.2	-12.7 \pm 0.0
Collagen (6)	Vycor 900°C	1.8 \pm 0.1	-12.6 \pm 0.1
Meat† (3)	Pyrex 550°C	2.1 \pm 0.3	-23.8 \pm 0.0
Meat† (3)	Vycor 900°C	2.3 \pm 0.0	-24.0 \pm 0.1

* Measured as CO₂ pressure per mg of sample.

† Freeze dried.

As with Sofer's (1980) results there is no significant difference in either the collagen CO₂ yields or $\delta^{13}\text{C}$ values. The observed difference of 0.2‰ for the meat values is not surprising as lipids were not extracted, so the meat

was potentially not as homogeneous as the collagen samples. The difference in these two methods is thus $\leq 0.2\%$. However, DeNiro's caution was heeded; the extra expense and trouble of using Vycor or quartz glass tubing at 900°C were taken to ensure definitely that no fractionation took place due to incomplete combustion. All measurements reported in this study were made in Vycor at 900°C .

Perhaps a more important consideration is that of proper preheating of the oven before the samples are inserted. As mentioned by Sofer (1980), failure to preheat the oven may lead to premature charring of some samples which can result in incomplete combustion and hence possible fractionation of the carbon. Heating to 900°C seems to combust even charred materials thereby avoiding the problem.

More recently Bombin and Muehlenbachs (1985) carried out a similar comparison and found no difference in results for samples combusted at 550°C in Pyrex, or at 700°C , 800°C , and 900°C in Vycor. Further investigation may show that borosilicate glass is sufficient provided the burning conditions are correct.

While the combustion method is very convenient the tubes have occasionally burst in the oven if too much sample has been used, if the sample has picked up moisture or has not been completely dried, or if the tubes have not been properly annealed.

Collagen extraction

In the previous chapter it was suggested that the most important step in dealing with variability was sample pretreatment. Consequently, sample extraction procedures were tested. Three variants of Longin's (1971) method, commonly used in radiocarbon laboratories, were compared. Six samples of

human bone were selected from different archaeological sites, representing different depositional circumstances. The samples were mechanically cleaned, ground to about 1mm, and subdivided into three portions. The first extraction method tested was Longin's (1971) original one. In this method the acid soluble inorganic portions of the bone, any acid soluble peptides, and free amino acid residues are first removed by extraction with 1N HCl for 18 minutes, at room temperature. After multiple filtration, or centrifugation, the remaining acid insoluble material, which includes undenatured, and hence insoluble collagen, is extracted for about 16 hours in hot (90°C) water. The heating denatures and partially hydrolyzes the intact collagen and thereby renders it soluble. The heated solution is maintained at about pH 3 to avoid dissolving any acid-insoluble contaminants. Only the denatured and partially hydrolyzed collagen extract, or gelatin, enters the solution. While bone protein includes more than collagen the other forms are not as stable as collagen and should come out in the HCl treatment stage. The heated solution is then filtered to remove insoluble residues, and the gelatin isolated by evaporation or freeze drying of the filtrate.

Although there was originally no evidence to indicate so, there is a chance that the 18 minute treatment might not completely remove all of the acid soluble contaminants, particularly soil humates (Bumsted 1984). Examination of this via further treatment of the residue from the first extractions with 1N and 0.25N HCl revealed that removal of inorganics, and possibly some organic materials, was not complete in the original process.

Method 2 is identical to Longin's (1971) except that the sample is additionally treated for about 20 hours with 0.5% NaOH, before the hot water extraction step, to remove base soluble contaminants. Originally the NaOH soak was introduced to collagen extractions to avoid contamination by humic

and fulvic acids (Häkansson, 1976; Bumsted 1984). Stevenson (1982) mentions that HCl extractions may remove up to 5% of the organics from soil samples, including some low molecular weight fulvic acids (ca. 200 daltons), which indicates that some are acid soluble. However, with the exception of these potential contaminants, all other humic and fulvic acids are soluble in alkali and not in acid (Stevenson 1982) so that maintenance of an acid environment should keep them out of solution throughout the process. Thus, repeated acid treatments should extract all of the soluble fulvic acids leaving behind only those that are insoluble.

The third method tested is a modification of Longin's that was suggested by P. Grootes (1980, personal communication). In this case demineralization and removal of all acid and water soluble materials is accomplished by serial extraction with 0.25N HCl to a stable pH, usually of about 1. This more gentle treatment is intended to remove all acid and water soluble materials, including free amino acids and soluble peptides, while minimizing gelatin loss. It is therefore more suitable for very small samples. In this method contamination by acid-insoluble materials such as humic and fulvic acids is avoided by maintaining a pH of 3 or lower at all times, the acid soluble materials already having been removed by the initial extraction to a stable pH. Gelatin is extracted as in Longin's method, by heating to 90°C for about 10 hours or longer, while maintaining a pH of about 3. Collagen that is denatured via this heating comes out into solution while the acid insoluble materials still remain in the residue (Häkansson 1976). As long as complete molecules of collagen or all of the large representative peptides (ca. 60,000 and 30,000 Daltons), resulting from the hydrolysis of complete collagen molecules, are brought into solution during this last step then we should not have to worry that the extraction of all of the gelatin has been completed. Further

extractions of the residue for 5 samples have yielded no more gelatin. Reproducibility for the Grootes method is shown by 6 separate extractions from the same bone which gave an average value of $\delta^{13}\text{C} = -20.65 \pm 0.09\text{‰}$ (1σ) (Chisholm et al 1983a). The comparison results are shown in table 4.

Table 4 Comparison of gelatin extraction methods (results are $\delta^{13}\text{C} \pm 0.1\text{‰}$).

Sample	Method 1 (Longin)	Method 2 (NaOH)	Method 3 (Grootes)
1	-16.0	-16.3	-16.1
2	-15.6	-15.7	-15.6
3	-13.6	-13.6	-13.5
4	-13.2	-13.2	-13.1
5	-21.5	-21.1	-21.5
6	-11.5	-10.8	-11.6

Due to the hygroscopic nature of the amino acids and peptides, yield measurements were not sufficiently precise for good comparison but in general, method 2 appeared to give lower yields than either of the other methods. The $\delta^{13}\text{C}$ values for methods 1 and 3 were virtually identical with a difference of 0.1‰ , about equal to the measurement precision. The samples treated with NaOH varied with respect to those otherwise treated by as much as 0.8‰ , as was found by DeNiro and Epstein (1981). This may be due to partial hydrolysis and preferential removal of portions of the intact collagen that had remained after the HCl extraction. Since $\delta^{13}\text{C}$ values may differ for the various amino acids (Abelson and Hoering 1961) differential removal of peptides or amino acids could bias the results unpredictably. Because of this

and because of the apparently reduced yields method 2 has been avoided and Grootes' method was adopted for use.

Determination of gelatin purity

The obvious check on the extraction method used is to carry out an analysis of extraction products. To do this the C/N values were determined (Table 5) for the samples shown in Table 4 and amino acid analyses were done on other human samples, of which five typical examples are shown in Table 6.

Table 5 C/N values, %C and %N for extraction method test samples. *

Sample	Method 1 (Longin)			Method 2 (NaOH)			Method 3 (Grootes)		
	%C	%N	C/N	%C	%N	C/N	%C	%N	C/N
1	32.0	11.5	2.79	15.9	5.9	2.78	40.3	14.1	2.87
2	36.4	13.2	2.76	19.5	7.0	2.81	37.0	13.2	2.82
3	37.2	13.1	2.85	27.8	10.0	2.79	43.6	15.6	2.80
4	33.2	11.7	2.82	21.2	7.6	2.78	44.2	15.9	2.79
5	31.1	11.0	2.83	20.2	7.1	2.84	41.4	14.7	2.82
6	26.9	9.5	2.83	15.6	5.6	2.81	37.3	13.1	2.85
Average	32.8	11.7	2.81	20.0	7.2	2.80	40.6	14.4	2.83
($\pm 1\sigma$)	3.8	1.4	0.03	4.4	1.6	0.02	3.1	1.2	0.03

Overall C/N average = 2.81 ± 0.03 (n=18).

* % C and % N are percent by weight.

The British Columbian samples used for the extraction method tests (Table 4) gave an average C/N value of $2.8 \pm 0.03\%$ (n=18), very close to the expected value of about 2.7 that was discussed in the previous chapter. There is no significant difference in C/N results for these samples whether treated with or not treated with NaOH (Table 5). Explanations for this difference in results from DeNiro's (1985) are difficult because he did not describe his C/N analysis methods (the B.C. ones were obtained by dry combustion). Although NaOH may remove amide nitrogen (Balian and Bowes 1977), it is possible that for some reason the carbon in these samples was reduced proportionally by the same amount, thus maintaining the C/N ratio. This problem would benefit from further investigation.

The close similarity of C/N results plus the small overall standard deviation shown for samples prepared by the three processes, which gave slightly different $\delta^{13}\text{C}$ results (Table 4), suggest that all of these samples are of equal purity. Thus the use of C/N values is not helpful in explaining the slight difference in $\delta^{13}\text{C}$ results found for the three extraction methods. Based on '%N' results (Table 5) the samples prepared by the Grootes' method appear to have the highest levels of protein, while the NaOH method yields the lowest. The '%C' results support this observation. Since there is no obvious disproportionate loss of nitrogen, or presence of contaminant carbon, it is likely that the lower '%C' and '%N' values indicate greater loss of collagen or peptides and amino acids from the Longin and NaOH treated samples, or the failure to remove all of the inorganic materials. Due to the hygroscopic nature of the gelatin, yield measurements were inconclusive. The non-carbon bearing components have not been identified.

Using the theoretical values of 50% C and 18.6% N for gelatin (Chapter 2) we can estimate the effects of contamination on the C/N value. A relative increase of carbon of 10%, due to the presence of nitrogen free compounds such as lipids or carbohydrates would yield a C/N value of 3.0. The average C/N value for the samples extracted by the Grootes method is 2.8 ± 0.03 which may indicate a relative addition of 4.3% (± 0.03) contaminant carbon. It could also indicate a relative loss of nitrogen of up to 5% due to sample processing, which is quite plausible as the NH_3 groups in proteins are quite labile, often leading to free NH_3 or NH_4^+ in amino acid hydrolysates.

To further check the product of the Grootes extraction, amino acid analysis of the gelatin has been carried out for a number of samples, of which five representative samples are shown (Table 6). It is interesting to note that the major components (hydroxyproline + proline, glycine, and alanine) for the NaOH-treated samples deviate further from the expected values than in samples extracted according to the Grootes method. This may be taken as further evidence that NaOH treatments modify the gelatin and thus may be detrimental. There is no evidence of amino acids such as ornithine, cystine or tryptophane, which are common in plant and other animal proteins (other than collagen) and should be present in at least small quantities if such contaminant proteins were present. In addition, the proportions of about 1/3 glycine and 1/5 hydroxyproline plus proline, match the characteristic pattern for collagen (Kuhn 1982; White et al 1978; Woodhead-Galloway 1982). On the basis of these analyses one may assume that the extraction product is gelatin from collagen and that it contains few or no other protein products. It is still necessary to identify and eliminate any other non-protein compounds that may remain in the gelatin. This is still being investigated.

Table 6 Amino acid analysis of 5 representative samples of gelatin.

Amino Acid	Expected Values *	Sample Values				
		Grootes Method			NaOH Method	
		069	049	058	201-5	201-C
Hydroxyproline plus Proline	21.6	22.2	19.6	20.1	18.2	18.6
Aspartic Acid	4.6	4.8	3.4	4.4	3.7	4.6
Threonine	1.6	2.0	2.0	1.5	1.3	1.3
Serine	3.1	3.1	3.0	2.5	2.1	2.3
Glycine	33.0	30.0	32.0	31.0	28.8	29.6
Glutamic Acid	7.5	7.1	6.7	6.8	6.0	6.5
Alanine	11.4	10.6	11.4	10.9	13.1	13.9
Valine	2.3	2.7	0.9	2.6	3.0	2.5
Methionine	0.6	0.3	0.4	0.2	0.4	0.4
Isoleucine	1.2	1.6	1.1	1.0	1.0	1.1
Leucine	2.5	3.1	2.5	2.5	2.5	2.4
Tyrosine	0.4	0.6	0.3	0.9	0.2	0.2
Phenylalanine	1.3	1.6	1.0	0.8	1.2	1.0
Lysine	3.5	0.8	2.7	4.3	4.3	2.8
Histidine	0.5	0.4	0.4	0.3	0.5	0.3
Arginine	5.2	4.7	4.8	4.3	5.4	4.0
Ammonia		4.4	7.8	4.3	8.3	8.8

* Kuhn (1982); individual residues are expressed as % of total residues. The error on the sample results is ca. $\pm 5\%$.

Tests of lipid extraction

None of the above techniques specifically removes any lipids that could be present in the samples. Lipids are known to have $\delta^{13}\text{C}$ values that differ by up to $7.9 \pm 1.6\text{‰}$ from those of protein in the same animal (DeNiro and Epstein 1978; Vogel 1978). If the C/N values shown in Table 5 do indicate a contamination by lipids as opposed to other compounds, of $4.3 \pm 0.03\%$, and if lipids are $7.9 \pm 1.6\text{‰}$ removed from protein as discussed previously, then we would expect the value for the protein samples to be in error by $0.3 \pm 1.6\text{‰}$.

The relative amounts of lipid and protein in bone and hence the average $\delta^{13}\text{C}$ value of the whole bone will depend upon the nutritional circumstances of the animal at death and upon the different diagenetic processes that operate after burial. The lipids are unlikely to be present in bone for long periods after burial as they will be physically removed by ground water. However, to avoid variability due to the possible presence of lipids it is wise to ensure they are removed entirely before measurement.

To determine the magnitude of this effect we (Chisholm et al 1983a) sampled bone from six individual modern deer (*Odocoileus* sp.), extracted collagen by the Grootes' method, and evaporated the resulting gelatin to dryness. Each sample was then split into two parts, one of which was extracted with hot water as in the Grootes' method. The other part was freeze dried to remove all water and then treated with a chloroform / methanol / water mixture (Bligh and Dyer 1959) to remove any lipids. To ensure that there were no lipids remaining in the gelatin, sub-samples of gelatin were again extracted with chloroform. No further lipids were observed. The residue was again dried and then subjected to the hot water extraction. The measurement results are given in Table 7. In general the values for the lipid-free samples are very slightly heavier isotopically than those that were not treated for lipids. This agrees generally with other studies (DeNiro and Epstein 1978; Vogel 1978). As the error on $\delta^{13}\text{C}$ measurements here is 0.08‰ the differences for samples 2, 4, 5 and 6 are not significant. The difference for samples 1 and 3 may indicate that some lipids were still present in these modern unextracted bone samples. The conclusion reached from these test results is that the Grootes method with lipid extraction successfully removed all of the non-gelatin components including lipids, from modern bone samples.

Table 7 Effect of removing lipids from modern *Odocoileus* sp. bone samples

Sample	$\delta^{13}\text{C}$ (‰) (Method 3)	$\delta^{13}\text{C}$ (‰) (Method 3 plus lipid extraction)	Difference (‰)
1	-21.5	-18.8	2.6
2	-20.4	-20.3	0.1
3	-21.4	-21.1	0.3
4	-21.2	-21.1	0.1
5	-20.5	-20.4	0.1
6	-21.0	-21.0	0.0

While modern bone contains high levels of lipids, which can be easily seen (and smelled) when the bone is cut, archaeological bone appears to lose lipids after even short periods of burial, at least in samples recovered from sites in British Columbia. Attempts to extract lipids from 10 samples of archaeological bone from various locales, and of various ages, in British Columbia yielded no detectable lipid residues. For that reason it was felt that lipid extraction was not necessary for archaeological bone samples obtained in British Columbia. Since depositional conditions will vary in other locales it will be necessary in other areas to test for lipid presence before assuming it is safe to omit lipid extraction from bone sample preparation. Lipids were not extracted from the British Columbian archaeological samples.

Variation in one individual's gelatin

Having decided upon the sample preparation technique it was possible to examine potential variation in the collagen itself (Table 8). To do this, sample splits were taken from the same bone for each of five adults and

separately extracted and measured. Samples were also taken from different bones for each of seven adults and separately extracted and measured. The humans sampled came from different sites on the coast and in the interior of British Columbia. None of the samples showed any evidence of alteration or diagenesis.

Table 8 Reproducibility of gelatin results for different extractions from the same individual.

<u>Sample</u>	<u>Number</u>	<u>Average Difference</u>	<u>Range</u>
Same bone	5 pairs	$0.1 \pm 0.06 \text{ ‰}$	0.07 - 0.2‰
Different bone	7 pairs	$0.2 \pm 0.13 \text{ ‰}$	0.06 - 0.3‰

These results indicate that the average difference between separate extractions and measurements of samples taken from the same bone is not significant, being within the measurement error of the technique. For different bones from the same individual the difference between results is only slightly greater than the measurement error. We can therefore assume that intra-individual differences in collagen $\delta^{13}\text{C}$ values are not significant for adults, but cannot confirm this for growing children yet. These results also indicate that we cannot make valid comparisons between individuals, or between single individuals and populations, that differ by 0.2‰ or less.

Preparation of dietary samples

The procedure followed in preparing dietary samples was to trim excess fats from the meat samples and then to freeze dry the meat or just to freeze it for storage. Initially, measurements were made on samples that had not been treated to remove lipids. Later examination of the literature, as

discussed in chapter 2, suggested that lipids should be extracted from dietary samples so the process was altered. Dried meat samples were homogenized and lipids extracted with a chloroform / methanol / water mixture (Bligh and Dyer 1959). This process, carried out in a separatory funnel, was repeated twice to ensure completeness. Since use of a third extraction step did not yield any further lipids the two step process was assumed to be sufficient. Samples were then oven dried and about 5 mg loaded into Vycor tubes for isotopic analysis. Samples used in the preliminary study were extracted and remeasured and are discussed in Chapter 4.

Discussion and Summary

Combustion of samples for measurement should be carried out at 900°C in Vycor tubes until such time as other acceptable methods are demonstrated. Gelatin extraction should be carried out using the Grootes method with no NaOH wash being used. To check quality of extractions C/N values and amino acid analysis results may be used, but they may not detect low levels of contamination. Further quality control methods must be developed. Lipids in bone may affect the results for modern bone samples and therefore must be extracted before combustion. In archaeological samples from British Columbia there was no evidence of lipids in the bone so the lipid extractions may be omitted, thus simplifying and speeding up the process.

The precision of analyses for the instrument used in this study is $\pm 0.08\%$. Instrumental error is therefore not going to contribute noticeably to uncertainties for the results. The variation in a set of samples is made up of 4 components: 1) that due to sample preparation and measurement, represented by the reproducibility of results for multiple extractions and measurements of the same bone of $\pm 0.1\%$; 2) any intra-individual variation

due to the consumer's metabolism and that is reflected in the gelatin, which is expressed by the intra-individual difference of $0.2 \pm 0.1\%$ in gelatin values, which will also incorporate any reproducibility uncertainty; 3) inter-individual variation in ' Δbc ', which will also reflect the consumers' metabolism and is discussed in Chapter 5; and 4) that introduced by the foods that the consumers ate, which will be discussed with the human results in Chapter 6. The overall uncertainty for a set of sample results will include all four of these variabilities, and if the first three can be determined will allow us to observe the variation within a group of humans that is attributable to differences in their average diets.

5 Determination of the diet - consumer increment ' Δbc '

As discussed in Chapter 2, one of the items of information necessary for the application of the technique is the value and the variability for the increment between a consumer and its diet, or ' Δbc '.

To determine the magnitude of ' Δbc ' four female mice and four female rats were fed on a diet of Purina Rodent Laboratory Chow #5001 (batch Nov 08 832) for about two weeks and then were bred. After weaning, the offspring were raised on Laboratory Chow from the same batch. Two individual mice were taken every ten days until day 100 when the remaining mice were also sacrificed. The rats were all grown to 150 days. Coincidentally a number of cat cadavers from another study became available. They had been fed a constant diet of Pamper cat food and Pacific Evaporated Milk, both from single lots. The cat ages varied from 0.5 to 90 days.

