PREHISTORIC DIET IN CENTRAL BAJA CALIFORNIA, MEXICO

by

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ABSTRACT

Analysis of stable carbon and nitrogen isotopes in human bone has proved to be a useful method of investigating prehistoric diet in many different environmental settings. This study attempts to apply the technique in the Central Desert region of the Baja California peninsula, where the aridity of the region and the wide variety of dietary resources present special difficulties.

This study comprises several separate analyses: 1) stable isotope measurements of a sample of prehistorically important food items, in order to determine whether they display the consistent isotope measurements required to make quantitative estimates of dietary composition; 2) stable isotope measurements on both the protein and mineral fractions of bone in a sample of mammals from the region, in order to test whether the size of the increment between these measurements is related to trophic level, and whether this increment is useful in making dietary estimates; and 3) stable isotope and radiocarbon measurements of a small sample of human burials from two sites, one on the Gulf of California coast and one inland, in order to determine whether these groups had significantly different diets, and to evaluate previous estimates of dietary composition based on archaeological and historical evidence.

The results show that carbon and nitrogen stable isotope measurements can distinguish between the major dietary resources. As expected, the increment between the protein and mineral fractions of animal bone is related to trophic level, although interpreting this increment in humans is complex due to their varied diet. The results from the two human collections indicate that both groups made nearly exclusive use of nearby resources rather than traveling widely on a seasonal round. Quantitative estimates of diet are not possible because of the large number of isotopically labeled resources. However, a comparison of the human isotope measurements with those of a series of experimentally calculated diets suggest that previous assessments of diet can be improved.

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1.0 INTRODUCTION

The central Baja California peninsula is one of the most isolated and arid regions in North America. It is distinguished from other North American deserts by a number of unusual endemic species and by a rich and varied marine fauna along both of its coasts. The region also reveals an extensive, largely uninvestigated archaeological record of perhaps unexpected complexity and richness given the harshness of the climate. The purpose of this study is to characterize the diet of the prehistoric inhabitants of this unique environmental setting using stable isotope analysis.

Analysis of stable carbon and nitrogen isotopes in human bone has proved useful in reconstructing prehistoric diet in a variety of environmental settings. The method relies on the fact that different dietary resource types often display characteristic relative abundances of these isotopes, and that these isotopic signatures are preserved in the tissues of humans that consume them. Thus the method may allow assessment of the general categories of foods important in the diet. However, the aridity of the Baja California environment, as well as the wide variety of isotopic signatures expected of dietary items from the region, present special difficulties for the method.

This research seeks to address these problems through several specific studies. First, it seeks to establish a background database for paleodietary studies in the region by making carbon and nitrogen stable isotope measurements on a variety of prehistorically important food species. Certain food species, such as agaves and cacti, are known to display highly variable stable isotope measurements in other environments. If stable isotope analysis is to be a useful method of investigating diet in this region, these dietary items must display consistent and predictable stable isotope signatures.

Second, this study attempts to test whether the size of the increment between the carbon isotope measurements in the protein (collagen) and mineral (apatite) fractions of animal

bone is related to trophic level, as it appears to be in simpler ecological contexts, and whether such measurements made on humans may thus be useful in refining estimates of the composition of diet. Previous studies have suggested that interpreting this increment in humans is complex because of their varied diet. However, it is hoped that this increment may be understood by experimentally predicting the increment resulting from a series of hypothetical diets.

Third, this study makes stable isotope and radiocarbon measurements on two small samples of human burials from shoreline sites on the Gulf of California coast and from an inland mountain range in the central peninsula. A comparison of average stable isotope measurements between these groups may allow a preliminary assessment of regional differences in diet. Direct estimates of dietary composition from stable isotope values are not expected to be possible, because of the large number of dietary resources with distinct isotopic signatures. However, this study uses the results of the dietary background survey to evaluate a previous estimate of dietary composition based on historical accounts; this is done by calculating the hypothetical stable isotope measurements resulting from the estimated diet and comparing these to the results from the human collections. In an attempt to refine the estimate, the predicted stable isotope measurements of a variety of hypothesized diets are calculated, keeping in mind nutritional and archaeological considerations.

2.0 BACKGROUND: BAJA CALIFORNIA

2.1 Environment

Baja California is a peninsula approximately 1200 km long, extending roughly northwest to southeast from the mouth of the Colorado River to its tip at Cabo San Lucas (Figure 2.1). Varying in width between 25 and 150 kilometers, it is bounded by the Pacific Ocean on the west and the Gulf of California on the east. Dissected granitic and volcanic ranges run the length of the peninsula, generally reaching their greatest height near the Gulf coast.

The climate is arid, with high temperatures year-round. The peninsula receives winter precipitation from temperate-latitude storms systems as well as summer precipitation from tropical cyclones or *chubascos*. Both of these, however, are sporadic and unpredictable, especially in the central peninsula, which is outside the normal range of either of these systems. This central desert region supports vegetation typical of Sonoran deserts, including numerous cacti, agaves, yuccas, creosote, mesquite and other leguminous trees, as well as several unusual endemic species such as *torote (Pachycormus discolor)* and *cirio (Idria columnaris)*. Permanent watercourses are absent in the central desert, although water is found year-round in springs and natural rock tanks.

Paleoclimatic data from the nearby Sonora coast indicate that the region's climate has been generally similar to today's throughout most of the Holocene, with a slightly cooler regime in the early Holocene (Van Devender et al. 1994).

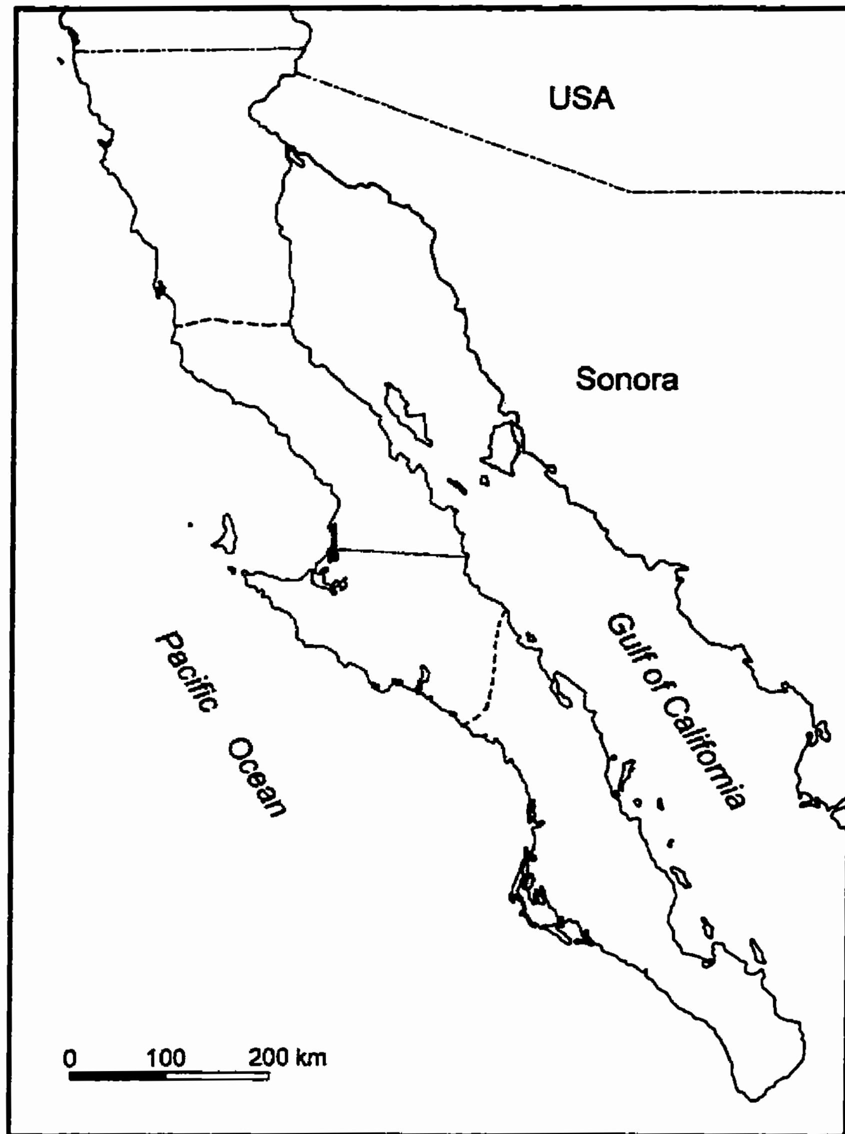

Figure 2.1. Baja California. Dashed lines show the boundary of the central desert region as defined by Aschmann (1959).

2.2 History

There has been little formal archaeological investigation on which to build a cultural chronology for the region, although human occupation of the area has a long history. Evidence of early Holocene occupation consists primarily of projectile points cross-dated

with Great Basin Paleoindian types (Aschmann 1952; Arnold 1957; Davis 1971); only a few possible Paleoindian occupation sites have been identified (Arnold 1957; Davis 1968).

Early and middle Archaic occupation is somewhat better documented. Based primarily on projectile point typologies, as well as obsidian hydration and radiocarbon measurements, Ritter (1985) tentatively proposes two Archaic periods for the Bahía Concepción region just to the south of the central desert: the Concepción tradition, lasting from about 5500 BC to 1000 BC, and the Coyote tradition, occurring from about 1000 BC to AD 1000. The first evidence for intensive exploitation of marine resources in this area appears during the latter period. Site distributions suggest the development of separate subsistence foci on marine and inland dietary resources during this time as well (Ritter 1985). A similar pattern is apparent in the Bahía de las Animas and Bahía de los Angeles areas to the north (Ritter 1995).

By far the best known prehistoric period is that of the Comondú culture, which is estimated to have originated around AD 1000, lasting into historic times as the material culture of the Cochimí people of the central peninsula (Massey 1966). This period is associated with a wide range of artifacts, including small, sometimes serrated projectile points, flat or shallow-basin grinding stones with small cobble manos, tubular stone pipes, *Olivella* beads, coiled basketry, and various types of netting. Late Comondú sites often contain mission period ceramics (Ritter et al. 1994). The large, figurative pictographs found in highland canyon sites in the region are most likely contemporaneous with the Comondú culture as well (Crosby 1984). Ritter (1995) sees evidence for further intensification of marine resource use on the Gulf coast during this period, including expanded reliance on more technology-intensive resources such as sea lions and sea turtles, and the development of formal east-west trade between some coastal and inland groups.

Jesuit missionaries established the first permanent European presence in the peninsula in the late 17th century. At the time of contact, the Cochimí people inhabited the central peninsula approximately between San Quintín and Bahía Concepción (Massey 1949). The Cochimí were foragers, organized in bands or *rancherías* of approximately 50 to 200 people traveling a seasonal round in a loosely held territory, generally gathering in summer around semi-permanent summer habitation sites and breaking into smaller units during the winter months. Economic and political organization was simple and egalitarian, in contrast to groups both to the north and south, which inhabited comparatively more productive environments.

While the missionaries produced a rich and detailed record of native society, language and economy (Venegas 1966; del Barco 1980; Baegert 1952; Clavigero 1937; Sales 1956), the missionaries also brought about the eventual extinction of the native population, primarily due to the introduction of smallpox and other diseases.

2.3 Evidence for prehistoric diet

Archaeological sites containing dietary residues are common in the region, but have rarely been subjected to archaeological analyses detailed enough to allow an assessment of the composition of native diet, much less regional or temporal differences in subsistence strategies. However, the missionaries' records are detailed enough that at least for the late prehistoric and historic periods, diet can be assessed with some accuracy.

The accounts emphasize the primary importance of vegetable foods in native diet. Indeed, plant foods such as agaves were often important in the economy of the missions. While terrestrial animal foods were eagerly sought after, they were a comparatively unreliable resource. For many groups, marine foods provided a more productive and reliable resource. However, many bands were oriented toward terrestrial foods and only made short seasonal trips to the coast to gather shellfish (Aschmann 1959:101). In some areas,

formalized trade may have developed between coastal and inland groups. However, Aschmann (1959) argues that most bands had direct access to a wide variety of resource types, if only seasonally.

A typical seasonal round would include a summer season of heavy reliance on cactus fruits, with a minor emphasis on annuals and legumes increasing in the fall. In winter and early spring agaves made up the main source of food, supplemented by spring-flowering herbaceous annuals. During this season of scarcity, inland-oriented groups would come to the coast to harvest shellfish, while marine-oriented groups would hunt and gather spring-flowering annuals in the highlands (Ritter 1985). In any given year, however, these patterns could vary greatly according to environmental conditions, and the unpredictability of most resources required a great deal of seasonal mobility.

Vegetable foods

Missionary accounts uniformly stress the importance of agaves (*Agave* spp.) as a dietary staple. When the plant was in bloom, the flower bud could be eaten after brief cooking, but most often the heart of the plant was harvested and rendered edible only after roasting in pits for a minimum of a day. Because the agave heart could be harvested year-round, it assumed much greater importance during the spring, when other plant foods were generally unavailable.

Of the many cactus species growing in the central desert, the most important was the *pitaya dulce* (*Lemairocereus thurberi*). Widespread in the central desert south of San Borja, its fruit is ripe for a short period in the summer. Native populations would make heavy use of this resource when available, gathering in large groups and taking part in a variety of religious and social activities. While this resource was of great social importance, it was less important in quantitative terms because it was not stored beyond its brief ripening period. Other important cacti include *pitaya agria* (*Machareocereus gummosus*), which ripens in the fall, and *cardón (Pachycereus pringlei*), ripening in the

spring and early summer. These do not appear to have made up a significant part of diet, except in the northern part of the central desert where *pitaya dulce* is absent. The fruits of *nopal* (*Platyopuntia* spp.), *garambullo* (*Lophocereus schottii*), and *viznaga* (*Ferocactus* spp.) were also of minor importance.

Leguminous trees are widespread in the central desert; the most important of these were *dipúa* and *palo brea* (*Cercidium* spp.). The pods of these species ripen one or two months after seasonal rains. The spring ripening was particularly important in alleviating the season of scarcity in the winter and early spring. Several varieties of mesquite (*Prosopis* spp.) are also found throughout the central desert. These ripen in the fall, and because they tap groundwater are a comparatively reliable resource regardless of variations in rainfall. However, there is little historic reference to the use of the plant.

The seeds of herbaceous plants also figure widely in missionary accounts of diet. However, the flowering of these species is highly dependent on rainfall and thus probably did not make up a major proportion of diet. The most important of these are the chenopods and amaranths, referred to collectively as *bledo*. Other important herbaceous annuals include *verdolaga* (*Portulaca* sp.) and *teda* (*Antigonon leptopus*).

Other plants were also of occasional importance, including the fruits of palms (*Washingtonia* and *Erythea* spp.), roots (*Amoreuxia* sp. and possibly *Manihot* sp.), *datilillo* (*Yucca valida*), the wild fig or *zalate* (*Ficus palmeri*), a berry known as *frutilla* (*Lycium* sp.) and various grass seeds.

Animal foods

Mission accounts indicate that smaller fauna such as snakes, lizards, rodents, insects, and insect larvae were much more important than larger game such as rabbits, deer, and antelope, although the latter were much sought after. Animals were often consumed completely, including bones and hide. Scavenging was also common. Coyotes, foxes, and

badgers appear to have been largely ignored. Hunting equipment included long bows, curved throwing sticks, nets and snares (Aschmann 1959:68).

The extensive shell middens on both coasts attest to the importance of mollusks in the diet. On the Gulf coast, the most important bivalve genera include the bivalves *Chione*, *Cardita*, *Protothaca*, and *Dosinia*, as well as snails such as *Turbo* (Ritter et al. 1994). Important resources in the cooler Pacific waters include bivalves such as *Chione*, *Tivella*, as well as abalone (*Haliotis*) and lobster (*Panulirus*). The availability of these species, however, varies widely throughout both coasts; many productive coastal areas also appear to have been largely ignored due to the lack of available water nearby (Aschmann 1959:98).

Fish were caught by netting, trapping and poisoning in tidal zones as well as hook-and-line or spear fishing from tule balsas in open water. Sea turtles (*Chelonia* spp., *Demochelys* sp.) and sea mammals such as sea lions and sea otters were also hunted from balsas. No means of hunting cetaceans existed, although beached whales provided an occasional windfall. Seabirds and their eggs were of minor importance.

<u>Estimates of dietary composition</u>

Based on historical accounts, Aschmann (1959) has made a quantitative estimate of the average composition of native diet during the late prehistoric and historic periods (Table 2.1). While he assumed that coastal and inland groups would have had widely divergent diets, he intended the estimate as an average of diet throughout the region. The percentages are given in terms of estimated "food values" (i.e., caloric content); vegetable foods would generally increase in importance if the percentages were expressed as bulk or weight. While this estimate is largely based on subjective impressions, it provides a useful starting point for further understanding of prehistoric diet.

This study attempts to evaluate Aschmann's dietary estimate, as well as the general picture of dietary adaptation and seasonal mobility provided by archaeological and historical evidence, through stable isotope analysis of two small collections of human burials from inland and coastal sites in the region. However, as discussed below, the Baja California environment presents serious difficulties for stable isotope analysis.

Table 2.1. Aschmann's estimate of dietary composition.

Category	%
Vegetable stuffs	**57**
Agave	28
Cactus fruits	12
Leguminous seeds and fruits	6
Other small seeds	6
Roots	3
Other items	2
Land animals	**18**
Rodents and reptiles	8
Insects	5
Deer and other large mammals	4
Birds	1
Marine animals	**25**
Shellfish	11
Fish	5
Sea mammals	5
Turtles	2
Sea birds	1
Bird and turtle eggs	1

3.0 BACKGROUND: STABLE ISOTOPE ANALYSIS

Stable isotope analysis relies on the fact that different dietary resources often have characteristic relative abundances of common atomic isotopes, and that these isotopic signatures are preserved in the tissues of humans that consume them. Isotopic analysis of human tissues thus allows assessment of the general categories of foods that were important in prehistoric diets.

For example, carbon has two stable isotopes, ^{13}C and ^{12}C, with average relative abundances of about 1% and 99% respectively. Similarly, nitrogen has two stable isotopes, ^{15}N and ^{14}N, with relative proportions of about 3% and 97%. Different dietary resources have characteristic proportions of these isotopes that depart slightly from the averages. These differences are easily measured by mass spectrometry, and are typically expressed relative to a laboratory standard in the δ notation:

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

where R is the ratio of the heavier isotope to the lighter one. While this study employs $\delta^{13}C$ and $\delta^{15}N$ measurements, similar δ values for hydrogen, oxygen, strontium and sulfur isotopes have also been used in paleodietary and paleoenvironmental studies.

3.1 Stable isotopes in nature

Carbon

Atmospheric CO_2, with an average $\delta^{13}C$ of –8‰, enters terrestrial foodwebs during plant photosynthesis. Most plants reduce CO_2 to carbohydrate using the C_3 metabolic pathway, which discriminates heavily against $^{13}CO_2$ and thus produces average values around –26‰. By contrast, plants using the C_4 pathway discriminate less against the heavier isotope and thus give average values of –12.5‰ (Smith and Epstein 1971). C_4 plants are

most common in arid environments, and include grasses and herbaceous plants such as amaranths and chenopods. Finally, crassulacean acid metabolism (CAM) plants, comprising succulents such as cacti, agaves and yuccas, can shift photosynthetic modes according to environmental conditions (Bender et al. 1973). In arid environments such as Baja California, CAM plants tend to have $\delta^{13}C$ values close to those of C_4 plants (Eickmeier and Bender 1976).

The $\delta^{13}C$ values of plants are also dependent on several additional factors, including the local $\delta^{13}C$ value of air, temperature, irradiance, water stress (Tieszen 1991). Average plant $\delta^{13}C$ values are higher at low latitudes and in hot environments (Stuiver and Braziunas 1987). In arid environments, plant $\delta^{13}C$ values may vary seasonally by up to 3‰ (Ehleringer et al. 1992). In addition, different tissues of plants, as well as different dietary fractions (i.e. lipids, carbohydrates, proteins) within those tissues, may vary greatly in their $\delta^{13}C$ values (DeNiro and Epstein 1978; Tieszen 1991).

The isotopic signature of plants is preserved throughout the animal foodwebs they support. With each step up in trophic level, there appears to be a slight enrichment in average $\delta^{13}C$ values, estimated in one study at 0.8 ± 1.1‰ (DeNiro and Epstein 1978). However, the $\delta^{13}C$ values of animal tissues are widely disparate, reflecting internal fractionation during tissue synthesis as well as preferential uptake of different components of the diet into specific tissues

Marine carbon enters foodwebs as bicarbonate, which has a $\delta^{13}C$ of around 0‰. Marine plants approximate the C_3 pathway, resulting in values averaging –20‰. These values may vary regionally as a result of water temperature, local upwelling currents, and stream runoff containing terrestrial carbonates (Smith and Epstein 1971; Haines and Montague 1979). In marine animals, there is a slight enrichment in $\delta^{13}C$ with trophic level similar to that of terrestrial animals (Sealy 1986).

Nitrogen

Because atmospheric nitrogen is unreactive, it is not absorbed directly by plants, but is obtained as soil ammonium and nitrates; some plants also host symbiotic bacteria that fix atmospheric N_2 (Shearer and Kohl 1986). Thus the $\delta^{15}N$ values of nitrogen-fixing plants such as legumes tend to reflect atmospheric nitrogen, while non-nitrogen fixing plants reflect the $\delta^{15}N$ value of the soil in which they grow. Soil $\delta^{15}N$ values are generally enriched over atmospheric nitrogen, but depend on a variety of environmental factors, including temperature, rainfall, salinity, and altitude. Soils in hot, dry climates tend to have more enriched $\delta^{15}N$ values than those in cool, temperate regions. Thus non-nitrogen fixing plants often have significantly higher $\delta^{15}N$ values than nitrogen fixers, but this separation is extremely variable and in many environments there is overlap (Shearer et al. 1983; Heaton et al. 1986).

Animals take up nitrogen as dietary protein. Laboratory experiments, as well as studies of natural ecosystems, show an enrichment of +2 to +6 ‰ in most body tissues relative to diet (Schoeninger and DeNiro 1984; Ambrose and DeNiro 1986; Sealy et al. 1987). This effect is large enough to differentiate animals at different trophic levels. Animal $\delta^{15}N$ values are dependent on climatic and physiological factors; average $\delta^{15}N$, as well as trophic enrichment, appear to increase greatly in arid environments (Sealy et al. 1987; Ambrose 1991). Because nitrogen is excreted as ^{15}N-depleted urinary urea, average $\delta^{15}N$ also appears to depend on water-retention efficiency, with drought-tolerant animals more enriched (Ambrose 1991). These factors substantially complicate dietary interpretation using $\delta^{15}N$ values.

