

University of Nevada, Reno

Assessing the Utility of Dental Calculus in Paleodietary Analysis: A Case Study from West Mexico

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by

Kirk Schmitz

Dr. G. Richard Scott / Thesis Advisor

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We recommend that the thesis
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Kirk Schmitz

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G. Richard Scott, Advisor

Christopher von Nagy, Committee Member

Christopher S. Jazwa, Committee Member

Simon R. Poulson, Graduate School Representative

David W. Zeh, Ph.D., Dean, Graduate School

May, 2018

Abstract

Using dental calculus from 16 individuals, stable isotope analysis demonstrates the role of C4 plants in the diet of the inhabitants of the West Mexican site of El Opeño. When put in a broader context with diverse world populations processed by the Nevada Stable Isotope Lab, samples from West Mexico correspond well with those of known maize agriculturalists. Patterned differences in stable isotope values demonstrate the ability of calculus based paleodietary studies to identify shifts towards increased exploitation of C4 plants through $\delta^{13}\text{C}$ values and the exploitation of high trophic level resources through $\delta^{15}\text{N}$ values. Sample treatment with HCl results in lower atomic carbon to nitrogen (C/N) ratios, and lighter $\delta^{13}\text{C}$ values in all instances. Correlations between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and C/N ratios in the 474 individuals from world samples indicate that diagenetic alterations are more likely in samples with C/N ratios > 12 , as suggested by Eerkens et al. (2014). Excluding samples above the acceptable threshold of 12 impacts mean $\delta^{13}\text{C}$ values, moving them closer to expected dietary signatures.

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1.1 Introduction

This study addresses the diet of the Formative period site of El Opeño, located in the modern state of Michoacán (see Figure 1.1). It represents the first of its kind in the region and joins a small but growing number of isotopic studies aimed at elucidating the origins of maize agriculture. Furthermore, it demonstrates the utility of bulk dental calculus as a proxy in paleodietary research. Examining the current sample in the larger context of the work done at the Nevada Stable Isotope Lab reveals patterns that pertain to all past and future stable isotopic studies on dental calculus.