For each individual, bone samples were taken and hand cleaned. The bones were ground, lipids were removed and the collagen was extracted using the Grootes' method. Samples of the Laboratory Chow, Pamper, and Pacific Milk had their lipids removed and were oven dried for analysis. Sample analysis was as described previously for this study. The results of the analysis are summarized in Table 9.

Since the mice and rats ate only rodent chow and water, the rodent chow value is applicable as their diet value. However, the cats ate a mixture of cat food and milk. The proportion of the two could, and probably did, vary between individual cats, thereby introducing some variability into the cat results. Since the cat food and milk have different values it was necessary to assume a weighted average for the cat's diet. Since the protein content of the evaporated milk is only about 8% (Watt and Merrill 1963:39) it is likely that the majority of the cat's protein is derived from the cat food. In this case it

is assumed that milk contributed 20% of the cat's dietary protein. Because of this assumed value it is not wise to include the cat results in the overall average for ' $\Delta\delta\text{C}$ '. They provide a general indication of ' $\Delta\delta\text{C}$ ' for the cats.

Table 9 Results of the ' $\Delta\delta\text{C}$ ' study (± 1 standard deviation)

<u>Diet</u>	<u>Diet</u> $\delta^{13}\text{C}$ (‰)	<u>Consumer</u> <u>Species</u>	<u>Consumer gelatin</u> $\delta^{13}\text{C}$ (‰)	<u>'$\Delta\delta\text{C}$'</u> [†] $\delta^{13}\text{C}$ (‰)
Rodent chow (n=4)	-19.7 \pm 0.7	Mouse (n=20)	-15.1 \pm 0.6	4.6 \pm 0.4
		Rat (n=11)	<u>-15.4 \pm 0.5</u>	<u>4.3 \pm 0.4</u>
Combined		(n=31)	-15.2 \pm 0.5	4.5 \pm 0.4
Cat food (n=2)	-18.4 \pm 0.1			
Milk (n=1)	<u>-23.4 \pm 0.02</u> *			
Weighted avg. **	-19.4 \pm 0.1	Cat (n=16)	-15.0 \pm 0.9	<u>4.4 \pm 0.3</u>

[†] result given is the difference between diet and consumer mean values.

* error stated is the average for 2 analysis runs of 10 counts each.

** the weighted average value for the cat diet assumes that cats are obtaining about 20% of their protein from the milk.

In addition to these results there were nine that were isotopically lighter and over 2 standard deviations away from the mean for their species. As the person who prepared the samples mentioned that six of them might still have some lipids remaining, sub-samples from all of the outliers were re-extracted with chloroform. Nine samples yielded traces of what appeared to be lipid residue. Three other samples were very small and yielded little CO₂ for analysis. Values for these 3 samples were considered suspect

(T. Saling, University of Washington, 1986, personal communication). While these 12 samples were excluded, any other samples with outlying values have been included in the data set. In spite of this it is clear that the mice and rats are similar ($.1 < p < .375$) and may be combined, giving a value for ' Δc ' of $4.5 \pm 0.4\%$. This is between the values suggested by DeNiro and Epstein (1981, 1978) for mice and by van der Merwe and Vogel (1978) for ungulates (see Table 2), and is lower than the value of 5% used initially in this study. Recently van der Merwe (1986; Table 1) has reported a ' Δc ' value of 4.7% for a small South African sample of modern ungulates and the carnivores that would be consuming them. This means that ' Δc ' values of about 4.5% have been observed for mice, rats, cats and large mammals. Because the metabolic chemistry of mice, rats, cats, ungulates, humans, and other vertebrates differs very little, the value of ' Δc ' = $4.5 \pm 0.4\%$ should also apply to humans. For convenience, and since human data is not available, this value is rounded to ' Δc ' = $4.5 \pm 0.5\%$.

The variability of $\pm 0.4\%$ observed here suggests that there are inter-individual differences in ' Δc ' for mice and rats. Since we have no results from controlled studies on humans we do not know the extent to which inter-individual variability may exist for them. Inter-individual variability in ' Δc ' for humans will contribute to the uncertainties for human population results. If the uncertainty for the human populations is small, as suggested by Lovell et al's (1986) results, then we can be assured that the inter-individual variability is also small. This will be discussed further with the human results of this study, in the next chapter.

In summary, the measurements reported here indicate the best value for ' Δc ' is $4.5 \pm 0.5\%$. This value will be used later for determining dietary proportions.

6 The applicability of the technique, particularly in British Columbia.

As discussed in Chapter 2 the absence of consumers known to have subsisted exclusively on either marine or terrestrial diets means that the human end-points cannot be determined directly. Therefore, in order to relate human $\delta^{13}\text{C}$ results to palaeodiet in British Columbia we first need to know the average values and the variabilities of the marine and terrestrial diet alternatives, and the value and variability of ' Δdc ' for humans. Given this information we may also estimate the end-points for the humans. Before examining the results it is first necessary to know whether the observations are normally distributed and hence random and allow statistical comparison.

6.1 Dietary species

Preliminary analyses

When this project was initiated it was not known whether the isotopic approach was applicable to marine versus terrestrial comparisons; therefore, a preliminary study was carried out (Chisholm et al 1982, 1983).

Examination of the ethnographic and archaeological literature produced a list of those species which occurred frequently, and hence were most likely used as dietary meat. Potential dietary species were divided into those from the marine and littoral, and those from the terrestrial environments. While some species are represented in archaeological deposits the ethnographic record indicates that for various reasons they were not normally used as food. Examples are: reptiles, owls, ravens, bush rats and mice, muskrat, mink, fisher, marten, fox, wolverine, bobcat, cougar, wolves, coyotes, river otters, sharks and killer whales (Barnett, 1955; Duff, 1952; Suttles, 1955, 1974; Teit, 1956). These species would have been sufficiently uncommon that they

need not be measured for this study. Both dog and dogfish remains have been found but it isn't clear whether they were eaten.

Samples of muscle tissue from animals on the list were obtained for 19 species from British Columbia coastal waters, and from 19 terrestrial species from various areas of British Columbia. For analysis the muscle tissue samples were freeze dried, but lipids were not removed. Sample combustion and measurement was as described in chapter 4.

The mean value and standard error found for the flesh of the terrestrial animal population was $-25.7 \pm 0.3\%$ and for the marine animal population was $-17.8 \pm 0.3\%$, giving a difference of $7.9 \pm 0.4\%$ (Chisholm et al 1982, 1983). The difference of ca. 8‰ between oceanic and atmospheric carbon reservoirs indicated by Stuiver (1983) is thus preserved at this level in the food chains. For this reason and because the values for consumers were known to reflect their diet it was concluded that $\delta^{13}\text{C}$ measurements may be used to determine the relative proportions of marine- and terrestrial-based protein in aboriginal diets on the British Columbia coast.

Dietary sample requirements and sample selection

The preliminary results have demonstrated a difference in $\delta^{13}\text{C}$ values between species from the marine and from the terrestrial environments but they have not accounted for the potential variations in reservoirs, herbivores and food chains that were discussed in chapter 2. To account for such variations it is necessary to select samples to determine whether geographic differences in the $\delta^{13}\text{C}$ values of food species are a factor or not. Specifically, samples of the same species must be examined from different geographic areas. Because fewer terrestrial species and trophic levels have been reported from archaeological deposits, and since there are no humans

from northern interior areas sampled in this study, it should be sufficient to compare a representative cross section of species from northern interior areas with a similar set of samples from southern interior areas. The situation is more complex for marine species due to potentially greater variations in the local environments. In areas near large rivers there may be an influx of terrestrial runoff materials, including organic carbon debris, that could affect the local species. Such influences are probably greater in enclosed areas, like the Gulf of Georgia, than in more open areas, like Prince Rupert. Accordingly, a number of areas should be sampled, i.e., 1) the Prince Rupert, Skeena River and Nass River area, 2) the Queen Charlotte Island area, 3) the Namu area, 4) the West Coast of Vancouver Island, and 5) the Gulf of Georgia. These coincide with the areas from which the human samples discussed in Chapter 8 were obtained.

There may be differences along the length of longer coastal inlets that affect localized species, particularly shellfish. Terrestrial biogenic carbon may alter CO₂ isotopic ratios and fresh water will alter the salinity which may affect shellfish size and numbers (Barnes 1984). If possible it is advisable to sample such localized species at a few sites along the length of such an inlet.

It is possible to group the food species into categories that share similar diets, or types of diets, such as: terrestrial herbivores, marine herbivores, and marine carnivores. In at least one area a number of species in each category must be sampled to ensure that there are no intra-group differences. If there are none it will be permissible to sample only a few representatives from a group from each remaining area, using the same species in each area, preferably the most commonly used ones. For terrestrial species, the major sample can be taken in either the southern or northern portions of the

province. The comprehensive marine species sample should be obtained from an area not influenced by runoff from large rivers.

During the breeding season birds tend to suffer stress which interferes with growth and weight build up, and for marine - terrestrial consumers this effect restricts growth to the marine, or non-breeding, portion of their cycle (K. Hobson, SFU, personal communication). Therefore their $\delta^{13}\text{C}$ values may reflect a more marine diet average. This factor, plus the low levels of human use of birds reported means that their terrestrial contribution should be minimal. They should be sampled in the same manner as the other species.

Most commonly used fish species and sea mammals are migratory and should not reflect local variations in $\delta^{13}\text{C}$ value, if any exist. To ensure this it will be sufficient to sample two separate regions; a southern and a northern one. Differences between the sampled areas will be best detected through examination of the local shellfish species.

These criteria define an ideal dietary species sample for isotopic paleodiet studies in British Columbia. There are likely to be difficulties in obtaining some of the samples. For example, sea mammals are not commonly available as they are not hunted any more. Contamination by carbon-containing materials such as sewage and terrestrial biogenic wastes in coastal inlets and in the Gulf of Georgia may interfere with obtaining shellfish samples for comparing different environments.

Dietary sample results

Samples for this study were obtained by a number of means, including field collection, obtaining samples from hunters, trappers and fishermen, and purchasing samples from fish markets. Some samples were taken from specimens being prepared for the Archaeology Department faunal collection,

while others were obtained from the British Columbia Provincial Museum, and through the Canadian Wildlife Service.

Forty six of the dietary samples used in the preliminary study and 73 additional ones were extracted to remove lipids as discussed in chapters 3 and 4. Since lipid content may vary, often quite widely, between individuals and between species, and since lipids are depleted in ^{13}C relative to protein and carbohydrates, the expected results were 1) slightly heavier $\delta^{13}\text{C}$ values for the extracted samples when compared to their unextracted equivalents, and 2) a reduction in the variation in the average values for the diet alternative groups. Since marine species generally are higher in lipid content than terrestrial species their change should be greater. Results for the 46 extracted versus non-extracted pairs are shown in Table 10.

It is clear that extraction of the lipids did not produce the expected results. Samples that had been freeze dried and then extracted gave results that were significantly displaced in the opposite direction to expectation. Marine samples that had not been freeze dried before extraction appeared to respond as predicted but the shift of 0.9‰ in $\delta^{13}\text{C}$ values was not statistically significant. The terrestrial samples that had not been freeze dried exhibited no shift. The effect of freeze drying on the non-extracted samples was also not statistically significant, suggesting that freeze drying does not affect the protein carbon itself. In sum, the freeze dried, lipid extracted samples differed significantly from all others and showed an increase in variability. All other sample groups were statistically similar.

At the moment we can only speculate as to the reason for these results. No lipids were evident after 2 extractions i.e., a third extraction step produced no more lipids. Discussion with G. Buono Core of the Chemistry Department at Simon Fraser University led to one possible explanation for the

unexpected results. It is possible that there were reactions between the methanol and the meat carbohydrates, lipids, and/or protein. Such reactions are common, catalyzed by the presence of trace amounts of acids in the chloroform that is used for the lipid extraction. Apparently chloroform commonly contains traces of acid, and should be passed over basic alumina to remove them before use. As all of the freeze dried samples were stored together and then processed together this is a possibility. Use of a different batch of chloroform on the other extracted samples may explain the difference between the freeze dried and non-freeze dried samples.

Table 10 Results ($\% \pm 1\sigma$) for the 46 paired samples used to test lipid extraction.

<u>Description</u>	<u>(n=)</u>	<u>non extracted</u>	<u>extracted</u>	<u>shift *</u>
Terrestrial species				
Freeze dried	(16)	-25.7 \pm 1.6	-27.8 \pm 1.8	-2.1 \pm 1.3
not freeze dried	(8)	-24.5 \pm 1.2	-24.4 \pm 1.8	+0.1 \pm 1.0
Fresh water species				
freeze dried	(2)	-29.1 & -31.9	-31.0 & 34.9	-1.9 & -3.0
Marine species				
freeze dried	(5)	-17.4 \pm 1.4	-19.7 \pm 1.9	-2.3 \pm 0.8
not freeze dried	(9)	-18.0 \pm 1.4	-17.1 \pm 1.5	+0.9 \pm 2.1
Marine/Terrestrial birds				
freeze dried	(2)	-20.8 & -14.9	-23.3 & -16.2	-2.5 & -1.3
not freeze dried	(2)	-19.2 & -19.3	-18.0 & -19.2	1.2 & 0.1
Steelhead				
Freeze dried	(2)	-20.7 & -21.5	-22.3 & -22.6	-1.6 & -1.1

* values given are the averages and standard deviations (at 1σ) of the differences for individual pairs.

Of course another possible explanation is operator error. Some lipids are soluble in methanol and some in chloroform. If a mistake was made in the proportions of the two it is possible that not all of the lipids were removed. Since different lipids have different $\delta^{13}\text{C}$ values (DeNiro 1977, Galimov 1985) this could bias the results for the diet samples. However, since the solvents were mixed separately for each sample and not in a batch this explanation is unlikely. As the method of Bligh and Dyer was designed for extracting lipids for analysis and not for removing contaminants from protein samples, it may be that a different method would be more appropriate. These are factors that may explain the unexpected results, but further sample collection and more detailed chemical investigation than has been done here will be necessary to solve the problem.

These unexpected results introduce a problem to the study. The preceding discussions suggest strongly that it is necessary to remove lipids from dietary samples in order to minimize uncertainty in the dietary alternative values. The obvious procedure to follow here would be to go back to the original flesh samples and redo the lipid extractions with new solvents and without freeze drying the samples first. Unfortunately they are no longer available so new samples must be obtained and measured. In addition it will be necessary to check the lipid extraction method and to identify the various components extracted and left behind, and perhaps to test other lipid extraction methods. As this will entail a long delay it was decided to omit all of the lipid-extracted samples from consideration at this time. This means that the sample numbers are reduced to 103 and their utility restricted. In that very few diet samples reported in the literature have had their lipids extracted before measurement these results will be comparable

to other studies. In spite of the difficulties with diet samples some preliminary geographic comparisons can be made (Table 11).

Before making such comparisons it is necessary to consider which values should be used to describe the variability in the results. Since we are interested in comparing a population of diet species that will be sampled many times by a number of different humans the variability in the human diet is best represented by the standard errors ($\bar{\sigma}$) for the diet alternative averages and not by their standard deviations.

Table 11 North - South comparison for modern terrestrial diet samples.

	Northern samples	Southern samples
<u>Description</u>	<u>$\delta^{13} (\text{‰} \pm 1\bar{\sigma}) (n=)$</u>	<u>$\delta^{13} (\text{‰} \pm 1\bar{\sigma}) (n=)$</u>
Herbivores	-26.1 \pm 0.3 (17)	-25.1 \pm 0.5 (11)
Omnivores		-23.9 & -24.7
Birds	-25.5 \pm 0.5 (9)	-24.8 \pm 0.7 (6)
Freshwater species	<u>-27.1 & -31.9</u>	<u>-29.1 & -27.3</u>
MEAN	-26.1 \pm 0.3	-25.2 \pm 0.4
Steelhead	-20.7 & -21.5	-22.3 & -23.2

The difference in mean values for the two regions is greater than $2\bar{\sigma}$ which means that the results for the two areas are different and that it is not permissible to combine the results for the northern and southern areas to obtain an average value for the terrestrial diet alternative (Table 13). This geographical difference reinforces the idea that we must sample foods in the study areas and cannot rely on data from other areas.

One reason that southern samples are isotopically heavier may be because herbivores are eating small quantities of C₄ species. Teeri and Stowe (1977)

indicate that grasses in the southern dry areas, such as the Thompson River and Okanagan Valleys from where these samples were recovered, may be as much as 6‰ C₄. Other possible causes of the north - south difference may be that the lipid content of the animals is different in the two areas, or that the regional climate may affect local reservoir and/or plant, and thus herbivore, $\delta^{13}\text{C}$ values.

Additional variation in results could arise from the incorporation of steelhead in the diet. They spend part of their life in the ocean and part in rivers; hence they have intermediate values which could bias consumer results away from the terrestrial towards the marine average. This type of error would be eliminated only if steelhead were not a diet choice.

A higher lipid content would explain the isotopically lighter value for the freshwater species (trout and river mussels), but until the lipid extraction problem is solved this must remain speculative. These species yield isotopically lighter values than either marine or other terrestrial species and if present in human diets may bias results towards the terrestrial alternative they represent. Thus they will not give a marine bias to consumer results but could bias marine consumer results towards terrestrial values.

Geographic comparisons for the marine diet samples along the British Columbia coast are more limited due to the loss of data; however, some comparison is possible (Table 12).

When the combined results for each of the areas are compared only Central Coast halibut and two clams from Tsawassen Beach differ significantly from the others. This permits the results to be combined and gives a modern marine average and standard error for the whole coast of $-17.7 \pm 0.2\text{‰}$ (n=44), including the two clams from Tsawassen. These clams exhibit an isotopically lighter value which likely results from the presence of

terrestrial biogenic carbon in the Fraser River outwash. (This difference is evident in the rejected samples also.) The reason for the difference in halibut from the Central Coast is not known.

Table 12 Modern marine diet sample results ($\text{‰} \pm 1\sigma$) by regions

<u>Gulf of Georgia</u>	<u>Vancouver Island</u>	<u>Central Coast</u>	<u>Queen Charlottes</u>	<u>Prince Rupert</u>
Fish -17.7 \pm 0.4 (18)	-17.8 \pm 0.7 (4)	-15.0 & -15.7		-16.1 & -19.2
Sea Mammals -17.1	-16.4, -18.1, -18.4			
Shellfish -18.0 \pm 0.4 (4) (-20.6 & -21.2)*		-17.7 \pm 0.2 (8)	-16.5 & -16.8	
<u>All marine species: averages</u> -17.7 \pm 0.3 (23)	-17.7 \pm 0.4 (7)	-17.3 \pm 0.4 (10)	-16.7 (2)	-17.7 (2)

* these two samples were clams from Tsawassen Beach near the mouth of the Fraser River, and are not included in the averages.

All the usable values from the analysis (Appendix A) are summarized in Table 13. According to this data the best available modern average values for British Columbia are $\delta^{13}\text{C} = -17.7 \pm 0.2\text{‰}$ for a human diet based solely on marine foods, $-26.1 \pm 0.3\text{‰}$ for one composed solely of terrestrial foods from northern British Columbia, and $-25.2 \pm 0.4\text{‰}$ for a similar terrestrial diet in southern British Columbia. It is significant that the difference between marine and terrestrial alternatives reflects the difference of 8‰ between atmospheric and oceanic carbon observed for the North Pacific (Stuiver 1983).

Table 13 Summary of modern diet sample results for British Columbia.