Nitrogen in marine foodwebs is derived from production of ammonium and nitrates by N_2-fixing bacteria and blue-green algae. Dissolved N_2 has a higher $\delta^{15}N$ than atmospheric N_2, and this is reflected in the elevated $\delta^{15}N$ values of marine foodwebs. Marine foodwebs have more complex trophic systems than terrestrial ones, so marine $\delta^{15}N$ values are more variable than in terrestrial systems, but the amount of trophic enrichment is similar to that

observed in terrestrial animals (Wada 1980; Minagawa and Wada 1984; Schoeninger and DeNiro 1984).

3.2 Isotopes and human diet

Archaeologists have used these isotopic differences between dietary resources to assess marine versus terrestrial resource use in a variety of settings (Tauber 1981; Chisholm et al. 1983a; Sealy and van der Merwe 1986; Walker and DeNiro 1986), and to document the spread of C_4 domesticates such as maize into temperate regions (van der Merwe and Vogel 1978; Bender et al. 1981; Schwarcz et al. 1985). Most of these studies are limited to observations of trends over space or time in human δ values, or to qualitative assessments of diet based on comparisons of human δ values to those of animals with known dietary habits. A few studies have used isotope measurements to make quantitative estimates of dietary composition (e.g. Chisholm et al. 1983a). However, inferring directly from human δ values to actual proportions of foods requires a detailed understanding of the behavior of isotopes in natural ecosystems which is only now emerging.

At a minimum, dietary estimates require knowledge of 1) the average isotopic and nutritional composition of important dietary resources, 2) systematic differences between dietary δ values and the δ values of the human tissues under study, and 3) differences between the δ values of living human tissues and the δ values of what is recovered and measured in the laboratory. Having established each of these, dietary composition is estimated by interpolating human δ values between the δ values of dietary resources. Each of these issues is discussed further below.

3.3 Characterizing dietary resources

For isotopic analysis, important foods in prehistoric diets are typically identified by employing zooarchaeological, archaeobotanical, ethnographic or other evidence. Isotopic estimates of diet require that the δ values of these foods group into a few distinct alternatives, or "end points." Average δ values for these end points may be generally known, but since the δ values of dietary items are influenced by a wide range of environmental factors, accurate estimates of diet require characterization of locally important dietary items.

Because of the potential for variation between tissues in dietary δ values, it is preferable to sample the portion of the dietary item that was actually eaten. However, this is not always possible. Often archaeological bone is the only type of sample available; for example, in Baja California many prehistorically important food species are now endangered or extinct. In this case, if there is a consistent relationship between the tissue analyzed and the dietary tissue of interest, a correction factor can be applied to approximate the dietary tissue. For example, bone collagen $\delta^{13}C$ is enriched by about +2.3‰ relative to muscle protein (Lee-Thorp et al. 1989; Tieszen and Fagre 1993a).

Another difficulty in analyzing modern dietary specimens is the difference in $\delta^{13}C$ of modern analogues of food species as compared to their archaeological counterparts, due to change through time in atmospheric $\delta^{13}C$ resulting from burning of fossil fuels. The δ values of modern samples must be adjusted to reflect this change. Several lines of evidence have produced measurements of this effect. Samples of air trapped in Antarctic and Greenland ice cores (Friedli et al. 1984, 1986) in conjunction with direct atmospheric $\delta^{13}C$ measurements (Siegenthaler and Oeschger 1987) suggest a total depletion of about –1.5‰ since AD 1740. Measurements on cellulose from stored and archaeologically recovered maize grains (Marino and McElroy 1991; Tieszen and Fagre 1993b) as well as tree ring

cellulose (Freyer 1986) all show a similar depletion of –1.5‰ from preindustrial times to the present.

Having identified end point δ values, the disproportionate influence of different end point resources on human δ values must also be assessed. Because different dietary fractions (i.e. protein, lipid and carbohydrate) are often taken up preferentially into various human tissues (Ambrose and Norr 1993), different foods can have unequal influence on human tissue δ values. Thus, if actual proportions of foods in the diet are to be estimated, a weighting factor is needed that can quantify the importance of the dietary item to the tissue being measured. For example, the protein portion of bone (collagen) takes up carbon and nitrogen from dietary protein preferentially (Ambrose and Norr 1993), so the influence of a given dietary resource on collagen δ values can be approximated by determining its protein content. Also, because different dietary fractions may have different δ values, it is preferable to measure only the protein fraction of a dietary item when comparing it to collagen δ values. Thus, for comparison with collagen $\delta^{13}C$ values, animal flesh samples should be treated to remove lipids, which are isotopically depleted relative to muscle protein.

By contrast, the mineral fraction of bone (apatite) appears to reflect whole diet rather than any specific dietary fraction (Ambrose and Norr 1993), so the influence of a dietary resource on apatite $\delta^{13}C$ can be best estimated by determining its caloric content. Thus samples intended to explain apatite $\delta^{13}C$ are perhaps best submitted untreated, as an approximation of total energy.

3.4 Diet-to-tissue increments and isotopic variation in human tissues

Dietary end point δ values cannot be compared directly to human δ values, because human tissues show characteristic isotopic enrichments relative to diet. In order to estimate dietary composition, an isotopic increment between diet and tissue can be applied to the

end points, in effect simulating the δ values of populations of humans subsisting entirely from each end point. Accurate estimates of these diet-to-tissue increments are critical for dietary interpretation because of the large systematic effect they can have on dietary estimates.

Diet-to-collagen increment (Δ_{D-C})

Estimates of the isotopic increment between diet and bone collagen (Δ_{D-C}) in $\delta^{13}C$ have varied widely, in part because of uncertainty over what part of the diet collagen $\delta^{13}C$ measures. Most studies have assumed that collagen $\delta^{13}C$ reflects whole diet (Schwarcz et al. 1985; Schoeninger 1989; Spielmann et al. 1990), while a few studies have assumed that collagen $\delta^{13}C$ reflects dietary protein only (Chisholm et al. 1983a). Experimental studies show that collagen takes up dietary protein preferentially, with a small contribution by non-protein carbon that varies inversely with the amount and quality of protein in the diet (Ambrose and Norr 1993; Tieszen and Fagre 1993a). Thus, as an approximation, $\Delta^{13}C_{D-C}$ may be most usefully defined as the increment between dietary protein and collagen $\delta^{13}C$, but in very low-protein diets this simplification breaks down, resulting in highly variable values for the increment, especially when protein and non-protein dietary fractions have different isotopic signatures. In experimental studies, where all dietary fractions have approximately the same isotopic signature $\Delta^{13}C_{D-C}$ is about +4.5‰ (Ambrose and Norr 1993); this is in good agreement with values estimated for animals and humans in field studies (Vogel and van der Merwe 1977; Sullivan and Krueger 1981; Lee-Thorp et al. 1989).

Less is known about $\Delta^{15}N_{D-C}$. Both muscle and bone collagen are elevated above diet by 3 to 4‰, with no discernible difference between the two tissues (DeNiro and Epstein 1981; Sealy et al. 1987). However, this increment is dependent on a variety of climatic and physiological factors, and is expected to be larger in arid environments, and in drought-tolerant animals (Sealy et al. 1987; Ambrose 1991). Because dietary nitrogen comes

almost exclusively from protein, $\Delta^{15}N_{D-C}$ should also be understood as the increment between dietary protein and collagen.

Diet-to-apatite increment ($\Delta^{13}C_{D-A}$)

Carbonate in bone apatite is derived from blood bicarbonates, which are produced during energy metabolism. Thus bone apatite should theoretically reflect the portion of the diet used for energy (Sullivan and Krueger 1981; Lee-Thorp et al. 1989). Blood bicarbonates are expelled from the body as respiratory CO_2. The fractionation between these two phases results in a characteristic enrichment ($\Delta^{13}C_{D-A}$) of 10-12‰ over diet (DeNiro and Epstein 1978; Sullivan and Krueger 1981; Ambrose and Norr 1993). Ambrose and Norr (1993) show that bone apatite $\delta^{13}C$ values of laboratory animals accurately reflect the average isotopic composition of whole diet better than any specific dietary fraction, so $\Delta^{13}C_{D-A}$ is best defined as the increment between whole diet and apatite δ values.

Collagen-to-apatite increment (Δ_{C-A})

The increment between collagen and bone apatite $\delta^{13}C$ values (here designated Δ_{C-A}) depends on the dietary fractions that each tissue preferentially incorporates. As discussed above, collagen has been shown to incorporate dietary protein preferentially, while bone apatite reflects whole diet. Krueger and Sullivan (1984) and Lee-Thorp et al. (1989) argue that because of differences between herbivores and carnivores in dietary sources of carbon, it should be possible to use Δ_{C-A} as an indicator of trophic level. Based on a $\Delta^{13}C_{D-C}$ of +5‰ and a $\Delta^{13}C_{D-A}$ of +12‰, the Δ_{C-A} value of herbivores should be about +7‰. Carnivore collagen should be enriched over herbivore flesh protein by 5‰, and thus over herbivore diet by about 8‰. (Note that this results in a trophic increment in collagen $\delta^{13}C$ of +3‰, which is larger than has been observed in most field situations.) Carnivore apatite $\delta^{13}C$ is derived from lipids as well as protein in herbivore flesh (depleted by 2‰ and enriched by 3‰ relative to diet, respectively), resulting in an average enrichment of 12‰ over herbivore diet. Thus, carnivore Δ_{C-A} should be about +4‰ (Figure 3.1). Finally, omnivore Δ_{C-A}, including that of humans, should be intermediate between these.

Lee-Thorp et al. (1989) provide data for African wild animal populations which support the model convincingly. However, their data on human populations in southern Africa show a Δ_{C-A} smaller than that of the carnivores. This is most likely because the model does not account for diets in which different dietary fractions have different isotopic signatures. For example, a contribution of marine protein to a primarily C_3-based diet would tend to elevate collagen $\delta^{13}C$ values relative to apatite $\delta^{13}C$, thus reducing Δ_{C-A}. Experimental studies (Ambrose and Norr 1993) also show that Δ_{C-A} can be made to vary by changing the isotopic composition of different dietary fractions. Because of this effect, Δ_{C-A} cannot be used as a simple indicator of trophic level. However, it may still be useful in refining dietary estimates when dietary composition is already approximately known, through collagen δ values or other means.

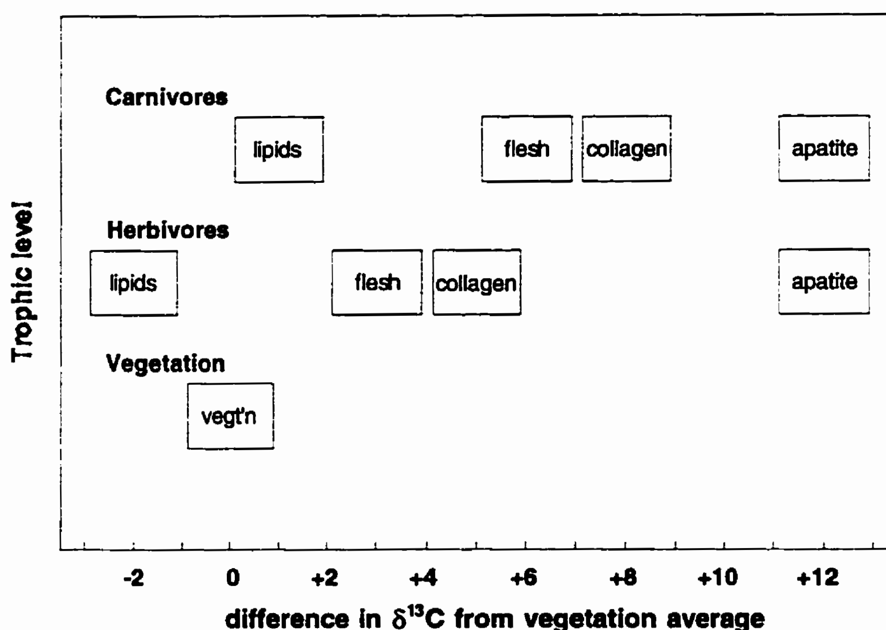

Figure 3.1. The Lee-Thorp et al. (1989) model of collagen-apatite increments (Δ_{C-A}) in herbivores and carnivores in a simple isotopic ecosystem.

Variability in diet-to-tissue increments

Some variation between individuals in diet-to-tissue increments might be expected due to sex, age, or other metabolic effects. Thus quantifying endogenous sources of variability is important in assessing the accuracy of dietary estimates. Unfortunately these have not been extensively studied, except through empirical observation of archaeological populations.

Laboratory animals have shown no discernible sex differences in collagen $\delta^{13}C$ or $\delta^{15}N$ values (DeNiro and Schoeninger 1983). Similarly, in an archaeological study, Lovell et al. (1986) observed no variation related to age or sex in collagen $\delta^{13}C$ values, indicating that most observed correlations between δ values and age or sex are probably best explained as actual differences in diet. However, infants may show trophic enrichment in $\delta^{15}N$ due to breastfeeding (Katzenberg 1990, 1993).

In archaeological studies, the total range of variation in observed δ values subsumes several sources of variability, including actual differences in diet as well as any endogenous variability in diet-to-tissue increments and measurement error. The fact that studies have shown standard deviations in collagen $\delta^{13}C$ as low as ±0.3‰ (Lovell et al. 1986) suggests that the range of variation in diet-to-tissue increments is extremely small. In addition, this small range of variability indicates that the long turnover time of bone tissues produces a long-term average of diet, despite a potentially very large range of dietary δ values.

Nutrition and tissue turnover

Dietary estimates proceed on the assumption that a diet-to-tissue increment can be accurately measured relative to a macronutrient (such as protein, in the case of collagen), and further that the rate of tissue turnover is proportional to consumption of that nutrient, regardless of the overall nutritional adequacy of diet. This may be an oversimplification. For example, collagen deposition is mediated not only by protein availability but also by a

variety of other nutrients such as vitamin D, calcium and phosphorus (Raisz and Kream 1983a, 1983b). If diet quality varies substantially over the course of a year, it is possible that collagen turnover may proceed partially independent of protein intake, biasing the dietary signal toward certain times of year. Parkington (1991) cites studies showing that acidosis induced by excessive protein can temporarily accelerate bone turnover. Ambrose (1993) suggests that protein-induced acidosis may also solubilize recently deposited collagen, eradicating part of the dietary signal deposited during a seasonal round. Little work has been done that can address these concerns.

3.5 Preparation and measurement of collagen and apatite

Another challenge for paleodietary studies is to adequately control for the effects of diagenesis on archaeological bone. Because collagen is resistant to chemical alteration, it is an ideal material for isotopic analysis. It may, however, give poor yields and anomalous δ values in some situations, due to excessive breakdown of the protein structure or to the presence of lipids and humic contaminants. Because of these problems, different laboratory extraction procedures may produce somewhat different results. Apatite has proved much more susceptible to diagenesis than collagen, due to the easy exchange of carbonate with groundwater ions, and thus apatite $\delta^{13}C$ values have met with less general acceptance. Recent research has shown that pretreatment, particularly of tooth enamel apatite, can produce reliable measurements (Lee-Thorp and van der Merwe 1991; Sillen 1989).

Collagen preparation

Collagen is the major organic constituent of bone (Boskey and Posner 1984). Its fibrous structure gives it great tensile strength as well as exceptional resistance to chemical alteration and decay. Collagen is metabolically very stable (Stenhouse and Baxter 1979), its low rate of turnover tending to average the effects of seasonal or episodic changes in diet.

Most methods of extracting collagen from bone follow the acid solubilization procedure (Longin 1971; Chisholm et al. 1983b; Brown et al. 1988), in which bone is demineralized in dilute hydrocholoric acid, the protein residue solubilized by heating, then filtered and dried. Many laboratories soak the residue in a dilute sodium hydroxide solution following demineralization to remove lipids and base-soluble contaminants such as humic acids. Brown et al. (1988) suggest ultrafiltration after solubilization to remove smaller peptides and contaminants. After freeze-drying (lyophilization), the yield of dry extract as a percentage of bone weight can be used as a measure of preservation. Fresh bone gives yields of up to 20%. Extracts with very low yields (under 1%) can give anomalous δ values (Ambrose 1990; Bada et al. 1989). The acid solubilization procedure does not chemically characterize the resulting extract, so the use of the term "collagen" implies an assumption about its composition.

The use of the sodium hydroxide soak is problematic because it has been shown to alter $\delta^{13}C$ values by up to 1‰, as well as reducing the collagen yield (Liden et al. 1995). Ambrose (1990) and others argue that a sodium hydroxide wash is necessary to remove lipids and base-soluble contaminants such as humic acids. However, if the solution is maintained at an acid pH throughout the extraction process, base-soluble contaminants should not solubilize during the extraction, and should thus be removed during filtration (Chisholm 1989).

Removing bone lipids, which can significantly alter collagen $\delta^{13}C$ values, has also proved problematic. For modern bone samples, Chisholm et al. (1983b) recommend a methanol-chloroform-water extraction (Bligh and Dyer 1959), although the organic solvents must be completely removed before analysis. Others have relied on the sodium hydroxide soak (Ambrose 1990), but Liden et al. (1995) show that in addition to its other drawbacks, it is ineffective in removing lipids. Archaeological bone is usually so poor in lipids that it does not require extraction (Chisholm 1989).

Prior to isotope measurement, the collagen extract is converted to CO_2 and N_2 gas by combustion in sealed quartz tubes, or using an elemental analyzer. The ratio of carbon to nitrogen in the collagen extract can be determined by cryogenic distillation of the gas sample, or by the elemental analyzer. This ratio can be used as a check of the purity of the sample. Pure collagen has a C/N ratio by weight of approximately 2.8:1, so samples which deviate significantly from this value are most likely contaminated (DeNiro 1985; Ambrose 1990). However, a C/N ratio in agreement with that of collagen does not assure that the sample is free from contamination.

<u>Apatite preparation</u>

The mineral fraction of bone is a poorly crystalline calcium phosphate salt similar in structure to hydroxyapatite (Termine and Lundy 1973; Sillen 1989). Carbon makes up about 2% of bone apatite by weight, occurring as carbonate ions substituted in the phosphate and hydroxyl positions of the crystal structure, and as adsorbed carbonate on crystal surfaces (Termine and Lundy 1973; LeGeros and Tung 1983; Chickerur et al. 1980).

Bone apatite is prepared for analysis using the method developed by McCrea (1950) and refined by Krueger and Sullivan (1984) and Lee-Thorp et al (1989). Typically, protein is removed from samples with sodium hypochlorite or another solvent, then treated with acetic acid to remove diagenetic carbonates. The treated mineral is then reacted with phosphoric acid under vacuum. The produced CO_2 is collected and purified by cryogenic distillation, measured by manometry, and sealed in glass tubes for measurement. (Again, the use of the term "apatite" implies that the composition of the treated mineral is known, but it is rarely determined directly.) The difficulty with the method lies in the pretreatment stage. Despite intensive recent research into bone apatite diagenesis, a satisfactory method for removal of diagenetic carbonates has yet to be developed.

Structural defects in biogenic apatite render it highly prone to recrystallization, during which contaminant carbonates can be incorporated. Biogenic apatite may recrystallize as other minerals such as calcite, francolite, and brushite, or as highly crystalline diagenetic apatite, all of which can be detected using infrared spectroscopy and x-ray diffraction (Hassan et al. 1977; Lee-Thorp and van der Merwe 1991; Sillen 1989). Other methods of detecting recrystallization include measurement of Ca/P ratios (Sillen 1989) and microscopic examination of histological structure (Schoeninger et al. 1989). All of these lines of evidence seem to indicate that the relationship between time and recrystallization is nonlinear, with the stability of biogenic apatite largely dependent on the preservation of the organic phase (Sillen 1989).

While the above are useful methods of detecting diagenesis, the main concern is with counteracting its effects. Carbonates adsorbed on crystal surfaces are much more prone to diagenesis, and more soluble, than those incorporated in the crystal structure (Termine and Lundy 1973). In addition, diagenetic apatite is often higher in carbonates and therefore more soluble than biogenic apatite. Pretreatment methods thus rely on these differences in solubility in an attempt to remove adsorbed carbonates and diagenetic apatite. However, it is difficult to determine when the contaminant carbonates have been satisfactorily removed, without dissolving too much of the crystal structure of the biological apatite. Excessive treatment can also cause dissolved carbonates to recrystallize in solution as brushite, which can be detected using infrared spectrometry (Lee-Thorp and van der Merwe 1991). Ambrose (1993) suggests that treated bone apatite should contain between 0.6 and 1.3 % carbon by weight and that values outside these limits may indicate overtreated or incompletely treated samples.

Stable isotope measurement

For isotope measurement, samples are admitted to an isotope ratio mass spectrometer as gas, either after sealed-tube combustion and distillation, or after combustion in an elemental analyzer. The sample gas is admitted to a high-vacuum chamber, ionized, and

accelerated through a curved pathway by a magnetic field. This separates the gas stream by mass, allowing separate detection of the gas streams containing each isotope. The precision of the analysis is usually better than 0.2‰.

3.6 Calculating dietary proportions

Having established the average δ values of dietary end points in a given region, and having determined a diet-to-tissue increment to be applied to each of these end points in order to allow comparison with human δ values, quantitative estimates of diet can theoretically be made by a linear interpolation of human tissue δ values between the corrected end points (Figure 3.2). The equation for this interpolation is

$$\delta_j = \Sigma p_i w_{ij}(\delta_i + \Delta_j) \;/\; \Sigma p_i w_{ij}$$

where δ_j is the δ value of a given human tissue *j*, p_i is the proportion in the diet of each end point *i*, δ_i is the average end point δ value, Δ_j is the diet-to-tissue increment, and w_{ij} is a weighting factor describing the end point's relative influence on the tissue δ value (i.e., protein content for collagen $\delta^{13}C$ and $\delta^{15}N$, caloric content for apatite $\delta^{13}C$) (Schwarcz 1991; Spielmann et al. 1990). Theoretically, in a system of *n* dietary end points, relative proportions of each could be estimated by measuring *n*-1 isotopes and solving the above equation for p_i for each isotope (Schwarcz 1991).

The uncertainty in these proportion estimates depends on the variability both in average human δ values and in each of the end points. Because the tissue-corrected end point values describe the δ values expected in humans consuming end point resources, and because the long turnover time of human tissues tends to average the isotopic composition of the diet, these corrected end points should display less variability than the dietary end points; hence, dietary estimates may be possible in some cases even when the range of δ values observed in dietary items is very large. However, in practice, quantitative dietary

estimates have not been attempted with more than two end points, and the complexity of natural ecosystems probably precludes multiple-end point estimates except under the best of circumstances.

An alternative approach is to use the equation above to attempt by trial and error to produce a combination of dietary resources (of known δ_i and w_{ij}) that best approximate observed human δ values (e.g. Spielmann et al. 1990). While in complex ecosystems many different diets can result in the same δ values, this method allows the experimenter to place limits on dietary proportions that reflect a plausible nutritional and archaeological reality. In central Baja California this is likely to be the only productive way of investigating diet.