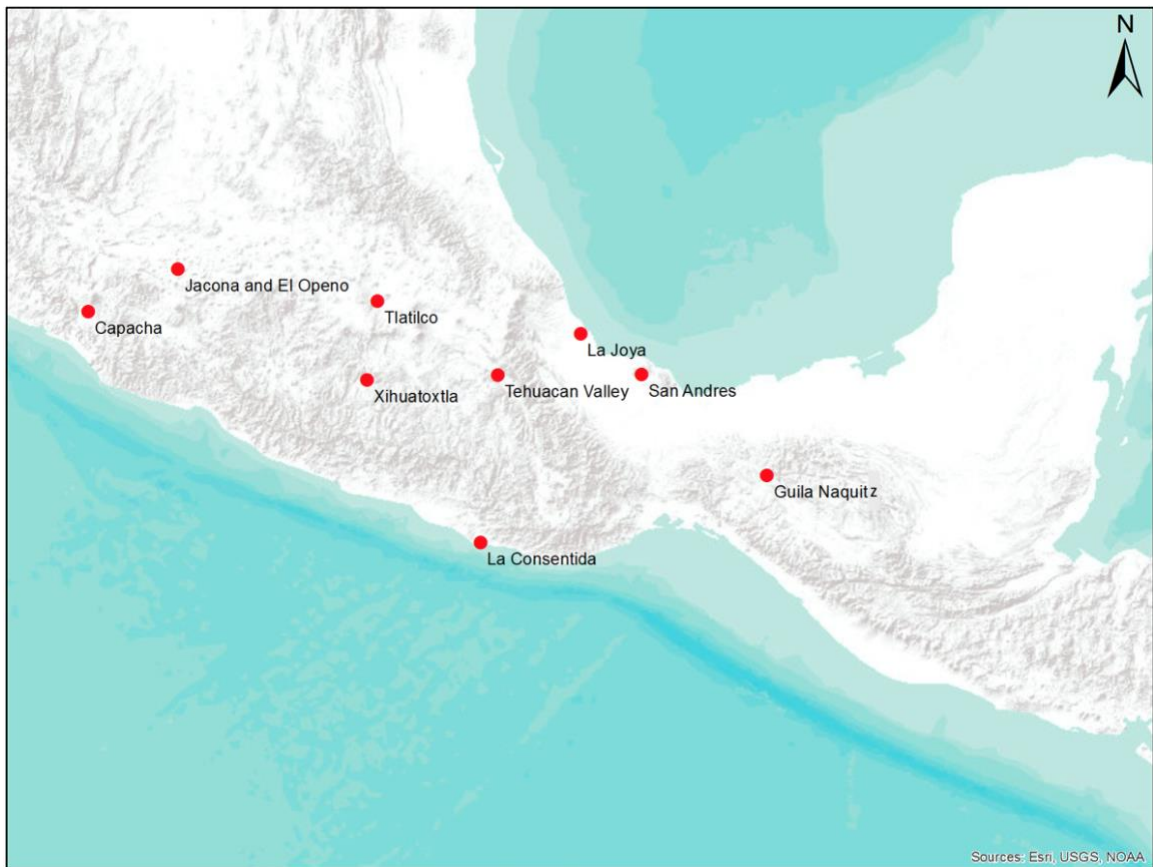


Figure 1.1. List of select locations mentioned Chapter 1

Archaeologically, Michoacán is included in the culture area often known as West Mexico, but the region is ill-defined and under-represented in the literature. It is often defined as the Pacific Coast of Mexico from Michoacán to Sinaloa (Olay 2015), although various definitions, including that used by the Instituto Nacional de Antropología e Historia (INAH) in Mexico, include the state of Guerrero as well (Pollard 1997: 348). Whether the definition includes the entire state of Guerrero or not, the geographic region that encompasses the Balsas River drainage is included in the culture area.

The beginning of the Formative period is often placed at 2000 B.C. (all dates cited in this thesis represent calendar years, radiocarbon dates are calibrated) in traditional progressionist classification systems (see Sanders and Price 1968 for example). Voorhies (2012: 342) contends the Formative period began, in its earliest manifestations, around 1900 B.C. but the start date should vary regionally. Lesure (2011: 10) stresses the importance of looking at the timespan between 3500 B.C. and 500 B.C. as a period of transition, or a time when the boundary between Terminal Archaic and Early Formative lifeways had not yet solidified. On one side, there were mobile hunter-gatherers and low-level food producers (Smith 2001). On the other was the Mesoamerican cultural package, with permanent villages, ceramic production, and full-scale maize agriculture.

El Opeño is a complex of 12 tombs near the modern town of Jacona. Radiocarbon dates obtained from material found in three tombs excavated during the 1991 season returned a range of 1740 to 900 B.C. with the greatest concentration of dates falling between 1310 and 1110 B.C. (Oliveros and Ríos 1993: 47). As yet, no habitations or domestic buildings associated with the culture that built the tombs have been located (Oliveros 2009: 24). While this dearth of domestic contexts means nothing is known of

the settlement pattern of those individuals (255 in the five tombs excavated by Oliveros in his 1970 and 1991 field seasons) whose remains ended up in the tombs, the planned layout of the subterranean crypts and the wide range of dates recovered from them represent a degree of sedentism, at least in mortuary practices. Whether a greater exploitation of maize preceded this increased sedentism as it did in other parts of Mesoamerica during this period is unclear, but that is the expectation.

Genetic data (Doebley et al. 1984; Matsuoka et al. 2002), as well as the results of recent archaeological investigations (Piperno et al. 2009; Ranere et al. 2009) have highlighted the importance of West Mexico in the domestication of *Zea mays* subspecies *mays* (maize) during the Archaic period. Hunter-gatherers were cultivating *Zea* by 7000 B.C., at which point it already exhibited signs of domestication, such as softening of the pericarp and increased rigidity of the rachis (Piperno et al. 2009). Despite this early focus on the domestication of maize, full-scale maize agriculture did not occur in many areas until much later. Rosenswig et al. (2015: 90) argue that, based on evidence from the Gulf Coast and the Soconusco region, food production began to make up a larger portion of the calories in the Mesoamerican diet by around 1900 B.C., but it was not until after 1000 B.C. that maize became the focus of food production.

Given the geographic and temporal context of El Opeño in the heartland of the wild progenitor of maize and on the cusp of a regional shift towards food production and sedentism, analysis of paleodiet of the remains found in the tombs of El Opeño functions as an interesting insight into the behavior of Formative period West Mexicans during this important transition. While maize is not the only species the inhabitants of ancient Mexico domesticated, it became an important component of the diet during the Formative

period and is readily detectable using stable isotope analysis and will be the focus of the analysis in this thesis.

Scott and Poulson (2012) initially demonstrated the utility of analyzing stable isotope values from dental calculus in paleodietary studies. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values obtained from the remains of 15 individuals from El Opeño using this method serve as a direct proxy for paleodiet during the utilization of the site during the Formative period. Comparison with stable isotope values of calculus from other populations around the world put the degree of reliance on C_4 plants (e.g., maize; less negative $\delta^{13}\text{C}$ values) of the occupants of El Opeño in a global context. Furthermore, this thesis advances some general observations based on data derived from calculus-based isotopic studies of populations outside of West Mexico conducted by the Nevada Stable Isotope Lab, and on the strengths, limitations, and unknown considerations in the use of dental calculus for paleodietary studies.