<u>Description of samples</u>	<u>n</u>	<u>$\delta^{13}\text{C}$ (‰ $\pm 1\sigma$) †</u>
A) The marine diet alternative:		
Marine mammals	4	-17.5 \pm 0.5
Marine fish and shrimp	26	-17.5 \pm 0.3
Littoral shellfish	16	-18.1 \pm 0.3
POPULATION MEAN		-17.7 \pm 0.2
B) The northern terrestrial diet alternative:		
Terrestrial mammals	15	-26.0 \pm 0.3
Terrestrial birds	9	-25.5 \pm 0.5
Fresh water species	2	-27.1 & -31.9
POPULATION MEAN		-26.1 \pm 0.3
C) The southern terrestrial diet alternative:		
Terrestrial mammals	13 *	-24.9 \pm 0.4
Terrestrial birds	6	-24.8 \pm 0.7
Fresh water species	2	-29.1 & -27.3
POPULATION MEAN		-25.2 \pm 0.4
D) Species found in both coastal and interior areas:		
Steelhead	4	-22.6 \pm 0.4
Salmon **	8	-17.7 \pm 0.5
Marine - Terrestrial birds	3	-19.8 \pm 0.5

† values given with standard errors at 1σ .

* including two black bear from the southern region.

** these have also been included with the marine fish and shrimp. They are shown separately here because they are found in the interior rivers.

It is also interesting to note that the 8‰ difference also applies to those samples that gave the questionable results (Table 10). $\delta^{13}\text{C}$ results for the diet alternatives were tested using the Shapiro-Wilk test (Conover 1980: 363ff) and were found to be normally distributed.

Since marine and terrestrial species are separated by about 8‰ they can be clearly distinguished on the basis of $\delta^{13}\text{C}$ values, and since consumers, including humans, have been shown to reflect their diets it is possible to use $\delta^{13}\text{C}$ values for people in coastal and interior riverine situations to determine whether they obtained their protein from either marine or terrestrial sources.

It must be noted that these are modern samples of the diet species, so that for consideration as prehistoric diet analogs a correction of about $+0.7 \pm 0.2\text{‰}$ must be made to account for fossil fuel effects, as discussed on page 15. As a result the $\delta^{13}\text{C}$ value for a prehistoric marine-based diet would be -17.0‰ , for a prehistoric terrestrial-based diet in northern British Columbia it would be -25.4‰ , and for southern interior British Columbia it would be -24.5‰ . The uncertainty on the fossil fuel induced shift will not affect the variability for the diet samples so the standard deviations and errors of the modern sample results (Table 13) are taken to be representative of the prehistoric diet alternative values.

6.2 Human population results in British Columbia

In Chapter 2 the information necessary to establish the applicability of the technique was outlined. We already know the modern dietary average values and ' Δdc ' and have some information on individual human variability but we also need to know the variability associated with human populations. This will tell us if comparisons of the human groups are permissible and give us information on the amount of variability that may be attributable to

inter-individual differences in $\delta^{13}\text{C}$ and to variation in the local diets. Before proceeding it is necessary to know whether the observations are normally distributed and hence random and thereby allow statistical comparison.

As with the dietary species, $\delta^{13}\text{C}$ results for all human groups in the study area containing more than 7 individuals were tested for normality of distribution using the Shapiro-Wilk test (Conover 1980: 363ff). All sample sets are normally distributed except one, from EISx 1 (Part II, figure 3), which is a marginal case at the .05 level. It is skewed slightly, but was considered to be close enough to normal to permit unpaired 't' testing. The acceptance level applied here for unpaired 't' test results is the commonly used level of $.05 < p \leq .1$. The fact that the human results are normally distributed with small standard deviations (of about 0.2 to 0.7‰) means that the variations are random, and that the population may be characterized by a relatively small number of individuals, say 5 or 6.

Any individual human values more than 2 standard deviations (2σ) from the mean for their particular sample set are considered as outliers and are discussed later in this chapter. They have been omitted from any statistical testing, but are listed in the tables and in Appendix B.

For prehistoric coastal humans (Part II, Table 19), who would have subsisted primarily on marine species, there are three groups that include 14 or more individuals. Their average values and standard deviations (at 1σ) are $-13.3 \pm 0.6\text{‰}$ ($n=28$) for EISx 1 adults, $-13.0 \pm 0.5\text{‰}$ ($n=43$) for DeRt 2 adults, and $-13.5 \pm 0.3\text{‰}$ ($n=14$) for DgRr 1 adults. The average and standard deviation for the total coastal adult sample is $-13.3 \pm 0.7\text{‰}$ ($n=151$, $1\bar{\sigma} = \pm 0.1\text{‰}$).

In the interior there are two prehistoric groups with slightly lower standard deviations (Part II, Table 20), i.e., the Lillooet area adults with an average of $-15.5 \pm 0.2\text{‰}$ ($n=9$) and the Thompson River area adults with an

average of $-15.8 \pm 0.3\text{‰}$ ($n=14$). That the standard deviations for coastal people are greater than for interior people should not be surprising because there are more marine species available as diet alternatives. In addition, marine diet species values themselves may be expected to be more variable as some marine species migrate through and exploit more environments and carbon reservoirs than terrestrial species do. It is possible that some people were biased in their food choice, selecting species with heavier isotopic values.

Standard deviations for the two interior groups and for the DgRr 1 adults are essentially the same as those for the southern interior terrestrial- and coastal marine-diet populations, of ± 0.3 and $\pm 0.2\text{‰}$ respectively. Removing the variability in results due to systematic and intra-individual metabolic factors, of about $\pm 0.2\text{‰}$ (page 55), leaves little residual uncertainty for the interior area and DgRr 1 samples, and presumably for the samples reported by Lovell et al (1986). This suggests that: 1) these people were living on monotonous diets and 2) inter-individual variability in ' $\Delta\delta\text{C}$ ' for the humans is small, essentially zero. The rats and mice discussed in Chapter 5 both exhibited a variability on ' $\Delta\delta\text{C}$ ' of $\pm 0.4\text{‰}$ and if we assume that the analytical and intra-individual variability is the same as for humans then the residual variability for the rodents would be about 0.2%, which is higher than for the humans. We would expect populations of consumers to show less variability than their diets because the consumers select foods and average the $\delta^{13}\text{C}$ values for a large number of meals. Consumers at higher trophic levels, such as humans, would benefit from the averaging effects of consumers at lower trophic levels, such as rodents.

As discussed in Chapter 3 intra-individual differences in gelatin values of $0.2 \pm 0.1\text{‰}$ have been found which means that comparisons of individuals

that differ by less than that will be invalid. This will apply to comparisons of single individuals with each other as well as to the comparisons of single individuals with larger sample sets. Barring sample contamination, differences that are greater than 0.2‰ will indicate dietary differences for those individuals.

There are three results, between -20.0 and -19.0‰, for prehistoric people from interior areas (DgQd Y, DIQu Y, and EbPw 1) where the diet likely included small amounts of salmon. They do not provide a terrestrial end-point value but do indicate that it will be -20.0‰ or less.

The prehistoric coastal population average of -13.3 ± 0.1 ‰ is 6.7‰ from the most extreme interior result, of -20.0‰, which is the same as the postulated southern terrestrial prehistoric end-point that will be discussed in the next section. This is less than but reflects the marine - terrestrial diet difference of 7.5‰ for southern British Columbia, indicating that humans consuming marine and C₃ terrestrial based diets can be distinguished. It also suggests that the prehistoric coastal people relied heavily on marine species.

A number of results are more than 2σ removed from the mean for the majority of the samples at the particular site(s) in question. Some are isotopically lighter by as much as 4‰ and represent bone samples that were reported in field notes to be charred or that were likely charred by either cremation or post-depositional heating. With two exceptions they did not show any visible evidence of burning, which brings up the possibility of using isotope analysis results to identify cremated or partially cremated remains. It has been shown by DeNiro et al (1985) that charring of bone causes changes in $\delta^{13}\text{C}$ values. As a result the warning given by Chisholm et al (1983a) to avoid burned bone has been substantiated and such samples have been disregarded. Barring contamination or charring of samples the isotopically-

lighter outlying values represent individuals who were obtaining more of their protein from terrestrial sources. This may be because they were visitors from some other area, it could result from individual dietary preferences or food taboos. Status differences, which may be indicated by burial goods, could also be responsible for the diet differences that are suggested by the $\delta^{13}\text{C}$ results. These samples are discussed further in Part II.

6.3 Interpreting the $\delta^{13}\text{C}$ results for humans

As discussed earlier, if a human samples both C3 and marine-based diet alternatives, the $\delta^{13}\text{C}$ value for its bone gelatin should lie between the human marine and terrestrial end-points. A simple linear interpolation, locating the sample value between the end-points, will result in a proportion estimate for the relative amounts of C3 and marine species in that consumer's diet. In order to do this it is necessary to know three pieces of information, the best value for each of the end-points and for the sample individual or population.

The prehistoric human end-point values are obtained by adding the value for ' Δdc ' to the modern diet alternative values and then correcting to account for the effects of fossil fuel carbon. (The uncertainties on the end-points will be discussed in the following section.) The best available value for ' Δdc ' has already been determined to be 4.5‰ (Chapter 5). The best values for the diet alternatives are the mean values shown in Table 13. Thus, modern Southern British Columbia dwellers obtaining all of their protein from terrestrial species should have gelatin $\delta^{13}\text{C}$ values of -20.7‰, and modern northern dwellers living on terrestrial foods exclusively would have a gelatin value of -21.6‰. A coastal dweller getting all of his protein from marine species should yield a gelatin value of -13.2‰. When the correction for fossil fuel carbon effects of about +0.7‰ is applied these values become

-20.0‰, -20.9‰, and -12.5‰ for prehistoric southern terrestrial, northern terrestrial, and marine end-points respectively. However, since this correction value is only known approximately, it is advisable to use rounded values of -20‰, -21‰, and -12.5‰ for these end-points.

For comparison, the northern prehistoric end-point is consistent with the terrestrial values of about -21.5‰, reported by Bender et al (1981) and Schwarcz et al (1985) for Archaic period individuals in eastern North America, who likely ate less meat and more C₃ plant foods than people on the Pacific Coast. The southern prehistoric end-point is slightly heavier isotopically, and over 1σ from the northern end-point. It is also equivalent to the value of -20‰ originally used by Chisholm et al (1982, 1983) and by Vogel and van der Merwe (1977) for prehistoric C₃-diet consumers. A rounded value of δ¹³C = -13‰ was used for prehistoric marine diet consumers by Chisholm et al (1982, 1983) based on results reported by Tauber (1981) and diet species measurements taken on the British Columbia coast.

Errors on the proportion determinations

When making proportion estimates we are locating either the value for an individual consumer or the mean for a population of consumers, in relation to the mean values for the two end-points. The distribution of results around the mean is of interest when comparing sample sets to each other, and in determining the degree to which diets vary, but is not important when estimating the proportions. Instead, we are interested in the accuracy, or reproducibility, with which the means are determined; or the standard error of the means, in this case at 1σ.

Since the end-points represent hypothetical populations who would have eaten monotonous diets we need to know the standard errors (1σ) typical of

such populations. For a prehistoric population subsisting on a monotonous, or nearly monotonous diet, Lovell et al (1986) observed a variability (1σ) of $\pm 0.3\%$, giving a standard error ($1\bar{\sigma}$) of 0.04% . As mentioned above, three prehistoric British Columbia populations, for whom the diet was likely quite monotonous, have standard deviations of 0.2 or 0.3% , and give standard errors of 0.07 to 0.08% . Therefore, a rounded value of $\pm 0.1\%$ has been combined with the uncertainty on the correction for fossil fuel carbon, of $\pm 0.2\%$ (Page 16), to obtain an uncertainty on the prehistoric end-points of $\pm 0.2\%$.

Since an isolated individual does not necessarily fall on the mean for the unknown population he came from, his uncertainty is best represented by the variability of that hypothetical population. This is best approximated by the variability of a typical population subsisting on a monotonous diet, of 0.3% (1σ). For populations the best uncertainty value to use will be the standard error ($1\bar{\sigma}$) since we are concerned with the accuracy, or reproducibility, of the mean for the subject population. This value may vary for different populations according to their size, but in this study ranges between 0.1 and 0.3% (Tables 19 and 20). Larger standard errors may also reflect greater variability in the diet of those particular populations.

For the estimates of proportions the uncertainties are calculated from: 1) the uncertainty on each of the two prehistoric human end-points, and 2) either the uncertainty for an individual or the standard error of the study population. For the individuals examined here this yields a calculated uncertainty on the proportion determinations of $\pm 6\%$ when taken over the 7.5% range between the prehistoric marine and southern-interior terrestrial end-points. Populations studied here have standard errors that range from ± 0.1 to $\pm 0.3\%$ which yield a range of uncertainties on the proportion estimates of ± 3 to 6% when taken over the same range. The uncertainty for an

individual is higher than for some populations because, as mentioned above, we cannot accurately locate an individual in relation to the mean for his population.

The uncertainty on ' $\Delta\delta\text{C}$ ', of $\pm 0.5\%$, suggests that the end-points may shift. If they do, both will shift in the same direction simultaneously, there will be no expansion or contraction of the range. This will have the effect of shifting the whole range up or down by up to 0.5%. This is equivalent to an additional uncertainty on the proportion estimates of 7%, thereby increasing the overall uncertainty on the proportions to about $\pm 9\%$ for an individual, and to about ± 8 to 9% for the populations studied here. In view of the assumptions made here, particularly about ' $\Delta\delta\text{C}$ ' and fossil fuel effects, it is probably wise to use a rounded off error of about $\pm 10\%$ for estimates of the relative amounts of C3 and marine species in prehistoric consumer's diets; for both individuals and populations.

Summary

The dietary alternative samples and the human samples were found to be normally distributed. Their $\delta^{13}\text{C}$ results are summarized in the next chapter.

Although the sample set was reduced in size, results of lipid extraction tests carried out on the dietary alternative species did not show a decrease in variability for extracted samples when compared to unextracted samples. Possibly the importance of lipids as a contributor to variability was over estimated. Unfortunately lipid extractions of dietary species were flawed so that it was not possible to determine for certain whether there would be a shift in the dietary alternative values due to lipid extraction. Nine marine species samples from which lipids were extracted did show an average shift of $+0.9\%$. This is one area where further research will be required.

7 Conclusions about the technique and its application, particularly in British Columbia

The preceding chapters indicate that stable carbon isotopic analysis results can be used to distinguish between marine and terrestrial dietary species. Since the $\delta^{13}\text{C}$ values for consumers reflect the $\delta^{13}\text{C}$ values of their diets, and since bone collagen preserves isotopic information, $\delta^{13}\text{C}$ values will also be useful for determining the relative amounts of marine and terrestrial protein in the diet of prehistoric people.

In Chapter 2 it was suggested that in order to apply this technique in paleodiet studies we need to know that: 1) the diet alternatives differ in the study area, 2) there is a constant and known increment between a consumer's $\delta^{13}\text{C}$ value and its average diet value, 3) metabolic differences are small enough that they don't interfere with any comparisons we wish to make, and 4) the material analyzed retains the required information. In addition, the analytical methods need to be reliable and must not introduce any errors or additional uncertainties to the results. These questions have been answered and the results are summarized here.

The technique

The stated precision of isotopic determinations, of $\pm 0.08\%$ for the instrument used here, is such that it will not contribute significantly to the uncertainties in dietary analyses.

Test results indicate that collagen extraction is best carried out using the Grootes' modification to Longin's (1971) method, without any NaOH treatment. Use of C/N values to check sample purity is not sufficient. Better methods must be developed and applied. At the moment the best indicator for gelatin purity seems to be a combination of C/N values and amino acid

analysis results, which have indicated here that the Grootes' method is the most reliable of those tested. The reproducibility of this extraction method was found to be $\pm 0.1\%$ on measurements for 6 separately extracted samples from the same piece of bone.

Gelatin samples must be carefully and completely dried before combustion. Until clearly demonstrated otherwise, extracted samples should be combusted in Vycor tubes at 900°C for 2 hours to ensure complete combustion of carbon to form CO_2 for analysis.

Isotopic variation found in gelatins should be due to dietary differences and not metabolic ones. Age and sex have been shown by Lovell et al (1986) to not influence $\delta^{13}\text{C}$ results. Tests here showed that the variation in gelatin for separate samples from the same bone is $0.1 \pm 0.1\%$ and that for samples from different bones of the same individual the variation is $0.2 \pm 0.1\%$. Thus the location of the bone sampled does not affect isotope results, except possibly in cases where different bones record changes in an individual's diet as he grows. However, these intra-individual gelatin differences of up to 0.2% preclude comparisons between individuals, or between individuals and populations where the difference is $\leq 0.2\%$. They will also contribute to the variability in population results.

It has been demonstrated by a number of workers that the isotope ratio for an individual consumer reflects that of his diet. Interpretation of results requires knowledge of the $\delta^{13}\text{C}$ increment between the bone gelatin from a consumer and that consumer's diet, i.e., ' Δdc '. At present the most appropriate value for ' Δdc ' is $+4.5 \pm 0.5\%$.

The technique as outlined here presents useful and important data but there are still a few areas requiring further investigation, in particular, standardization of methodology and the adoption of appropriate laboratory

standards. Further methods of checking sample preparation and collagen extraction need to be developed, and the collagen enrichment, or ' $\Delta\delta\text{C}$ ', value must be investigated for more species and larger sample sets.

Diet alternative values in British Columbia

Selection of dietary samples for analysis must take geographic, temporal and environmental variations into consideration. Samples must be of the tissues eaten. While it may be of interest to know about the species contributing to herbivore and/or carnivore diets, we really only need to know about the species that our target population was eating.

Marine and terrestrial diet alternatives can be clearly distinguished from each other on the basis of $\delta^{13}\text{C}$ values. The best value for a purely marine modern diet in this region is $\delta^{13}\text{C} = -17.7 \pm 0.2\text{‰}$ and for a prehistoric terrestrial diet is $-26.1 \pm 0.3\text{‰}$ in Northern British Columbia, and $-25.2 \pm 0.4\text{‰}$ in Southern British Columbia. The separation of about 8‰ is similar to that reported between the atmospheric and oceanic carbon reservoirs for the North Pacific region. The variabilities of ± 0.2 to $\pm 0.4\text{‰}$ are small enough that they will not interfere with comparisons between the diet alternatives.

Within the limits of the present samples there are no significant geographic differences for species, or groups of similar species, in British Columbian marine environments. The terrestrial diet sample averages for the northern and southern regions of British Columbia differed by $0.9 \pm 0.5\text{‰}$.

Results for human populations

Applying ' $\Delta\delta\text{C}$ ' = $4.5 \pm 0.5\text{‰}$ to the diet alternative values, and correcting for the effects of fossil fuel carbon, has enabled derivation of expected values for the prehistoric human consumers of purely marine- and purely

terrestrial-based diets. The end-points for prehistoric British Columbia dwellers obtaining all of their protein from marine species should yield a $\delta^{13}\text{C}$ value of $-12.5 \pm 0.2\text{‰}$. For a population getting all of its protein from terrestrial species the value should be $-21.0 \pm 0.2\text{‰}$ in northern British Columbia and $-20.0 \pm 0.2\text{‰}$ in southern British Columbia. These values will be used in Part II for proportion determinations.

Observed values for humans from British Columbia range from $-13.3 \pm 0.1\text{‰}$ for the coast population average to -20.0 for an individual in the interior, approximating the range between the expected end-points. This range is less than the 7.5‰ difference between the two diet alternative averages, but the humans did not represent end-point populations, i.e., they were not consumers of exclusively marine or terrestrial diets. The standard deviations, at 1σ , for the human populations were between 0.2 and 0.7‰ . This means that populations that are separated by greater amounts may be safely compared to each other. Standard errors for the populations were ± 0.1 to $\pm 0.3\text{‰}$.

Human population standard deviations for interior and coastal dwellers are as low as ± 0.2 and $\pm 0.3\text{‰}$ respectively. These results indicate that the combined metabolic effects and diet differences within the populations are no higher than 0.2 or 0.3‰ . Variabilities higher than that should result from greater variability in the diets or in the diet species.

Proportion determinations

Because the human samples were prepared differently the problems with the dietary alternative data will not interfere with the analysis of the human data. Human $\delta^{13}\text{C}$ values can be compared before diet proportions have been determined. In fact they should be, because determination of the diet

proportions will introduce greater uncertainty to the human results due to the uncertainties associated with the diet alternative averages and with ' Δdc '.