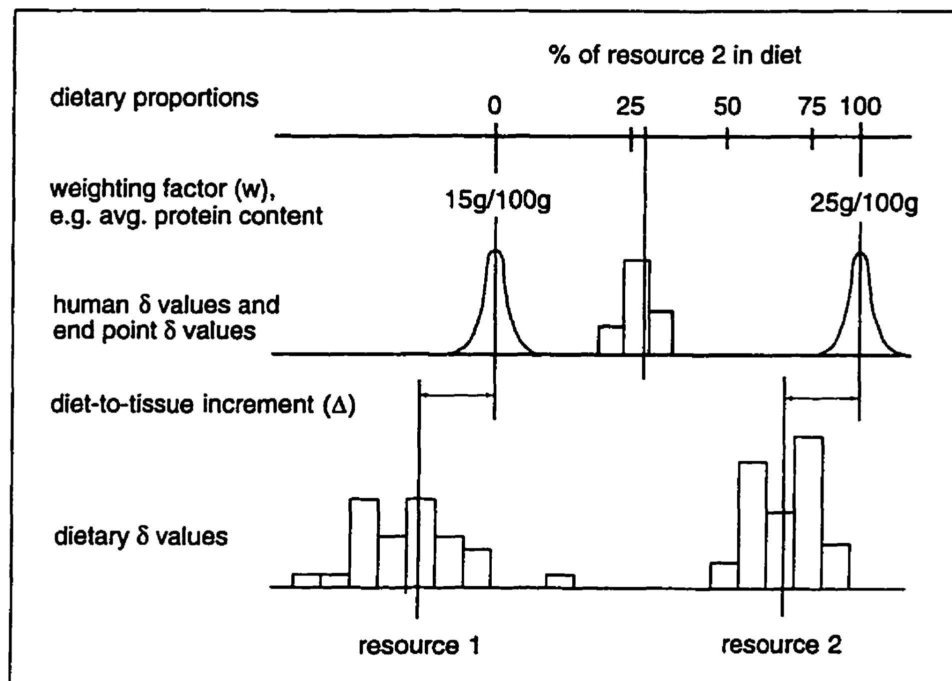

Figure 3.2. Estimating dietary proportions in a two-end point isotopic ecosystem. The bottom line shows frequency distributions of δ values for two isotopically distinct dietary alternatives. The middle line shows a frequency distribution of δ values in a sample of humans, as well as the end point values expected of humans consuming each resource exclusively (note the reduction in variability expected of end point consumers). The top line shows an interpolation of the human δ values between the two end points, after applying a weighting factor to each end point. In this example, the proportion of resource 2 in the diet is estimated at about 30%.

This research employs this approach by conducting three separate studies: 1) by finding appropriate end point δ values through a background study of dietary items; 2) by finding whether current understanding of diet-to-tissue increments holds true in the region, through collagen and apatite stable isotope measurements on a sample of mammals from the region; and 3) by using the above to estimate the collagen $\delta^{13}C$, $\delta^{15}N$ and $\Delta_{C\text{-}A}$ of a range of hypothesized diets, in an attempt to approximate observed δ values in two small collections of human burials.

4.0 MATERIALS AND METHODS

4.1 Sampling methods

Samples for dietary background survey and evaluation of Δ_{C-A}

Samples of plants and animals were chosen to satisfy two goals: first, to characterize as wide as possible a range of dietary items, in order to investigate the structure of the isotopic ecosystem in the region; and second, to characterize the bone collagen and apatite δ values of a sample of mammal species with various habitats and feeding habits, in order to evaluate the model of trophic differences collagen-apatite spacing (Δ_{C-A}) set forth by Lee-Thorp et al. (1989).

Identification of prehistorically important dietary items relied heavily on Aschmann (1959). Items were selected for sampling based on their quantitative importance as estimated by Aschmann, as well as on their relative influence on human tissue δ values (i.e., animal foods are emphasized because of their disproportionate influence on human collagen δ values). Because of practical limitations on the size of the dietary sample, sample selection was generally not intended to assess systematic variation throughout the study area due to altitude, soil type, ground cover, vegetation community, or other factors. A small variation in soil and plant $\delta^{13}C$ values in response to latitude has been observed in the study area (Amundson et al. 1994), so a larger sample of agaves was selected in order to evaluate this.

Sampling was completed during three short trips through the study area in July 1995, January 1996, and June 1996. Sampling was opportunistic, although an effort was made to collect several samples of each taxon from widely dispersed locations, in an attempt to capture the widest possible range of isotopic variability in each. Some taxa, however, are only represented by one or two samples. Plant samples were taken in the field, using Roberts (1989) and Wiggins (1980) to aid identification. Animal samples were taken from

the field, from museum collections at the Museo de Naturaleza y Cultura at Bahía de los Angeles, from the Glendale Community College Sea of Cortez Research Station at Bahía de los Angeles, and from archaeological collections made during the Proyecto Arte Rupestre de Baja California Sur in the Sierra de San Francisco. In all cases the sample provenance was recorded as accurately as possible, although samples from the museum collections could not be precisely located. Samples and their provenance are listed in Tables 4.1 and Table 4.2. Figure 4.1 shows sampling locations.

Several potentially important resource types are not represented in the sample, including herbaceous grasses, root vegetables, palm fruits, insects, and most importantly, marine samples from the Pacific coast, which may have significantly different δ values than those from the Gulf. However, it was felt that Pacific coast species probably did not make up a large percentage of the diet in either of the human collections measured in this study.

Some dietary items are represented by tissues other than what was eaten. For example, *pitaya* fruits, which were important during the short summer ripening season, are here represented only by samples of stems from the same species. As discussed above, there may be some variation in δ values between plant tissues. Also, many animals are represented here by bone rather than flesh. This is not a serious problem, as a correction factor can be applied. However, many of the bones available for analysis, especially those from archaeological collections, were fragmentary and could not be identified to species.

Animal flesh samples were submitted for isotopic analysis after extracting lipids, using the method described below. Because of the low expected protein and lipid content of most of the plant samples, these were submitted untreated. However, since legumes were expected to be high in protein, these were submitted both untreated and after lipid extraction.

Table 4.1. Background survey samples. See Table 4.2 for key to collection localities.

Group	Sample	Species	Part	Loc'n	
Plant samples					
Family Agavaceae	BDS-35	*Agave deserti*	heart	PP	
	BDS-36	*Agave deserti*	heart	PP	
	BDS-37	*Agave shawii*	leaf	PP	a
	BDS-42	*Agave deserti*	heart	CV	
	BDS-43	*Agave deserti*	heart	CV	
	BDS-56	*Agave deserti*	heart	SE	
	BDS-57	*Agave deserti*	heart	SE	
	BDS-63	*Agave deserti*	heart	SW	
	BDS-64	*Agave deserti*	heart	SW	
Family Bromeliaceae	BDS-23	*Yucca valida*	fruit	VC	b
	BDS-26	*Yucca valida*	fruit	VC	b
Family Cactacecae	BDS-24	*Machareocereus gummosus*	stem	VC	a
	BDS-25	*Machareocereus gummosus*	stem	VC	a
	BDS-27	*Pachycereus pringlei*	stem	VC	a
	BDS-28	*Pachycereus pringlei*	fruit	VC	
	BDS-30	*Lophocereus schottii*	fruit	VC	
	BDS-31	*Opuntia cholla*	fruit	VC	
	BDS-33	*Ferocactus sp.*	fruit	VC	
	BDS-59	*Lemairocereus thurberi*	stem	SE	a
	BDS-62	*Lemairocereus thurberi*	stem	SW	
	BDS-67	*Platyopuntia sp.*	fruit	SF	
	BDS-68	*Platyopuntia sp.*	fruit	SF	
Family Fabaceae	BDS-13	*Cercidium microphyllum*	seeds	MN	
	BDS-21	*Acacia greggii*	seeds	EA	
	BDS-34	*Prosopis glandulosa*	seeds	VC	
	BDS-48	*Prosopis sp.*	seeds	SI	
	BDS-51	*Prosopis sp.*	seeds	SI	
	BDS-93	*Prosopis sp.*	seeds	BA	
Family Moraceae	BDS-61	*Ficus palmeri*	fruit	SW	
Animal samples					
Seabirds	BDS-91	*Pelecanus occidentalis*	bone	MC	
	BDS-96	*Pelecanus occidentalis*	bone	MC	
Fish	BDS-97	Family Balistidae	bone	MC	
	BDS-98	Family Balistidae	bone	MC	
Sea turtles	BDS-86	*Chelonia sp.*	bone	MC	
	BDS-95	*Dermochelys sp.*	bone	MC	
Marine mammals	BDS-81	*Zalophus californianus*	bone	MC	
	BDS-82	*Zalophus californianus*	bone	MC	
	BDS-99	*Zalophus californianus*	bone	MC	
	BDS-92	Family Delphinidae	bone	MC	

Group	Sample	Species	Part	Loc'n	
Marine invertebrates	BDS-6	*Turbo fluctuosus*	flesh	SO	
	BDS-7	*Turbo fluctuosus*	flesh	SO	
	BDS-9	*Chione sp.*	flesh	PG	
	BDS-10	*Chione sp.*	flesh	PG	
	BDS-46	*Saxidomus sp.*	flesh	LO	c
	BDS-47	*Trachycardium sp.*	flesh	LO	c
Terrestrial herbivores	BDS-11	*Lepus sp.*	flesh	EP	
	BDS-12	*Lepus sp.*	flesh	EP	
	BDS-38	Order Artiodactyla	bone	AC	d
	BDS-39	Order Artiodactyla	bone	AC	d
	BDS-70	Order Artiodactyla	bone	AC	d
	BDS-71	Order Artiodactyla	bone	AC	d
	BDS-72	Order Artiodactyla	bone	AC	d
	BDS-73	Order Artiodactyla	bone	AC	d
	BDS-83	*Ovis canadensis*	bone	MC	
	BDS-85	*Odocoileus hemionus*	bone	MC	
	BDS-88	*Ovis canadensis*	bone	MC	
Terrestrial omnivore	BDS-84	*Taxidea taxus*	bone	MC	
Terrestrial carnivores	BDS-89	*Canis latrans*	bone	MC	
	BDS-100	*Canis latrans*	bone	MC	
	BDS-101	*Canis latrans*	bone	MC	
Terrestrial reptile	BDS-87	*Sauromalus obesus*	bone	MC	

a) note tissue used in place of edible part; b) unripe; c) specimens collected south of study area; d) archaeological specimens.

Table 4.2. Sample collection localities.

Code	Locality	Date
AC	Archaeological collections from Proyecto Arte Rupestre, Sierra de San Francisco.	
BA	Sandy, gently sloping foothils near Bahia de los Angeles.	1/96
CV	Boulder fields 5 km north of Cataviña; sandy, sparsely vegetated.	1/96
EA	Heavily vegetated drainage between Pozo Alemán and El Arco.	7/95
EP	Sandy, gently sloping, moderately vegetated area around Rancho El Progreso.	7/95
LO	Intertidal zone of beach immediately south of Loreto River.	1/96
MC	Museum collections at Bahía de los Angeles.	
MN	Rocky, heavily vegetated flat at the northern foot of Mesa El Carmen.	7/95
PG	Intertidal flats at Punta la Gringa.	7/95
PP	30 km north of Punta Prieta, in rocky, dissected, sparsely vegetated uplands.	7/95
SE	Rocky, moderately sloping, sparsely vegetated foothils 15 km E of San Ignacio.	1/96
SF	Rocky, steeply sloping, moderately vegetated uplands in Sierra de San Francisco	1/96
SI	Sandy, heavily vegetated flat immediately S of San Ignacio.	1/96
SO	Intertidal zone at Punta San Francisquito.	7/95
SW	Sparsely vegetated rocky slope on west flank of Sierra de San Francisco.	1/96
VC	Vizcaíno Desert floor, sandy, sparse to moderate cover	7/95

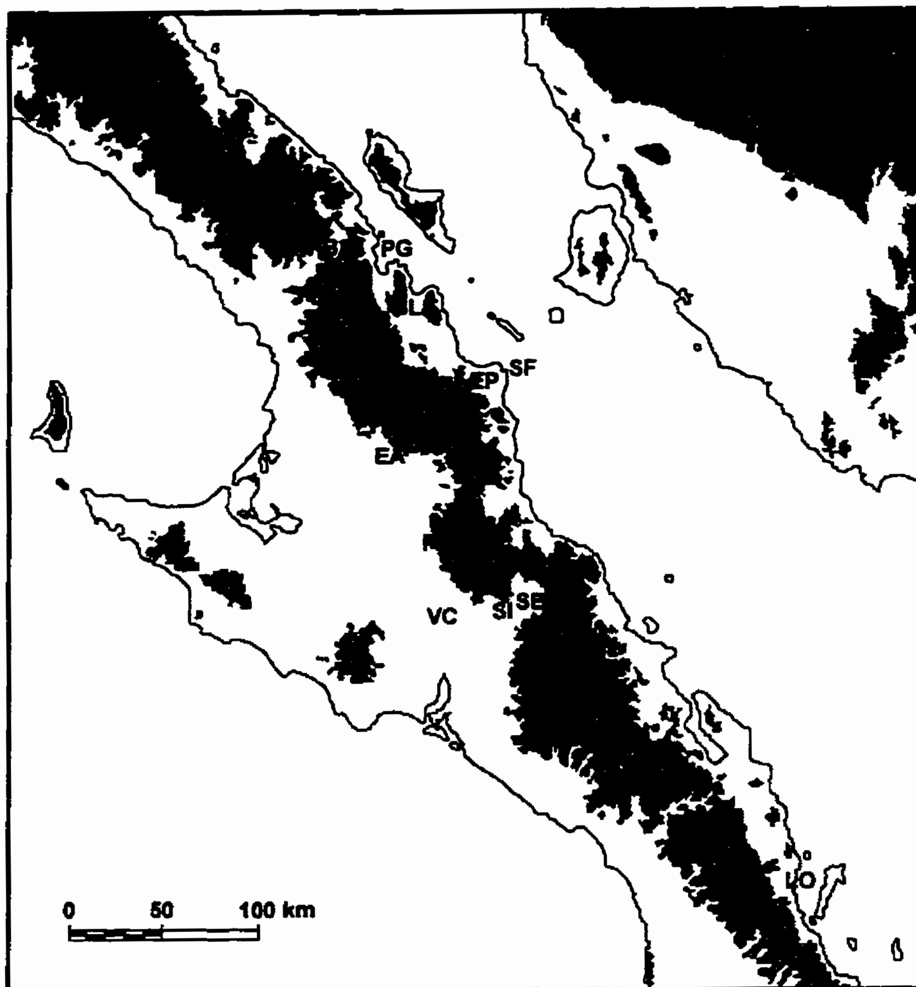

Figure 4.1. Sample collection localities. See table 4.2 for key to codes. Also shown are site locations for the Bahía de las Animas (BLA) and Sierra de San Francisco (PAR) human collections.

<u>Human samples</u>

Samples of human burials were collected from two areas: the coast of Bahía Las Animas, a bay on the north-central Gulf coast; and from the Sierra de San Francisco, an isolated volcanic range approximately 150 km to the south in the central peninsula (Figure 4.1). Selection of these samples was determined primarily by availability.

The coastal collection originates from two adjacent archaeological sites on the western shore of Bahía de las Animas. The sites are bouldery, barren hillsides whose principal features are primary and secondary human burials in walled rock cavities, some of which

are associated with bivalve shells and animal bones. Many of the burials are exposed, highly weathered, and appear to have been recently disturbed. Some of the burials show evidence of cremation. The sites also show cleared rock enclosures and polished rock surfaces of unknown, probably ritual, significance. Habitation debris such as flaked and ground stone is rare or absent (Ritter 1995).

A total of seven samples was collected from these sites during the 1994 and 1995 field seasons of Eric Ritter's ongoing archaeological research in the region (Ritter 1995). Subsamples of all seven samples were subjected to collagen extraction, as described below; of these, all were submitted for stable isotope measurement, and two for radiocarbon measurement. All seven samples were also submitted for stable isotope measurement of bone apatite. Samples are listed in Table 4.3 below.

The inland collection originates from a mortuary cave site in the Sierra de San Francisco. The cave was located during archaeological survey for the Proyecto Arte Rupestre de Baja California Sur, a rock art documentation project directed by the Instituto Nacional de Antropología e Historia (INAH). The cave has been extensively disturbed and as a result the remains are fragmentary and commingled. The assemblage consists of approximately fifty postcranial bones and fragments, most in an excellent state of preservation. Many of the bones are coated with red pigment. Patchy black staining of uncertain origin is also apparent on many of the bones. Ochre-painted secondary burials are a well-known feature of the Las Palmas culture of the Cape region of the peninsula (Massey 1966); this assemblage appears to represent an unusual occurrence of this burial tradition outside its known geographic and temporal range. However, a detailed analysis of the archaeological significance of this assemblage awaits further research.

Sample selection from the assemblage focused on the most common elements, in order to produce an estimate of the minimum number of individuals represented in the collection. Seventeen samples were subjected to collagen extraction, as described below; of these, all were submitted for stable isotope measurement, and five were submitted for radiocarbon

measurement. Bone subsamples for which sufficient material remained after collagen preparation were also subjected to bone apatite preparation and stable isotope measurement. Samples are listed in Table 4.3.

Table 4.3. Human samples. Samples selected for collagen stable isotope measurement, apatite stable isotope measurement and radiocarbon measurement are indicated in columns "C," "A," and "R" respectively.

Sample	Site	Feature	Element	C	A	R	
Bahía de las Animas samples							
BLA-1	UC-BC-44	Feature 19 / Burial 20	Cranial fragment	x	x		
BLA-2	UC-BC-44	Feature 10 / Burial 12	Right radius	x	x		
BLA-3	UC-BC-44	Feature 16 / Burial 17	Cranial fragment	x	x		a
BLA-4	UC-BC-44	Feature 18 / Burial 19	Left scapular fragment	x	x		
BLA-5	UC-BC-44	Feature 3 / Burial 3	Distal right femur	x	x	x	b
BLA-6	UC-BC-44	Feature 3 / Burial 4	Right humerus	x	x		b
BLA-7	UC-BC-45	Burial 2	Left tibia	x	x	x	
Sierra de San Francisco samples							
PAR-1			Left tibia	x		x	
PAR-2			Right tibia	x			
PAR-3			Right tibia	x	x		c
PAR-4			Left tibia	x		x	c
PAR-5			Left tibia	x		x	c
PAR-6			Left femur	x			
PAR-7			Right femur	x			c
PAR-8			Left femur	x	x		
PAR-9			Right femur	x			c
PAR-10			Right femur	x			
PAR-11			Left humerus	x			c
PAR-12			Right humerus	x			c
PAR-13			Left humerus	x		x	c
PAR-14			Left humerus	x	x		c
PAR-15			Right humerus	x	x		c
PAR-16			Left tibia	x			c
PAR-17			Left os coxae	x	x	x	c

a) This sample appears calcined, possibly as a result of cremation.
b) The burials in Feature 3 are commingled, comprising at least two individuals (Molto 1995).
c) Samples show red pigment.

4.2 Analytical methods

Untreated (bulk) sample preparation

Most plant samples were submitted for stable isotope analysis without chemical pretreatment. Each sample was cleaned carefully, dried in an oven at 50° C, and crushed to a powder in a mortar and pestle.

Lipid extraction

Samples were first thoroughly cleaned, then homogenized by freezing in liquid nitrogen and crushing with mortar and pestle. Approximately 100 mg of tissue was used for plant tissue extractions, and about 1 g of tissue was used for animal extractions. Each sample was added to a 50 ml centrifuge tube with 10 ml of a 2:1 methanol:chloroform solution and enough distilled water to equal 12.7 ml total, including the sample volume. (Chloroform was first washed with distilled water in a separatory funnel.) The solution was thoroughly agitated with a vortexer and allowed to stand for 15 minutes. The tube was then centrifuged and the supernatant decanted. Another 10 ml of the methanol:chloroform solution was added, vortexed, allowed to stand, centrifuged and decanted. The treated sample was allowed to evaporate to dryness, rinsed with distilled water, frozen and lyophilized.

Collagen extraction

All bone specimens were carefully cleaned before sampling. For each specimen, an area of thick cortical bone was chosen, taking care to avoid cracked, weathered or pigmented areas if possible. After removal of a thin surface layer of bone, bone shavings were removed with a small handheld electric drill. Smaller bone samples were powdered by freezing in liquid nitrogen and crushing in a steel percussion mortar. Approximately 100 mg of bone powder was used in each extraction. Modern bone samples were treated to remove lipids before protein extraction, using the lipid extraction technique described above.

Each sample was accurately weighed and admitted to a 15 ml glass funnel fitted with a stopcock and sintered glass plate, over which was placed a Whatman GF/A glass-fibre filter. The bone was demineralized by adding approximately 10 ml of 0.25 N HCl to the funnel and letting stand at room temperature for 24 hours. The HCl was then drained off under vacuum, replaced with fresh solution, and drained off after another 24 hours. The remaining material was solubilized by adding approximately 10 ml of 0.01 N HCl to the funnel and heating to 58° C for 24 hours. The solubilized protein solution was then drained off under vacuum into an Amicon brand Centriprep-30 ultrafiltration device with a 30kD (30,000 mw) cutoff. After centrifugation, the filtrate was discarded. Filtration was repeated twice after topping the protein solution with distilled water. The filtered collagen extract was then admitted to a tared vial and lyophilized. The resulting dry extract was weighed in order to calculate the percent yield from the bone sample.

Apatite sample preparation

Powdered bone samples were produced as for collagen extraction, either by drilling or by freezing in liquid nitrogen and crushing in a steel percussion mortar. Approximately 100 mg of powdered bone, weighed accurately, was added to a 15 ml centrifuge tube and deproteinated by adding approximately 15 ml of 1.5% sodium hypochlorite solution. The reaction was allowed to continue for approximately 72 hours, with daily vortexing. Following this the solution was decanted and the sample was rinsed to neutrality with distilled water, lyophilized, and weighed. A subsample of the deproteinated bone, weighed to within ± 0.5 mg of 50 mg, was added to a clean 15 ml centrifuge tube and treated to remove diagenetic carbonates by adding 10 ml of 1M acetic acid solution. The reaction was allowed to continue for 24 hours. The sample was again rinsed to neutrality, lyophilized and weighed to determine the amount of sample remaining. (Where less than 50 mg of sample was available for the acetic acid treatment, the volume of solution was adjusted so that the proportion of reactants was the same for all samples.)

To collect carbon as CO_2 gas from the sample, approximately 10 mg of the dry, prepared sample was weighed and introduced to one arm of a two-armed carbonate reaction vessel (McCrea 1950). The other arm was loaded with an excess (approximately 3 ml) of 85% phosphoric acid. The vessel was evacuated to 10^{-5} torr or less on a high vacuum line and sealed. The reactants were combined and the vessel placed in a dry block heater at 105° C for 25 minutes, with periodic agitation to ensure complete reaction. The vessel was then reattached to the high vacuum line. The evolved gas was passed through a dry ice/isopropanol trap and collected on a cold finger cooled with liquid nitrogen. After freezing, the sample was pumped free of noncondensible gases for five minutes. The gas was frozen into a second cold finger, warmed, and the quantity of CO_2 measured by manometry. Finally, the gas was frozen into a collection tube and sealed with a propane/oxygen torch. The sealed tube was sent to the isotope ratio mass spectrometer for measurement.