1.2 Culture Area Background

1.2.1 Evidence for the Evolution of Maize in West Mexico

Zea mays ssp. *mays* (maize) is a domesticated member of the Panicoideae subfamily. Congeners of maize inhabit a number of environments from Mexico down through Central America, and resemble maize morphologically in that they are stalky, seed-bearing grasses. Some are perennial and one is tetraploid (Doebly 1990: 8). These traits set them apart from teosinte (as the close relatives of maize are called, including the ancestor from which maize was domesticated) and maize, which are annual, diploid, and can crossbreed readily with each other (Benz 2006). Teosinte has two subspecies conspecific to maize that played an important role in its evolution: Balsas teosinte (*Z.*

mays ssp. *parviglumis*), native to the lowland drainages of the Balsas River Valley, and highland teosinte (*Z. mays* ssp. *mexicana*), whose natural range includes the highlands valleys of Tehuacán, Oaxaca and the Basin of Mexico (Ramos-Madrigal et al. 2016). Matsuoka et al. (2002) demonstrated through gene mapping of 99 microsatellite loci in maize and its conspecifics that *parviglumis* has considerable genetic overlap with modern maize, indicating a single origin for maize from this lowland cousin. Furthermore, the authors produced an estimate on the time of divergence based on a normal mutation rate. They calculated that approximately 9188 years would have been necessary to account for the dramatic morphological and genetic changes apparent between maize and its ancestor (Matsuoka et al. 2002: 6083).

Matsuoka et al.'s calculations were corroborated by the discovery of ground stone artifacts in Xihuatoxtla rockshelter in the Iguala Valley (Ranere et al. 2009). While no macroremains (cobs, leaves, stalks, roots etc.) were preserved at the site, starch grains were recovered from several of the flaked stone and ground stone tools indirectly dated to 7050 - 6660 cal B.C. (Piperno et al. 2009: 5021). Microremains (pollen, phytoliths, starches) are made of harder materials and often preserve better than macroremains. At Xihuatoxtla, residues including starch grains were extracted from several of the flaked and ground stone tools. While teosinte does not occur naturally in the valley today (Piperno et al. 2007), it is near the natural range of Balsas teosinte and it is possible that past conditions allowed for its occurrence there. Fortunately, the size and morphology of maize starch grains make them diagnostic from those of the teosinte conspecifics, so it can be said with confidence that the microremains found in the Iguala Valley were of maize and not a relict population of teosinte. Piperno et al. (2009: 5019) found that maize

was the dominant taxon on every tool for Xihuatoxtla that yielded residues, accounting for 90% of the starch grains present. Furthermore, phytoliths collected from the tools and the sediments in immediate contact with them demonstrated a recognizable degree of domestication already present in the maize exploited by the occupants of Xihuatoxtla rockshelter. Phytoliths are siliceous bodies produced by plants to provide structure and, like starch grains, are diagnostic of taxonomy based on morphology (Piperno and Pearsall 1993: 339). At Xihuatoxtla, the phytoliths that make up the hard cases covering the kernels, which are present in teosinte but absent in domesticated maize, were missing from the assemblage (Piperno et al. 2009: 5022).

1.2.2 Archaic - Formative Transition Period

The domestication of maize set the stage for the cultural transition Lesure (2011) describes, which took place between 3500 B.C. and 500 B.C and includes the increased exploitation of domesticates. Exploitation of the annual grass was probably seasonal and was opportunistically included in the schedule already in place for hunter-gatherer microbands when advantageous (Flannery 1968). The cave site of Guilá Naquitz in the Valley of Oaxaca provides an example of hunter-gatherer behavior on the Archaic side of the transition. Earlier dry season living surfaces contained the remains of deer (*Odocoileus*), peccary (*Pecari*), and cottontail (*Sylvilagus*) as well as masticated maguey (*Agave*) quids. Rainy season surfaces yielded large quantities of acorns (*Quercus*) and pine nuts (*Pinus*), both processed and unprocessed, mesquite bean pods (*Prosopis*), and various succulent and tree fruits (Marcus and Flannery 1996). Three maize cobs excavated from ash lenses in the cave's living surfaces between the clearly Archaic lower

deposits and later Formative period ceramic scatters are the earliest directly dated maize macroremains (Piperno and Flannery 2001). These cobs yielded radiocarbon dates of 4300 BC and are unmistakably, if only partially, domesticated (Benz 2001).