The proportion determinations will be made on the basis of the value given above for ' Δdc ' and the diet alternatives. The uncertainty on the proportion estimates ranges from ± 3 to $\pm 6\%$, with the possibility that the end-points may shift together by 0.5% . In view of the assumptions that have been made here it has been considered wise to use a rounded off uncertainty on the proportion estimates of $\pm 10\%$.

Summary

The questions posed earlier have been answered and the technique may be applied to marine - terrestrial comparisons, at least in British Columbia. We now know that the marine and terrestrial dietary alternatives are separated by 7.5 to 8.4% in British Columbia, and that their variabilities, of 0.2 to 0.4% , are small enough that they do not obscure that difference. The increment between a consumer's gelatin and its diet is about $4.5 \pm 0.5\%$ for a number of species that have metabolisms similar to humans; therefore, it can be assumed that the human value is similar.

Low overall isotopic variabilities associated with human populations in British Columbia indicate that the contribution of metabolic effects, analytical errors, and variability in average diets is less than 0.3% . Human population mean values that vary by greater than 0.3% will result from greater variability in average diets.

Part II Prehistoric Diet in British Columbia

1 Archaeological evidence for paleodiet in British Columbia

The Coast

The Pacific Coast of British Columbia has been inhabited for at least 9700 years, and probably longer (Carlson 1979; Fladmark 1975, 1982). Because of the high Coast Range mountains, which dip steeply into the ocean in many areas, access to terrestrial resources is often restricted and difficult. While terrestrial species are often available in delta areas, on coastal flats, and by following river valleys inland, the obvious focus for subsistence has been the marine environment. This is indicated by the many large shell middens found along the coast and by recovered artifacts which include items such as hooks, net sinkers and cordage used in fishing and sea mammal hunting, and by features such as fish weirs and traps of stone and wood. There is little doubt that British Columbia coastal people relied heavily upon the marine environment for their subsistence. The question is - how heavily, and for how long?

Prior to about 5500 BP the British Columbia Coast may be subdivided into two cultural traditions, one extending north from Vancouver Island, and one extending south from the northern end of Vancouver Island into Washington State. The Northern tradition, or Early Coast Microblade Complex (Fladmark 1975, 1982) is represented at Namu, on the Queen Charlotte Islands, and is similar to that found in sites around the Gulf of Alaska. Unfortunately organic preservation is virtually non-existent for this time period and there are no faunal assemblages that can be definitely associated with it. It is generally assumed that the people of this tradition were strongly marine oriented in their subsistence strategies.

The southern tradition or 'Old Cordilleran Pattern' (Fladmark 1982; Matson 1976) is represented at the Bear Cove (Vancouver Island), Glenrose, Olcott and Milliken sites. Again organic preservation is poor and the organic remains that do occur come at the end of the period and may not be associated with it (Fladmark 1982). It is generally thought that the Old Cordilleran subsistence was more generalized initially but developed an increasing emphasis on marine and riverine resources (Bryan 1963; Burley 1980; Matson 1976; Mitchell 1971). Grabert (1979) suggests that the adaptation to maritime environments was perhaps only seasonal. Unfortunately the absence of both faunal and human bone leaves these arguments unresolved.

A further complication for this early time period is the absence of shell midden sites dating before about 5000 BP. Whether this results from a lack of shellfish, a lack of interest in shellfish, or the disappearance of sites due to rising sea levels is not clear. The result is that we lack any definite faunal evidence with which to interpret subsistence for this period. However, if we can determine the degree of reliance of these people on marine resources via isotopic analysis then we may be able to address these problems. Evidence of differences in the reliance on marine foods between the northern and southern areas, and of increasing levels of marine food usage through time would support the pattern outlined above. It is unlikely that we will be able to use isotopic analysis to determine whether the marine species used were exploited on a seasonal basis as we will be seeing a long term average for individuals in our analytical results.

After about 5500 BP the distinction between northern and southern areas seems to have disappeared and Fladmark (1982) groups the various phases along the British Columbia Coast into a 'Developmental Stage'. This is subdivided into an 'Early Substage' equivalent to the Charles Phase, or the St.

Mungo and Mayne phases, (ca. 5500 - 3500 BP) of the Gulf of Georgia region; a 'Middle Substage', temporally equivalent to the Locarno Beach and Marpole phases, (ca. 3500 - 1500 BP); and a 'Late Substage', equivalent to the developed Coast Salish or Gulf of Georgia phases (post 1500 BP). Two characteristics of interest to this study are the appearance of shell middens in the 'Early Substage' and the appearance and growth of specialized fishing and sea mammal hunting technologies, as evidenced by harpoons, bone hook barbs, and the like. This 'sudden appearance' may be due to the fact that the shell midden environment affords good organic preservation so that faunal evidence is available for these substages. While only a few analyses are available (eg, Boehm 1973; Conover 1978; Ham 1982, 1976; Matson 1976), remains of fish, molluscs, sea mammals, land mammals and birds are reported.

The 'Middle Substage' is the time period in which intensive salmon fishing appeared as a subsistence base (Burley 1980; Grabert 1984), a period heralded by the "full development of complex and diversified fishing and sea mammal hunting equipment, generally similar to that of the ethnographic period" (Fladmark 1982). This is evident in the abundant samples of bone harpoons and hook barbs, fishnets, canoe paddles, and the like, that have been recovered, especially from waterlogged sites (Bernick 1983; Borden 1976; Inglis 1976). Ground slate knives, commonly associated with fish processing, are frequently reported, but for whatever reason, poor recovery, real absence of samples etc., fish remains themselves have not been reported.

The 'Late Developmental Substage' extending from ca. 1500 BP to the ethnographic period, continued and refined the previous substages. There seems to be no major change in the presence of subsistence-related items, only changes in their design and manufacture.

The subsistence base of the Developmental Stage appears to have been quite stable. Major differences that might be found lie in the transition from the preceding lithic stages to the Developmental Stage, and in the North - South dichotomy expressed in the earlier stages. Presumably the transition to the Developmental Stage did not happen overnight, so we may see a gradual increase in use of marine resources, particularly with "a coast wide stabilization of sea levels" and an associated increase in the productivity of salmon, and presumably shellfish (Fladmark 1982).

Until recently, archaeological descriptions of subsistence in this region tended to be scanty at best, which makes it difficult to determine the degree of dietary reliance of the prehistoric Northwest coast people upon either marine or terrestrial species. There are numerous lists of modern species available in the local areas and of species recovered from site excavations (e.g., Capes 1964; Chapman 1982; Charlton 1977; Fladmark 1970; Haggarty and Sendey 1976; Mitchell 1971, 1963; Munsell 1976; Percy 1974; Seymour 1976; Smith 1907; Trace 1981). Some studies (Bryan 1963; Carlson 1976; Galdikas Brindamour 1972; Onat 1976; Simonsen 1973; Stryd 1969) have included bone counts and in some cases weights for various species, but in reality are little more than species lists, since no relative comparisons of results were possible or attempted. An exception is that of Simonsen (1973) who ranked the remains from two levels of sites on the Central Coast and found evidence of change in faunal remains that he related to change in population density on the sites.

There are now a number of zooarchaeological studies providing varying degrees of quantified data of use in determining diet proportions. Savage (1973) compared sea and land mammal remains recovered from the Boardwalk Site (Prince Rupert) and the Yuquot Site (Nootka Sound, Vancouver Island). He

observed a shift from a predominance of land mammals in the earlier levels (from ca. 4200 BP at Yuquot and ca. 3500 BP at Prince Rupert) to a predominance of sea mammals in the upper levels (approaching recent times) at both sites. Conover (1978) compared the exploitation of the coastal forest and littoral zones at Namu. She found that before ca. 1000 BP the majority of mammal remains were from the forest zone. Unfortunately both of these studies neglect fish and shellfish species and may thus lead to incorrect interpretations.

C. Carlson (1979) found that 78% of mammal bones at the Bear Cove Site (Vancouver Island) were marine, but could not compare other species.

Boucher (1976) provides bone counts for sea and land mammals, fish, and birds for the Helen Point Site (Mayne Island, Gulf Islands) that show an increase from ca. 20% fish in the Mayne Phase (=Early Developmental Substage) to ca. 75% fish in the San Juan Phase (AD 1250 - 1850). The results of this study may also be somewhat biased because Boucher did not deal with shellfish.

Boucher also carried out an analysis of the faunal remains from the McNaughton Island and Kwatna Sites on the Central Coast for Pomeroy (1980). She calculated MNI, determined ages of individuals where possible, and determined bone usage. Results indicated a greater emphasis on land mammals than sea mammals for Kwatna (an inner coast site) and an increase in sea mammal and salmon usage in more recent times for McNaughton Island (an outer island site). Stryd (1969) also noticed more land mammal use in inner coast sites in the area.

Matson's (1976) report on the Glenrose Cannery Site included determinations of age, seasonality, MNI and meat weights for mammals

(Imamoto 1976), and for shellfish (Ham 1976). Casteel (1976) was concerned with comparing recovery methods for fish remains.

Work at Yuquot (Vancouver Island) by Clarke and Clarke (1980), McAllister (1980), and Rick (1980) included determinations of MNI and meat weights for mammals, birds, fish, and shellfish, as well as seasonality for shellfish. Changes were observed in the ratio of epifauna (rock dwelling species) to infauna (sand and gravel dwelling species), but the major subsistence focus remained constantly and heavily marine. At nearby Hesquiat, Calvert (1980) and Haggarty (1982) found no evidence for environmental or dietary change with marine species comprising about 90% of the recovered faunal remains.

At Ozette, faunal data were used primarily to construct models of the relationship between faunal distributions and social and economic behaviours (Friedman 1980; Huelsbeck 1981; Wessen 1981) but were not used to determine species proportions.

Ham (1982) summarizes literature data which suggest that in the Georgia Strait area, land mammals were the most commonly occurring species in the Old Cordilleran, Locarno, Marpole, and Gulf of Georgia phases, while fish were second. In the Charles phase fish were most common while land mammals ranked second. His analysis of Crescent Beach material (Ham 1982) supports this interpretation.

For the Shoemaker Bay Site near Alberni (Vancouver Island), Calvert and Crockford (1982) carried out an analysis of two site components, dating from before ca. 1700 BP for one, and after 1700 BP for the other (McMillan and St. Claire 1982). Using NISP and MNI figures and meat weight estimates they found an increase in shellfish and herring use that may correlate with increasing site activity. Otherwise there were no changes in subsistence species, the major ones being deer, ducks, and salmon.

Difficulties that may affect all of these studies are that the throwing of fish bones back into the water (Jenness n.d.), poor fish bone preservation, drying of fish and shellfish away from site areas, and recovery biases may lead to low estimates of the importance of fish in the diet. Whether this is the case or not, it is difficult to make accurate and precise dietary proportion estimates from the incomplete zooarchaeological data.

Ethnographic descriptions of subsistence along the coast provide much detail on the species eaten, methods of harvest or capture, preparation of food species, seasonal availability and procurement patterns, division of labour, taboos, etc., (Barnett 1955; Boas 1909, 1921; Drucker 1951; Garfield and Wingert 1951; Jenness n.d.; Kraus 1885; McIlwraith 1948; Niblack 1890; Singh 1966; Suttles 1951; and others). However, except for statements such as "salmon of one kind or another was the main food of the Saanich, as it was of all other Coast Salish Indians" (Jenness n.d.), or that the Indians of the Gulf of Georgia region "subsist largely upon fish and shellfish" (Smith 1907), we are told little about the actual relative importance of various foods to the local inhabitants. As Belcher (1985) points out, shellfish were often denigrated or ignored in the ethnographic literature. Fladmark (1975) surveyed a number of these sources and produced a rank ordering of faunal resources for the whole coast, in which marine species occupy the first six and the eighth rankings (Fladmark 1975: table III).

Murdock's (1967) estimate of about 45 - 55 percent marine foods in British Columbia coastal diets appears to be the only proportion estimate available from the ethnographic literature. Recently, in response to the initial reporting of this study (Chisholm et al 1983), Folan (1984) estimated diet proportions for the Nootka, using data from Jewitt's (1807, 1896)

reports of his captivity by them. His results supported the estimate of ca. 90% marine species in local diets made by Chisholm et al (1983).

We can see that there are three major questions relating to coastal subsistence: 1) what were the relative proportions of marine and terrestrial protein in the peoples diet; 2) was there a North - South difference in subsistence base coinciding with observed lithic differences; and 3) was there a change in adaptation at about 5000 BP, from a more land-oriented hunting pattern to a more marine-oriented hunting, fishing, and gathering pattern, as suggested by change in artifact assemblages (Borden 1975; Boucher 1976; Bryan 1963; Burley 1980; Carlson 1960; Grabert and Larsen 1975; Hester and Nelson 1978; Hobler and Carlson n.d.; Matson 1976; Mitchell 1971; and others), or was there no change, with the appearance of midden sites being contingent upon the sea levels stabilizing at about that time (Fladmark 1975)?

In addition, there are a number of other questions that may be addressed through isotope ratio determinations, if the results permit. Differences between age classes or sexes of the people from within a site, a group of sites or a population may indicate differences in diet that are culturally or behaviourally induced. The absence of such differences in the humans studied by Lovell et al (1986) suggests that if differences do occur, they are not likely to result from the consumer's metabolism. The presence of sexual differences may lead to interpretations of sexual division of labour, or may relate to food taboos etc. Differences between age groups may result from the children spending much of their time with their mothers, eating what the mothers eat and foraging for edible treats such as berries. The ethnographic literature does not provide much information in this regard.

If burials from both inland and coastal locations, or inner coast and outer island sites, show the same isotope ratios then those people would have been eating the same average diets. This could be because they are from the same population but were buried in different sites that the people had occupied during their seasonal rounds. Similarities could also result from the trading of foods between two groups of people. People who exhibit different diets than the majority of their neighbours may well be intrusive to an area, perhaps as immigrants, raiders, visitors, etc.

Mitchell (1971) and Burley (1980) refer to four differing subsistence strategies for the Gulf of Georgia region, including northern Gulf diversified fishermen, central and southern Gulf river fishermen, Straits reef net fishermen, and Puget Sound diversified fishermen. The differences are related in part to the ease of access to the Fraser River salmon runs (Burley 1980) and are geographically differentiated. These differences will not be visible isotopically, however, because the subsistence base itself does not differ, only the techniques of capture do.

The Interior

Archaeological work in Interior British Columbia has concentrated on pit house sites, particularly in the area around Lillooet in the middle Fraser River area and in the valley of the South Thompson River. Based ultimately on the Lochnore - Nesikep sequence developed by Sanger (1967, 1970) Fladmark (1982) divides interior prehistory into three major divisions. The Early Period, prior to ca. 8000 BP, is poorly known and has yielded no faunal evidence yet. The oldest dated human remains, at 8250 ± 115 BP (S-1737), are those of the burial from Gore Creek in the South Thompson Valley near Kamloops (Cybulski et al 1981). The Middle Period extends from ca. 8000 BP

to ca. 3500/3000 BP and is characterized in part by the presence of microblades. The Late Period from ca. 3500/3000 BP to the present is characterized by the general disappearance of microblades and the increase in pit house numbers. Use of salmon was felt to increase after ca. 2000 BP (Palmer 1975; Stryd 1981; Wilson 1980), although C. Carlson (1980) felt that the evidence from the South Thompson Valley would not substantiate this. More recently this sequence has been applied in different locales and refined by new data (eg, Rousseau 1984; Stryd 1973; Wilson 1980).

In the interior of British Columbia archaeological knowledge of subsistence is less developed than for the coast. Archaeological reports (Blake 1976; C. Carlson 1980; Copp 1979; Kusmer n.d.; Rousseau 1984; Stryd 1973; Williams 1982; Wilson 1980) provide lists of the species available in the site areas and in some cases recovered from the sites. To date sample size and recovery techniques have limited faunal analyses to NISP (number of individual specimens) counts, and higher levels of inference have not been possible.

Our knowledge of British Columbia Interior subsistence has been derived primarily from ethnographic accounts. Palmer (1975) has provided an extensive summary of earlier reports (e.g., Boas 1890, 1908; Dawson 1891; Ray 1939; Teit 1909) relating to the Shuswap, determining that they subsisted primarily on deer, elk, salmon, service berries and dog tooth violet roots, with diversification to include small quantities of a great many other plants and animals. Plant species used in the interior included one species of cactus (*Opuntia* sp.), a CAM species, but no C₄ species were noted (Palmer 1975; Turner 1978). Dawson (1891), Hill Tout (1907), and Teit (1930, 1909, 1906, 1900) recorded that river dwellers utilized the anadromous salmon, and reported on techniques of capture. Consequently, although we know that

salmon were present and used, we really have no idea of how important they were to the prehistoric economies of the interior dwellers. We do know that the largest human population concentrations coincide with areas where salmon availability was high (Kew 1976; Sneed 1971).

Ham (1975), Kew (1976), and Sneed (1971) have presented arguments regarding the seasonal availability of salmon and have attempted to derive seasonal behaviour patterns for the prehistoric interior people from such data. Kew (1976) has suggested that there is a gradient of natural accessibility of salmon, proceeding from the sea to the upper reaches of the interior rivers. He suggests that where the salmon are most accessible they are the least valuable, and where they are the least accessible they are the most valuable. The salmon available to the Chilcotin people were less plentiful and of poorer quality than they were to the neighbouring Interior Salish (Kew 1976). A similar situation applied for the Carrier and their neighbours along the salmon bearing Skeena River (Montgomery 1978). Stryd (personal communication) has suggested that salmon use decreased for locales further upstream on the Fraser and Thompson River systems. Teit (1909) mentions strife between different groups over fishing places and hunting grounds. Territorial behaviour of this nature might show in the isotopic record if some groups were restricted in their access to salmon by such competition.

Regional differences may also be expected between different tribal groups and areas. Teit (1909) found that for the South Lillooet people, salmon fishing was the most important industry and that it occupied a more prominent position than for most interior tribes, including the northern Lillooet branch. Similarly, the lower Thompson people relied on salmon more than their upstream relatives (Teit 1909). In the Okanagan area salmon were

scarce in Okanagan Lake and in the upper Okanagan River (Teit 1930) so people occasionally travelled out of the region to obtain and prepare them. Farther south, in eastern Washington, the Flathead Indians hunted buffalo and spent less time fishing, although salmon did occur around Spokane (Teit 1930). To the east the Kutenai included bison in their diet and took some salmon from the Columbia River although the quantity varied with distance from the river (Turney-High 1941).

According to Palmer (1975) the principal structural elements of Plateau Culture include a semi-nomadic riverine settlement pattern, sexual division of labour, loosely-structured intertribal trade networks etc. These factors may be reflected in dietary differences.

In the interior of British Columbia it is not clear when salmon runs were re-established after the retreat of Pleistocene ice and hence when people began obtaining salmon from the many salmon streams throughout the interior (Copp 1979; Fladmark 1975). Salmon are reported for Kettle Falls on the Columbia River system from ca. 9000 BP (Chance et al 1977).

A present day consideration is that of the continuity of the salmon fishery in the interior. This is of interest in legal arguments regarding aboriginal fishing rights. Evidence obtained from sources other than oral tradition and ethnographies would be useful to clarify this issue.

Human samples

To address these questions about subsistence requires samples of human bone representing all of the time periods and geographical areas of interest. Samples should represent different environments, such as open coast areas, sheltered inner coast and inlet locations etc., from both the northern and southern coastal zones, as well as the Gulf of Georgia. In addition, it is

desirable to sample both females and males as well as both children and adults, to see if there might be sex or age related differences in diet.

Similar coverage is desirable for the interior regions of British Columbia. Samples should be selected to cover the different time periods and geographic areas that have been discussed. Different sites along salmon rivers and sites away from those rivers should be sampled.

While it is possible to detail the human samples that might provide answers to most of our questions, it is unlikely that all of those samples will be available. We are forced to rely mainly on museum collections, with a few samples coming from field research projects from time to time.

2 Human results from British Columbia

The human bone samples analysed in this study were obtained primarily from museum collections. A few were provided by excavators. The sites from which the samples originated are shown in Figures 3 and 4. Age and sex data were obtained from the archaeologists that provided the samples.