This apatite preparation method was previously evaluated by experimental tests on approximately thirty subsamples from animal bone specimens used in the dietary survey. These tests examined the effects of different acetic acid solution concentrations, volumes and treatment times on sample attrition, gas yield and $\delta^{13}C$. Results are presented in Appendix B.

Stable isotope measurement

Stable isotope measurements were made at the Department of Oceanography, University of British Columbia, using a VG Micromass Prism isotope ratio mass spectrometer fitted with a CHN elemental analyzer. Solid samples were combusted to CO_2 and N_2 gas in the elemental analyzer, which also determined sample carbon and nitrogen content by weight. $\delta^{13}C$ values were reported relative to the VPDB fossil belemnite standard, while $\delta^{15}N$ was reported relative to atmospheric N_2. Measurement precision and accuracy for the stable isotope ratios obtained were better than 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$, primarily

limited by sample inhomogeneity and processing, not by the measurement itself (Bente Nielsen, UBC, pers. comm. 1995).

Radiocarbon measurement

Subsamples of selected collagen extracts were submitted to the Center for Accelerator Mass Spectrometry (CAMS) at the Lawrence Livermore National Laboratory for AMS radiocarbon measurements. At CAMS, samples were combusted to CO_2 in sealed quartz tubes, reduced to a graphite coating on particles of iron or cobalt catalyst, and introduced into the accelerator for measurement. Sample preparation backgrounds were subtracted based on preparations of ^{14}C-free coal, and scaled relative to sample size. The radiocarbon ages thus determined were corrected for isotopic fractionation using the results from the stable isotope measurements. Conventional radiocarbon ages were calibrated to calendar dates using the CALIB 3.03 computer program (Stuiver and Reimer 1993), the results of which are presented and discussed in Appendix C.

Correction of δ values for dietary samples

As discussed in section 3.3 above, the $\delta^{13}C$ values of dietary samples required correction for intertissue fractionation effects, modern depletion of atmospheric $\delta^{13}C$, or both, according to the nature of the sample. The figures used for this study to obtain the corrected values were +1.5 ‰ for depletion of atmospheric $\delta^{13}C$ and –2.25 ‰ for correcting bone $\delta^{13}C$ to muscle protein $\delta^{13}C$. No corrections were applied to $\delta^{15}N$ values.

Determination of the minimum number of individuals in the PAR collection

Because the remains in the PAR collection are commingled, the isotopic variability in the collection could not be accurately assessed without finding the minimum number of individuals (MNI) represented. Collagen $\delta^{13}C$ and $\delta^{15}N$ values, radiocarbon measurements and osteometric traits were used in conjunction to arrive at an MNI estimate; the methods used are discussed in Appendix D.

5.0 RESULTS

Dietary background survey

Results of the dietary background survey are shown in Table 5.1 below. $\delta^{13}C$ measurements have been corrected if necessary for intertissue fractionation effects and modern depletion of atmospheric $\delta^{13}C$, as discussed above. All collagen samples reported in Table 5.1 gave percent collagen yields and C/N ratios close to those of unaltered bone. The raw data for these measurements are presented in Appendix A.

Table 5.1. Dietary background survey results. The column labeled $\delta^{13}C$ (u) shows corrected $\delta^{13}C$ values for untreated dietary samples; the column labeled $\delta^{13}C$ (l) shows corrected $\delta^{13}C$ values for lipid-free dietary samples.

Sample	Taxon	Mat'l	Loc'n	$\delta^{13}C$ (u)	$\delta^{13}C$ (l)	$\delta^{15}N$	
Plant samples							
Family Agavaceae							
BDS-35	*Agave deserti*	heart	PP	-12.3		1.9	
BDS-36	*Agave deserti*	heart	PP	-12.1		1.6	
BDS-37	*Agave shawii*	leaf	PP	-12.1		9.4	a
BDS-42	*Agave deserti*	heart	CV	-11.5		3.1	
BDS-43	*Agave deserti*	heart	CV	-11.4		5.2	
BDS-56	*Agave deserti*	heart	SE	-8.8		2.7	
BDS-57	*Agave deserti*	heart	SE	-10.0		2.4	
BDS-63	*Agave deserti*	heart	SW	-10.1		1.1	
BDS-64	*Agave deserti*	heart	SW	-10.0		2.0	
	mean ± sd			***-10.9***		***3.3***	
				± 1.2		***± 2.6***	
Family Bromeliaceae							
BDS-23	*Yucca valida*	fruit	VC	-10.5		5.7	
BDS-26	*Yucca valida*	fruit	VC	-9.9		4.2	
	mean			***-10.2***		***5.0***	
Family Cactaceae							
BDS-24	*Machareocereus gummosus*	stem	VC	-11.9		12.4	a
BDS-25	*Machareocereus gummosus*	stem	VC	-11.9		7.4	a
BDS-27	*Pachycereus pringlei*	stem	VC	-11.4		6.7	a
BDS-28	*Pachycereus pringlei*	fruit	VC	-10.4		10.4	
BDS-30	*Lophocereus schottii*	fruit	VC	-11.1		9.5	
BDS-31	*Opuntia cholla*	fruit	VC	-11.5		8.3	

Sample	Taxon	Mat'l	Loc'n	$\delta^{13}C$ (u)	$\delta^{13}C$ (l)	$\delta^{15}N$	
BDS-33	*Ferocactus sp.*	fruit	VC	-11.9		6.7	
BDS-59	*Lemairocereus thurberi*	stem	SE	-10.7		7.3	a
BDS-62	*Lemairocereus thurberi*	stem	SW	-10.0		6.0	
BDS-67	*Opuntia sp.*	fruit	SF	-11.6		7.0	
BDS-68	*Opuntia sp.*	fruit	SF	-10.7		2.3	
	mean ± sd			***-11.2***		***7.6***	
				± 0.7		***± 2.6***	
<u>All CAM plants</u>							
	mean ± sd			***-11.0***		***5.6***	
				± 0.9		***± 3.2***	
<u>Family Fabaceae</u>							
BDS-13	*Cercidium microphyllum*	seeds	MN	-21.6	-21.8	7.0	
BDS-21	*Acacia greggii*	seeds	EA	-23.0	-22.5	6.1	
BDS-34	*Prosopis glandulosa*	seeds	VC	-20.9	-20.8	1.3	
BDS-48	*Prosopis sp.*	seeds	SI	-23.0	-22.3	-0.3	
BDS-51	*Prosopis sp.*	seeds	SI	-23.5	-22.4	-0.8	
BDS-93	*Prosopis sp.*	seeds	BA	-20.2	-20.0	4.8	
	mean ± sd			***-22.0***	***-21.6***	***3.0***	
				± 1.3	***± 1.0***	***± 3.4***	
<u>Family Moraceae</u>							
BDS-61	*Ficus palmeri*	fruit	SW	-26.0		4.4	
Animal samples							
<u>Seabirds</u>							
BDS-91	*Pelecanus occidentalis*	bone	MC		-13.3	17.1	
BDS-96	*Pelecanus occidentalis*	bone	MC		-13.4	16.8	
	mean				***-13.4***	***17.0***	
<u>Fish</u>							
BDS-97	Family Balistidae	bone	MC		-11.9	16.9	
BDS-98	Family Balistidae	bone	MC		-11.7	17.7	
	mean				***-11.8***	***17.3***	
<u>Sea turtles</u>							
BDS-86	*Chelonia sp.*	bone	MC		-15.5	16.2	
BDS-95	*Dermochelys coriacea*	bone	MC		-14.2	20.2	
	mean				***-14.9***	***18.2***	
<u>Cetacean</u>							
BDS-92	Family Delphinidae	bone	MC		-12.6	16.0	
<u>Pinnipeds</u>							
BDS-81	*Zalophus californianus*	bone	MC		-12.6	21.1	
BDS-82	*Zalophus californianus*	bone	MC		-13.4	20.6	
BDS-99	*Zalophus californianus*	bone	MC		-13.8	22.4	
	mean ± sd				***-13.3***	***21.4***	
					± 0.6	***± 0.9***	
<u>All marine vertebrates</u>							
	mean ± sd				***-13.2***	***18.5***	
					± 1.1	***± 2.3***	

Sample	Taxon	Mat'l	Loc'n	$\delta^{13}C$ (u)	$\delta^{13}C$ (l)	$\delta^{15}N$	
<u>Marine invertebrates</u>							
BDS-6	*Turbo fluctuosus*	flesh	SO		-10.1	11.6	
BDS-7	*Turbo fluctuosus*	flesh	SO		-9.7	11.6	
BDS-9	*Chione sp.*	flesh	PG		-12.7	12.6	
BDS-10	*Chione sp.*	flesh	PG		-12.6	10.9	
BDS-46	*Saxidomus sp.*	flesh	LO		-15.3	11.9	
BDS-47	*Trachycardium sp.*	flesh	LO		-14.4	11.7	
	mean ± sd				***-12.5 ± 2.2***	***11.7 ± 0.5***	
<u>Terrestrial herbivores</u>							
BDS-11	*Lepus sp.*	flesh	EP		-18.4	12.5	
BDS-12	*Lepus sp.*	flesh	EP		-19.3	10.2	
BDS-38	Order Artiodactyla	bone	AC		-15.9	8.5	b
BDS-39	Order Artiodactyla	bone	AC		-17.2	8.2	b
BDS-70	Order Artiodactyla	bone	AC		-16.9	10.0	b
BDS-71	Order Artiodactyla	bone	AC		-18.9	8.8	b
BDS-72	Order Artiodactyla	bone	AC		-13.8	8.9	b
BDS-73	Order Artiodactyla	bone	AC		-17.7	8.2	b
BDS-83	*Ovis canadensis*	bone	MC		-15.1	7.5	b
BDS-85	*Odocoileus hemionus*	bone	MC		-16.7	12.5	
BDS-88	*Ovis canadensis*	bone	MC		-17.8	7.1	
	mean ± sd				***-17.1 ± 1.6***	***9.3 ± 1.8***	
<u>Terrestrial omnivore</u>							
BDS-84	*Taxidea taxus*	bone	MC		-17.7	10.7	
<u>Terrestrial carnivores</u>							
BDS-89	*Canis latrans*	bone	MC		-15.6	15.9	
BDS-100	*Canis latrans*	bone	MC		-13.9	13.8	
BDS-101	*Canis latrans*	bone	MC		-14.7	14.1	
	mean ± sd				***-14.7 ± 0.8***	***14.6 ± 1.1***	
<u>Terrestrial reptile</u>							
BDS-87	*Sauromalus obesus*	bone	MC		-18.5	5.9	

a) Tissue used as proxy for edible tissue. No intertissue correction applied.
b) Archaeological specimen. Samples were not subjected to lipid extraction, but are assumed to be lipid-free.

Collagen and apatite stable isotope measurements

Results of collagen and apatite stable isotope measurements on the human collections, as well as on nonhuman species measured for determination of the collagen-apatite increment (Δ_{C-A}), are presented in Table 5.2 below. $\delta^{13}C$ measurements on modern specimens have been corrected for depletion of atmospheric $\delta^{13}C$. Results of radiocarbon measurement on selected human collagen samples are also presented. Calibrations of conventional radiocarbon ages to calendar dates are presented and discussed in Appendix C. Raw data for all measurements are presented in Appendix A.

All collagen samples, with the exception of BLA-3, produced percent yields and C/N ratios close to those of fresh bone. BLA-3, with a burned appearance and a collagen yield of 0.7%, should be viewed with caution since such low yields may sometimes produce anomalous measurements. However, it gives an acceptable C:N ratio of 2.8, so it will be retained for further analysis. All apatite samples gave gas yields close to those of fresh, unaltered bone. However, sample PAR-8 was found to contain contaminant gases and was unmeasurable.

Table 5.2. Results of bone collagen $\delta^{13}C$, apatite $\delta^{13}C$, Δ_{C-A}, collagen $\delta^{15}N$ and ^{14}C measurement.

Sample	Taxon	$\delta^{13}C$ (c)	$\delta^{13}C$ (a)	Δ_{C-A}	$\delta^{15}N$	^{14}C age BP	
Bahía de las Animas burials							
BLA-1		-9.3	-4.8	4.5	22.1		
BLA-2		-9.0	-3.9	5.1	21.3		
BLA-3		-9.1	-3.3	5.8	18.2		a
BLA-4		-9.2	-3.5	5.7	19.0		
BLA-5		-9.4	-4.4	5.0	22.8	970 ± 50	
BLA-6		-9.1	-4.4	4.7	21.3		
BLA-7		-8.7	-2.4	6.3	20.3	490 ± 70	
	mean ± sd	***-9.1***	***-3.8***	***5.3***	***20.7***		
		± 0.2	***± 0.8***	***± 0.7***	***± 1.7***		
Sierra de San Francisco burials							
PAR-1		-9.5			12.7	3090 ± 60	b
PAR-2		-9.3			11.5		
PAR-3		-10.5	-1.7	8.8	11.9		b
PAR-4		-10.1			12.3	3250 ± 60	
PAR-5		-10.4			11.8	3090 ± 60	
PAR-6		-10.8			12.6		b

Sample	Taxon	$\delta^{13}C$ (c)	$\delta^{13}C$ (a)	Δ_{C-A}	$\delta^{15}N$	^{14}C age BP	
PAR-7		-11.8			12.0		
PAR-8		-9.2	n/m	n/m	12.6		b,c
PAR-9		-9.3			12.4		
PAR-10		-9.5			12.2		b
PAR-11		-10.8			13.1		
PAR-12		-10.4			12.4		b
PAR-13		-9.7			12.4	3260 ± 60	b
PAR-14		-9.2	-3.6	5.6	13.2		
PAR-15		-10.4	-4.1	6.4	12.8		b
PAR-16		-10.1			12.7		
PAR-17		-9.9	-4.0	6.0	12.4	3380 ± 50	b
	mean ± sd	***-10.1***	***-3.4***	***6.7***	***12.4***		
		± 0.9	***± 1.1***	***± 1.5***	***± 0.6***		
Marine mammals							
BDS-81	*Zalophus californianus*	-10.3	-6.4	3.9	21.1		
BDS-82	*Zalophus californianus*	-11.2	-10.0	1.2	20.6		
BDS-92	Family Delphinidae	-10.4	-8.7	1.7	16.0		
BDS-99	*Zalophus californianus*	-11.6	-10.3	1.3	22.4		
	mean ± sd	***-10.9***	***-8.9***	***2.0***	***20.0***		
		± 0.6	***± 1.8***	***± 1.3***	***± 2.8***		
Terrestrial herbivores							
BDS-38	Order Artiodactyla	-13.6	-6.2	7.4	8.5		
BDS-39	Order Artiodactyla	-14.9	-7.7	7.2	8.2		
BDS-70	Order Artiodactyla	-14.7			10.0		
BDS-71	Order Artiodactyla	-16.6			8.8		
BDS-72	Order Artiodactyla	-11.5			8.9		
BDS-73	Order Artiodactyla	-15.4			8.2		
BDS-83	*Ovis canadensis*	-12.9	-5.5	7.4	7.5		
BDS-85	*Odocoileus hemionus*	-14.5	-7.6	6.9	12.5		
BDS-88	*Ovis canadensis*	-15.6	-8.2	7.4	7.1		
	mean ± sd	***-14.4***	***-7.0***	***7.3***	***8.9***		
		± 1.5	***± 1.1***	***± 0.2***	***± 1.6***		
Terrestrial omnivore							
BDS-84	*Taxidea taxus*	-15.4	-12.3	3.1	10.7		
Terrestrial carnivores							
BDS-89	*Canis latrans*	-13.3	-8.9	4.4	15.9		
BDS-100	*Canis latrans*	-11.6	-8.4	3.2	13.8		
BDS-101	*Canis latrans*	-12.4	-8.3	4.1	14.1		
	mean ± sd	***-12.4***	***-8.5***	***3.9***	***14.6***		
		± 0.9	***± 0.3***	***± 0.6***	***± 1.1***		

a) Low collagen yield.
b) Does not contribute to MNI estimate as determined in Appendix D. Mean and standard deviation for PAR collagen δ values are shown for specimens contributing to MNI estimate; the mean for all PAR specimens is –10.1±0.7‰ for $\delta^{13}C$ and –12.4±0.5‰ for $\delta^{15}N$.
c) Apatite $\delta^{13}C$ not measurable.

6.0 DISCUSSION

6.1 Background survey results

The CAM plants sampled here include two yuccas, nine agaves, and eleven cacti. These show corrected $\delta^{13}C$ values averaging –11.0 ± 0.9 ‰, with no significant differences between any of the families. The enriched average value agrees with previous studies showing that most CAM plants do not utilize the C_3 pathway in their native habitats (Eickmeier and Bender 1976). Agaves, which were selected so that they would have wide enough geographical representation to test trends in $\delta^{13}C$ within the study area, show a statistically significant enrichment in the southern collection locations relative to northern ones (–9.7 ± 0.6‰ vs. –11.9 ± 0.4‰, respectively; $p<0.001$). This result is in agreement with a previous study showing progressive enrichment in soil and CAM plant $\delta^{13}C$ values as one moves south through the Central Desert (Amundson et al. 1994).

The $\delta^{15}N$ values of CAM plants are much more variable (5.6 ± 3.2‰) and do not appear to be correlated with location. Agaves have significantly lower average values than cacti (3.3 ± 2.6‰ vs. 7.6 ± 2.6‰, respectively; $p=0.001$), although the ranges overlap. Of particular note are highly enriched outliers in each group (e.g. BDS-24, -28 ,-37). These samples were collected nearby others with more characteristic values, so the source of this variation is unexplained.

The six legumes sampled here show an average $\delta^{13}C$ of –22.0 ± 1.3‰. These values are comparatively enriched relative to most C_3 plants, but are in general agreement with latitude- and climate-related trends observed in tree cellulose $\delta^{13}C$ (Stuiver and Braziunas 1987). Because of the high protein content of the legumes, these samples were prepared both with and without lipid extraction in order to test the effects of this procedure. The average of the lipid-extracted samples is –21.6 ± 1.0 ‰, which is significantly heavier than the unextracted samples (paired $t=2.36$; $p=0.062$), as expected (Tieszen 1991). However,

the magnitude of change is small enough that routine lipid extractions of plant samples are probably unnecessary.

The $\delta^{15}N$ values of the legumes are quite variable (3.0 ± 3.4‰). The large range of values may be due to differences in atmospheric N_2 fixation. A previous study of *Prosopis* trees in the northern Central Desert area found leaf $\delta^{15}N$ values averaging 8.9‰, indicating that many legumes in the region do not fix significant amounts of N_2 (Shearer et al. 1983). The same study found that *Prosopis* grown under conditions of forced N_2-fixation gave average leaf $\delta^{15}N$ values of –1.3‰, close to the minimum values observed here. A larger sample of leguminous species might reveal geographic patterning in legume $\delta^{15}N$ due to differences in N_2-fixation.

A single wild fig (*zalate*), with a $\delta^{13}C$ of –26.0‰, is significantly lighter than the legumes ($p \cong 0.03$), suggesting large potential differences between different C_3 species. However, this species is not expected to have made up a large part of diet, and is not considered in the diet estimates made below. The $\delta^{15}N$ of 4.4‰ is comparable to those of the legumes.

The sample of herbivorous mammals includes nine artiodactyls, both modern and archaeological, and two lagomorphs. The average herbivore $\delta^{13}C$ value of –17.1 ± 1.6‰ appears to reflect a mixture of C_3 and CAM foods in the diet. Given the large range of $\delta^{13}C$ values in the local vegetation, the potential range of variability in feeding habits and the wide geographic and temporal range of the samples selected, the variability in $\delta^{13}C$ is strikingly small. The herbivore $\delta^{15}N$ value of 9.3 ± 1.8‰ is generally enriched above those of the sampled plants, reflecting trophic enrichment. Again, variability in $\delta^{15}N$ values is low despite the large range of $\delta^{15}N$ values in the local vegetation. While the lagomorphs show both a lighter average $\delta^{13}C$ and higher average $\delta^{15}N$ than the artiodactyls, these differences do not reach statistical significance at $\alpha=0.05$ ($p=0.09$ and $p=0.08$, respectively). A larger sample may reveal isotopic patterning between species; however,

the difference may also result from error in the estimate of intertissue fractionation between muscle protein and bone, since all the artiodactyl samples were prepared from bone rather than flesh.

Bone collagen from a single reptile was also measured. Its $\delta^{13}C$ and $\delta^{15}N$ values are statistically indistinguishable from those of the herbivores. Again, a larger sample of reptiles may reveal isotopic differences between reptiles and herbivores not apparent here. While reptiles undoubtedly made up at least a minor portion of diet, they are not considered separately from herbivorous mammals in the dietary estimates below.

Terrestrial carnivores sampled here include three coyotes. These animals are not expected to have made up a significant portion of the diet, and were primarily sampled for the evaluation of $\Delta_{C\text{-}A}$ (see Section 6.2 below). However, they can be used to assess the trophic increment in $\delta^{13}C$ and $\delta^{15}N$. The average enrichment in $\delta^{15}N$ between herbivores and carnivores of $5.3 \pm 2.1‰$ is similar to the large trophic increment observed in arid environments elsewhere (Ambrose and DeNiro 1986). The $\delta^{13}C$ values are enriched over herbivore values by $2.4 \pm 1.8‰$ ($p=0.04$), which is somewhat larger than previously reported values for trophic enrichment of $\delta^{13}C$ (DeNiro and Epstein 1978).

A single badger was also measured. While its diet is assumed to be omnivorous, its collagen $\delta^{13}C$ and $\delta^{15}N$ values are not distinguishable from those of the herbivores ($p\cong0.6$ and $\cong0.3$, respectively).

The marine invertebrates sampled for this study include two gastropods and four bivalves. The average $\delta^{15}N$ of $11.7 \pm 0.6‰$ does not distinguish this group from terrestrial mammals. While the average $\delta^{13}C$ value of -12.5 ± 2.2 is significantly enriched over that of terrestrial animals, there is a great deal of variability within the group; gastropods show significantly enriched values relative to the bivalves (-9.8 ± 0.3 vs. $-13.7 \pm 1.3‰$,

respectively; p=0.02). Again, this points to the possibility of taxonomic patterning within this group, although a larger sample would demonstrate this more conclusively.

The marine vertebrates sampled for this study include two fish, three pinnipeds, a cetacean, two seabirds and two sea turtles, all prepared from bone collagen. The average $\delta^{13}C$ value for all samples is -13.2 ± 1.1‰. While the mean $\delta^{13}C$ values for fish and reptiles are both significantly different from the remainder of the samples (-11.8‰, p=0.03 and -14.9‰, p=0.01 respectively), the sample is not large enough to confidently evaluate systematic differences between species. While $\delta^{15}N$ values range widely (18.5 ± 2.3‰), only pinnipeds have significantly different values than the remainder of the samples (21.3 ± 0.9‰ vs. 17.3 ± 1.4‰ respectively; p= 0.002); this reflects the elevated trophic position of these species.