The most complete assemblage of early domesticated maize comes from the caves of the Tehuacán Valley in the highland state of Puebla. Long and et al. (1989) directly dated the oldest macroremains to 3500 BC, and the rest of the roughly 20,000 partial or intact cobs excavated during the project represent a continuous sample of maize in the Tehuacán Valley up to AD 1500. With the exception of one four-rowed cob, like the cob from Guilá Naquitz, the maize from the earliest phase of the Tehuacán assemblage (Coxcatlan phase) is polystichous (i.e. more than two ranks), indicating continuous improvement on the caloric yield of domesticated maize.

The macroremains assemblage of the Tehuacan Valley is unrivaled; however, there is evidence for the increasing use of maize throughout Mesoamerica in the form of microremains. Pope et al. (2001) found evidence of slash and burn agriculture as early as 5300 BC in the Gulf Coast site of San Andres. Although no species of *Zea* are native to the Gulf Coast, the authors reported *Zea* pollen grains, as well as a spike in the occurrence of charcoal in a series of sediment cores and excavations. In their reanalysis of the sediment cores, Pohl et al. (2007: 6872) determined that the phytolith taxa of the pre-maize bearing strata pointed towards an old-growth forest. Following the introduction of maize to the region, large quantities of arboreal charcoal are present. There is also an increase in phytoliths of the Panacoideae subfamily, many with evidence of charring themselves. This pattern is indicative of a shift from woodlands to grasslands. Manipulation of the environment of this type was not confined to the Gulf Coast. Piperno

(2006: 289) identified a similar pattern from two sites in the Balsas River Valley, where maize dominated the pollen assemblage from the lowest stratum of the sediment core, dated to approximately 2500 BC, and large chunks of charcoal are present throughout. Piperno et al. (2007: 8) found evidence of deforestation and cultivation in the watershed of Ixytacyola, near Xihuatoxtla rockshelter in northern Guerrero at 5200 BC, around the same time as it is noted in the record at San Andres. The pattern is repeated, albeit later in time than the Gulf Coast and West Mexico in the Soconusco region of the Pacific Coast of Mexico (modern state of Chiapas). Kennett et al. (2010) noted the same dramatic increase in charcoal between 2150 and 1850 BC, along with a pronounced uptick in pollen from so-called “disturbance taxa,” such as weeds of the *Chenopodium* and *Amaranthus* genera. These taxa favor disturbed ground like agricultural fields and an increase in the abundance of their pollen indicates a shift from arboreal conditions to grassy conditions. Piperno et al. (2009) have demonstrated the antiquity of domesticated maize is profound (ca. 6700 BC), but full-scale production of food probably did not occur until much later as indicated by the *en masse* conversion of forests to grasslands through slash and burn agricultural practices.

Based on microremains, Rosenswig et al. (2015) argue that, although maize microremains were present in the region back to 4550 B.C. (Kennett et al. 2010: 3407), maize was not a significant portion of the diet in the Soconusco until after 1000 B.C. during the Middle Formative. VanDerwarker and Kruger (2012) came to a similar conclusion for the site of La Joya in the Tuxtla Mountains, near the Gulf Coast based on the density of paleobotanical remains. Their conclusion corroborated an earlier study (Arnold 2009), which points to two lines of evidence indicating a reliance on wild

resources as opposed to maize agriculture. Arnold (2009) proposes that settlement patterns shifted from habitation in riparian zones until around 900 B.C., to higher ground, away from the traditionally exploited freshwater resources during the Middle Formative. A second shift occurred concomitantly with the shift in settlement. This change was in the material culture, from mortar and pestles dominating the ground stone assemblage, to the two-handed mano and metate, which are closely associated with the processing of maize. These three studies reflect a recent trend in Mesoamerican archaeology, a suggestion that maize agriculture may not have been as intricately tied to the early part of the Formative Mexico as was previously believed, at least not in the regions on the peripheries of the Mexican Altiplano.

1.2.3 El Opeño

The assumption of the subsistence of the inhabitants of El Opeño before 1000 B.C. is that they were agriculturalists. Oliveros (2009: 16 translation mine) says that they were “...a society dedicated to agriculture, fishing, hunting and gathering.” This statement is based more on inference than direct evidence. Almost nothing is known of the domestic context of the individuals who built the tombs of El Opeño.

The 1991 field season led by Oliveros began with the goal of uncovering domestic architecture to the north of known tombs. A trench placed by Oliveros to test the stratigraphy turned up an impression of one circular structure. This structure is assumed, based on the shape of the imprint, to have been a wattle and daub habitation, although neither post-holes nor living surfaces were identified (Oliveros 2009: 27). The one piece of direct evidence for maize use at the site was the imprint of one maize cob located

within the circular structure. The lack of carbon-bearing material in the structure, or the cob prevented absolute dating to put these imprints in context with the tombs located below them. Because of the lack of domestic middens or living surfaces, the only evidence available for the dietary behavior of the inhabitants of El Opeño is that offered by the material culture represented in the mortuary offerings and in the physical remains themselves.