Sample preparation and analysis were as described in chapters 3 and 4. Results for the larger sample groups have been discussed in Part I (Chapter 6) when the applicability of the technique was considered. This chapter considers all of the human results, examining any differences and/or similarities that may occur, and relating them to the archaeology of the region when possible.

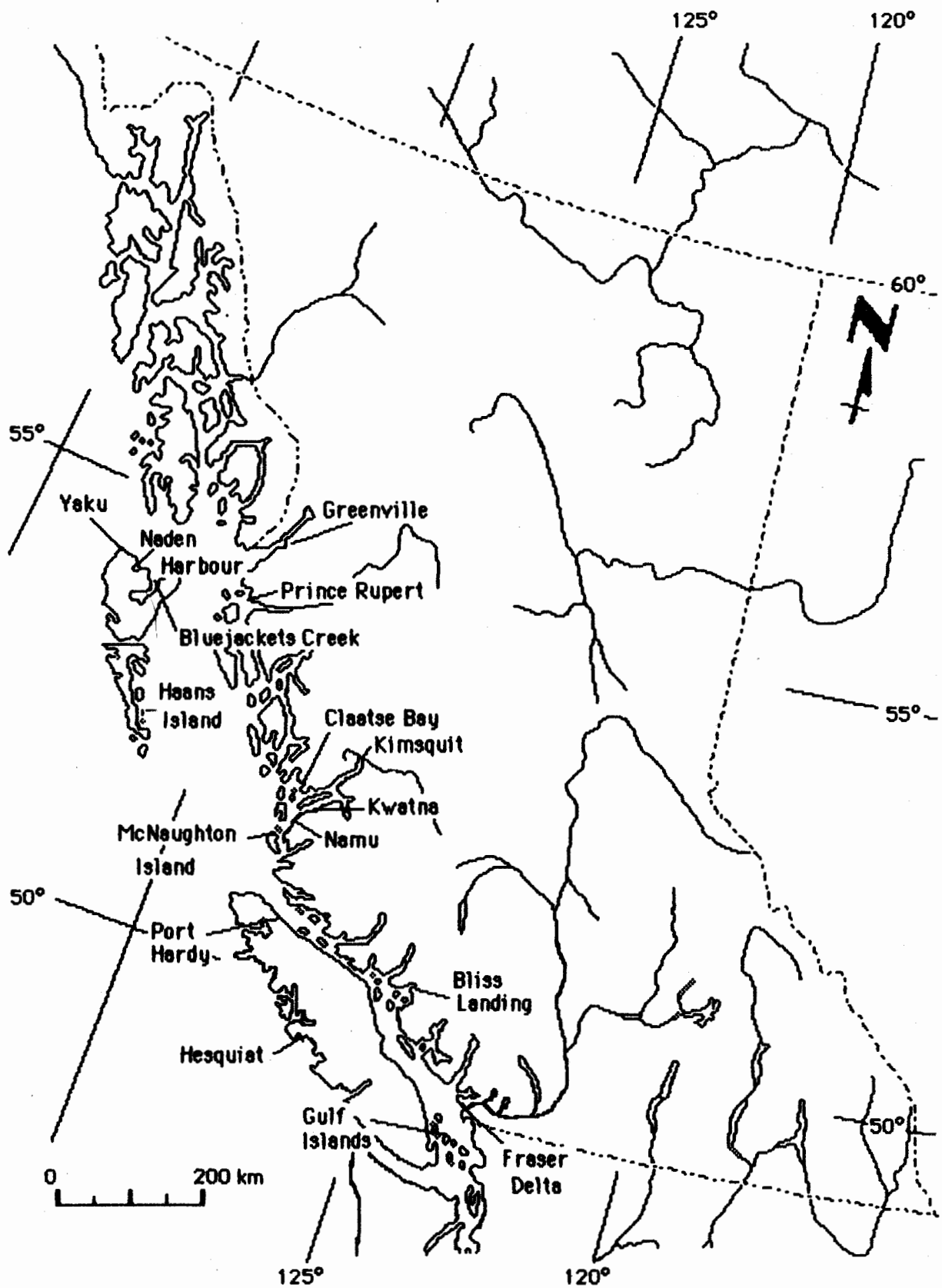
The results and the comparisons are presented region by region, compared within regions, and then compared between regions. Proportion determinations are discussed after the regional and other comparisons have been made.

2.1 The coastal results

The Namu - Central Coast people

For the Central Coast region there were a total of 48 individuals sampled, 38 of which are from one site, EISx 1 (Namu) while the remaining 10 are from seven other sites (EaSe 2, EeSu 5, EITb 10, FaSu 6, FaSu 9, FcSx 1, FeSr 10). Of these 47, nine are immature individuals under ten years old. Teenage individuals have been classified as adults on the assumption that by age ten their diet has been like that of the adults for long enough to mask possible early differences, and because their $\delta^{13}\text{C}$ values do not differ from those of the adults.

Figure 2 Locations of coastal sites sampled.



Examination of the EISx 1 $\delta^{13}\text{C}$ results for adults (Appendix B) reveals that only 2 samples are more than 2σ away from the mean values for the other samples of the same sex and time period. One individual was cremated or burned post deposition and yielded a value of -17.0‰ . The second outlier, at -14.9‰ , is isotopically lighter; the bone does not appear to have been burned so the result suggests a higher terrestrial protein intake. This individual is believed to pre-date 5600 BP (Curtin 1984) and thus may hint at a higher reliance on terrestrial protein before then. This is highly speculative since there is only one improperly dated sample. As discussed previously, it is possible that this individual was from an area where terrestrial species were more important, he preferred terrestrial species over marine species, he practised food taboos, or was of different status.

While mean values and standard errors of $-13.5 \pm 0.2\text{‰}$ for adult females and $-13.0 \pm 0.1\text{‰}$ for males from EISx 1 differ significantly from each other ($.005 < p \leq .01$), neither group differs across time periods, from ca. 5600 - 1000 BP ($.375 < p \leq .4$, or better).

Results for 7 of the 9 adult samples from other nearby sites (EaSe 2, Bliss Landing; EITb 10, McNaughton Island; FaSu 6 and 9, Kwatna; FeSr 10, Kimsquit) are within 1σ of the averages for the temporally closest EISx 1 adult groups, and have been combined with the EISx 1 adults to obtain an average for the Central Coast area (Table 14). One male from EaSe 2, at -12.6‰ , is within 2σ of the mean for the comparable EISx 1 samples and was also combined with the EISx 1 samples. The last individual, a female from EeSu 5 near Port Hardy at the north end of Vancouver Island, at -11.9‰ , is more than 2.7σ removed from the mean for the 13 females at EISx 1. While there is the possibility of finding a result that far removed in a normally distributed sample set, in this case the probability is .004; therefore she was

not likely from the same population as the females at EISx 1. A tentative explanation for this difference and the isotopically heavier value is that, out of necessity or by choice, this woman had been relying more on species that were from higher trophic levels, and possibly lighter isotopically, and avoiding species from lower levels of marine food chains.

Table 14 Combined Central Coast results ($\pm 1\sigma$).

A) Adults:

<u>Site and Time period</u>	<u>All Adults*</u> $\delta^{13}\text{C}$ (‰) (n=)	<u>Females</u> $\delta^{13}\text{C}$ (‰) (n=)	<u>Males</u> $\delta^{13}\text{C}$ (‰) (n=)
Central Coast 5600-70 BP	-13.3 \pm 0.6 (38)	-13.5 \pm 0.6 (13)	-13.0 \pm 0.3 (13)
> 5600	-14.9 (1)		
EeSu 5, 2950 - 2350	-11.9 (1)	-11.9 (1)	

B) Children

<u>Time period</u>	<u>(0 - 2.5 years)</u> $\delta^{13}\text{C}$ (‰)	<u>(2.5 - 6 years)</u> $\delta^{13}\text{C}$ (‰)
5600-1000	-11.9 & -12.4	-14.1, -14.5, & -16.9
< 1000		-13.4 (1)

* including those for which the sex could not be determined

The children from EISx 1 are of some interest. The 6 - 8 year olds do not differ from the adult samples ($p > .4$) and may therefore be combined with

them. One child from FcSx 1, at -13.2‰ , is of unknown age and thus cannot be compared properly although it does fit with the adults.

The two youngest children (0 - 2.5 years old) have values indicating they ate less terrestrial protein than the older children, or than their mothers. Of these two, one is over 2σ and the other is almost 2σ removed from the female adult average value for EISx 1. These will be discussed later in the summary section following the coastal results.

One older child (2.5 - 6 years old), at -16.9‰ shows a higher terrestrial intake than both the female and male adults. Two others are 0.6 and 1.0‰ lighter isotopically than the female adults, and may also have been eating more terrestrial foods than them. A possible explanation for this would be that the children were eating quantities of berries or other plant foods while out playing, or foraging with their mothers. It is also possible that the child at -16.9‰ was represented by heated bone. One other older child from <1000 BP is within 0.1‰ of the female average.

The Prince Rupert Area samples

Seven individuals from 3 sites (GbTo 31, 33, 36) near Prince Rupert, and from GgTj 6 (Greenville) at the mouth of the Nass River were measured (Table 15).

Table 15 Prince Rupert area individual results

<u>Location</u>	<u>Time Period</u>	<u>$\delta^{13}\text{C}$ (‰)</u>
Prince Rupert Harbour	3000 - 1750	-12.7, -13.0, -14.1 -13.2, -13.9
Greenville	?	<u>-13.5, -14.0</u>
AVERAGE ($\pm 1\sigma$)		-13.5 \pm 0.5

The variability for these samples is essentially the same as those for DeRt 2 and EISx 1. Therefore the diet at Prince Rupert was no more varied than at DeRt 2 and EISx 1.

The Queen Charlotte Islands results

The Queen Charlotte Island results are problematic because 4 of the 15 individuals examined gave results that are at least two standard deviations lighter than the mean value for the other 11 samples (Table 16). Since 2 of the outliers date to 4890 ± 150 and 1230 ± 60 BP, they are not recent individuals who may have been eating non-traditional foods. The other two outlying samples were not dated. The difference is not related to age, sex, or date, and aside from the possibility of heating of the bone, has no obvious explanation other than the individuals may have used more terrestrial foods.

Table 16 Queen Charlotte Island results

<u>Site</u>	<u>Date (BP)</u>	<u>$\delta^{13}\text{C}$ (‰)</u>	<u>Outliers (‰)</u>
FIUa 4	4900 - 4500	-13.0, -14.1, -15.2 <u>-15.4</u>	-17.9; -18.4
AVERAGE ($\pm 1\sigma$)		-14.4 \pm 1.1	
Others	< 4500	-12.2, -12.4, -13.1 -13.3 -13.3, -13.5 <u>-13.9</u>	-19.1; -18.8
AVERAGE ($\pm 1\sigma$)		-13.1 \pm 0.6	

There was no evidence of charring of the bone, and the available data on the burials does not provide any information regarding charring for any of these outliers. It is possible the bones were heated for long enough at a lower temperature to induce a change in $\delta^{13}\text{C}$ values without visibly altering the bone. (It might be worthwhile to investigate the possibility that amino acid racemization analysis could be used to detect whether bones had been heated or not.) The outlying values are about 4‰ lighter than the average for the other Queen Charlotte samples. On a range of about 8‰ between marine and terrestrial consumer values this represents a difference in marine species intake of about 50%. As this is not too likely for people in the Queen Charlottes the most likely explanation for the shift is heating of the bone.

As shown in Table 16 the main sample of 11 individuals may be split into two categories, those from FIUa 4 (Bluejackets Creek) dating to about 4900 - 4500 BP, and those from other sites that are of more recent date. The standard deviation for the more recent samples is equivalent to that for the Central Coast and Gulf Islands people (Table 19). From this it is concluded that the diet of these Queen Charlotte Islanders was no more varied than it was for the Central Coast and Gulf Island dwellers. However, the older FIUa 4 samples were more varied, possibly because of heating of the bones, particularly for the samples at -15.2 and -15.4‰, or possibly because the diet was in fact more varied. Averaged results for the older FIUa 4 samples indicate a greater reliance on terrestrial species than for the more recent individuals. In view of the small number of individuals, and of the outliers, this may suggest but cannot be taken as a reliable indicator of diet change.

Hesquiat results

Five individuals were examined from DiSo 1 and 9 at Hesquiat, on the west coast of Vancouver Island. One appeared charred upon inspection and yielded a value of -19.2% . The other four samples are summarized in the following table. The outlier yielded a C/N value of 3.0 which indicates the possibility of up to about 14% contaminant carbon (see pp. 50-52).

Table 17 Hesquiat results

<u>Date (BP)</u>	<u>$\delta^{13}\text{C}$ (‰)</u>	<u>Outlier (‰)</u>
1350 - 1100	-12.5 & -12.6	-14.4
600 - 450	<u>-12.5</u>	
Average	-12.5	

Age at death and sex of the Hesquiat individuals were not determined, except to note that they were all adults.

Gulf of Georgia sites

Samples were obtained from sites in the Gulf Islands and nearby areas and from the Fraser Delta region, including DhRr 6 (Belcarra) on Burrard Inlet (Table 18).

For samples from DeRt 2 (Pender Canal) there is no difference in $\delta^{13}\text{C}$ averages between males and females. Three individuals, from DcRu 52, DeRt 1 and DfRu 42, were all within 1σ of the DeRt 2 mean and were included with the DeRt 2 samples as part of the Gulf Islands sample.

While the age at death of the DeRt 2 children has so far only been roughly determined, 2 of the 3 appear to be very young, possibly equivalent to the 0 -

2.5 year group from EISx 1. While one of these DeRt 2 children, at -12.8‰ is not significantly different than the females, the other at -12.0‰ is 1‰ heavier isotopically than, and is more than 2σ removed from, the average for the females. These results will be discussed further in the following section. The third child may be a little older and at -13.3‰ is within 1σ of the females.

Table 18 Gulf of Georgia area results (\pm standard error).

A) Adults:

<u>Location</u>	<u>All Adults</u> <u>$\delta^{13}\text{C}$ (‰) (n=)</u>	<u>Females</u> <u>$\delta^{13}\text{C}$ (‰) (n=)</u>	<u>Male</u> <u>$\delta^{13}\text{C}$ (‰) (n=)</u>
Gulf Islands	-13.0 ± 0.1 (46)	-13.0 ± 0.1 (14)	-13.0 ± 0.1 (16)
DfRu 8	-13.4 ± 0.2 (8)	-13.3 ± 0.3 (4)	-12.9 & -14.2
Fraser Delta	-13.6 ± 0.1 (34)	-13.9 ± 0.2 (9)	-13.4 ± 0.1 (9)

B) Children

<u>Location</u>	<u>$\delta^{13}\text{C}$ (‰)</u>
DeRt 2	$-12.0, -12.8$ & -13.3
Fraser Delta	-14.0 & -14.2

(**N.B.** Gulf of Georgia area outliers: Gulf Islands: $-16.5, -17.4, -18.7$; DfRu 8: -14.9 ; Fraser Delta: $-19.5, -15.9, 20.8$)

Average values for the 8 individuals from DfRu 8 (Helen Point, Mayne Island) differ by 0.4‰ from the other Gulf Islanders, which although small is statistically significant ($.005 < p \leq .01$). Three possible explanations for this difference come to mind: 1) the DfRu 8 samples date earlier than the other

Gulf Island individuals and the results reflect a diet change; 2) the DfRu 8 results reflect a seasonal round in which these people used more terrestrial dietary protein than the other Gulf Islanders did; and 3) the DfRu 8 people were obtaining their protein from isotopically lighter foods either from species that obtained their carbon from different reservoirs, or from species at different trophic levels of the food chains than those that the other Gulf Islanders were exploiting, assuming that the trophic level difference reported by DeNiro and Epstein (1978) is valid. Since Boucher (1976) found a shift from ca. 20% fish in the Mayne Phase to ca. 75% fish in the subsequent phases, diet change must be considered a possibility. However, other sample sets from the coast, from DeRt 2, and from the Fraser Delta area show no significant temporal shift in the degree of reliance on marine species over the same time period as covered by the DfRu 8 individuals so this would likely be a localized phenomenon. A complication is the absence of data on shellfish use, and of well dated samples for DfRu 8. Perhaps a better explanation is that the DfRu 8 individuals represent people who came to the site from somewhere else as part of their seasonal round. In this case the people would have obtained terrestrial species at some other location in their round. Although there is a large temporal jump involved, some support for this argument is found in Jenness (n.d.) who observed that during ethnographic times people from around Duncan, on Vancouver Island, came to fish on Mayne Island since they did not have access to the Fraser River salmon runs as did other groups in the Saanich and Gulf Islands areas. These people may not have had as easy access to marine resources as those from DeRt 2 or from the Fraser Delta. Another possibility is that the DfRu 8 individuals came from the Fraser Delta area since the two groups are similar ($.1 < p \leq .375$), their averages differing by only 0.2%.

When results for all of the sites from the Fraser Delta region were averaged together the females exhibited a significantly higher ($.01 < p \leq .025$) reliance on terrestrial species than the males did. Since the males and females from DgRr 1 (Crescent Beach) and DgRs 1 (Beach Grove), the only sites where male and female remains were recovered together, weren't significantly different ($.1 < p \leq .375$) the difference should be discounted. This discrepancy may result from the low sample numbers analyzed, it may result from the inclusion of some individuals who lived adjacent to and some who lived away from the Fraser River with its load of biogenic terrestrial carbon, or it may result from differences in diet. Results for children from these two sites did not differ significantly from the adults.

The major difference in results for the Gulf of Georgia region is between the Gulf Island sites and those for the Fraser Delta ($p \leq .0005$). The delta people were isotopically lighter on average than the Gulf Islanders by 0.6‰. This suggests that the delta dwellers obtained more of their protein from terrestrial species. This should not be too surprising since the delta dwellers would have had greater access to the terrestrial species that inhabited the flats of the lower Fraser Valley region. Another possibility is that the shellfish in the Fraser Delta area were isotopically lighter due to the presence of terrestrial biogenic carbon in the river outwash, and were eaten in sufficient quantity as to bias the human results.

The outliers reported for this region are also of interest. According to the field notes two individuals, burials 85-1 from DeRt 1 ($\delta^{13}\text{C} = -18.7\text{‰}$) and 85-9b ($\delta^{13}\text{C} = -16.5\text{‰}$) from DeRt 2 were probably burned, although this was not evident upon visual inspection. DeRt 2 burial 84-43a was found near an ash lens area but showed no evidence of burning other than its $\delta^{13}\text{C}$ result of -17.4‰ . By fortunate accident different bones from each of two DeRt 2

samples were measured. Burial 85-13 gave values of -12.4 and -15.4‰ and burial 85-7 gave results of -12.9 and -16.5‰. As both individuals were from areas where heating was a possibility the difference could result from heating part of an individual's skeleton while other parts were not heated.

The remaining outliers showed no evidence of burning and site data does not mention it as a possibility. If burning or heating can be eliminated as a possibility then the explanation for the outliers is that those individuals were eating a diet that differed from, and was more terrestrial than the other coastal individuals.

Summary and discussion of Coastal results

There are a number of general observations that can be made about the results for the whole British Columbia coastal zone. While the overall average value for coastal adults, of $-13.3 \pm 0.1\text{‰}$, is 0.8‰ away from the postulated end-point for prehistoric consumers of a purely marine diet (Chapter 6), it is clear that the coastal dwelling adults were obtaining most of their protein from marine species.

A number of results are more than 2σ from the mean for the majority of samples. Some of these are isotopically lighter by as much as 4‰ and probably represent bone samples that were charred by either cremation or post-depositional heating. With two exceptions they did not show any visible evidence of burning, which brings up the possibility of using isotope ratios to identify cremated remains. Barring contamination or charring of samples, the isotopically-lighter outlying values represent individuals who were obtaining more of their protein from terrestrial species. This may be because they were visitors from interior areas of the province, or it could result from avoidance of marine species in their diets for some reason.

Table 19 Summary of coastal results ($\delta^{13}\text{C}\%$ average \pm standard error).