Pinnipeds from archaeological sites on the coast of southern California (DeNiro 1985) show significantly different $\delta^{13}C$ and $\delta^{15}N$ values than the pinnipeds measured in this study ($\delta^{13}C$ of -15.1 ± 0.6 vs. -13.3 ± 0.6‰; $\delta^{15}N$ of 17.9 ± 1.0 vs. 21.4 ± 0.9‰; $p<0.001$ for both isotopes). Assuming similar dietary habits in Pacific and Gulf pinnipeds, this points to the possibility of significant differences in average δ values between the two coasts. However, the difference may also be at least partially related to differences in collagen extraction methods. Whether marine animals from the Baja California Pacific coast are expected to have similar values is unknown. The humans in this study are not expected to have made much use of Pacific resources, but the possibility of differences in $\delta^{15}N$ values between the coasts would substantially complicate dietary interpretation in a larger-scale study of the peninsula.

In general, the dietary sample shows encouraging results and agrees with previously observed trends in both $\delta^{13}C$ and $\delta^{15}N$ values. Average values for each of the end points defined here cluster reasonably well, with little overlap between dietary categories. However, because there are so many different isotopic signatures in evidence, dietary

interpretation will be difficult. In addition, there appears to be some geographic and taxonomic patterning within each of the categories, so the assumption of random variability around end point δ values required by dietary estimates may not be strictly warranted. The major dietary resources and their average $\delta^{13}C$ and $\delta^{15}N$ values are shown in Figure 6.1.

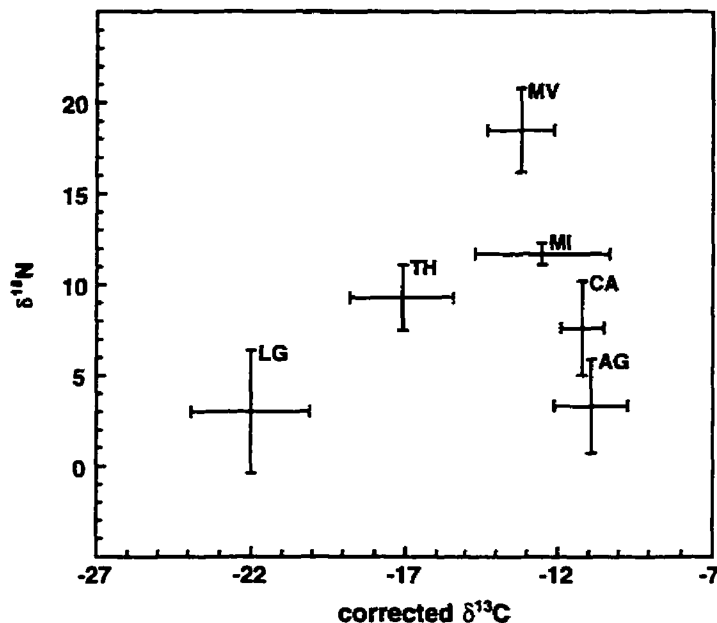

Figure 6.1. Bivariate plot of average $\delta^{13}C$ and $\delta^{15}N$ values of the six major dietary end points identified in this study. The end points are labeled LG, legumes; CA, cactus fruits; AG, agaves; TH, terrestrial herbivores; MI, marine invertebrates; MV, marine vertebrates. Error bars show standard deviations.

6.2 Collagen-apatite increment ($\Delta_{C\text{-}A}$)

Species sampled in order to evaluate $\Delta_{C\text{-}A}$ include terrestrial herbivores, represented by five artiodactyls; terrestrial carnivores, represented by three coyotes; an omnivorous badger; and marine carnivores, including three sea lions and a dolphin. Results of apatite $\delta^{13}C$ measurement and $\Delta_{C\text{-}A}$ are empirically very encouraging, and suggest that diagenetic effects on the bone apatite values are minor.

The artiodactyl apatite $\delta^{13}C$ values average $-7.0 \pm 1.1‰$. The $\Delta_{C\text{-}A}$ clusters better than does $\delta^{13}C$, and at $7.3 \pm 0.2‰$, is in excellent agreement with the Lee-Thorp et al. (1989) model. This small variability in $\Delta_{C\text{-}A}$, despite the wide range of $\delta^{13}C$ values in the artiodactyl diet, lends substantial support to the Lee-Thorp model, and suggests that $\Delta_{C\text{-}A}$ is basically stable at a given trophic level regardless of the composition of overall diet.

The carnivore apatite $\delta^{13}C$ values average $-8.5 \pm 0.3‰$, giving a $\Delta_{C\text{-}A}$ of $3.9 \pm 0.6‰$. These results are also in general agreement with the Lee-Thorp model and indicates $\Delta_{C\text{-}A}$ is stable within a trophic level. While carnivore $\Delta_{C\text{-}A}$ behaves as predicted, it should be noted that both the collagen and apatite $\delta^{13}C$ values are somewhat more depleted than the Lee-Thorp model predicts. Interestingly, while the badger apatite $\delta^{13}C$ of $-12.3‰$ is much lighter than those of the canids, and while its collagen $\delta^{13}C$ and $\delta^{15}N$ values resemble those of herbivores, its $\Delta_{C\text{-}A}$ of is not distinguishable from that of the carnivores ($p \cong 0.4$).

The apatite $\delta^{13}C$ and $\Delta_{C\text{-}A}$ of marine mammals do not cluster particularly well, at $-8.9 \pm 1.8‰$ and $2.0 \pm 1.3‰$, respectively. Sample BDS-81, with a $\Delta_{C\text{-}A}$ of $3.9‰$, appears to be somewhat anomalous. While the average $\Delta_{C\text{-}A}$ is quite small, it appears to meet the general expectations of the Lee-Thorp model. However, little is known about using $\Delta_{C\text{-}A}$ to characterize marine diets. While marine trophic systems are more complex than terrestrial ones, it is not clear whether the Lee-Thorp model indicates that $\Delta_{C\text{-}A}$ should be further reduced in trophic levels above that of primary carnivores. The small $\Delta_{C\text{-}A}$ of the marine carnivores relative to terrestrial carnivores may result from a generally greater dietary importance of lipids, which act to reduce $\Delta_{C\text{-}A}$.

Human apatite values present an interesting contrast to the animal values. In the Bahía de las Animas collection, the $\Delta_{C\text{-}A}$ of $5.3 \pm 0.6‰$ is larger than that of marine carnivores or of terrestrial carnivores, despite the close similarity of the collagen δ values to those of marine carnivores. Similarly, in the Sierra de San Francisco collection, the $\Delta_{C\text{-}A}$ of $6.7 \pm$

1.4‰ is closer to that of herbivores than carnivores, even though the enrichment in $\delta^{15}N$ values might itself suggest a trophic level enrichment more typical of carnivores (see section 6.3 below). Figure 6.2 illustrates the relationships between collagen $\delta^{13}C$, $\delta^{15}N$, and Δ_{C-A} of the trophic categories sampled.

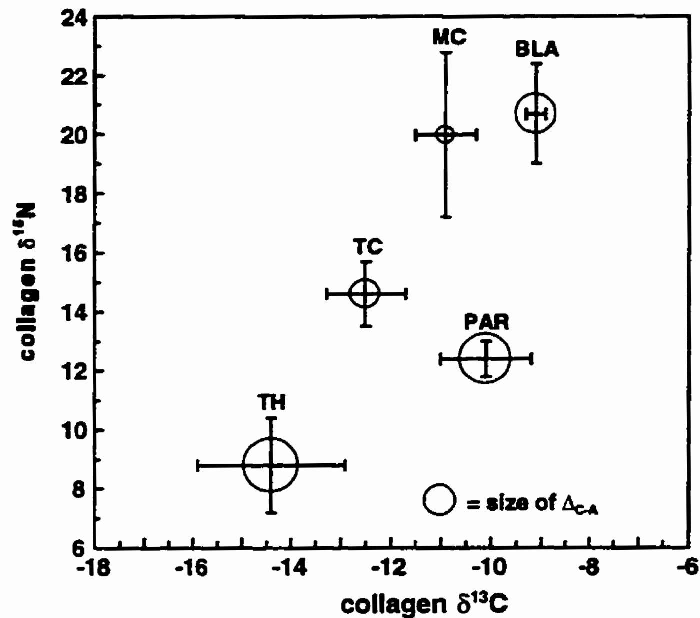

Figure 6.2. Bivariate plot of collagen $\delta^{13}C$, $\delta^{15}N$, and collagen-apatite spacing (Δ_{C-A}). Categories shown are terrestrial herbivores (TH), terrestrial carnivores (TC), marine carnivores (MC), and the Bahía de las Animas (BLA) and Sierra de San Francisco (PAR) human collections. Error bars indicate standard deviations. The circles represent the size of the average collagen-apatite spacing (Δ_{C-A}) for each category.

The reduced Δ_{C-A} of carnivores in the Lee-Thorp model results from the isotopically depleted animal lipids used in energy metabolism. In a multi-endpoint system, humans may draw metabolic energy from dietary resources with different $\delta^{13}C$ signatures and thus display unusual Δ_{C-A} values. For example, Lee-Thorp et al. (1989) observed a smaller Δ_{C-A} in humans than expected in a C_3-based system, presumably because enriched marine protein influenced collagen $\delta^{13}C$ values while most energy came from more depleted terrestrial C_3 plants. In Baja California, the large Δ_{C-A} could result from the influence of

enriched CAM species (i.e., agaves, cacti) on apatite $\delta^{13}C$, with collagen $\delta^{13}C$ controlled largely by the more depleted animal end points, including marine foods. This complexity means that Δ_{C-A} cannot be used in humans as a straightforward estimator of trophic level; however, by calculating the expected collagen and apatite δ values of a range of hypothetical diets, the resulting Δ_{C-A} can be found and compared to human Δ_{C-A} values.

6.3 Human samples

General observations

The collagen $\delta^{13}C$ values in the Bahía de las Animas collection are tightly clustered at –9.1 ± 0.2‰, reflecting the enriched $\delta^{13}C$ values of both marine resources and CAM plants. The fact that a human population can exhibit such small variability despite large isotopic variability in the presumed diet strongly supports the argument that the isotopic variability of end point consumers is less than that of the foodwebs from which they sample. Conversely, assuming a diet with a uniform isotopic composition, this value can serve as an empirical estimate of maximum variability in diet-to-tissue increments in a human population; this value is lower than some experimental estimates (DeNiro and Schoeninger 1983).

At 20.7 ± 1.7‰, the $\delta^{15}N$ values of the Bahía de las Animas collection are more variable but are extremely high, indicating heavy reliance on marine sources of protein, particularly higher trophic level species such as fish, sea turtles, and pinnipeds. The large variability in $\delta^{15}N$ values parallels that in marine dietary resources, and may to some degree reflect differences between individuals in the composition of diet not apparent in the $\delta^{13}C$ values.

The two radiocarbon measurements on the collection show that these interments date from the late prehistoric Comondú period. As discussed in Appendix C, the calibration of these measurements to calendar dates is problematic. Sample BLA-7 appears to date from the terminal prehistoric or protohistoric, perhaps some time after 1700 cal AD.

As discussed in Appendix D, the MNI of the Sierra de San Francisco collection is estimated at eight. At $-10.1 \pm 0.9‰$ and $12.4 \pm 0.6‰$, the mean $\delta^{13}C$ and $\delta^{15}N$ values for the samples contributing to the MNI estimate are better clustered than any of the dietary end points, again suggesting a trophic averaging effect. (The mean $\delta^{13}C$ and $\delta^{15}N$ of all seventeen specimens is the same as above, but gives slightly smaller standard deviations of $\pm 0.7‰$ and $\pm 0.5‰$, respectively.)

The comparatively low average $\delta^{15}N$ in the Sierra de San Francisco collection seems to indicate terrestrial species as the primary source of dietary protein, with perhaps some contribution by low trophic-level marine foods such as shellfish and bivalves. A higher proportion of terrestrial foods is not unexpected in this inland location, but the difference between the two collections suggests a surprising degree of dietary specialization given the easy availability of both marine and terrestrial resources to both groups. Whether this difference is due to long-standing differentiation between coastal and inland groups or to intensification of marine resource use in the late prehistoric can't be proven, since the collections aren't coeval. Climate change is unlikely to have contributed to the difference (Van Devender et al. 1994).

Five radiocarbon dates were taken from the collection. The calibrated dates, as discussed in Appendix C, substantially predate the Comondú culture, so it is possible that the general dietary adaptation of these people was quite different than that described in historic accounts. However, environmental conditions are expected to have been similar (Van Devender et al. 1994). The close agreement in the dates suggests the possibility that the burials may have been deposited during a single event, although since sample PAR-17 is statistically distinct from PAR-4 and PAR-5 it appears that interments may have been added to the site over at least a short period of time.

Other human collections from the Cape region are comparable to the results of this study, but are generally more variable (Figure 6.3). Molto and Kennedy (1991) measured eight

individuals, all from locations on or near the Gulf coast throughout the peninsula. The $\delta^{13}C$ and $\delta^{15}N$ values are generally more enriched in the northern locations; the most enriched value is from Bahía de los Angeles, to the immediate north of Bahía de las Animas. Molto and Fujita (1995) measured five Las Palmas burials from La Matancita, a mortuary cave near Todos Santos in the Cape region of the peninsula. The variability in the collection is somewhat greater than either group measured for this study. Some additional source of variability may be present in the Cape region, such as gender- or status-based differences in diet, greater variability in CAM plant $\delta^{13}C$ values in the milder climate, or contribution by both Gulf and Pacific marine resources. Different collagen extraction methods may also be partially responsible.

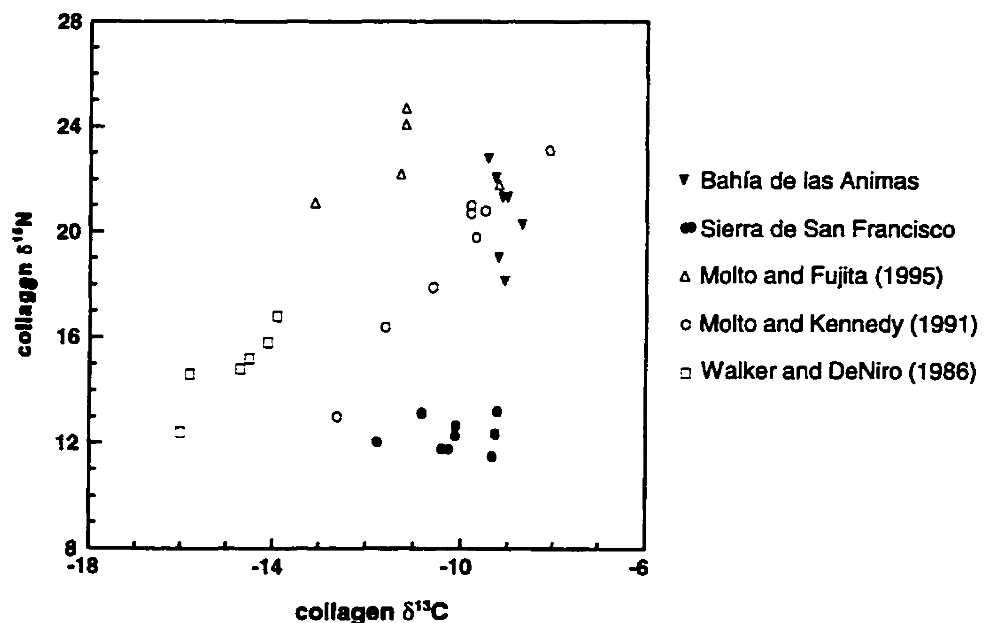

Figure 6.3. Scatterplot of human collagen $\delta^{13}C$ and $\delta^{15}N$ values from Baja California and coastal southern California. In the Sierra de San Francisco collection, only individuals contributing to the MNI estimate are shown. Values from Walker and DeNiro (1986) are averages by site.

Values from a variety of sites in coastal southern California (Walker and DeNiro 1986) are also shown here, as an illustration of the strong effect of regional differences in dietary δ values on human δ values. The most enriched values are from coastal sites on the Channel Islands, which undoubtedly record a very high proportion of marine resources in the diet, yet both $\delta^{13}C$ and $\delta^{15}N$ values are much lower than on the Gulf coast. These values may serve as a general indicator of the δ values to be expected from marine-oriented groups on the Pacific coast of Baja California.

Diet reconstruction

There are at least six major dietary end points in the isotopic ecosystem of Baja California. Thus, directly estimating dietary proportions from human δ values is impossible, because any of a large number of end point combinations could produce the observed values. However, some of these are more plausible than others, both archaeologically and nutritionally, and this discussion attempts to reconcile observed δ values with these considerations by comparing the expected δ values of a range of different postulated diets to those observed in the human samples.

The predicted $\delta^{13}C$ and $\delta^{15}N$ values of the hypothetical diets are calculated using the mixing equation described in section 3.6. The δ value for each dietary end point (δ_i) is estimated using the average values from the background survey. Despite the observed geographic and species-related patterning within some of the end points, this study will ignore this and treat observed variation as if it were random about a single end point value. Also, some potential resources, such as Pacific marine foods and herbaceous annuals, are not reflected in the end point values used here, but it is assumed that the most important resources are represented.

A diet-to-tissue increment (Δ_{ij}) is added to each of these end points. For collagen, a value of +4.5‰ is used for both $\Delta^{13}C_{D\text{-}C}$ and $\Delta^{15}N_{D\text{-}C}$ values. As discussed above, while $\Delta^{13}C_{D\text{-}C}$ is relatively well known, $\Delta^{15}N_{D\text{-}C}$ is expected to vary depending on a variety of climatic and

physiological factors, and thus can be only roughly estimated here. The observed increment in collagen $\delta^{15}N$ between herbivores and carnivores in this study is somewhat larger than this, but it is unclear whether these species can serve as proxies for human $\Delta^{15}N_{D-C}$.

Finding the increment between bulk diet and apatite ($\Delta^{13}C_{D-A}$) also presents difficulties, because it varies according to the nutrient composition of each end point. Thus an appropriate increment is determined empirically for each end point, by adding the observed animal Δ_{C-A} values to the $\Delta^{13}C_{D-C}$ value of +4.5‰. For the terrestrial herbivore end point, the Δ_{C-A} of +3.9‰ for herbivore consumers (i.e., carnivores) plus $\Delta^{13}C_{D-C}$ gives an increment of about +8.5‰. For the marine end points, the observed marine carnivore Δ_{C-A} of 2.0 gives an increment of +6.5‰. For plant foods, the observed herbivore Δ_{C-A} of 7.3‰ gives an increment for plant foods of about +12‰, a figure which agrees with experimentally determined values of $\Delta^{13}C_{D-A}$ in herbivorous lab animals. The increment-corrected end point δ values are shown at the top of Table 6.1.

The metabolic weighting factors for each end point and tissue (w_{ij}) are taken from food composition data. The influence of each dietary end point on human collagen $\delta^{13}C$ and $\delta^{15}N$ values is estimated from its average protein content per 100 g, while apatite $\delta^{13}C$ values are weighted by average caloric content per 100 g. Data for plant foods is taken from a nutritional study of prehistorically important plant foods from highland Mexico (Robson and Elias 1986), while the values used for animal food end points are averages of analogous species in the U.S. Department of Agriculture food composition database (USDA 1996). These values are listed at the top of Table 6.1.

The experimental δ values calculated here do not reflect uncertainty in the end point δ values, in the diet-to-tissue increments applied, or in the weighting factors used. If any of these are substantially in error, the predicted δ values will be in error also. The increment

in $\delta^{15}N$ values is particularly problematic because of climatic and physiological effects on the diet-to-tissue increment. Because of these problems, the estimates presented here should be considered tentative.

First, the estimate of dietary proportions proposed by Aschmann (1959) is approximated as closely as possible, excluding minor components of diet and those for which no δ values are available, and the resulting δ value calculated. (Note that Aschmann's percentages are originally given in terms of "food values" or calories, but are adjusted here to reflect unprocessed weight; this is, of course, only one way of expressing dietary importance.) As Table 6.1 shows, the δ values produced by the Aschmann estimate do not match either human collection very closely, although they are an approximate average of the two. Given that Aschmann intended the estimate as an average of inland and coastal subsistence, the estimate appears surprisingly accurate.

Next, a variety of hypothetical diets is also calculated in an attempt to more closely match the human δ values while using Aschmann's proportion estimates as a general guide. For experimental diet sets A and C, the importance of each plant food end point is held in the fixed relative proportions suggested by the Aschmann estimate, while varying the absolute importance of plant foods in the diet (Table 6.1). This reduces the number of variable end points to three, so that a point estimate of the importance of each animal end point can then be made by solving the mixing equation for collagen $\delta^{13}C$ and $\delta^{15}N$. Where the observed human δ values cannot be matched exactly, the proportions are allocated so as to most closely approximate the observed collagen $\delta^{13}C$ and $\delta^{15}N$ values (as measured by the summed z-score of the calculated δ values relative to the human δ values; see Spielmann et al. 1990).

These experimental diets keep plant foods in fixed relative proportions, but significant variations from the proportions indicated by Aschmann's estimate might also be possible. Because agaves and cacti are low in protein, changing the dietary proportions of these

items relative to one another has little effect on predicted collagen δ values; because they have similar $\delta^{13}C$ values, changing their relative proportions has little effect on apatite $\delta^{13}C$. Legumes, by contrast, are high in both protein and calories, so experimentally changing their importance relative to other plant foods might produce significant changes in both collagen and apatite δ values. To investigate this, a second set of diets (sets B and D) is calculated in order to assess the effects of changing relative proportions of plant foods, while keeping the absolute proportion of plants in the diet at a fixed level (50% for set B, 90% for set D). Again, the proportions of animal foods in each diet are allocated to best approximate the human $\delta^{13}C$ and $\delta^{15}N$ values.

The observed δ values of the Bahía de las Animas collection can be reproduced in a number of different ways. Diets A2 through A5 all match the collagen $\delta^{13}C$ and $\delta^{15}N$ values exactly, and A1 nearly does so. This illustrates the small influence of plant foods on collagen δ values under the assumptions of the mixing equation. However, since $\Delta_{C\text{-}A}$ is related to trophic level, it is more sensitive to these changes. Diets A3 and A4 (50% and 60% plants respectively) produce the best match with the observed $\Delta_{C\text{-}A}$ value. However, as diet set B shows, $\Delta_{C\text{-}A}$ also varies in response to the proportion of legumes in the diet when the proportion of plant foods is held constant, so it cannot be understood as a simple measure of the quantity of plant foods in the diet. As legumes increase in importance, the percentage of plant foods giving the best match with observed $\Delta_{C\text{-}A}$ also increases. However, assuming a relatively minor input from legumes (i.e., not more than 10%), the experimental diets indicate that the proportion of plant foods in the diet is probably somewhere between 45% and 65%. In all diets, the proportion of terrestrial animal foods is close to zero. Marine foods consist largely of higher trophic level species (fish, seabirds, pinnipeds, and sea turtles). However, this conflicts somewhat with the archaeological picture, since shellfish remains appear to be much more abundant in archaeological sites than marine animal bone (Ritter 1995). A detailed zooarchaeological study in the region may help to resolve this issue.