The structure of the tombs is likely a precursor to the shaft tomb (*tumbas de tiro*) complex that became characteristic of parts of West Mexico. Stairways lead to passageways, which terminate in openings sealed with flagstones. Oliveros (2009: 36) found the seals intact but noted the accumulation of fine sediments within the tombs. During seasons of heavy precipitation, the stairways and sloped floors of the passageways served as funnels, directing water to the tombs. Oliveros (2009: 34) describes an olla near the entrance of Tomb 5 in which 36 layers of ultra-fine sediments had accumulated after filtering through the seal over the entranceway to the main chamber. Rather than fearing contamination, Oliveros (2009: 34, translation mine) remarked that the sediments served to "... protect their precious contents against time."

Beyond the sealed entrances of the tombs are the burial chambers. Within the burial chambers, Oliveros excavated lithic material, both flaked and ground. Stone vessels and beads were found amongst the piles of bones and scattered throughout the chambers. Ceramics were the dominant class of artifact. They include utilitarian vessels, and symbolic and ritual artifacts, such as whistles and figurines.

The lithic assemblage offers few clues as to the extent of plant exploitation. The majority of the ground stone artifacts consists of mortars and one-handed pestles. These

artifacts could be used to process plant material, such as seeds, or maize kernels, but they were also used to grind non-food substances like ochre or cinnabar. While residue analyses on the grinding surfaces have not yet been performed, the most common type of ceramic figurine depicts females with elaborate designs painted or tattooed on their bodies, suggesting pigments were regularly used (Oliveros 2009: 61). There is one larger slab metate of the type used with a two-handed mano, often associated with efficient processing of maize (Hepp et al. 2017: 707).

Flaked stone tools make up a large part of the lithic assemblage. Oliveros describes four groups of projectile points, totaling 102 examples (2009: Table 5). Faunal remains of various species are present, including deer and peccary, a profile reminiscent of that of hunter-gatherer occupation of Guilá Naquitz (Marcus and Flannery 1996). Faunal elements include modified awls, needles, and decorative pendants (Oliveros 2009: 155). That is not to say that the taxa represented in the faunal assemblage did not make up a portion of the diet at El Opeño. However, the objects that make up the mortuary offerings at the site cannot be considered analogous to a domestic midden.

The ceramic assemblage, specifically the form and type of the constituent vessels, reflects both exploitation of plant materials and ties with other known Formative period sites. The dominant types of vessels were *ollas*, which are vessels with wide necks and rimmed openings, and bowls. A subtype of bowl relevant to the conversation is the *molcajete*, also known as the grater bowl. Grater bowls have incisions or depressions on the inside surface that are used for processing food. Hepp et al. (2017: 710) suggest an increase in the frequency of grater bowls offered as grave goods relative to *ollas* at the contemporaneous site of La Consentida on the coast of Oaxaca may have been related to

a shift in the way maize was processed and consumed, from liquid form (something similar to *atole*), to the production of corn flour (*masa*). The form of the vessels is similar to assemblages across Mesoamerica, but the specific style links the ceramics of El Opeño with two specific ceramic traditions. Kelly first reported on a ceramic tradition associated with burials at a number of sites in the state of Colima (1980). Absolute dating of Capacha sites overlaps with the occupation of El Opeño (Pollard 1997: 358). Kelley (1980: 31) remarks that the similarity between the two traditions is strong to the point of being almost indistinguishable from one another. Stylistic similarities also link some of the vessels from El Opeño with those of Tlatilco in the Basin of Mexico (Oliveros 2009: 84).

In addition to the similarity of style in the vessels, the ceramic figurines at the site also tie El Opeño stylistically to Tlatilco (Oliveros 2009: 58). These figurines are remarkable in the way they represent the physiognomy of the individuals of El Opeño. The ceramic figurines portray individuals with dental modification and cranial deformation, which is also evident in the skeletal remains. Cranial deformation indicates ties between El Opeño and the Capacha culture of Colima (Pompa 1980). It is also reminiscent of similar practices at the site of Tlatilco in the state of Mexico (Joyce 2001). Given the presumed bias in the material record towards ritual, as opposed to domestic behavior, isotopic analysis of the remains of El Opeño themselves offer an alternative avenue of investigation into paleodiet at the site.