<u>Area</u>	<u>All Adults*(n=)</u>	<u>Females (n=)</u>	<u>Males (n=)</u>
A) Sites near river deltas:			
Fraser Delta	-13.6 \pm 0.1 (33)	-13.9 \pm 0.2 (9)	-13.4 \pm 0.1 (10)
Prince Rupert	<u>-13.5 \pm0.2 (7)</u>		
Average	-13.6 \pm 0.1 (40)		
B) Sites away from river deltas:			
Central Coast	-13.3 \pm 0.1 (38)	-13.5 \pm 0.2 (13)	-13.0 \pm 0.1 (13)
Queen Charlotte Is.	-13.1 \pm 0.2 (7)		
Hesquiat	-12.5 \pm 0.1 (3)		
Gulf Islands	<u>-13.0 \pm0.1 (46)</u>	<u>-13.0 \pm0.1 (14)</u>	<u>-13.0 \pm0.1 (16)</u>
Average	-13.1 \pm 0.1 (94)	-13.2 \pm 0.1 (27)	-13.0 \pm 0.1 (29)
C) DfRu 8	<u>-13.4 \pm0.2 (8)</u>	<u>-13.3 \pm0.3 (4)</u>	<u>-12.9 & -14.2</u>
Overall Averages	-13.3 \pm 0.1 (151)	-13.3 \pm 0.1 (40)	-13.1 \pm 0.1 (40)

* Including adults for which sex was not determined.

There are no significant temporal shifts in $\delta^{13}\text{C}$ results, and hence in dietary protein proportions, for any of the regions sampled. There may have been a slightly higher reliance on terrestrial species before about 4500 BP at FIUa 4 (Bluejackets Creek) in the Queen Charlotte Islands and before about 5600 BP at EISx 1 (Namu), but this must remain speculative due to the small number of individuals sampled. Earlier samples were not available.

As can be seen in Table 19 people from sites with better access to inland areas (i.e., Prince Rupert on the Skeena River, and the Fraser Delta sites) are significantly lighter isotopically ($p \leq .0005$), which may indicate a greater reliance on terrestrial species than at sites in the other areas sampled, (i.e., Hesquiat on Vancouver Island's west coast, the Central Coast sites, the Queen Charlotte Island sites, and the Gulf Island area sites - except for DfRu 8). The difference of 0.5‰ in the averages could also indicate the presence of biogenic terrestrial carbon in the dietary samples from the delta areas, particularly in shellfish. This difference applies to female, male, and combined adult groupings. The average for people from DfRu 8 fell between those for the two groups but was slightly closer to that of the delta people ($.1 < p \leq .375$) than to the Gulf Islanders ($.05 < p \leq .1$).

One individual from EeSu 5, at Port Hardy near the north end of Vancouver Island, is about 1‰ heavier and was likely more reliant on marine species than people from any of the other areas. Three individuals from Hesquiat, on Vancouver Island's West Coast, are 0.8‰ heavier isotopically than the coastal average, indicating a more marine diet, and coincide with the estimated marine end-point. If higher trophic levels are isotopically heavier as suggested by other workers (page 11) then this result may reflect greater use of marine mammals in the local diet than at other coastal sites.

As discussed above, with the exception of the EISx 1 people, differences between females and males are not statistically significant. EISx 1 females were isotopically lighter than the EISx 1 males indicating a greater terrestrial protein intake.

Of the children whose age at death was well known (Table 14), 3 out of 4 aged 2.5 to 6 years were isotopically lighter and thus ate more terrestrial

protein than their mothers. Two children of unspecified age from the Fraser Delta gave results that indicate more terrestrial species in their diet.

Of two children from EISx 1 younger than 2.5 years, and two children from DeRt 2 that were likely younger than 2.5 years, 3 gave results that appear less terrestrial than the females. Two were more than 2σ , and one was almost 2σ removed from the female adult averages at their respective sites. It is interesting to speculate that this effect may be the result of these children still being breast fed, or having just recently been weaned. We know that milk from cows feeding on C3 plant pasturage is enriched in ^{13}C relative to the pasturage by as much as 2.9‰ (Minson et al 1975). If human milk is also isotopically heavier relative to the mother's diet, then the infants will be eating food that is isotopically heavier than that the mothers eat. The infants will then give collagen results that are slightly heavier isotopically than the mother's, as is the case for these children. A similar pattern is found for the mice and the cats examined in the ' Δdc ' study reported in Chapter 5. Three out of four mice under 30 days of age had values that were almost 2σ heavier isotopically than the average for all of the mice, and the younger cats were also isotopically heavier than those over 20 days of age, with the exception of neonates, which were scattered through the range (unpublished data, study in progress).

The problem with this argument is that it contradicts the evidence presented by 12 children of about the same age from the Gray site in the Canadian Plains (Lovell et al 1986). Although the evidence from only two EISx 1 children is not strong, it is possible that the 6 Gray site children in the 0 - 1 year age group were too young to show any shift from their birth values, which should be the same as their mothers'. The 6 children in the 2 - 4 year age group may have been weaned earlier than the EISx 1 children so that any

isotopic shift that may have accompanied nursing may have already disappeared. There were no Gray site children in the 1 - 2 year age class. Another possible explanation is that the EISx 1 mothers' diets were composed of isotopically heavier foods during the time they were nursing. This would have to be for an extended period of time because if it were only for a short time period the dietary shift would be averaged out and not be evident in either the children or the mothers. Since the females are not significantly different than the total adult average this explanation is unlikely. Yet another possible explanation is that the infants' bone, since it is fast growing, contains significant quantities of immature collagen and tropocollagen. It is not known whether these collagens differ isotopically from mature collagen or not.

Since the EISx 1 sample is only two individuals this discussion must remain speculative and is put forward as a suggestion for further investigation. This question could be investigated further via a controlled study using mother's milk and protein samples from the mother's newborn offspring, perhaps hair or finger nails, and by examining the isotopic behaviour of immature forms of collagen.

The standard deviations for the coastal sites examined are ± 0.5 to $\pm 0.6\%$, of which the portion attributable to dietary inhomogeneity would be ± 0.3 to $\pm 0.4\%$. Taking this variability over the range of 7.5% between the marine and terrestrial consumer extremes suggests that the coastal diets varied by only about 5% in marine species content. When all of the coastal adults are combined the standard deviation is $\pm 0.4\%$ which would represent a variability in marine species intake of $\pm 5\%$. On average the males vary in diet by only $\pm 0.1\%$ while the females vary by 0.3%. The difference is not considered significant.

2.2 Interior results

Subsequent to the interior study reported by Lovell et al (n.d.) a further 30 samples, including 12 from EbRi 7 (the Nicoamen River site), were analyzed. The site locations are shown in Figure 4 and the results of both groups of samples are listed in the appendices and discussed below.

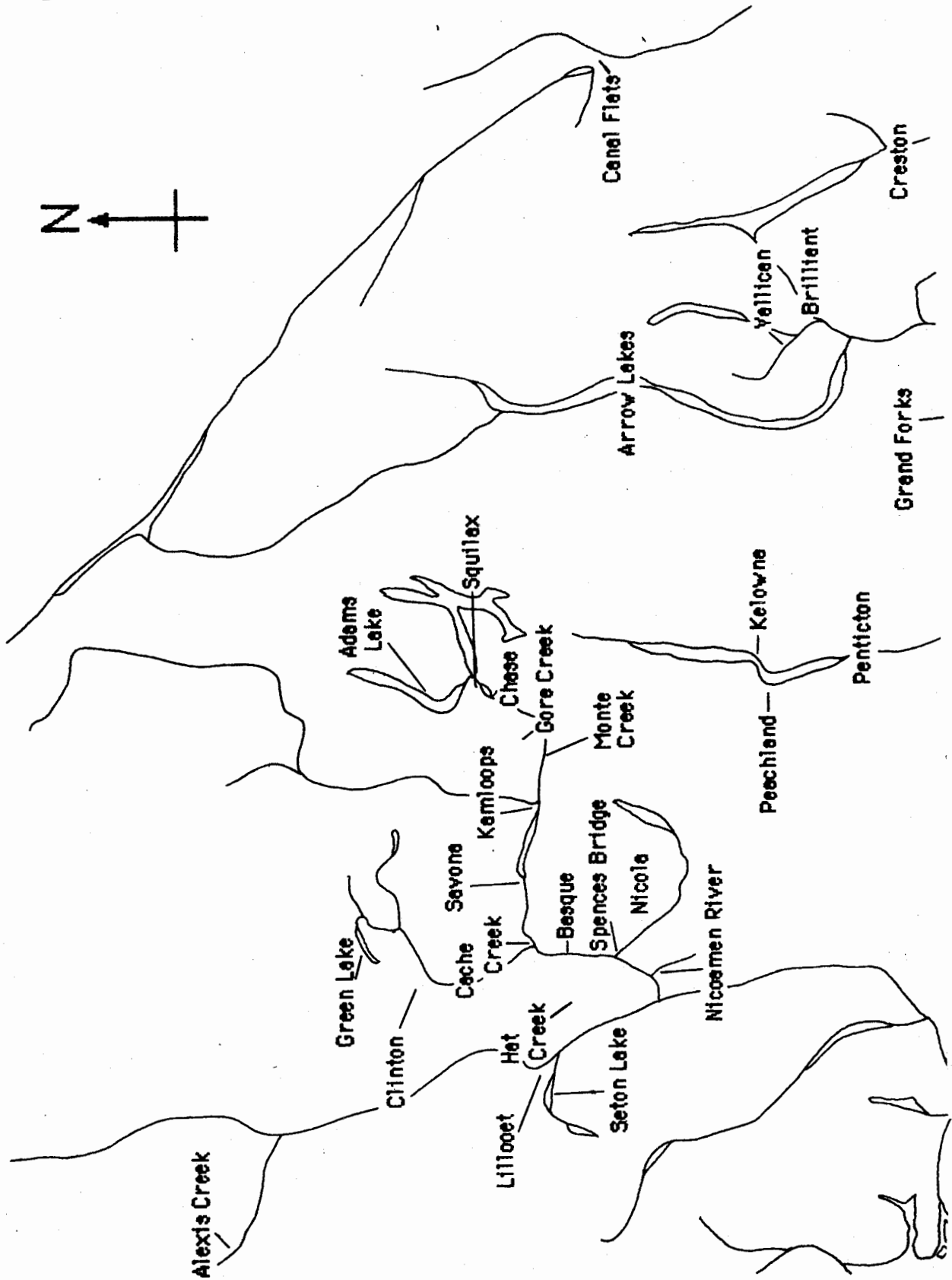
Generally, the people who lived near the salmon runs of the Fraser and Thompson Rivers yielded values around -15 to -17‰ which is about midway in the range of values reported in this study. It is also about midway between the expected end-points of -20 and -12.5‰ for prehistoric consumers of purely terrestrial- and purely marine-based diet discussed in Part I, Chapter 6. This suggests that the people in the two river valleys obtained about half of their protein from salmon, since it was the only marine species available.

For individuals that lived in areas removed from the salmon rivers and from the coast the values tend more towards the terrestrial end of the spectrum.

A number of similarities exist between results and they are summarized in Tables 20 & 21. For example, results for females and males from sites along the Fraser River within about a 5 km radius of Lillooet (EdRi 22, EeRi 6, EeRi 18, 19, 80, 167, 169, 192) are within 2σ of their average value of $-15.5 \pm 0.2\text{‰}$ and are thus grouped together. One child from EeRk 4 in this area, over 2σ from the adult average, is lighter isotopically by 0.6‰ and hence more terrestrial in dietary protein intake than the adults. One adult from the Lillooet area gave a value of -19.3‰ but showed no evidence of heated bone.

Seven adults from EbRi 7 (Nicoamen River) gave an average value of $-15.8 \pm 0.2\text{‰}$. There were also three outliers from the same site that gave results that were about 4 to 8‰ lighter isotopically, and showed no evidence of heating. Since two of these samples, at -23.7 and -22.0‰ fell outside of the observed range for the other British Columbia interior samples, and were also

Figure 3 Locations of interior sites sampled.



outside the expected range discussed in Part I, Chapter 6, it is unlikely that their differences were caused by diet. Heating of the bone is a more likely explanation for this difference.

Table 20 Interior results ($\pm 1\sigma$)

A) Adults:

<u>Location (Date)</u>	<u>$\delta^{13}\text{C}$ (‰) (n=)</u>	<u>Location (Date)</u>	<u>$\delta^{13}\text{C}$ (‰) (n=)</u>
Lillooet area (<1600)	-15.5 \pm 0.1 (9)	Seton Lake (2420)	-14.9
Thompson River	-15.8 \pm 0.1 (14)	Gore Creek * (8250)	-19.4
S. Thompson R. (\leq 1500)	-16.4 \pm 0.1 (7)	Adams River	-16.9 & -16.9
Clinton area (ca. 4950)	-16.7, -17.1, -17.2	Alexis Creek	-18.2
Kelowna	-19.0, -19.2, -17.5	Peachland	-16.7
Penticton	-15.7	Grand Forks	-16.9 & -17.1
Creston	-20.0	Canal flats	-19.4
Vallican	-15.7 & -16.4	Arrow Lakes	-16.1
Brilliant	-14.6, -15.7, -16.9, -18.4		

B) Children

<u>Location</u>	<u>$\delta^{13}\text{C}$ (‰)</u>	<u>Location</u>	<u>$\delta^{13}\text{C}$ (‰) (n=)</u>
Lillooet area	-16.1	Thompson River	-15.9 \pm 0.3 (5)
South Thompson	-15.9 & -18.3	Green Lake	-17.6
Alexis Creek	-18.2	Vallican	-16.8 & -17.0

(N.B. Interior area outliers: Lillooet area: -19.3; Thompson River area: -14.6, -22.0, -23.7, -20.9, -19.4; S. Thompson River area: -20.0, -13.2, -17.6; Thompson River area children: -17.7, -19.0.)

One EbRi 7 female adult gave a result of -14.6% which indicates more marine species in her diet. She could have been a visitor from closer to the coast, or a local individual who ate more salmon. All of the other individuals from nearby sites along the Thompson River or its tributaries (EaRf 6, Nicola R.; EdRh Y 53, Spences Bridge; EdRh 1, Cache Creek; EdRk 3, Hat Creek) are within 2σ of the average for the EbRi 7 adults and have been combined under the heading 'Adults: Thompson River' (Table 20). For these sites the females and males are statistically similar.

The 5 Thompson River area children, all from one site, are of interest. Three are term neonates (Skinner, 1986, personal communication) in which case they would be expected to have results very similar to their mothers. They would have drawn their protein-forming amino acids from the same pool as their mothers and would not yet have been influenced by their mother's milk. A slight difference may be expected since a mother's value would be a longtime average while an infant would reflect its mother's diet during her pregnancy. In fact, the mean for the children does not differ significantly from that for the females, who perhaps were their mothers, but the children's variability is greater. Their results therefore do not support arguments that suggest we can see the effects of weaning.

The grouped Thompson River adults are only 0.3% lighter, but are statistically significantly different than those from the Lillooet area sites ($.0005 < p \leq .005$). Within the group of Thompson River sites examined here there is no observable gradient in $\delta^{13}\text{C}$ values, and hence in salmon utilization. The Lillooet people on the Fraser River were using slightly more salmon than the Thompson River people, possibly due to greater quantities and better quality of the fish being available, perhaps due to tribal restrictions or

territorial access controls, or because there were fewer or poorer fishing areas along the Thompson.

Farther up the Thompson system, beyond the confluence of the Bonaparte River, individuals from Savona, Kamloops (EdRc 61, EeRb 10, EeRc 8, etc.), and Lee Creek are all within 0.3‰ of their average and have been grouped with two similar individuals from EeQw 1 (Chase) as 'Adults: South Thompson' (Table 20). Male - female differences could not be tested due to sample size, and the children were ambiguous, one from Kamloops being isotopically heavier and one from Chase being lighter than the adults. There were three outliers, of which two were isotopically lighter, and one from EeRb 10 that may have been burned. A second is 1.2‰ lighter which may indicate a higher intake of terrestrial species. The third outlier yielded an isotopically heavier result of -13.2‰ which is only 0.1‰ from the coastal average, suggesting that this individual may have been a visitor from the coast.

Individuals from EfQv 10 (Squilax) and EgQw Y66 (Adams Lake) differed by 0.5‰, which was significant ($.005 < p \leq .01$), and were not combined with those from the South Thompson. Within the group of South Thompson River people there was no observable gradient, statistical or otherwise, in $\delta^{13}\text{C}$ values and hence in salmon usage between adjacent sites. The grouped South Thompson River people were at least 0.6‰ removed from and hence differed significantly from those of the Thompson River or Lillooet area sites ($p \leq .0005$) and thus were likely using less salmon than the other two groups.

One of the more interesting samples from the South Thompson area is the EeQw 48 (Gore Creek) burial (Cybulski et al 1981). This individual, was radiocarbon dated to 8250 ± 115 BP (S 1737) and has a $\delta^{13}\text{C}$ value of -19.4‰ (Chisholm and Nelson 1983). This value is close to the terrestrial diet end of the range observed for consumers here hence it is concluded that this

individual ate mostly terrestrial protein. This agrees with Cybulski et al's (1981) conclusion that this man followed an inland hunting adaptation. Whether this was due to a shortage of salmon in local rivers, or because this individual was a visitor from elsewhere, or because he didn't like salmon, cannot be determined from the available evidence. While it appears that his dietary protein intake was about $8 \pm 10\%$ salmon (Table 22) the $\delta^{13}\text{C}$ result could also reflect the presence of some C4 species, or of C4 eating herbivores, particularly if the individual came from some warmer and more arid grassland region, or if there were more C4 plant species present in the region at that time. Unfortunately the ecology of the area is not well known for that time period.

Results for three individuals, two from EiRm 1 dating to 4950 ± 170 BP, from the Clinton area indicate a lower, but still significant reliance on salmon. They are of interest because they suggest that salmon constituted a large portion of the protein intake as far back as about 5000 BP, possibly as much as $40 \pm 10\%$ (Table 22). A child, probably dating to less than about 500 BP from EiRh 4 (Green Lake) in the same general area exhibited a similar reliance on salmon.

While significant differences are not apparent between adjacent sites along the Thompson and the South Thompson Rivers, they become apparent when the results for sites on or near each of the two rivers are grouped, and also when they are compared with the Lillooet area sites on the Fraser River. People from site clusters farther upstream show less reliance on salmon than those on or close to the major salmon runs of the Fraser River. We would expect the South Thompson River runs to be somewhat smaller than those of the Thompson because of the escapements into the North Thompson and other tributary rivers.

These observations are in general agreement with those of Lovell et al (n.d.) which suggest a trend towards less salmon usage for upstream sites. When Lovell et al's (n.d.) sample results are grouped as done here the differences between adjacent sites are obscured by the measurement errors and standard deviations for the results from individual sites.

In other interior areas the same pattern prevails, i.e., individuals who likely had greater access to salmon show greater intake of marine protein. Three individuals from the Okanagan Valley show decreasing salmon use with increasing distance from the salmon rivers to the south in Washington State. Near the confluence of the Kootenay River with the Columbia River people from DhQj 1 (Brilliant) show greater salmon use than people from DjQj 1 (Vallican) on the tributary Slocan River. An individual from EaQl 10 on the Arrow Lakes used even less salmon. This trend is consistent with decreasing salmon use by people who had less access to the annual fishery at Kettle Falls. Individuals from sites such as DgQd Y (Creston), DgQo 2 (Grand Forks) and EbPw 1 (Canal Flats) would have had still less access to salmon, which is reflected in their isotopic ratios.

The samples from Brilliant and Vallican, although of only 3 and 2 individuals respectively, suggest that there was greater variability in diet than for the Lillooet and Thompson River people. It has been suggested that interior people who did not live near the major salmon rivers often travelled to fish at locations such as Kettle Falls (Chance et al 1977) and the Adams River area (Mohs 1980, personal communication). With people coming from a number of areas where salmon availability differed it would be reasonable to expect differences in diet and hence in $\delta^{13}\text{C}$ values for people who died and were buried near such gathering centers. Brilliant, at the confluence of the Kootenay and Columbia Rivers, may have been such a site, Vallican is nearby.