Finding a diet that accurately reflects the Sierra de San Francisco collection δ values is rather more difficult. None of the experimental diets matches the observed values exactly. Of the diets in which plant foods are kept in the proportions indicated by the Aschmann estimate (set C) the diet that comes closest to a match has an implausibly high proportion of plant foods and probably inadequate dietary protein as well (C1, 95%). In addition, the Δ_{C-A} values resulting from the diets in set C are higher than the observed value, increasing as the proportion of plant foods increases. However, the best matches with the observed δ values indicate a higher proportion of plant foods than Aschmann estimates, perhaps between 75% and 90%. Diet set D, at a constant 90% plant foods, shows the strong effects on Δ_{C-A} of changing proportions of legumes. Diet D3, with a greater proportion of legumes than Aschmann suggests, shows the best overall match with observed $\delta^{13}C$, $\delta^{15}N$ and Δ_{C-A}, and is perhaps the most plausible experimental diet overall. However, this diet places greater emphasis on marine invertebrates than on terrestrial herbivores, which is perhaps a questionable result.

The fact that the Sierra de San Francisco values cannot be easily modeled suggests problems with the assumptions used in calculating the diets. One possibility is that these people made significant use of a dietary resource not represented in the six-end point model. The seeds of C_4 annuals such as amaranths, which are high in protein and should display $\delta^{13}C$ and $\delta^{15}N$ values similar to those of CAM plants, are a likely candidate. There may also be errors in the estimated end point values or in the increments applied. In particular, the predicted collagen $\delta^{15}N$ values are dependent on a very poorly known diet-to-tissue increment. Finally, the low-protein diets suggested here indicate that collagen $\delta^{13}C$ may incorporate non-protein carbon to some extent. This study cannot resolve these problems.

Table 6.1. Calculated δ values of the Aschmann (1959) diet estimate and of a range of hypothesized diets, as compared to the human collections. Experimental diet sets A and C are calculated by holding plant end points in the fixed relative proportions suggested by Aschmann (1959) and varying the total quantity of plant foods in the diet. Animal foods are allocated so as to best approximate the observed δ values. Proportions may not add exactly to 100% due to rounding error.

	dietary proportions of end point foods						predicted values		
	Agaves	Cactus fruits	Legumes	Terrestrial herbivores	Marine invertebrates	Marine vertebrates			
g protein / 100 g	0.3	1.1	5.7	22	13	19			
kilocalories / 100 g	125	40	300	125	90	120			
end point $\delta^{13}C$ (c)	-6.4	-6.7	-17.5	-12.6	-8.0	-8.7			
end point $\delta^{15}N$ (c)	7.8	12.1	7.5	13.8	16.2	23.0			
end point $\delta^{13}C$ (a)	1.1	0.8	-10.0	-8.6	-6.0	-6.7	$\delta^{13}C$	$\delta^{15}N$	
end point Δ_{C-A}	7.5	7.5	7.5	4.0	2.0	2.0	(c)	(c)	Δ_{C-A}
<u>Aschmann estimate</u>	26	35	2	14	12	11	**-10.2**	**16.7**	**6.8**
Bahía de las Animas							**-9.1**	**20.7**	**5.3**
<u>Experimental diet A</u>									
A1 (80% plants)	33	44	2	2	0	18	-9.1	20.2	7.1
A2 (70% plants)	29	39	2	3	1	26	-9.1	20.7	6.4
A3 (60% plants)	25	33	2	4	4	32	-9.1	20.7	5.6
A4 (50% plants)	20	28	2	5	7	38	-9.1	20.7	5.0
A5 (40% plants)	16	22	2	6	10	44	-9.1	20.7	4.4
<u>Experimental diet B</u>									
B1 (0% legumes)	21	29	0	6	6	38	-9.1	20.7	5.3
A4 (2% legumes)	20	28	2	5	7	38	-9.1	20.7	5.0
B2 (3% legumes)	20	27	3	4	7	38	-9.1	20.7	4.7
B3 (6% legumes)	19	25	6	3	9	39	-9.1	20.7	4.2
Sierra de San Francisco							**-10.1**	**12.4**	**6.7**
<u>Experimental diet C</u>									
C1 (95% plants)	39	53	3	3	2	0	-10.2	12.6	9.4
C2 (90% plants)	37	50	3	7	3	0	-10.7	13.2	9.4
C3 (80% plants)	33	44	2	15	5	0	-11.3	13.7	9.0
C4 (70% plants)	29	39	2	23	7	0	-11.6	13.8	8.4
C5 (60% plants)	25	33	2	31	9	0	-11.7	13.9	7.7
<u>Experimental diet D</u>									
D1 (0% legumes)	38	52	0	9	1	0	-10.9	13.3	10.5
C2 (3% legumes)	37	50	3	7	3	0	-10.7	13.2	9.4
D2 (6% legumes)	36	48	6	4	6	0	-10.6	13.1	8.5
D3 (9% legumes)	34	47	9	2	8	0	-10.4	12.9	7.6

7.0 CONCLUSIONS

Several general conclusions can be made from this study. First, the dietary background survey shows that previously observed trends in stable isotope measurements of different resource types hold true in the arid environment of the central peninsula, and that prehistorically important dietary resources can all be distinguished on the basis of carbon and nitrogen isotope measurements. However, the large number of isotopically labeled resources seriously complicates dietary interpretation. The data also suggest some location- and species-related trends in stable isotope values which could complicate dietary estimates. A larger-scale study of dietary items might resolve these problems.

Second, carbon isotope measurements on mammals from the region support the argument that the increment between collagen and apatite carbon isotope values is related to trophic level, despite the isotopically complex Baja California environment and despite methodological difficulties with bone apatite measurement. However, interpreting this increment in humans is complex because of their varied diet.

Third, the large isotopic difference between the two human collections suggests great variety in possible adaptations to the central desert environment. Because radiocarbon results show that the two collections are not coeval, it cannot be argued that separate coastal and inland-focused populations necessarily coexisted throughout prehistory. However, the fact that either group could concentrate on locally available resources to such a degree is perhaps surprising in the face of historical accounts which stress the marginal nature of the subsistence base.

Finally, estimates of the predicted stable isotope values of a range of hypothesized diets allow several tentative refinements of these observations. For the inland population, the experimental diets suggest that vegetable foods made up about 75% to 90% of the diet, including a high proportion of legumes, perhaps up to 10%. Marine foods made up no

more than 5% to 10% of the diet; these consisted of easily collected invertebrates gathered during short seasonal trips, rather than higher trophic-level marine foods such as fish, pinnipeds and sea turtles, which would have required greater expertise and technology to exploit. In contrast, the coastal population made heavy use of these resources, approaching 40% of diet. Plant foods were generally less important in the diet, making up between 40% and 60% of diet. Terrestrial animal resources were almost entirely absent from the coastal diet.

However, because of the many remaining uncertainties in this analysis, these conclusions must be considered preliminary. To gain a clearer understanding of the isotopic ecosystem of the central peninsula, further study in the region will need to explore geographic variation in the stable isotope values of dietary resources, and expand the scope of the study to include dietary resources not sampled here, such as Pacific marine foods and C_4 annuals. In addition, some problems with stable isotope analysis remain to be resolved if diet is to be assessed with greater confidence; these include uncertainty over the dietary sources of bone collagen carbon in low-protein diets, effects of seasonally variable diets on the rate of bone tissue turnover, and the effects of arid climates on nitrogen stable isotope measurements.

If these issues can be resolved, further sampling of humans from the region could serve to better document differences in diet between coastal and inland populations, and perhaps changes over time in subsistence patterns within both areas.

APPENDICES

Appendix A: Results

Table A.1. Results of untreated dietary sample analysis. The column "$\delta^{13}C$ (u)" shows the uncorrected measurement, while the "$\delta^{13}C$ (c)" column shows the measurement after applying a fossil-fuel correction of +1.5‰. Reported $\delta^{13}C$ values are correct to within ±0.1‰; $\delta^{15}N$ values are correct to within ±0.2‰.

Sample	Taxon	Mat'l	%C	%N	C/N ratio	$\delta^{13}C$ (u)	$\delta^{13}C$ (c)	$\delta^{15}N$
BDS-13	*Cercidium microphyllum*	seeds	21.2	3.2	7	-23.13	-21.6	6.96
BDS-21	*Acacia greggii*	seeds	45.4	3.0	15	-24.50	-23.0	6.14
BDS-23	*Yucca valida*	fruit	45.4	3.0	15	-12.03	-10.5	5.74
BDS-24	*Machareocereus gummosus*	stem	44.6	0.3	172	-13.43	-11.9	12.43
BDS-25	*Machareocereus gummosus*	stem	39.9	0.4	108	-13.39	-11.9	7.38
BDS-26	*Yucca valida*	fruit	44.4	1.8	25	-11.40	-9.9	4.24
BDS-27	*Pachycereus pringlei*	stem	38.3	0.7	58	-12.94	-11.4	6.68
BDS-28	*Pachycereus pringlei*	fruit	36.5	2.9	13	-11.86	-10.4	10.37
BDS-30	*Lophocereus schottii*	fruit	45.5	2.6	18	-12.60	-11.1	9.52
BDS-31	*Opuntia cholla*	fruit	34.8	0.6	57	-12.97	-11.5	8.33
BDS-33	*Ferocactus sp.*	fruit	41.8	1.6	27	-13.38	-11.9	6.74
BDS-34	*Prosopis glandulosa*	seeds	44.5	5.4	8	-22.43	-20.9	1.28
BDS-35	*Agave deserti*	heart	47.3	0.6	73	-13.84	-12.3	1.90
BDS-36	*Agave deserti*	heart	40.4	0.6	73	-13.63	-12.1	1.64
BDS-37	*Agave shawii*	leaf	39.1	1.3	30	-13.59	-12.1	9.43
BDS-42	*Agave deserti*	heart	46.8	0.9	50	-13.02	-11.5	3.09
BDS-43	*Agave deserti*	heart	47.5	0.9	54	-12.93	-11.4	5.17
BDS-48	*Prosopis sp.*	seeds	46.0	3.0	15	-24.48	-23.0	-0.30
BDS-51	*Prosopis sp.*	seeds	45.8	7.0	7	-24.97	-23.5	-0.79
BDS-56	*Agave deserti*	heart	40.6	0.6	73	-10.31	-8.8	2.69
BDS-57	*Agave deserti*	heart	37.1	0.6	67	-11.48	-10.0	2.35
BDS-59	*Lemairocereus thurberi*	stem	48.1	0.3	150	-12.18	-10.7	7.26
BDS-61	*Ficus palmeri*	fruit	43.8	1.8	25	-27.46	-26.0	4.43
BDS-62	*Lemairocereus thurberi*	stem	44.3	0.3	130	-11.51	-10.0	5.99
BDS-63	*Agave deserti*	heart	38.6	1.1	35	-11.62	-10.1	1.14
BDS-64	*Agave deserti*	heart	41.0	1.2	34	-11.54	-10.0	2.02
BDS-67	*Platyopuntia sp.*	fruit	35.3	0.6	62	-13.15	-11.7	6.99
BDS-68	*Platyopuntia sp.*	fruit	36.5	1.3	28	-12.24	-10.7	2.31
BDS-93	*Prosopis sp.*	seeds	45.6	6.0	8	-21.69	-20.2	4.84

Table A.2. Results of lipid-extracted dietary sample analysis. The column "$\delta^{13}C$ (u)" shows the uncorrected measurement while the "$\delta^{13}C$ (c)" column shows the measurement after applying a fossil-fuel correction of +1.5‰. Reported $\delta^{13}C$ values are correct to within ±0.1‰; $\delta^{15}N$ values are correct to within ±0.2‰.

Sample	Taxon	Mat'l	%C	%N	C/N ratio	$\delta^{13}C$ (u)	$\delta^{13}C$ (c)	$\delta^{15}N$
BDS-13L	*Cercidium microphyllum*	seeds	40.0	6.7	6.0	-23.25	-21.8	
BDS-21L	*Acacia greggii*	seeds	41.8	3.2	13.1	-24.01	-22.5	
BDS-34L	*Prosopis glandulosa*	seeds	41.4	5.3	7.8	-22.27	-20.8	
BDS-48L	*Prosopis sp.*	seeds	42.2	5.7	7.4	-23.80	-22.3	
BDS-51L	*Prosopis sp.*	seeds	42.5	7.4	5.7	-23.91	-22.4	
BDS-93L	*Prosopis sp.*	seeds	40.2	3.8	10.6	-21.51	-20.0	
BDS-6L	*Turbo fluctuosus*	flesh	46.7	13.4	3.5	-11.61	-10.1	11.55
BDS-7L	*Turbo fluctuosus*	flesh	45.9	13.3	3.5	-11.18	-9.7	11.60
BDS-9L	*Chione sp.*	flesh	46.2	12.4	3.7	-14.17	-12.7	12.64
BDS-10L	*Chione sp.*	flesh	44.2	13.8	3.2	-14.13	-12.6	10.88
BDS-11L	*Lepus sp.*	flesh	46.4	14.0	3.3	-19.91	-18.4	12.49
BDS-12L	*Lepus sp.*	flesh	44.5	13.8	3.2	-20.79	-19.3	10.15
BDS-46L	*Saxidomus sp.*	flesh	48.1	14.5	3.3	-16.77	-15.3	11.93
BDS-47L	*Trachycardium sp.*	flesh	47.1	12.5	3.8	-15.88	-14.4	11.68

Table A.3. Results of bone collagen preparation and analysis. The column "$\delta^{13}C$ (u)" shows the uncorrected measurement; the "$\delta^{13}C$ (cc)" column shows the value after applying a fossil-fuel correction of +1.5 ‰ to modern samples, thus giving comparable collagen $\delta^{13}C$ values for all samples; the "$\delta^{13}C$ (cd)" column shows the fossil-fuel corrected collagen $\delta^{13}C$ values after applying a correction of –2.25 ‰ to the dietary samples, to give a value for dietary protein. Reported $\delta^{13}C$ values are correct to within ±0.1‰; $\delta^{15}N$ values are correct to within ±0.2‰.

Sample	Taxon	% yield	% C	% N	C/N ratio	$\delta^{13}C$ (u)	$\delta^{13}C$ (cc)	$\delta^{13}C$ (cd)	$\delta^{15}N$	
BDS-38B	Order Artiodactyla	7.2	43.7	14.9	2.9	-13.61		-15.9	8.51	a
BDS-39B	Order Artiodactyla	5.5	42.3	15.1	2.8	-14.90		-17.2	8.23	a
BDS-70B	Order Artiodactyla	6.4		15.7		-14.68		-16.9	10.00	a
BDS-71B	Order Artiodactyla	9.2		15.7		-16.62		-18.9	8.78	a
BDS-72B	Order Artiodactyla	4.7		15.4		-11.50		-13.8	8.89	a
BDS-73B	Order Artiodactyla	19.0		15.8		-15.44		-17.7	8.15	a
BDS-81B	*Zalophus californianus*	14.9	43.8	15.7	2.8	-11.80	-10.3	-12.6	21.11	
BDS-82B	*Zalophus californianus*	13.7	44.9	15.8	2.8	-12.70	-11.2	-13.5	20.60	
BDS-83B	*Ovis canadensis*	12.6	44.6	16.2	2.8	-14.39	-12.9	-15.1	7.46	
BDS-84B	*Taxidea taxus*	9.8	43.3	15.6	2.8	-16.95	-15.4	-17.7	10.67	
BDS-85B	*Odocoileus hemionus*	15.1	41.7	15.8	2.6	-15.99	-14.5	-16.7	12.49	
BDS-86B	*Chelonia sp.*	13.0	44.6	16.0	2.8	-14.78	-13.3	-15.5	16.22	
BDS-87B	*Sauromalus obesus*	11.5	39.2	15.8	2.5	-17.79	-16.3	-18.5	5.87	

Sample	Taxon	% yield	% C	% N	C/N ratio	$\delta^{13}C$ (u)	$\delta^{13}C$ (cc)	$\delta^{13}C$ (cd)	$\delta^{15}N$	
BDS-88B	*Ovis canadensis*	13.2	44.9	16.1	2.8	-17.06	-15.6	-17.8	7.08	
BDS-89B	*Canis latrans*	10.3	42.9	15.4	2.8	-14.83	-13.3	-15.6	15.91	
BDS-91B	*Pelecanus occidentalis*	5.2	44.4	15.9	2.8	-12.58	-11.1	-13.3	17.09	
BDS-92B	Family Delphinidae	4.8	44.4	15.8	2.8	-11.88	-10.4	-12.6	16.05	
BDS-95B	*Dermochelys sp.*	13.6	46.0	15.7	2.9	-13.43	-11.9	-14.2	20.18	
BDS-96B	*Pelecanus occidentalis*	4.0	40.7	15.7	2.6	-12.68	-11.2	-13.4	16.84	
BDS-97B	Family Balistidae	10.0	44.2	15.9	2.8	-11.12	-9.6	-11.9	16.92	
BDS-98B	Family Balistidae	12.8	44.4	16.8	2.6	-10.93	-9.4	-11.7	17.73	
BDS-99B	*Zalophus californianus*	6.3	44.9	16.0	2.8	-13.09	-11.6	-13.8	22.35	
BDS-100B	*Canis latrans*	9.0	44.9	15.8	2.8	-13.14	-11.6	-13.9	13.81	
BDS-101B	*Canis latrans*	10.3	45.7	16.1	2.8	-13.91	-12.4	-14.7	14.10	
BLA-1B		9.3	44.3	16.4	2.7	-9.27			22.06	
BLA-2B		11.5	44.5	15.8	2.8	-9.01			21.33	
BLA-3B		0.7	35.7	12.8	2.8	-9.07			18.16	b
BLA-4B		12.4	44.5	15.8	2.8	-9.21			19.04	
BLA-5B		8.3	44.8	16.5	2.7	-9.44			22.80	
BLA-6B		6.8	44.0	15.9	2.8	-9.12			21.33	
BLA-7B		10.3	45.0	16.6	2.7	-8.70			20.29	
PAR-1B		5.0	43.1	15.5	2.8	-9.50			12.67	
PAR-2B		5.3	41.2	14.7	2.8	-9.32			11.49	
PAR-3B		4.0	45.1	16.1	2.8	-10.46			11.88	
PAR-4B		3.7	45.6	15.9	2.9	-10.12			12.30	
PAR-5B		5.9	45.2	16.1	2.8	-10.40			11.78	
PAR-6B		9.2	45.2	15.8	2.9	-10.83			12.56	
PAR-7B		10.1	44.5	15.4	2.9	-11.76			12.05	
PAR-8B		6.1	44.9	16.4	2.7	-9.17			12.63	
PAR-9B		6.7	45.1	16.5	2.7	-9.26			12.36	
PAR-10B		6.1	43.7	15.8	2.8	-9.49			12.19	
PAR-11B		7.3	44.3	15.5	2.9	-10.82			13.13	
PAR-12B		4.9	43.1	14.6	3.0	-10.43			12.36	
PAR-13B		6.8	44.6	16.3	2.7	-9.73			12.44	
PAR-14B		6.5	43.8	15.9	2.8	-9.21			13.21	
PAR-15B		5.9	44.8	15.5	2.9	-10.41			12.85	
PAR-16B		6.8	45.5	15.8	2.9	-10.10			12.67	
PAR-17B		5.0	44.9	16.1	2.8	-9.93			12.43	

a) Archaeological dietary sample.
b) Note low collagen yield.

Table A.4. Results of bone apatite preparation and analysis. Columns "Dp %" and "Ac %" refer to the proportion of sample remaining after deproteination and acetic acid treatment, respectively; "H+/HAP" refers to the amount of H+ in solution relative to what would be needed to react the sample completely if it were composed of hydroxyapatite; "%C" reports the amount of carbon as a percent of sample weight. The column "$\delta^{13}C$ (u)" shows the uncorrected measurement while the "$\delta^{13}C$ (c)" column shows the measurement after applying a fossil-fuel correction of 1.5 ‰ to modern samples. Reported $\delta^{13}C$ values are correct to within ±0.1‰.

	Deproteination			Acid treatment					Collection			Results	
Sample	Wt. pre (mg)	Wt. post (mg)	Dp %	Wt. pre (mg)	Ac vol (ml)	H+/ HAP	Wt. post (ml)	Ac %	Wt. (mg)	µg C	% C	$\delta^{13}C$ (u)	$\delta^{13}C$ (c)
BDS-38C	100.5	62.8	62.5	49.9	10.0	14.4	27.4	55.0	7.36	63	0.9	-6.24	
BDS-39C	99.8	76.0	76.2	49.8	10.0	14.4	31.0	62.3	9.54	94	1.0	-7.67	
BDS-81C	100.3	71.0	70.8	50.7	10.0	14.1	24.9	49.1	7.89	69	0.9	-7.91	-6.4
BDS-82C	99.4	69.0	69.4	50.2	10.0	14.3	31.6	63.0	8.45	76	0.9	-11.46	-10.0
BDS-83C	101.1	73.8	73.0	50.2	10.0	14.3	29.6	59.0	8.09	83	1.0	-6.99	-5.5
BDS-84C	100.2	75.0	74.9	49.9	10.0	14.4	32.4	64.8	9.40	85	0.9	-13.79	-12.3
BDS-85C	98.8	64.2	64.9	50.2	10.0	14.3	29.9	59.6	8.64	80	0.9	-9.08	-7.6
BDS-86C	98.3	60.8	61.8	50.4	10.0	14.2	25.1	49.9	10.18	49	0.5	-8.90	-7.4
BDS-88C	100.6	67.3	66.9	49.6	10.0	14.5	28.5	57.4	8.34	89	1.1	-9.71	-8.2
BDS-89C	100.3	58.2	58.0	49.7	10.0	14.4	29.4	59.1	9.00	63	0.7	-10.37	-8.9
BDS-92C	100.4	73.1	72.8	50.1	10.0	14.3	33.0	65.8	10.84	110	1.0	-10.24	-8.7
BDS-95C	99.9	70.5	70.6	50.4	10.0	14.2	32.2	63.9	11.55	56	0.5	-10.34	-8.8
BDS-99C	100.9	68.5	67.9	49.7	10.0	14.4	32.7	65.8	6.33	49	0.8	-11.79	-10.3
BDS-100C	100.2	71.2	71.1	49.9	10.0	14.4	34.6	69.2	9.60	78	0.8	-9.85	-8.4
BDS-101C	99.7	68.4	68.7	50.3	10.0	14.3	28.6	56.8	7.74	72	0.9	-9.84	-8.3
BLA-1C	100.2	74.4	74.3	50.1	10.0	14.3	8.5	16.9	6.78	65	1.0	-4.79	
BLA-2C	100.5	72.3	71.9	49.6	10.0	14.5	13.1	26.3	7.32	67	0.9	-3.86	
BLA-3C	99.3	89.1	89.7	49.5	10.0	14.5	32.3	65.2	7.91	87	1.1	-3.29	
BLA-4C	99.6	76.6	76.8	49.5	10.0	14.5	29.9	60.4	7.41	78	1.1	-3.48	
BLA-5C	100.0	77.0	77.1	49.4	10.0	14.5	10.9	22.0	7.03	65	0.9	-4.35	
BLA-6C	100.4	73.0	72.7	50.2	10.0	14.3	9.8	19.6	6.24	60	1.0	-4.36	
BLA-7C	100.1	69.8	69.7	49.3	10.0	14.5	13.2	26.8	6.32	63	1.0	-2.44	
PAR-3C	100.0	75.8	75.8	50.3	10.0	14.3	3.3	6.6	1.85	18	1.0	-1.73	
PAR-8C	76.2	60.9	79.9	49.7	10.0	14.4	2.5	5.0	2.14	20	0.9	n/m	
PAR-14C	52.9	37.5	70.9	30.1	6.0	14.3	3.0	9.9	1.81	22	1.2	-3.65	
PAR-15C	85.0	60.1	70.7	50.2	10.0	14.3	6.6	13.2	5.17	51	1.0	-4.06	
PAR-17C	99.6	65.1	65.3	49.9	10.0	14.4	28.2	56.6	7.76	85	1.1	-3.96	

Table A.5. Radiocarbon measurement results. $\delta^{13}C$ values were determined separately (Table A.3). Radiocarbon concentration is given as fraction Modern, $D^{14}C$ and conventional radiocarbon years BP, using the Libby half-life of 5568 years and following the conventions of Stuiver and Polach (1977). Sample preparation backgrounds were subtracted based on preparations of ^{14}C-free coal, and scaled relative to sample size.