1.3 Isotopic Analysis in Paleodietary Studies

Archaeological studies have developed numerous methods for inferring the composition of past human diets. Although a focus on subsistence has its precedents in the earlier armchair anthropologists of the 19th century (e.g. Lewis Henry Morgan's *Ancient Society*), it was the archaeologists of the early to mid-20th century that began systematically recording the necessary data to make meaningful inferences about the resources being exploited by early human groups. V. Gordon Childe's excavations in the Near East, which led to his development of the Oasis Hypothesis and eventually his concept of a "Neolithic Revolution," focused on what material culture could reveal about subsistence patterns. He found that sickles and grinding stones indicative of exploitation of grasses found in Natufian period deposits suggested a radical shift from hunting and gathering during the Pleistocene towards greater reliance on lower-yield resources during the Holocene (Winterhalder and Kennett 2006: 6). Large scale excavations in Mexico in the Tehuacan Valley (MacNeish 1964) and the state of Oaxaca (Flannery 1986) are excellent examples of projects that painstakingly quantified faunal and paleobotanical remains to infer change in reliance on resources through time. These methods allow archaeologists to form a general idea about the subsistence patterns at a given place and time, but in the same way a direct radiocarbon date is a more reliable indicator of the temporality of an object than a date by association, the direct observation of stable isotope composition of human remains can be a more reliable indicator of that individual's diet than approximations derived from quantifications of material remains. More accurately, the information gained from one type of analysis is complementary to the other. While researchers have found uses for many isotopes in paleodietary analysis,

this study focuses on isotopes of two of the more commonly used elements, namely carbon and nitrogen.

1.3.1 Carbon

In the field of radiocarbon dating, observations that maize systematically returned dates that were too young prompted research into the isotopic composition of various grasses (Bender 1968). During photosynthesis, plants discriminate against heavier ^{13}C isotopes to varying degrees. They group into three photosynthetic pathways. Plants that utilize the C_3 pathway (or Calvin pathway) include trees, shrubs, and some grasses. Among these grasses are some of the most widely consumed grains on earth, namely oats (*Avena*), wheat (*Triticum*), and barley (*Hordeum*) (Bender 1968: 468). Maize, along with other members of the Panacoideae and Chloridoideae tropical grass subfamilies, such as millet (multiple genera), sugarcane (*Saccharum*), and sorghum (*Sorghum*) falls into the C_4 pathway (or Hatch-Slack pathway). This lesser discrimination against heavier isotopes was the reason for the too-young radiocarbon dates of C_4 plants. The third pathway is only utilized by xerophytic succulent plants and is known as the CAM (crassulacean acid metabolism) pathway (O'Leary 1981). The first two pathways are by far the most common and can be distinguished from one another by their $\delta^{13}\text{C}$ values.

Delta values are used in the analysis of many isotopes and are calculated by dividing the ratio of the heavier isotope to the lighter isotope (in the case of carbon ^{13}C to ^{12}C) in a sample to the ratio of the heavier isotope to the lighter isotope in a standard (PeeDee Belemnite is the standard used for the calculation of delta values in carbon). The resulting quotient is subtracted by one, multiplied by 1000 and expressed in parts per thousand (per

mil; ‰; see Equation 1.1). C₃ plants express isotopic values that range from -22‰ to -34‰. C₄ plants exhibit distinctively less negative values, with a range of -9‰ to -16‰ (Vogel and van der Merwe 1977: 239). Bender's (1968: 468) study found that both modern and archaeological examples of both the cobs and the kernels of maize yielded values between -10‰ and -12‰, closer to the heavier end of the C₄ range. Furthermore, differences in δ¹³C values are preserved up the food chain so that organisms whose diets are comprised of C₄ plants can be distinguished from those that subsist on C₃ plants (Sullivan and Krueger 1981: 334).

$$\left[\frac{^{13}\text{C}/^{12}\text{C Sample}}{^{13}\text{C}/^{12}\text{C VPDB}} - 1 \right] \times 1000 = \delta^{13}\text{C}$$

Equation 1.1. Standard for calculating δ¹³C

Given this diagnostic difference between the isotopic values of maize and C₃ plants, as well as the wide dispersal of maize based economies found in the New World before European contact, establishing the geographic extent of maize through time became the natural focus of early applications of stable isotope analysis to questions about paleodiet. Vogel and van der Merwe (1977) hypothesized that the shift from hunting and gathering to maize agriculture apparent in the archaeological record in the state of New York should be accompanied by less negative δ¹³C values in the associated human remains. The authors utilized collagen derived from individuals from four different sites. Two antedated the shift and two sites dated to after the appearance of a variety of maize in the archaeological record. The latest site in the study was dated to the Historic period and

people were unmistakably producing their own food. The other site assumed to postdate the transition, but predate the Historic period, had no material evidence for the exploitation of maize (Vogel and van der Merwe 1977: 239). While the $\delta^{13}\text{C}$ values of the individuals from the two earlier sites ranged from -18.9‰ to -21.3‰ (n=4), those of the two later sites exhibited distinctively heavier values, ranging from -13.5‰ to -16.6‰ (n=4) (Vogel and van der Merwe 1977: 241).