Interior results: summary

A number of general observations may be made about the British Columbia Interior dwellers. First, it is clear that a large portion of the dietary protein of the interior people came from salmon, about 40 to 60% for people living along the Fraser and Thompson River systems (Table 22).

A number of sample sets exhibit standard deviations of 0.3% or less, which serves as an indicator of the limits of resolution of this analytical approach and indicates that the diet of those people was essentially invariant. Unfortunately, the standard deviations here are still large enough to obscure small differences between adjacent sites within a region (e.g., the sites along the Thompson and the South Thompson Rivers) but are not too large to obscure differences between areas (e.g., between the Lillooet area and the Thompson River area). The evidence for these regional groupings indicates a decrease in salmon use for locations farther upstream on the Fraser and Thompson/South Thompson River systems. For sites away from the Fraser and Thompson River systems the diet appears to have been more varied. As the distance of sites from the major salmon runs increased, the people's reliance on salmon decreased.

The major problem encountered here in relation to the interior sites is the lack of well-dated remains for analysis. This makes it difficult to establish the time depth for salmon usage in the region. However, there is good evidence that salmon have been used in the interior for at least 5000 years and possibly longer. The two individuals from EiRm 7 (Clinton), dated to 4950 ± 170 BP (SFU 298), were definitely making use of marine species. The individual from near Seton Lake, dated to 2420 ± 80 (SFU 269), was even more reliant on marine species than the others in the interior region. The remaining interior people date from about 1600 BP up to about 100 BP and all

obtained a large portion of their protein from salmon. The EeQw 48 individual's result may indicate some use of salmon as far back as 8250 BP.

2.3 Proportion determinations

As mentioned earlier the benefit of the proportion estimates is in making comparisons with other archeological evidence that does not rely on $\delta^{13}\text{C}$ results. For example, faunal data relating to diet are usually presented as counts of species or as proportions of various species or groups of species present in an assemblage. When making comparisons with other regions of the world it may be also necessary to resort to proportion estimates. This is because human $\delta^{13}\text{C}$ results may be affected by local differences in dietary species values, thus equivalent values may indicate different proportions in different areas. The proportions and their uncertainties were determined as outlined in Part I, Chapter 6.

Table 21 Proportions of marine protein in diets ($\pm 10\%$): based on Coastal individual's averages.

A) Adults

<u>Location</u>	<u>% marine</u>	<u>Location</u>	<u>% marine</u>
Central Coast	89	Queen Charlotte Is.	92
Prince Rupert	87	Hesquiat	100
Gulf Islands	93	DfRu 8	88
Fraser Delta	85		

B) Children

Central Coast, 5600-1000 BP, Age 0 to 2.5 yrs	100 %
Age 2.5 to 6 yrs	81 %
< 1000 BP, Age 2.5 to 6 yrs	88 %

Table 22 Proportions of marine protein in diets ($\pm 10\%$): based on Interior individual's averages.

A) Adults:

<u>Location (Date)</u>	<u>% marine</u>	<u>Location (Date)</u>	<u>% marine</u>
Lillooet area (<1600)	60	Seton Lake (2420)	68
Thompson River	56	S. Thompson R. (≤ 1500)	48
Adams River	41	Gore Creek (8250)	8
Clinton area (ca. 4950)	40	Alexis Creek	24
Brilliant	48	Kelowna	19
Vallican	52	Peachland	44
Arrow Lakes	52	Penticton	57
Grand Forks	40	Canal Flats	8
Creston	0		

B) Children

Green Lake	32	Alexis Creek	24
Lillooet area	52	Vallican	41
Thompson River	55		
South Thompson	55 and 23		

British Columbian humans: summary and discussion

Results of this study indicate that since about 5500 BP people on the British Columbia coast have obtained most of their protein from marine species. There is no evidence prior to that time due to the lack of human samples. Individuals from EISx 1, on the central coast, and FIUa 4, in the Queen Charlotte Islands, may show a slightly greater reliance on terrestrial

species, before about 4500 - 5000 BP, but these results are inconclusive due to variability in results and uncertainty of some of the dates. Since about 5000 BP there has been no obvious change in marine - terrestrial proportions for any of the coastal areas sampled and there are no significant differences between areas for that time period. This pattern is in agreement with that outlined by Fladmark (1982) and discussed in Part II, Chapter 1 of this report.

There are a number of site- and area-specific interpretations that have been discussed in Part II, Chapter 1 to be considered. For example, the shift away from land mammals towards sea mammals observed by Savage (1973) at the Boardwalk site in Prince Rupert may be weakly related to the shift in $\delta^{13}\text{C}$ values observed for people from EISx 1 and FIUa 4. The problem with this comparison is that Savage's observed shift in diet proportions occurred after about 3500 BP while the shifts at EISx 1 and FIUa 4 occurred before about 5000 - 4500 BP. Unfortunately, the Prince Rupert humans examined here, which are only 0.2‰ lighter isotopically, are of little help since they date from about 2200-1700 BP. Results from EISx 1 contradict Conover's (1978) evidence for heavy terrestrial mammal usage at Namu. Inner- versus outer-coast site differences (Pomeroy 1980, Stryd 1969) in the Central Coast area are not evident in the data obtained and reported here. C. Carlson (1979) working on northern Vancouver Island, Calvert (1980) working at Hesquiat, the Yuquot workers (Clarke and Clarke 1980, McAllister 1980, Rick 1980), and Folan (1984) summarizing Jewitt's (1807, 1896) observations on the Nootka, all indicate a heavy reliance on marine species that is supported here.

Boucher's (1976) arguments for change from about 20% fish in the earlier periods at DfRu 8 are contrary to the evidence here that suggests a marine intake of $88 \pm 10\%$. Samples from more recent periods are not available. Ham's (1982) observation that terrestrial mammals are the most common

species exploited in the Gulf of Georgia region is in direct opposition to the conclusions of this study. It is not clear why these differences in interpretation between isotopic and faunal evidence exist, but the most likely reason is that the faunal record is incomplete and has misrepresented the actual subsistence pattern of the prehistoric people. This could result from a number of causes, such as differential preservation, differential recovery, and differential deposition of bone - due to either natural or cultural causes.

In the interior areas of British Columbia there is little subsistence data with which to compare these results. Detection of temporal change is not possible due to the lack of dated remains. However, it is clear that some salmon have been eaten within the study area since at least about 5000 BP.

Decreases in salmon availability and possibly in salmon quality, and hence desirability, for people who lived further upstream on the Fraser and Thompson River systems have been discussed by Kew (1976), Montgomery (1978), Stryd (personal communication), and Teit (1909, 1930). They are supported by the evidence here, of a progressive decrease in salmon consumption for individuals from locations farther upstream, or farther removed from the salmon streams. Although there are differences between the Lillooet and Thompson River areas, evidence from more sites in the affected areas will be necessary to deal with reported regional differences in salmon exploitation due to intertribal conflict (Teit 1909).

It is clear from the results and interpretations reported here that there is still more work that can be done in unravelling prehistoric diet in British Columbia. Acquisition of dated human samples, particularly from the interior areas, is necessary if we are to determine the length of time that salmon were being used. Similarly, older dated samples from the coast will be needed before the question of change in diet at about 5000 BP is determined.

Another question that must be dealt with is the presence of outliers. They suggest that we cannot rely on only one or two individuals to infer diet for the people at one site or in one region. While either intentional or accidental heating of bone is a possible cause of anomalous results, the outliers observed here could represent individuals that were visiting from other areas where the diets differed, they could represent food taboos, or perhaps status differences. The low standard deviations of about $\pm 0.2\%$ attained for some of the interior sample groups do suggest that a large number of individuals are not necessary to determine diet for a local population. A reasonable sample size might be 5 or 6 individuals per group.

The variability in results that is attributable to dietary inhomogeneity is about 0.2 - 0.3‰ greater for coastal people than it is for those living along the Fraser and Thompson River systems. This suggests that either the diet of coastal people was slightly more varied than that of the Fraser - Thompson people, or that the foods themselves showed greater variability in $\delta^{13}\text{C}$ results. Variability for people from other interior locations is still greater than for the coastal dwellers indicating that either their diet was more varied than that of either the coastal or the Fraser - Thompson people, or the diet species values were more varied in coastal areas.

Dietary differences relating to children have not been reported in the literature for either the coast or interior areas of British Columbia. The limited evidence on children's diet changes, from age 0 - 2.5 years to ages 2.5 - 6 years, and older, is not paralleled in Lovell et al's (1986) study of a large population from the Grey site in Saskatchewan, where no differences were observed. However, the possibility that weaning of children may be detectable through $\delta^{13}\text{C}$ results is hinted at, but not proven, by the data here and is an area that would be worthy of further investigation.

Another area for investigation is the possibility that seasonal behaviour may be evident via this analytical approach. This is one explanation suggested by the results for the DfRu 8 people, who differed from their Gulf Island neighbours. Seasonal behaviour investigations may possibly be carried out in regions and time periods where the seasonal round can be well defined and where both marine and terrestrial foods were available.

9 Conclusions

This study includes the first results to indicate that stable-carbon isotopic analysis can be used to distinguish between marine and terrestrial dietary alternative species. It also reports the results of a study of the increment between the isotope ratio of a consumer's bone gelatin and that of its average diet. Because the isotope ratios that are preserved in the consumers bone collagen reflect the average isotope ratios for their diets, the ratios may be used to determine the relative proportions of marine and terrestrial species in prehistoric human diet in coastal situations, and riverine situations where anadromous species are present.

Questions posed in the earlier chapters relating to the applicability of the technique have been answered and show that the technique may be applied to marine - terrestrial comparisons, at least in British Columbia. We know that the marine and terrestrial dietary alternatives are separated by about 8‰ in this region, and that their variabilities are small enough that they do not obscure that difference. ' Δdc ', the increment between a consumer's gelatin and its average diet, is about $4.5 \pm 0.5\text{‰}$ for a number of species that have metabolisms similar to humans; it has therefore been assumed that the human value is similar. Intra-individual variability, which includes analytical reproducibility, is $\pm 0.2\text{‰}$. This is the same as the standard deviation for some of the human populations studied indicating that inter-individual variability for those groups, which would include variability due to individual differences in ' Δdc ', was not detectable. Variability for populations that is greater than that should result from differences in individuals' diets within the population. Differences between populations that exceed this value would also result from differences in diet species values.

Terrestrial dietary alternatives in British Columbia show a difference, of 0.9‰, in average values between northern and southern area samples. Marine samples are incomplete but show no significant differences between areas.

Human results from this study indicate that prehistoric coastal dwellers in British Columbia have obtained around $89 \pm 10\%$ of their protein from marine species for about the last 5500 years. For the individuals examined there has been no significant change in this proportion during that time period for any or all of the sites from which samples were obtained. Small but statistically significant biases towards terrestrial protein were indicated for sites near the Fraser and Skeena river mouths, perhaps due to the influx of riverborne biogenic carbon, or perhaps due to easier access to open areas where terrestrial species could be exploited. Statistical results show that people from Helen Point on Mayne Island more closely resemble those from the Fraser Delta area people than they do their Gulf Island neighbours. This may indicate that the people inhabited the Helen Point site only seasonally.

People from interior salmon-bearing rivers obtained from ca. $40 \pm 10\%$ to $60 \pm 10\%$ of their protein from marine species, specifically salmon. This value decreases to as low as $0 \pm 10\%$ for people living away from the major salmon runs. People from sites further upstream on the salmon rivers used slightly less salmon than those around Lytton and Lillooet. Although well-dated samples are rare in the interior it appears that salmon use existed as far back as about 5000 BP, and that salmon may have been in use as far back as 8250 BP at Gore Creek on the South Thompson River.

Apparent differences between females and males are small and are statistically significant in only one instance. The females from EISx 1 (Namu) were isotopically lighter than the males indicating a slightly higher

reliance on terrestrial species than the males, possibly because they were involved in terrestrial foraging while men fished and hunted sea mammals.

Results for the few children analyzed varied with their age. Neonates from EbRi 7 gave results that varied considerably. Two children from Namu aged from 0 to 2.5 years gave results that were heavier than the female average, indicating either a more marine diet for the mothers, or possibly that these children had not been weaned yet. Older children in both the interior and coastal areas gave results that were lighter isotopically than the adults, indicating a greater intake of terrestrial protein.

Estimates of the relative proportion of marine species in prehistoric diets reported here suggest that until recently archaeological interpretations of subsistence have under-rated the importance of marine species in British Columbian prehistoric diet.

The approach used in this study should be applicable in similar coastal and riverine situations elsewhere in the world once the local dietary alternatives are identified and their average isotope ratios determined. It has the capability of detecting differences in dietary protein sources that may possibly be correlated with other aspects of human subsistence behaviour. In fact it has already been used to examine changes in subsistence technology reflected in representational art during the Late Magdalenian period in France (Hayden et al n.d.). How useful the technique will be for determining such things as seasonal behaviour, or status differences that are reflected in diet, or similar phenomena, still remains to be demonstrated. However, the potential is there for the technique to provide information that is useful in solving archaeological problems in areas where marine and terrestrial diet alternatives are present. It provides a very useful complement to other lines of archaeological analysis.

Appendix A Results for individual samples of dietary species (‰).**A** Gulf of Georgia region:

Species	Individual samples $\delta^{13}\text{C}$ (‰)		
	Unextracted	Extracted A*	Extracted B*
<i>Clupea pallasii</i> (Herring)	-18.8 -21.0 -18.1 -18.8 -17.1	-16.3 -17.5 -18.7	
<i>Hippoglossus stenolepis</i> (Halibut)	-18.3 -16.0	-18.1	
<i>Sebastes</i> spp. (Rockfish)	-15.6 -15.1 -15.8 -18.4		-17.4 -20.0
<i>Onchorhynchus</i> spp. (Salmon)	-16.6 -17.6 -19.9 -16.2 -17.1 -18.7	-16.1 -18.1	-18.5
<i>Pandalus</i> sp. (Shrimp)	-17.3 -17.6		-20.5
<i>Eumetopias jubata</i> (Northern Sea Lion)	-17.1	-18.8	
<i>Protothaca staminea</i> (Little Neck Clam)	-18.0 -18.3 -21.2		-19.4 -20.6
<i>Saxidomus giganteus</i> (Butter Clam)	-20.6		-21.0 -21.9 -29.8
<i>Cancer</i> spp. (Crab)	-17.2		
<i>Ostrea lurida</i> (Oyster)	-18.4		
<i>Anas platyrhynchos</i> (Mallard)			-28.2
<i>Anas acuta</i> (Pintail Duck)	-19.2	-18.0	
<i>Aythya americana</i> (Red Headed Duck)	-20.8		-23.3

Gulf of Georgia region: continued

Species	Individual samples $\delta^{13}\text{C}$ (‰)		
	Unextracted	Extracted A*	Extracted B*
<i>Aechmophorus occidentalis</i> (Western Grebe)	-19.3	-19.2	
<i>Melanitta perspicillata</i> (Surf Scoter)			-28.2
<i>Ardea herodias</i> (Great Blue Heron)	-14.9		-16.2

B Vancouver Island, west coast region:

Species	Individual samples $\delta^{13}\text{C}$ (‰)		
	Unextracted	Extracted A*	Extracted B*
<i>H. stenolepis</i> (Halibut)	-17.7	-16.0	
<i>Sebastes</i> spp. (Rockfish)	-18.3	-19.2	
<i>Onchorhynchus</i> spp. (Salmon)			-23.8 -25.3
<i>Thunnus alalunga</i> (Albacore)			-24.8 -24.7
<i>Callorhinus ursinus</i> (Northern Fur Seal)	-18.1		
<i>Phocaena vomerina</i> (Harbour Porpoise)	-16.4	-16.0	
<i>Physeter catodon</i> (Sperm Whale)	-18.4	-14.4	
<i>P. staminea</i> (Little Neck Clam)			-26.1 -21.4
			-21.8 -22.4
<i>S. giganteus</i> (Butter clam)			-22.8 -21.5
<i>Mytilus edulis</i> (Blue mussel)			-24.7
<i>Cancer</i> sp. (Crab)			-21.9

C Central Coast region:

<u>Species</u>	<u>Individual samples $\delta^{13}\text{C}$ (‰)</u>		
	Unextracted	Extracted A*	Extracted B*
<i>Sebastes</i> spp. (Rockfish)			-19.5 -19.7
<i>Onchorhynchus</i> spp. (Salmon)			-20.9
<i>H. stenolepis</i> (Halibut)	-15.0 -15.7		
<i>M. edulis</i> (Blue Mussel)	-16.8 -18.5		
	-17.8		
<i>Cancer</i> spp. (Crab)	-17.8 -18.1		
<i>S. giganteus</i> (Butter Clam)	-18.2 -16.9		
<i>P. staminea</i> (Little Neck Clam)	-17.8		-20.2 -20.9

D Prince Rupert region:

<u>Species</u>	<u>Individual samples $\delta^{13}\text{C}$ (‰)</u>		
	Unextracted	Extracted A*	Extracted B*
<i>Onchorhynchus</i> spp. (Salmon)	-19.2 -16.1		
<i>P. staminea</i> (Little Neck Clam)			-21.3 -20.3

E Queen Charlotte Islands region:

Species	Individual samples $\delta^{13}\text{C}$ (‰)		
	Unextracted	Extracted A*	Extracted B*
<i>Sebastes</i> spp. (Rockfish)			-19.9 -19.7
<i>H. stenolepis</i> (Halibut)			-20.0
<i>P. staminea</i> (Little Neck Clam)			-20.1 -20.6
			-24.1
<i>S. giganteus</i> (Butter Clam)			-21.3 -21.8
			-21.5
<i>Cancer</i> sp. (Crab)	-16.5		
<i>Tegula</i> sp. (Snail)	-16.8		

F Northern Interior region:

Species	Individual samples $\delta^{13}\text{C}$ (‰)		
	Unextracted	Extracted A*	Extracted B*
<i>Alces alces</i> (Moose)	-24.4 -24.8		-24.8 -28.6
	-25.3		-26.9
<i>Odocoileus hemionus</i> (Deer)			-27.8 -25.4
<i>Lepus americanus</i> (Snowshoe Hare)	-26.2 -27.5		
	-26.4 -26.2		-30.2 -30.6
	-28.1 -27.4		
<i>Castor canadensis</i> (Beaver)	-25.8 -26.6		-28.1
	-25.1 -24.8		
	-26.4 -24.9		

Northern Interior region: continued

<u>Species</u>	<u>Individual samples $\delta^{13}\text{C}$ (‰)</u>			
	Unextracted		Extracted A*	Extracted B*
<i>Bonasa umbellus</i> (Grouse)	-25.3	-26.6		-29.6 -29.4
	-26.6	-24.4		-27.7
	-24.3	-25.4		
	-25.0			
<i>Lagopus</i> sp. (Ptarmigan)	-23.6			-25.5
<i>Branta canadensis</i> (Canada Goose)	-28.0			
<i>Salvelinus malma</i> (Dolly Varden)	-31.9	-27.1		-34.9 -27.3
<i>Salvelinus</i> sp. (Lake Trout)				-30.3 -29.8
<i>Salmo gairdneri</i> (Steelhead)	-20.7	-21.5		-22.3 -22.6

6 Southern Interior region:

<u>Species</u>	<u>Individual samples $\delta^{13}\text{C}$ (‰)</u>			
	Unextracted		Extracted A*	Extracted B*
<i>Cervus canadensis</i> (Elk)	-24.7	-25.2	-26.0	-26.2
<i>O. hemionus</i> (Deer)	-24.6	-24.9		-28.6
<i>A. alces</i> (Moose)	-24.7	-24.7		-28.4 -28.4
<i>Rangifer</i> sp. (Caribou)	-22.4		-21.7	
<i>C. canadensis</i> (Beaver)	-24.7	-24.6	-22.6	-29.4 -27.0

Southern Interior region: continued

Species	Individual samples $\delta^{13}\text{C}$ (‰)		
	Unextracted	Extracted A*	Extracted B*
<i>L. americanus</i> (Snowshoe Hare)	-29.1		-30.4 -30.8 -29.1 -27.7
<i>Spermophilus</i> sp. (Ground Squirrel)	-26.0	-26.9	
<i>Ursus</i> sp. (Black Bear)	-23.9 -24.6	-23.8	-26.7
<i>B. umbellus</i> & <i>Dendragapus obscurus</i> (Ruffed and Blue Grouse)	-24.7		-26.7 -28.2
<i>B. canadensis</i> (Canada Goose)	-27.4		-30.5 -29.4
<i>Olor</i> sp. (Swan)	-23.8 -23.0	-23.8	-25.5
<i>A. platyrhynchos</i> (Mallard)	-24.0	-24.7	
<i>Gavia immer</i> (Loon)	-26.1	-25.8	
<i>S. gairdneri</i> (Steelhead)	-23.2 -22.3		
<i>S. malma</i> (Dolly Varden)	-27.3		-29.3 -29.2
<i>Salvelinus</i> sp. (Lake Trout)			-34.0 -29.3
<i>Margaritifera</i> sp. (River Mussel)	-29.1		-31.0

* 'Extracted A' refers to those samples that had lipids removed but that were not freeze dried, 'Extracted B' refers to those that were freeze dried before lipid removal.