Sample	CAMS #	$\delta^{13}C$	fraction Modern	$D^{14}C$	^{14}C age
BLA-5A	29412	-9.4	0.8860 ± 0.0049	-114.0 ± 4.9	970 ± 50
BLA-7A	29413	-8.7	0.9403 ± 0.0079	-59.7 ± 7.9	490 ± 70
PAR-1A	23462	-9.5	0.6803 ± 0.0044	-319.7 ± 4.4	3090 ± 60
PAR-4A	23463	-10.1	0.6669 ± 0.0043	-333.1 ± 4.3	3250 ± 60
PAR-5A	23464	-10.4	0.6809 ± 0.0043	-319.1 ± 4.3	3090 ± 60
PAR-13A	23465	-9.7	0.6661 ± 0.0043	-333.9 ± 4.3	3260 ± 60
PAR-17A	23466	-9.9	0.6567 ± 0.0036	-343.3 ± 3.6	3380 ± 50

Appendix B: Tests of bone apatite preparation methods

This appendix describes experiments designed to develop and test a method for analyzing bone apatite $\delta^{13}C$ values. While there is a large body of literature on bone apatite preparation methods, the method had not previously been attempted at the Simon Fraser University stable isotope laboratory, so it was felt that some independent testing was warranted. In addition, existing methods seemed to call for large amounts of bone and perhaps unnecessarily long reaction times. A method was desired that was relatively quick and could accommodate small samples.

Most apatite preparation recipes call for deproteinating the sample, usually using a weak sodium hypochlorite solution. Since bone proteins are not expected to react with phosphoric acid to produce CO_2, deproteination should theoretically not affect stable isotope measurements, but it can affect the gas yield as a proportion of sample weight, which is a useful measure of carbonate content. These experiments assess the effect of different deproteination times on gas yields, sample attrition, and $\delta^{13}C$.

Apatite preparation methods also call for treating the sample with a dilute acid, typically acetic acid, in an attempt to remove the more soluble adsorbed carbonates and diagenetic apatite, while leaving most of the biogenic apatite in place. However, there is no simple way of determining the appropriate length of treatment. The solubility of apatites depends on a wide range of factors, such as composition, sample surface area, solution pH, product ion concentration, and the extent to which other calcium phosphate salts precipitate during treatment (Hassan 1977; LeGeros and Tung 1983). These experiments test the effects of solution volume, pH and length of treatment on gas yields, sample attrition and $\delta^{13}C$.

Once treated, bone apatite carbonate is reduced to CO_2 in an evacuated vessel with phosphoric acid (McCrea 1950). Most recipes call for reacting the sample in the evacuated reaction vessel for set periods of time, most often at 25° C, without explaining why these

temperatures and reaction times are necessary. It appears that most recipes use protocols borrowed from $\delta^{18}O$ analysis, and that time could be saved by using a faster, hotter reaction when $\delta^{18}O$ values are not being measured. Using subsamples of a single treated bone sample, these experiments test the effects of different reaction times on $\delta^{13}C$ values.

<u>Methods</u>

Samples TCE-A through TCE-C and TCE-1 through TCE-30 were prepared from an artiodactyl bone (dietary study sample BDS-39) collected from an archaeological site in the Sierra de San Francisco, with a collagen $\delta^{13}C$ of -14.9 ‰, collagen yield of 5.5%, and C/N ratio of 2.8. Samples TCE-31 through TCE-35 were prepared from a modern canid bone (dietary sample BDS-101) collected in the vicinity of Bahía de los Angeles, with a collagen $\delta^{13}C$ of -13.6 ‰, collagen yield of 7.2% and a C/N ratio of 2.9. Clean whole bone was powdered either by drilling with a handheld electric drill (samples TCE-A through TCE-C and TCE-1) or by freezing and crushing in a percussion mortar (samples TCE-16 and subsequent).

To prepare a deproteinated sample, approximately 80-100 mg of powdered bone was weighed accurately in a 15 ml centrifuge tube. (Samples TCE-16 and subsequent were weighed as nearly as possible to 100 mg). Since a 1.5% sodium hypochlorite solution seemed to produce a strong reaction in powdered fresh bone, it was decided to use this strength for all samples. The sample was deproteinated using approximately 15 ml of solution, letting the reaction continue for 48 hr (TCE-1 through 15) or 72 hr (samples TCE-16 and later) with daily vortexing. Following this the sample was rinsed to neutrality. Samples TCE-16 and later were then lyophilized and weighed to determine the amount of sample attrition occurring during this step.

Acid-treated samples were prepared by reacting the deproteinated samples in the same tube with varying concentrations and volumes of acetic acid solutions. During the treatment the samples were sonicated or vortexed periodically to ensure complete reaction. The samples

were then rinsed to neutrality as above. For samples TCE-17 through TCE-24, a portion of the acetic acid solution was retained after treatment for pH determination. The treated samples were lyophilized and weighed for comparison against untreated weights.

To collect carbon as CO_2 gas from a sample, approximately 10 mg of the dry, prepared sample was weighed and introduced to one arm of a two-armed carbonate reaction vessel (McCrea 1950). The other arm was loaded with an excess (approx. 3 ml) of 85% phosphoric acid. The vessel was sealed and evacuated to 10^{-5} torr or less on a high vacuum line. The reactants were then combined and the vessel placed in a dry block heater at 105° C for a minimum of 20 minutes, with periodic agitation to ensure complete reaction. The vessel was agitated periodically to ensure that the sample reacted completely. When reaction was complete, the vessel was reattached to the high vacuum line. The evolved CO_2 was passed through a dry ice/isopropanol trap and collected on a cold finger cooled with liquid nitrogen. When frozen, the CO_2 was pumped free of noncondensible gases for five minutes. The gas was frozen into a second cold finger, warmed, and the quantity of CO_2 measured by manometry. Finally, the gas was frozen into a collection tube and sealed with a propane/oxygen torch. The sealed tube was sent to the isotope ratio mass spectrometer for measurement.

Results

Results for experimental samples are shown in Table B.1 below. Samples TCE-2 through TCE-5 are not shown in the table. These samples were sonicated during the acetic acid treatment and dissolved completely. Also included are samples BDS-39C and BDS-101C, which are the final samples prepared for inclusion in the dietary study, and which differ in some respects from the other samples, as discussed below.

Table B.1 Results of bone apatite preparation experiments. Columns "Dp %" and "Ac %" refer to the proportion of sample remaining after deproteination and acetic acid treatment, respectively; "H+/HAP" refers to the amount of H+ in solution relative to what would be needed to react the sample completely if it were composed of hydroxyapatite; "%C" reports the amount of carbon as a percent of sample weight.

		Deproteination				Acid treatment									Gas collection				Results	
	Sample	Wt. pre (mg)	Dp time (hr)	Wt. post (mg)	Dp %	Wt. pre (mg)	Ac M	pH pre	pH post	Ac time (hr)	Ac vol (ml)	H+/ HAP	Wt. post (mg)	Ac %	Wt. (mg)	Rx time (min)	µg C	% C	$\delta^{13}C$	
BDS-39	TCE-A		25							n/a					13.0	20	166	1.3		a
	TCE-B		50							n/a					11.9	20	166	1.4		a
	TCE-C		115							n/a					11.4	20	168	1.5		a
	TCE-1	67.1	48	49.2	73.3					n/a					12.7	20	179	1.4	-7.7	a
	TCE-16		n/a							n/a					14.3	20	134	0.9	-8.3	b
	TCE-17A	99.6	69	73.1	73.4	73.1	1.0	2.47	3.44	24	10	9.8	28.8	39.4	8.5	25	83	1.0	-8.7	c
	TCE-17B	99.6	69	73.1	73.4	73.1	1.0	2.47	3.44	24	10	9.8	28.8	39.4	7.9	180	78	1.0	-8.7	c
	TCE-17C	99.6	69	73.1	73.4	73.1	1.0	2.47	3.44	24	10	9.8	28.8	39.4	8.0	1200	83	1.0	-8.7	c
	TCE-18	100.2	69	73.4	73.2	73.4	0.5	2.62	3.69	24	10	4.9	39.2	53.4	8.5	25	85	1.0	-7.8	
	TCE-19	99.8	69	73.2	73.3	73.2	0.1	2.97	4.33	24	10	1.0	51.4	70.3	9.0	25	98	1.1	-7.6	
	TCE-20	100.4	69	74.0	73.7	74.0	0.05	3.12	4.65	24	10	0.5	55.5	75.1	8.1	25	89	1.1	-7.6	
	TCE-21	100.9	68	68.5	67.9	68.5	1.0	2.47	3.46	48	10	10.6	21.8	31.9	8.8	25	65	0.7	-8.1	
	TCE-22	99.8	68	60.2	60.3	60.2	1.0	2.47	3.58	24	5	6.0	29.9	49.7	8.8	25	89	1.0	-8.1	
	TCE-23	100.2	68	62.6	62.5	62.6	1.0	2.47	3.82	24	2	2.3	38.7	61.7	8.5	25	85	1.0	-8.4	
	TCE-24	100.5	68	60.1	59.8	60.1	5.0	1.95	2.62	24	10	60.0	24.3	40.4	8.2	25	51	0.6	-7.6	
	TCE-26	100.7	68	78.3	77.8	78.3	1.0			2	10	9.2	43.4	55.4	11.4	25	121	1.1	-7.7	
	TCE-27	100.3	68	79.7	79.5	79.7	1.0			4	10	9.0	39.4	49.4	10.8	25	114	1.1	-7.9	
	TCE-28	99.8	68	75.7	75.8					n/a					8.3	25	105	1.3	-7.5	a
	TCE-30		n/a							n/a					9.8	25	96	1.0	-7.8	d
	BDS-39C	99.8	68	76.1	76.2	49.8	1.0			24	10	14.4	31.0	62.3	9.5	25	94	1.0	-7.7	
BDS-101	TCE-31	99.6	69	63.6	63.8	63.6	1.0			1	10	11.2	49.4	77.8	9.5	25	98	1.0	-11.8	
	TCE-32	99.6	69	66.1	66.4					n/a					9.2	25	150	1.6	-9.0	a
	TCE-33	99.2	69	64.9	65.4	64.9	1.0			4	10	11.0	50.3	77.5	9.0	25	92	1.0	-10.3	
	TCE-34	99.3	69	64.9	65.3	64.9	1.0			24	10	11.0	41.5	63.9	8.9	25	87	1.0	-11.3	
	TCE-35		n/a							n/a					11.8	25	112	0.9	-8.6	b
	BDS-101C	99.7	68	68.4	68.7	50.3	1.0			24	10	14.3	28.6	56.8	7.7	25	72	0.9	-9.8	

a) deproteinated only; b) untreated bone; c) repeat gas collections from the same sample; d) washed with distilled water and lyophilized.

Effect of deproteination time on sample attrition, gas yield and $\delta^{13}C$

As expected, gas yields increased with length of deproteination. After 48 hours in the solution, little additional gas yield resulted even after doubling the amount of time in the solution, so this was judged sufficient for reasonably complete deproteination. For samples TCE-16 and subsequent, deproteination was allowed to continue for 72 hours in order to accommodate the generally less vigorous reaction in the samples prepared in the percussion mortar as opposed to the drilled samples. Faster deproteination times might result from using a stronger solution.

Sample attrition was not measured for samples deproteinated for less than 24 hours, but observations suggest that most sample attrition occurs within the first few minutes of the reaction. The average value for all samples, about 70%, reflects the composition of fresh bone (Ambrose 1993). The amount of observed sample attrition is somewhat variable, and may reflect some sample loss during rinsing. In particular, the samples prepared in the percussion mortar were difficult to decant without some sample loss.

Because there are few data points, it is difficult to assess the effect of deproteination on $\delta^{13}C$, but a gradual enrichment is apparent. Untreated bone sample TCE-16, with a $\delta^{13}C$ of –8.3 ‰, is quite different from sample TCE-30, with a $\delta^{13}C$ of -7.8 ‰, which was washed with distilled water and lyophilized. The latter is more comparable to the other deproteinated samples. The fact that washing with water has such a marked effect on $\delta^{13}C$ is unexplained, but it suggests that the samples contained isotopically light contaminants that are easily removed with water.

Effect of length of acetic acid treatment on sample attrition, gas yield and $\delta^{13}C$

Figure B.1 shows the relationship between length of acetic acid treatment and sample attrition. Initially, sample attrition during acetic acid treatment varied greatly without correlation with length of treatment, probably because of differences in sample surface area, as well as poor control over the relative volumes of sample and acid solution volume.

Samples TCE-16 and subsequent were prepared from a single bone preparation event, rather than drilled individually. Standardized solution volumes were also introduced at this point. Sample attrition is much more stable in these samples. Drilled samples are generally subject to much more rapid attrition than crushed ones.

In contrast, the gas yield is basically constant up to 24 hours of treatment (Figure B.2). Untreated bone gives a gas yield of about 1.0% carbon, deproteinated bone between 1.4% and 1.6%, and acid-treated bone about 1.0%. This agrees with Ambrose's (1993) observation that treated apatite should give between 0.6% and 1.3% carbonate carbon by weight. This suggests that a less stable carbonate phase (i.e. adsorbed carbonates) enters solution within the first few minutes of the acid soak, and that additional time in the solution affects carbonate in structural positions. By 48 hours in solution, gas yields have decreased to 0.7%, near the lower limit suggested by Ambrose (1993).

The trend in $\delta^{13}C$ is less easy to explain (Figure B.3). The gradual enrichment after 24 hours may reflect precipitation of a different calcium salt such as brushite from the solution. The general trends in gas yield and $\delta^{13}C$ are similar to those from Lee-Thorp et al. (1991), except that the time scale is greatly reduced, possibly because of differences in the ratio of sample weight to solution volume.

<u>Effect of acetic acid concentration on sample attrition, gas yield and $\delta^{13}C$</u>

During the acetic acid treatment, sample attrition appeared to be much greater than the literature suggests is appropriate. It was suspected that a weaker solution might solve this problem. To test the pH dependence of sample dissolution, five acetic acid solutions of varying concentration (5M, 1M, 0.5M, 0.1M and 0.05M) were prepared and reacted with deproteinated bone for 24 hours. The measured pH values of the solutions, with their effects on attrition, gas yield and $\delta^{13}C$ are shown in Figure B.4.

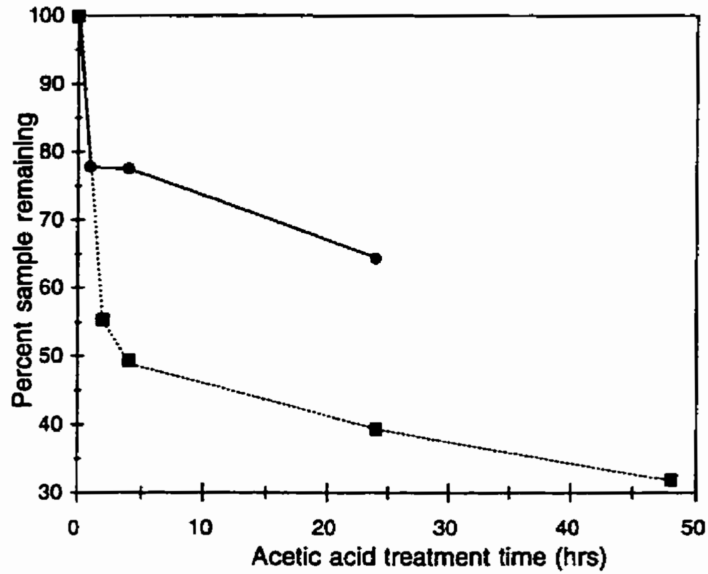

Figure B.1. Effect of length of acetic acid treatment on sample attrition. Shown are samples prepared from specimen BDS-39 (squares) and BDS-101(circles).

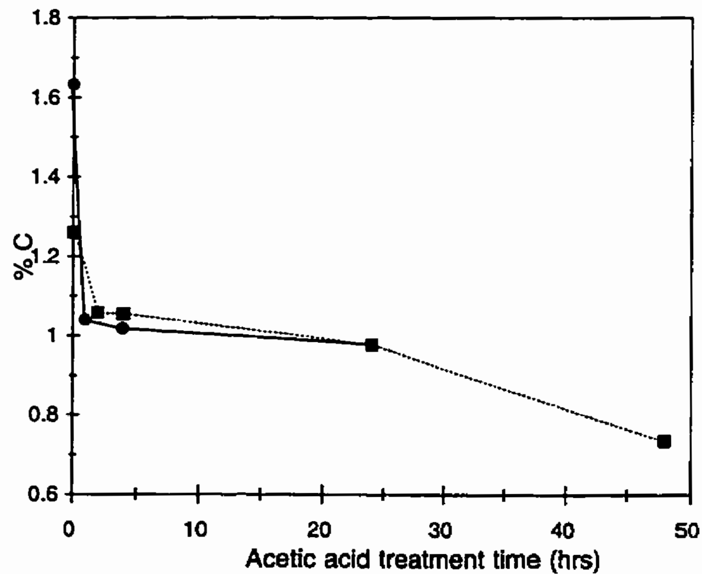

Figure B.2. Effect of length of acetic acid treatment on gas yields. Shown are samples prepared from bone specimens BDS-39 (squares) and BDS-101 (circles).

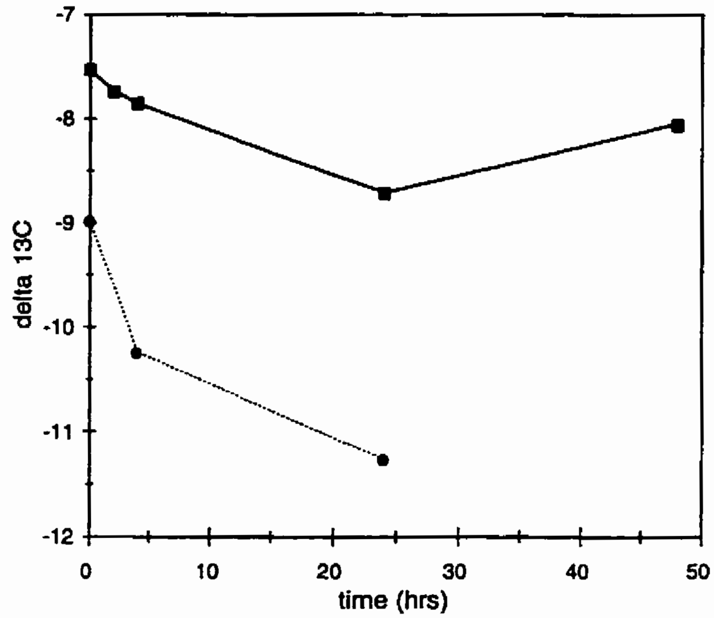

Figure B.3. Effect of acetic acid treatment on $\delta^{13}C$. Shown are samples prepared from specimens BDS-39 (squares) and BDS-101 (circles).

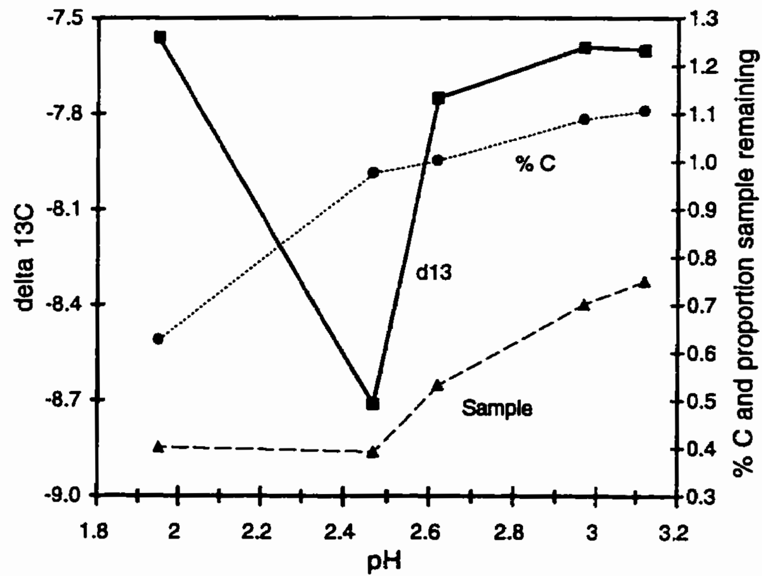

Figure B.4. Effect of solution pH on sample attrition, gas yield, and $\delta^{13}C$. Samples were prepared from artiodactyl bone BDS-39.