Chisholm et al. (1982) demonstrate the use of stable carbon isotope analyses to establish the relative proportions of marine and terrestrial protein sources in the diet (also see Walker and Deniro 1986). The study was conducted on a sample population from archaeological sites of the Canadian Pacific Coast (Chisholm et al. 1982: 1132). The principle underlying this study is that terrestrial plants and the animals that consume them ultimately derive the carbon in their systems from atmospheric CO_2 , while marine plants and the animals that consume them derive their carbon from a different source. The observed difference between these two sources is approximately 7‰. Stable carbon isotope values derived from the muscle tissue of marine and terrestrial fauna yielded mean $\delta^{13}\text{C}$ values of -25.7‰ and -17.8‰ respectively. This difference demonstrates the preservation of the variation of $\delta^{13}\text{C}$ values from their source, through the bottom trophic level plants to their consumers. Collagen of salmon eating individuals from the Ottawa Valley with a mean $\delta^{13}\text{C}$ value of -19.6‰ were similar to the mean value observed in Northern European C_3 consumers. Individuals from the interior of British Columbia exhibited a mean $\delta^{13}\text{C}$ value of -15.4‰ and coastal dwelling individuals a mean $\delta^{13}\text{C}$ value of -13.4‰ (Chisholm et al. 1982: 1131). Schwarcz et al. (2014) revisited the project to add stable nitrogen isotope data and additional individuals. The results are as

expected given the current understanding of the contribution of marine versus terrestrial protein sources to the $\delta^{15}\text{N}$ values of their consumers. The addition of stable nitrogen data confirmed that the coastal groups displaying less negative $\delta^{13}\text{C}$ values, indicating a high reliance on marine protein sources, also had $\delta^{15}\text{N}$ values higher than those groups from the interior.

The success of the study highlights the potential issues that can arise when looking at groups that have multiple potential sources of $\delta^{13}\text{C}$ variability. Inhabitants of Chiapas on the Pacific Coast of Mexico utilized the rich resources of the Pacific Ocean and maize leaf and cob phytoliths from a section of a core sample dating to 4500 B.C. indicate early experimentation with the production of C_4 plants (Kennett et al. 2010: 3407; Rosenswig et al. 2015). Stable isotope analyses by Blake et al. (1992) and later by Chisholm and Blake (2006) attempted to establish the point at which the inhabitants shifted from a coastal adaptation of exploiting shellfish and other marine resources towards a focus on maize production. In the original study (Blake et al. 1992), the collagen of earliest individuals, who were dated to the end of the Archaic period, had a mean $\delta^{13}\text{C}$ value of -10‰. Low $\delta^{15}\text{N}$ values (mean = 8.7‰) would suggest that the source of the heavier $\delta^{13}\text{C}$ values was not from heavy exploitation of marine resources, as was the case in Coastal British Columbia (Schwarcz et al. 2014). The lack of maize macroremains during this phase indicates that the production of maize was not high enough to be solely responsible for the heavy $\delta^{13}\text{C}$ values. Assuming that neither diagenesis nor improper methods were responsible for the isotopic values of the Archaic period individuals, the dearth of evidence for a strong maize component in the diet during that phase requires another resource with a similar isotopic profile. Chisholm and Blake (2006: 167) point out that

shrimp and crab, which are common in the estuarine environments of the Chiapas coast, produce a signature similar to maize because they are marine organisms of low trophic level. This calls attention to the risk of equifinality in isotopic studies and the utility of archaeological remains in constructing the most parsimonious explanation.

1.3.2 Nitrogen

Stable nitrogen isotope analyses were originally used alongside carbon to further evaluate the contributions of different types of plants to human diets. DeNiro and Epstein (1981) conducted controlled experiments in which organisms were raised on diets with known carbon and nitrogen isotopic compositions. Values in nitrogen isotope analyses are also expressed as a delta value ($\delta^{15}\text{N}$) where ^{15}N is the heavier of the two isotopes, ^{14}N is the lighter, and the ratio of the two isotopes present in N_2 gas the atmosphere is the universal standard to which the sample is compared (Equation 1.2). DeNiro and Epstein found in previous studies that organisms preferentially incorporate ^{15}N into their diet, leading to higher $\delta^{15}\text{N}$ values than those found in their diet (1981: 342). Certain types of plants have nitrogen fixing properties such as legumes within terrestrial systems, although there are a number of aquatic species that also fix nitrogen, leading to depleted $\delta^{15}\text{N}$ values (DeNiro and Epstein 1981: 346).

$$\left[\frac{^{15}\text{N}/^{14}\text{N Sample}}{^{15}\text{N}/^{14}\text{N AIR}} - 1 \right] \times 1000 = \delta^{15}\text{N}$$