Appendix B Individual Human sample results (‰).**A** Gulf of Georgia region

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Saltspring Island					
DcRu 52		Adult	?	610 ± 400	-13.3
Pender Island					
DeRt 1	85-1	Adult	?		-18.7
DeRt 1	85-3	Adult	?		-13.5
DeRt 2	58-4	Adult	?		-13.4
DeRt 2	84-5 a	Adult	F	3780±180	-13.7
DeRt 2	84-12	Mid Adult	M	5170±220	-13.5
DeRt 2	84-23	Young Adult	M?	4580±550	-13.1
DeRt 2	84-23 a	Young Adult	F?		-12.7
DeRt 2	84-27	Mid Adult	F?	3260±200	-13.3
DeRt 2	84-30 a	Old Adult	M?		-12.8
DeRt 2	84-31	Old Adult	F	4320±220	-12.4
DeRt 2	84-33	Adult	F?		-12.7
DeRt 2	84-34 a	Mid Adult	M?	2580±180	-13.0
DeRt 2	84-34 b	Adult	F	4320±150	-13.5
DeRt 2	84-34 c	Adult	?	3370±280	-12.8
DeRt 2	84-37	Old Adult	F	3140±200	-12.7
DeRt 2	84-41	Old Adult	M?	3990±160	-13.2
DeRt 2	84-42	Old Adult	M?	1090±130	-13.6
DeRt 2	84-43	Mid Adult	F?	3050±150	-17.4
DeRt 2	84-44	Old Adult	M	1420±90	-14.2
DeRt 2	84-46	Young Adult	M		-12.8
DeRt 2	84-47	Infant	?		-12.8
DeRt 2	85-1	Infant	?	ca. 4000	-12.0
DeRt 2	85-2	Adult	?	3000-2500	-12.8
DeRt 2	85-4	Young Adult	M	ca. 1000	-12.8
DeRt 2	85-7	Adult	F		-12.9
DeRt 2	85-7	Adult	?		-16.5
DeRt 2	85-8	Adult	?		-13.3
DeRt 2	85-9 a	Adult	M		-12.3
DeRt 2	85-9 a	Adult	M		-12.5
DeRt 2	85-9 b	Adult	?		-16.5
DeRt 2	85-12	Adult	?		-11.9
DeRt 2	85-13	Mid Adult	M		-12.4

Gulf of Georgia region, continued:

Site	Burial #	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Pender Island					
DeRt 2	85-13	Mid Adult	M		-15.4
DeRt 2	85-14	Adult	?		-12.1
DeRt 2	85-15	Mid Adult	F		-12.6
DeRt 2	85-16	Mid Adult	M		-12.3
DeRt 2	85-16	Mid Adult	M		-12.4
DeRt 2	85-17	Mid Adult	M		-12.7
DeRt 2	85-19	Adult	F		-12.9
DeRt 2	85-20	Adult	?		-12.5
DeRt 2	85-21	Adult	M		-13.0
DeRt 2	85-22	Adult	?		-12.8
DeRt 2	85-23	Adult	?		-13.2
DeRt 2	85-24	Adult	M		-13.0
DeRt 2	85-26	Adult	F		-12.6
DeRt 2	85-27	Immature	?		-13.3
DeRt 2	85-29 b	Adult	?		-12.7
DeRt 2	85-30	Adult	?		-12.8
DeRt 2	85-32	Adult	?		-12.7
DeRt 2	85-34	Adult	?		-13.7
DeRt 2	85-35	Adult	?		-12.7
DeRt 2	85-36	Mid Adult	F		-13.0
DeRt 2	85-37	Old Adult	F		-13.7
DeRt 2	85-38	Adult	M		-13.4
Fraser Delta					
DfRs 3	1	Teens	M		-20.8
DfRs3	2	Adult	M		-13.5
DfRs 3		Infant	?		-14.0
Vancouver Island					
DfRu42		44-59	F	1080±80	-12.6
Mayne Island					
DfRu 8	SFU 1	Old Adult	M	5000-3500	-14.2
DfRu 8	SFU 2	Adult	M	5000-3500	-12.9
DfRu 8	SFU 4B	Adult	?	5000-3500	-14.0
DfRu 8	SFU 5A	ca. 36	F	5000-3500	-14.9
DfRu 8	SFU 5B	15-18	?	5000-3500	-13.3
DfRu 8	SFU 6	Adult	F	5000-3500	-13.1
DfRu 8	SFU 7	14-17	F?	5000-3500	-12.8

Gulf of Georgia region, continued:

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Mayne Island					
DfRu 8	BCPM 1	17-20	F	5000-3500	-14.0
DfRu 8	BCPM 2	25-35	F	5000-3500	-13.2
Fraser Delta					
DgRr 1	1	Old Adult	M	3050-1550	-13.8
DgRr 1	3	Adult	M	3050-1550	-13.0
DgRr 1	4	Adult	F	3050-1550	-13.6
DgRr 1	5	Old Adult	M	3050-1550	-13.2
DgRr 1	6	Adult	M	3050-1550	-13.5
DgRr 1	7	Young Adult	F	3050-1550	-13.4
DgRr 1	8	Old Adult	M	3050-1550	-13.4
DgRr 1	10	Old Adult	M	3050-1550	-13.4
DgRr 1	13	Adult	F	5350-3050	-14.2
DgRr 1	15	Adult	F	5350-3050	-13.5
DgRr 1	16	Adult	F	2350-1550	-13.6
DgRr 1	20	Adult	?	3050-2350	-13.3
DgRr 1	23	Adult	?	3050-2350	-13.9
DgRr 1	27	Adult	?		-13.4
DgRs 1	10 a	Juvenile	?	3-2000	-15.0
DgRs 1	13	Adult	?	3-2000	-14.0
DgRs 1	14	17-25	F	3-2000	-14.9
DgRs 1	15 e	35-60	M	3-2000	-14.1
DgRs 1	17	Adult	?	3-2000	-19.5
DgRs 1	23 (ex 2)	22-40	F	2810±70	-13.9
DgRs 1	24 (ex 3)	Young Adult	M	2720±80	-12.8
DgRs 1	25 (ex 4)	Adult	F	3-2000	-14.4
DgRs 1	26 (ex 5)	Child	?	3-2000	-14.0
DgRs 1	27 (ex 6)	Adult	?	3-2000	-15.3
DgRs 1	33 (ex 12)	Infant	?	3-2000	-14.2
DgRs 1	29 (ex 8)	45-55	F	1770±120	-13.3
DhRr 6		Adult	?	ca. 2190	-13.3
DhRr 6		Adult	?	2190±90	-13.3
DgRr 2	3 Part 37	Adult	?		-13.3
DgRr 2	4 814	Adult	?		-13.3
DgRr 2	6 *MS 5	Adult	?		-13.0
DgRr 2	10 6214K	Adult	?		-12.7
DgRr 2	18 2368	Adult	?		-13.9

Gulf of Georgia region, continued:

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Fraser Delta					
DgRr 2	28 144	Adult	?		-12.7
DgRr 2	96 #2368	Adult	?		-13.8
DgRr 2	97 #2897	Adult	?		-13.6
White Rock	Victoria Av	Adult	?		-15.9

References: Beattie (1976) for DgRr 1; Carlson (1985) for DeRt 1 and 2, Carlson (1984, personal communication) for DfRu 8; Charlton (1977) for DhRr 6; Lazenby (1986, personal communication) for DeRt 2; Patenaude (1983, personal communication) for DgRr 2; Skinner (1984, personal communication) for DfRu 42; and excavation notes and lab analysis files in the physical anthropology laboratory at Simon Fraser University.

B Hesquiat (Vancouver Island):

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
DiSo 1	e.u. L.10	Adult	?	1,300-500	-12.5
DiSo 1	e.u.18 L.9a	Adult	?	1,300-500	-19.2
DiSo 9	e.u.2 L.2	Adult	?	2-1000	-12.5
DiSo 9	e.u.9 L.5b	Adult	?	2-1000	-14.4
DiSo 9	e.u.9 L.7c	Adult	?	2-1000	-12.6

Reference: Calvert (1980)

C Central Coast region:

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Bliss Landing					
EaSe 2	1	Young Adult	?	5350-3050	-13.3
EaSe 2	2	Old Adult	M	5350-3050	-12.6
EaSe 2	3	39-44	M	5350-3050	-13.2
Port Hardy					
EeSu 5	1	ca. 24	F	2950-2350	-11.9
Namu					
EISx 1	1-11-B	Old Adult	M?	5590±100	-12.9

Central Coast region, continued:

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Namu					
EISx 1	1-13D-1	Adult	?	>5600?	-14.9
EISx 1	2-11C-1	17-18	F	5-4000	-12.5
EISx 1	2-12E-1	20-35	F	4480±125	-13.8
EISx 1	4-B-1	6-7	?	5-4000	-13.1
EISx 1	4-C-1	Young Adult	M	5-4000	-13.1
EISx 1	4-G-1	Young Adult	F	5-4000	-13.5
EISx 1	4-G-2	Old Adult	M	4680±160	-13.4
EISx 1	4-G-3	ca. 17	?	5-4000	-13.4
EISx 1	4-G-4	Mid Adult	M	5-4000	-13.0
EISx 1	4-G-5	7-8	?	5-4000	-13.5
EISx 1	4-G-7	4.5-5.5	?	5-4000	-14.1
EISx 1	4-G-8	17-18	F?	4885±125	-13.6
EISx 1	4-G2-B2	Mid Adult	M	5-4000	-13.6
EISx 1	4-H	Mid Adult	M	5-4000	-12.5
EISx 1	4-I	15-16	?	5-4000	-13.0
EISx 1	4-J-1	Young Adult	F	4390±160	-13.0
EISx 1	4-K-1(1)	Adult	F	5-4000	-17.0
EISx 1	5-11P-1	Adult	M	5-4000	-12.7
EISx 1	5-11P-2	0-0.5	?	5-4000	-12.4
EISx 1	8-12A-1	Old Adult	F	5-4000	-13.9
EISx 1	9-0A-1	2.5-3.5	?	<1000	-13.4
EISx 1	9-1-22	0	?	2-1000	-11.7
EISx 1	9-1-24	14-16	?	2-1000	-14.5
EISx 1	9-1-39	ca. 18	F	2-1000	-13.3
EISx 1	9-3B-2	18-19	F?	3-2000	-14.5
EISx 1	11-1A-2	14-16	?	5-4000	-13.7
EISx 1	77-1	Mid Adult	?	5-4000	-13.0
EISx 1	77-2	Young Adult	M	4975±130	-12.8
EISx 1	77-3	Old Adult	M	5-4000	-12.9
EISx 1	77-4	Immature	?	5-4000	-14.5
EISx 1	77-6	15-17	F?	5-4000	-13.0
EISx 1	77-7	Mid Adult	M	5-4000	-12.6
EISx 1	77-8	3.5-4.5	?	5-4000	-16.9
EISx 1	77-9	Mid Adult	F	3-2000	-13.6
EISx 1	77-10	Young Adult	F	3-2000	-12.8
EISx 1	77-12	Young Adult	F?	5-4000	-14.7

Central Coast region, continued:

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Namu					
EISx 1	78-1	Young Adult	M	2530±160	-13.0
McNaughton Island					
EITb 10	1	Adult	?	3-1000	-13.9
Kwatna					
FaSu 6	1	Adult	?	4-100	-13.5
FaSu 9	3	Adult	?	150-70	-14.3
Claatse Bay					
FcSx 1	73-6(1)	Child	?	<1000	-13.2
FcSx 1	73-6(2)	Adult	?	<1000	-13.6
Kimsquit					
FeSr 10	7	Adult	F	600-200	-13.6

References: Beattie (1972) for EaSe 2; Carlson (1982, personal communication) for EITb 10; Chapman (1972) for EeSu 5; Curtin (1984) for EISx 1; Hobler (1982, personal communication) for FaSu 6 & 9 and FeSr 10; Keddie (1985, personal communication) for FcSx 1.

D Prince Rupert region:

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Prince Rupert Harbour					
GbTo 31	328	Adult	?	1750-1950	-14.1
GbTo 31	330	Adult	?	2250-2450	-13.9
GbTo 33	455	Adult	?	2000-2200	-13.0
GbTo 36	509	Adult	?	2800-3000	-13.2
GbTo 36	515	Adult	?	1750-2250	-12.7
Greenville					
GgTj 6	2D-48	Adult	?		-13.5
GgTj 6	3D-23	Adult	?		-14.0

References: Cybulski (1981, personal communication).

E Queen Charlotte Islands:

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Haans Island					
FgTw 5	73-14	Adult	?		-13.1
Bluejackets Creek					
FIUa 4	2	Adult	?		-15.4
FIUa 4	4	Adult	?	4500±145	-14.1
FIUa 4	7	Adult	?		-18.4
FIUa 4	15	Adult	?	4890±150	-17.9
FIUa 4	17	Adult	?		-13.0
FIUa 4	20	Adult	?	4900±155	-15.2
Naden Harbour					
FIUd Y	s59-23	Adult	?		-12.4
GaUd 1	57-6(1)	Adult	?		-19.1
GaUd 1	57-6(2)	Adult	?		-13.9
Yaku - Langara Island area					
GaUa 2	83-29	Old Adult	M	1230±60	-18.8
GaUa 3	73-24	Adult	?		-12.2
GbUg 2	71-101	Adult	?		-13.5
GbUg Y	s47-4(1)	Adult	?		-13.3
GbUg Y	s47-4(2)	Adult	?		-13.3

References: Fladmark (1970) for dates; Keddle (1985, personal communication).

F British Columbia Interior region:

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Alexis Ck.		Child	?		-18.2
Alexis Ck.		Adult	?		-16.2
Creston					
DgQd Y	1	Adult	M		-20.0
Grand Forks					
DgQo 2	1	Adult	?		-17.1
DgQo 2	2	Adult	?		-16.9
Brilliant					
DhQj 1	1	Adult	?		-14.6
DhQj 1	3	Adult	?		-15.7

F British Columbia Interior region:

Site	Burial #	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Brilliant					
DhQj 1	4	Adult	?		-16.9
Vallican					
DjQj 1	1	Infant	?		-16.8
DjQj 1	2	Foetal?	?		-17.0
DjQj 1	3	Adult	?	1250±120	-18.4
DjQj 1	4	Adult	?	1250±120	-16.4
DjQj 1	5	Adult	?		-15.7
Kelowna					
DIQu Y	1	Adult	F		-19.0
DIQu Y	2	Adult	F		-17.5
EaQu 6		Adult	F		-19.2
Arrow Lakes					
EaQl 10		Adult	?		-16.1
Nicola					
EaRf 6	B 78	Adult	?		-16.1
Canal Flats					
EbPw 1		Adult	?		-19.4
Nicoamen River					
EbRi 7	1	Adult	M		-23.7
EbRi 7	2	Adult	F		-22.0
EbRi 7	3	Mid teens	F	ca. 740	-15.7
EbRi 7	4	11 y.	F	ca. 740	-15.5
EbRi 7	5	5 y.	?	ca. 740	-16.0
EbRi 7	6	Adult	F	740 ±130	-15.8
EbRi 7	8	Adult	F	ca. 740	-15.9
EbRi 7	8 b	Adult	?		-19.4
EbRi 7	9	Adult	F		-15.8
EbRi 7	10	0	?		-16.6
EbRi 7	11	0	?		-15.8
EbRi 7	12	9 mo.	?	historic	-16.2
EbRi 7	13	Adult	M		-15.7
EbRi 7	14	Late teens	F		-14.6
EbRi 7	16	Adult	M		-16.2
EbRi 7	18	0	?		-15.1
Monte Creek					
EdQx 20		Adult	?		-16.5

British Columbia Interior region, continued:

Site	Burial *	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Cache Creek					
EdRh 1	A	Adult	?		-16.3
EdRh 1	F	Adult	?		-15.8
EdRh 1	H	Child	?		-17.7
EdRh 1	Ha	Foetal	?		-19.0
EdRh 1	I	Adult	?		-15.6
Basque					
EdRh 26		Adult	?		-20.9
Spences Bridge					
EdRh Y 53		Adult	F		-15.7
Hat Creek					
EdRk 3	B 1	Adult	?		-16.1
EdRk 3	B 2	Adult	?		-15.3
Lillooet					
EdRl 22		Adult	M	n.d.	-15.6
EeRk 4	19-B1-1	Infant	?	1250±100	-16.1
EeRl 6	6-B1-1	Young Adult	F	1800-1400	-15.4
EeRl 18		Adult	F	Historic	-15.2
EeRl 19	B1-1	Adult	M	450±70	-15.6
EeRl 19	B2-25	Adult	F	450±70	-15.5
EeRl 80		45-60	F	Historic	-15.8
EeRl 167		Adult	F	L. Prehist.	-15.1
EeRl 169		Adult	M	Historic	-15.4
EeRl 192	4	Adult	?		-15.6
?		Adult	?		-19.3
Chase					
EeQw 1	B 3	Adult	M	7-200	-16.1
EeQw 1	B 4	Adult	M	7-200	-16.5
EeQw 1	B 5	Child	?	7-200	-18.3
Kamloops					
Ed(e)Rc 61		Adult	?		-13.2
EeRb 10		Adult	?	ca. 3000	-20.0
EeRc 8	1	Adult	M	ca. 1500	-17.6
?	CCB 8	Adult	F		-16.4
?	CCB 14	Child	?		-15.9
?	CCB 18	Adult	F		-16.3

British Columbia Interior region, continued:

Site	Burial #	Age	Sex	Date	$\delta^{13}\text{C}$ (‰)
Gore Creek					
EeQw 48		Adult	M	8250±115	-19.4
Squilax - Adams Lake					
EfQv 10		Adult	F		-16.9
EgQw Y 66		Adult	M		-16.9
Clinton					
EgRk 2	CC B-12	Adult	?		-16.7
EiRm 7	A	Young Adult	M	4950±170	-17.2
EiRm 7	B	Adult	?M	4950±170	-17.1
Green Lake					
EiRh 4	B 79	ca. 5 yrs	?		-17.6
Penticton					
EjQv Y65		Adult	?		-15.7
Peachland					
EkQw Y1981		5-10	?	<500	-16.7
Lee Creek					
?	CCB 30	Adult	M		-16.5
Savona					
?	CCB 1	Adult	F		-16.4
Seton Lk.					
?		Adult	?		-14.9

References: Cybulski et al (1981) for EeQw 48; McKendry (1982, personal communication) for EiRm 7; Mohs (1982, personal communication) for DjQj 1 and EdRh 26; Pokotylo (1983, personal communication) for EdRk 3 and EeQw 1; Rousseau (1985, personal communication) for EeRb 10; Skinner (1986) for EbRi 7; Keddie (1985, personal communication) and Stryd (1982, personal communication) for the remaining sites.

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