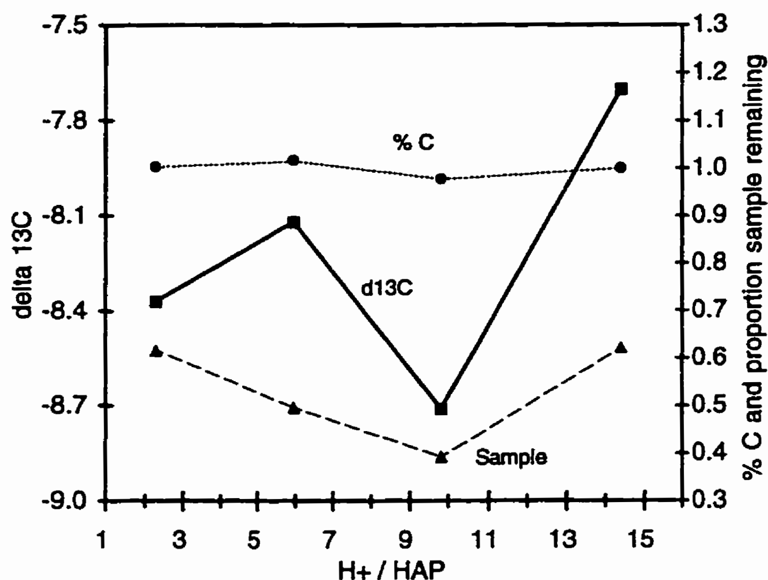

Figure B.5. Effect of different reactant volumes on sample attrition, gas yield and $\delta^{13}C$. Samples were prepared from artiodactyl bone BDS-39.

The results show a general pH-dependent trend in sample attrition, gas yield and $\delta^{13}C$, except for the 5M solution. This confirms the observation that high concentrations of acetic acid give generally unreliable results (Lee-Thorp and van der Merwe 1991). It also tends to confirm the observation that calcium salts can re-precipitate under certain conditions (i.e., high concentrations and long exposures).

Effect of reactant volumes on sample attrition, gas yield and $\delta^{13}C$

Another possible reason for the rapid attrition of these samples was the high ratio of solution weight to sample volume relative to other recipes (75 mg in 10 ml solution vs. up to 1 g in 40 ml solution). The concentration of product ions in solution might be expected to slow the dissolution reaction when the ratio of solution to sample is smaller. To test this, samples were reacted with varying volumes of 1M solution (10 ml, 5 ml, 2 ml). The arbitrary ratio "H+/HAP" is the amount of H+ ion in solution relative to what would be

needed to react the sample completely if it were composed of pure hydroxyapatite (Figure B.5). Also included in this graph is sample BDS-39C, with a standardized sample weight of 50 mg, solution volume of 50 ml, and H+/HAP of 14.4, as discussed below.

Gas yield is constant, suggesting that this factor is largely dependent on initial pH. For sample attrition and $\delta^{13}C$, there appears to be no explanation for the trend. Sample TCE-17, with a H+/HAP of 9.8, may be anomalous.

Effect of reaction time on gas yield and $\delta^{13}C$

Three subsamples of sample TCE-17 were prepared using reaction times of 20 minutes, 120 minutes, and 1800 minutes. The $\delta^{13}C$ values are nearly identical. This suggests that not only is the short reaction time appropriate, but that the gas collection procedure is highly reproducible, and that the observed variation in $\delta^{13}C$ results instead from pretreatment.

Changes to the preparation procedure for dietary and human samples

Because of the profound effect of H+/HAP on $\delta^{13}C$ values, it was decided after finishing the experiments that the volume of deproteinated sample needed to be controlled more carefully. The procedure was changed for dietary and human samples so that subsequent to the deproteination and lyophilization step, a separate centrifuge tube was prepared for the acid treatment and a subsample of the deproteinated bone, weighed as nearly as possible to 50 mg, was introduced to it with the acid solution. This allowed H+/HAP to remain constant at about 14.4 regardless of the amount of sample attrition occurring during the previous step. This seemed particularly appropriate for the archaeological samples, in which attrition during deproteination could be expected to vary greatly. This ratio was higher than the averages measured in the experiments (9-10), and unfortunately this change appears to have had a strong effect on measured $\delta^{13}C$. However, it was felt that as long as the ratio was held constant between measurements, that they should be basically comparable. The results of the dietary and human samples seem to meet most theoretical expectations, but it should be cautioned that the method used to obtain them has not been fully developed.

Conclusions

These experiments illustrate some serious difficulties. The basic problem with the technique explored here is that the treatment methods must settle for controlling systematic effects on $\delta^{13}C$ rather than eliminating them, so measured $\delta^{13}C$ is to some extent dependent on arbitrary procedural decisions. Nevertheless, with adequate controls, measurements appear to be reproducible.

The acetic acid treatment is the most important factor affecting apatite $\delta^{13}C$ values. Extreme care must be taken to hold solution concentration, reaction time, solution/sample ratio and sample surface area as constant as possible to ensure comparability between values. Preparing powdered samples in a rock mill might allow better control over this. Solution pH might be better controlled by preparing acetate buffers rather than acid solutions. Finally, a technique such as that described by Sillen (1989), in which samples are repeatedly rinsed with fresh solution, may minimize problems with recrystallization.

The gas collection procedure described here is highly reproducible. There appear to be no problems with high reaction temperatures. If measurement of carbonate $\delta^{18}O$ values was desired, however, a standardized reaction temperature as described in McCrea (1950) would be required, as well as the use of 100% phosphoric acid. $\delta^{18}O$ analysis could open up a potentially very interesting line of research (e.g. Verano and DeNiro 1993).

Appendix C: Radiocarbon calibration

Radiocarbon dating human bone in areas where marine foods are important is problematic because of differences in ^{14}C activity between marine and atmospheric sources of carbon. While the conventional radiocarbon age as defined by Stuiver and Polach (1977) incorporates a correction for isotopic fractionation, it does not correct for differences in ^{14}C activity between the different original sources of carbon. The oceans, for example, are depleted in ^{14}C because of the long residence time of carbon in deep ocean waters. Because of this, present-day marine samples have a radiocarbon age of several centuries. The magnitude of this marine reservoir effect is dependent on the extent of mixing with atmospheric ^{14}C as well as upwelling of deep ocean water, and thus varies through time and between different regions. Stuiver et al. (1986) calculate a global calibration curve for the marine "mixed layer" (0-75 m depth), by modeling the diffusion of atmospheric ^{14}C into the oceans. This marine curve parallels long-term changes in global atmospheric ^{14}C activity. Local variation from this curve is reflected by the term ΔR, comprising regional variations in ^{14}C activity in both the atmosphere and the ocean (Stuiver and Braziunas 1993). ΔR is typically determined by finding the radiocarbon ages of marine samples of known historical age. While ΔR can be expected to vary as a result of climatic change, as an approximation it can be assumed to be constant through time. Local marine calibration curves are thus produced by adding a constant ΔR term to the global curve.

Human burials in Baja California archaeological sites present an additional complication for dating because they contain a mixture of marine and terrestrial carbon. Mixed samples can be calibrated by interpolating between the atmospheric and marine calibration curves (Stuiver and Reimer 1993), but an accurate estimate of the proportion of marine carbon in the diet is required. In addition, ΔR values are different on the Pacific and Gulf coasts (Stuiver et al. 1986; Ingram and Southon 1996), so if resources from both coasts were significant in an individual's diet, these must be quantified separately. To complicate

matters, the Gulf is an area of strong upwelling with one of the largest ΔR values measured worldwide.

Bahía de las Animas samples

It is apparent from the stable isotope results, as well as the archaeological context, that marine foods were the dominant source of protein on the prehistoric Bahia de las Animas coast. Using the figures for ΔR given below (Table C.1), the two samples have been calibrated using various levels of marine carbon. The response of varying levels of dietary marine carbon on the 1 and 2 σ ranges (CALIB Method B) of both samples is illustrated in Figure C.1.

Table C.1 ΔR determinations on marine shells in the Gulf of California.

Location	Historical age	^{14}C age	ΔR
Bahia Kino, Sonora	1935	993 ± 53	514 ± 53
Isla Carmen, Baja California Sur	1911	1001 ± 54	531 ± 54
Isla Carmen, Baja California Sur	1940	860 ± 50	387 ± 53
Guaymas, Sonora	1940	910 ± 60	436 ± 56
Mean ± minimum error			470 ± 30

Sample BLA-7 presents an additional problem for radiocarbon calibration. Its radiocarbon age of 490 ± 70 BP is substantially younger than the apparent age of present-day marine samples from the Gulf. Given the presumed historical age of this burial of no later than about 200 cal BP (1750 cal AD), this measurement sets an upper limit on the contribution of marine carbon. At 40% marine, the lower boundary of the 1σ range is 265 cal BP (Figure C.1). Higher proportions cannot be calibrated due to the effects of bomb ^{14}C on the marine calibration curve.

This would seem to disagree with the stable isotope results, in particular $\delta^{15}N$ values, which appear to indicate a higher proportion of marine protein in the diet. The discrepancy is unexplained; however, this problem illustrates that if the historical age of burials can be determined through other means (e.g., through radiocarbon dating of burial associations) that radiocarbon dates could be used to estimate dietary composition. Indeed, in Baja California ^{14}C may be a better marine/terrestrial paleodietary indicator than either $\delta^{13}C$ or $\delta^{15}N$.

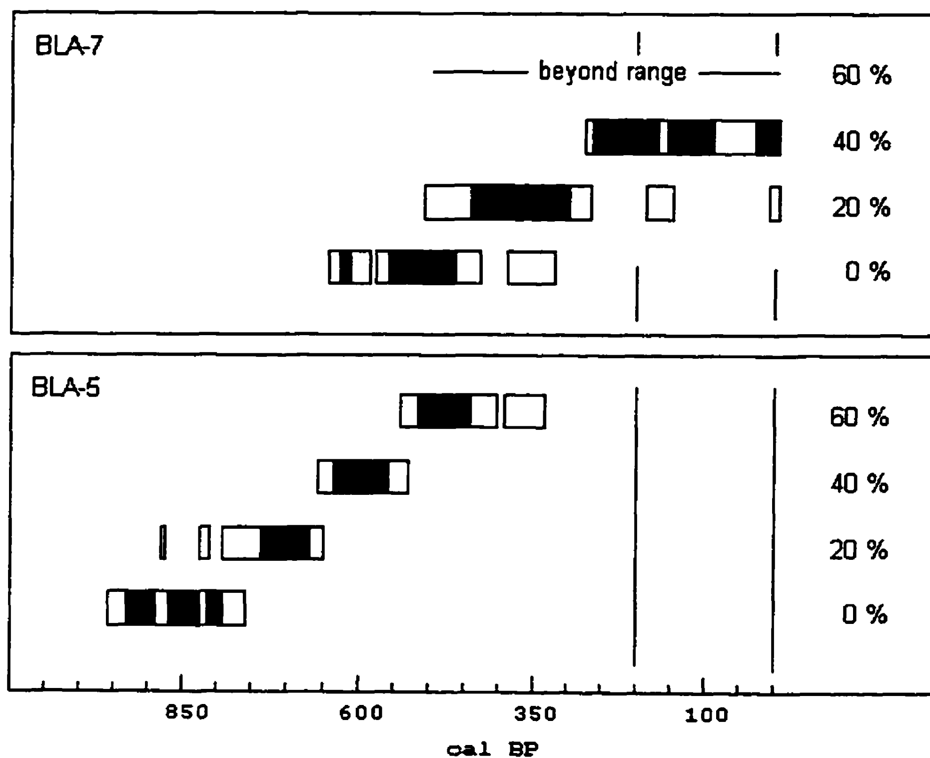

Figure C.1. Response of Bahía de las Animas sample calibrations to various percentages of dietary marine carbon. Solid areas are the 1 σ range; open areas are the 2 σ range. Vertical lines demarcate 0 BP and the approximate end of the prehistoric period at about 200 cal BP.

Table C.2 Radiocarbon results and calibrations for collagen samples BLA-5 and BLA-7. $\delta^{13}C$ values have been determined separately (Table A.3). Calibrations were calculated using the CALIB 3.03c program (Stuiver and Reimer 1993) for varying percentages of dietary marine carbon, using the bidecadal atmospheric and marine atmospheric calibration curves and a ΔR of 470 ± 30. An asterisk indicates that the range boundary cannot be specified due to bomb ^{14}C. Calibrated ages are accurate to no more than ±10 years.

Sample (CAMS #)	$\delta^{13}C$	^{14}C age	% marine	Cal Age BP	Ranges (CALIB Method B)			
					1 σ	p	2 σ	p
BLA-5A (29412)	-9.4	970 ±50	0	918	932-890 871-826 815-792	.40 .41 .20	959-759	1.00
			20	697	738-668	1.00	882-878 826-813 794-649	.00 .01 .99
			40	625 604 566	634-557	1.00	658-531	1.00
			60	489	517-442	1.00	541-401 391-333	.91 .09
BLA-7A (29413)	-8.7	490 ±70	0	517	627-612 557-464	.07 .93	644-585 575-427 387-320	.13 .77 .11
			20	315	440-296	1.00	508-266 185-148 7-0*	.96 .04 .01
			40	245 222 140 9	265-170 154-90 28-0*	.51 .34 .15	275-0*	1.00
			60	Beyond range				

Sierra de San Francisco samples

The calibration of these samples is rather more straightforward. The stable isotopic evidence indicates that marine foods were of minor importance in the diet of these individuals, thus the calibration curve used here is the atmospheric bidecadal curve. Sample PAR-17 is statistically distinct from both PAR-1 and PAR-5 at $\alpha=0.05$, so it appears that interments may have been added to the site over at least a short period of time.

Table C.3. Radiocarbon results and calibrations for Sierra de San Francisco collagen samples. $\delta^{13}C$ values have been determined separately (Table A.3). Calibrations were calculated with the CALIB 3.03c program (Stuiver and Reimer 1993) using the bidecadal atmospheric calibration curve. Calibrated ages are accurate to no more than ±10 years.

Sample (CAMS #)	$\delta^{13}C$	^{14}C age	Cal ages BP	Ranges (CALIB Method B)			
				1 σ	p	2 σ	p
PAR-1A (23462)	-9.5	3090 ±60	3336 3286 3274	3361-3242 3237-3217	.87 .13	3450-3431 3406-3141 3139-3111 3093-3086	.02 .95 .02 .01
PAR-4A (23463)	-10.1	3250 ±60	3465	3551-3513 3482-3383	.23 .77	3622-3604 3595-3357	.02 .98
PAR-5A (23464)	-10.4	3090 ±60	3336 3286 3274	3361-3242 3237-3217	.87 .13	3450-3431 3406-3141 3139-3111 3093-3086	.02 .95 .02 .01
PAR-13A (23465)	-9.7	3260 ±60	3467	3555-3507 3490-3393	.31 .69	3623-3601 3599-3366	.04 .96
PAR-17A (23466)	-9.9	3380 ±50	3626	3688-3660 3659-3559 3502-3498	.19 .79 .02	3802-3799 3712-3472	.00 1.00

Appendix D: Determination of MNI in the Sierra de San Francisco collection

Because the remains in the Sierra de San Francisco collection are commingled, one of the goals of this study is to determine how many individuals are represented in the assemblage. It would be misleading to treat the variability in the measurements as if it were that of a population of individuals, since some of the measurements are likely to be from the same individual. Thus a conservative estimate of the minimum number of individuals (MNI) in the assemblage is required. Several lines of evidence, including anatomical relationships, stable isotope values, and radiocarbon dates, can be used to make an MNI estimate. Each of these types of evidence can suggest or preclude a possible skeletal "match" between any two samples. Considering these criteria together should result in a higher and more accurate MNI estimate than would be otherwise possible. The methods used in applying these criteria are discussed briefly below.

Anatomical data

A brief osteological study has been made of the collection (Sanchez 1995). A more detailed study might indicate additional pairs of elements that do not match one another, but for this study the only characteristics considered were side and overall robusticity. When considering robusticity only obviously mismatched right-left pairs of the same element were excluded from matching.

Radiocarbon dates

Five of the specimens (PAR-1, PAR-4, PAR-5 and PAR-17) were submitted for radiocarbon measurement (Appendix A). The pairs PAR-1/PAR-17 and PAR-5/PAR-17 are statistically distinct at α=0.05 (Stuiver and Reimer 1993), thus these pairs were excluded from matching.

Stable isotope measurements

Using isotope measurements to test for possible matches between samples requires a realistic estimate of the range of variation in $\delta^{13}C$ and $\delta^{15}N$ values within an individual. Unfortunately, the range of intraskeletal variation in humans is not well known, and may vary as a result of seasonal or long-term changes in diet. Lovell et al. (1986) observed a standard deviation in $\delta^{13}C$ values between individuals of 0.3‰, suggesting that variation within a single individual on a monotonous diet should be somewhat smaller than this. DeNiro and Schoeninger (1983) found standard deviations of 0.3‰ in both $\delta^{13}C$ and $\delta^{15}N$ in a small sample of laboratory animals.

For this study a provisional estimate of the standard deviation of intraskeletal variability in bone collagen was made at ±0.2‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$, including measurement error. This gives a standard deviation of the difference between any two samples of 0.28‰ for $\delta^{13}C$ and 0.43‰ for $\delta^{15}N$. Sample pairs differing by more than two standard deviations were deemed not to match.

Table D.1. Matrix showing possible matches between sample pairs based on stable isotope measurements, anatomical relationships, and radiocarbon measurements.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	N															
3	N	N														
4	N	N	N													
5	N	N	Y	N												
6	N	N	Y	N	Y											
7	N	N	N	N	N	N										
8	Y	N	N	N	N	N	N									
9	Y	N	N	N	N	N	N	Y								
10	Y	Y	N	N	N	N	N	Y	N							
11	N	N	N	N	N	Y	N	N	N	N						
12	N	N	Y	Y	Y	Y	N	N	N	N	Y					
13	Y	N	N	Y	N	N	N	Y	Y	Y	N	N				
14	Y	N	N	N	N	N	N	Y	N	N	N	N	N			
15	N	N	N	Y	N	Y	N	N	N	N	Y	N	N	N		
16	N	N	N	N	N	N	N	N	N	N	N	Y	Y	N	Y	
17	N	N	Y	Y	N	N	N	N	N	Y	N	Y	Y	N	Y	Y

MNI estimates

To calculate MNI, a matrix was produced summing the results of pair-by-pair comparisons for each of the above criteria (Table D.1). Possible matches were those allowed by all three criteria. To reduce the matrix to an MNI estimate, possible matches were listed for each specimen, and the largest combination of mutually unmatched specimens found by process of elimination. No effort was made to specifically identify elements from the same individual.

Results

Results are shown in Table D.2 below. Possible matches are shown, but nothing is implied about the relative likelihood of these specimens actually being from the same individual. The MNI is eight, a surprisingly large result given the size of the collection. It should be stressed again that the MNI estimate is based on conjectured values for isotopic intraskeletal variation, and that using different estimates of these values would result in a substantially different MNI. However, it is felt that the values used here are conservative.

Although not tabulated here, the same process of elimination that produced the above results shows that the MNI represented by the five radiocarbon dates is three (PAR-1, PAR-4 and PAR-5) and that the MNI of the four apatite $\delta^{13}C$ values is also three (PAR-3, PAR-14, and PAR-15).

Table D.2. Specimens contributing to the MNI estimate and possible matches.

specimen	possible matches
2	10
4	12, 13, 15, 17
5	3, 6, 12
7	none
9	1, 8, 13
11	6, 12, 15
14	1, 8
16	12, 13, 15, 17

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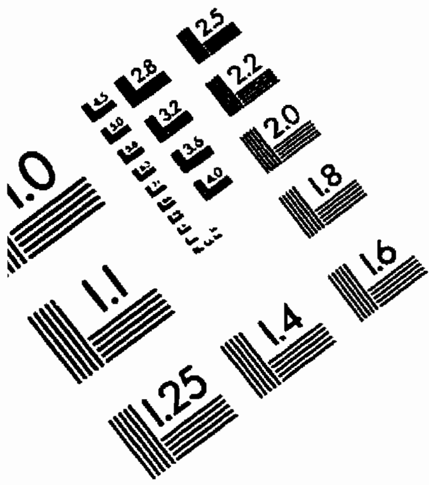
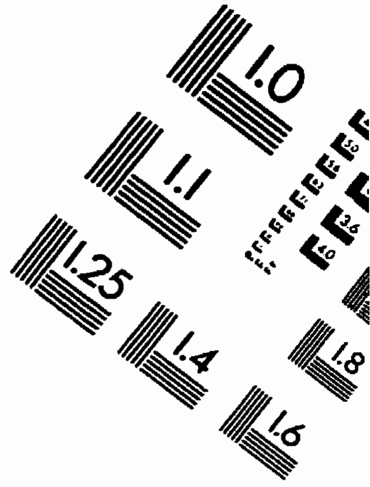
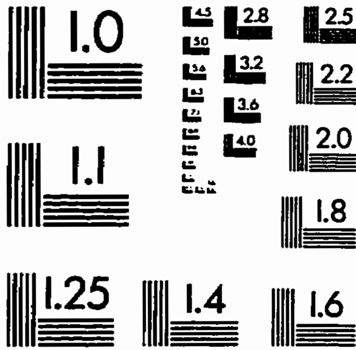
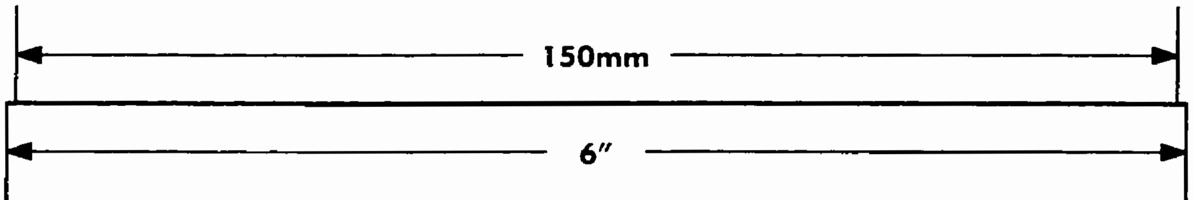

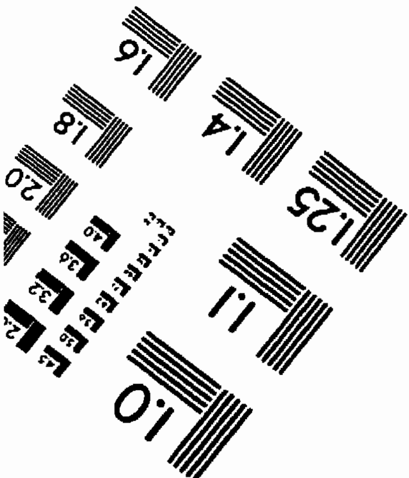
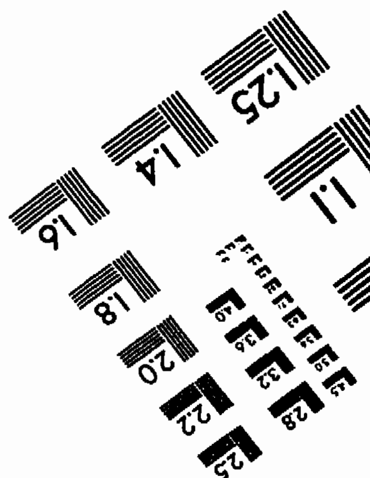

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