Equation 1.2. Standard for calculating $\delta^{15}\text{N}$

Given this observation, DeNiro and Epstein (1981) proposed that stable nitrogen isotope compositions could be used to supplement stable carbon isotope values to reconstruct the diet of humans. MacNeish (1967) built a reconstruction of the subsistence patterns of inhabitants of the Tehuacan Valley of Mexico through time derived from paleobotanical and faunal remains found in middens and paleofeces. Based on this reconstruction, DeNiro and Epstein (1981) calculated expected isotopic signatures for each archaeological phase. They compared these expected values against observed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of collagen from 12 individuals representing four phases. While shaped ground stone artifacts are present in deposits dating to the earliest phase (MacNeish 1971), MacNeish estimated, based on the archaeological remains that meat made up 54% of the diet at this time, with the C_3 and CAM plants making up the majority of the remainder of the calories (Webster 1986). DeNiro and Epstein's expectation for the $\delta^{13}\text{C}$ value of this phase, based on the archaeological reconstruction was approximately -18‰ and the observed collagen value, once corrected by -3.9‰ for the fractionation between the diet and collagen, fell within 1‰ of each other.

Unfortunately, the addition of $\delta^{15}\text{N}$ values did not accomplish what the authors intended. Nonetheless, this study demonstrates a significant strength of stable isotopic analysis of paleodiet, which is that it can correct biases imposed by the material record. Based on the archaeological data, the estimated $\delta^{13}\text{C}$ values for the Coxcatlan phase were estimated to be lighter than those of the preceding El Riego phase (DeNiro and Epstein 1981: Figure 4). However, the dry caves from which the paleobotanical and faunal remains were excavated represented seasonal camps of microbands, either as dry-season hunting camps or as rainy-season shelters for people tending their fields (Webster 1986).

Therefore, the material record would demonstrate a seasonal diet, whereas stable isotope values in collagen would be the result of the overall diet. Contrary to the expected value for the Coxcatlan phase, the observed collagen $\delta^{13}\text{C}$ value was 7‰ heavier. The most parsimonious explanation is the increase of the exploitation of C_4 plants. The Coxcatlan phase also coincides with the first appearance of maize macroremains in the archaeological record. Thus, the $\delta^{13}\text{C}$ values of collagen more accurately reflect a trend towards increasing food production than the archaeological reconstruction based on material remains.

The study of trophic levels has been another focus of stable nitrogen isotope analyses. Organisms preferentially fix the heavier ^{15}N that they consume. Thus, it will have a $\delta^{15}\text{N}$ signature slightly heavier than its diet. The biological process of fixing nitrogen at each successive level of the food chain allows for the investigation of the effects of status on access to different types of food using $\delta^{15}\text{N}$ values as a proxy for consumption of meat. This type of analysis has its roots in early studies such as White and Schwarcz (1989) who concluded that the only individual from the site of Lamanai, Belize who was buried in a tomb had increased access to marine resources because his $\delta^{15}\text{N}$ values were closer to those recorded from fishermen from the Bahamas than to any other individual at the site. The questions that such analyses have been used to answer have increased in complexity and scale. Metcalfe et al. (2009) use a similar methodology to look at the phenomena of hierarchical structures and heterarchical structures at the site of Chau Hiix, a secondary site located near Lamanai.

Adding another dimension to the analysis of status, the authors examine how $\delta^{15}\text{N}$ values at Lamnai compare to those of the other nearby sites. They hypothesize that, on a

regional level, if diet was based on a hierarchical system where components were different as well as ranked, individuals of higher status would consume a diet with more desirable resources, and high-status individuals at different sites would have similar diets. However, if regional diets were heterarchical phenomena where the constituent components of the system were different but unranked, the isotopic profile would be different between sites, but would not display the same differences on an intra-site level (Metcalf et al. 2009: 16-17). They found that when the general diet of the site of Chau Hiix was compared to the general diets of the other sites in the region, it was higher in $\delta^{15}\text{N}$ values than the others, possibly implying a greater access to meat on average than other sites in the region, but there was a distinct hierarchical component to diet between social classes within all of the sites used in the study (Metcalf et al. 2009: 33).

1.4 Isotope Biomaterials

While soft tissue can be recovered in regions with extreme preservation (Aufderheide et al. 1994), historically, archaeologists have used two biomaterials for isotopic analysis: bone apatite and collagen. Different information can be obtained from these materials depending on the source of the material. Information from dental enamel and dentin represents a different period in the life of an organism than does bone derived apatite or collagen. Recently, the development of stable isotope methodologies for the analysis of dental calculus has drawn significant attention (Eerkens et al. 2014; Price et al. 2018; Salazar-García 2014). As with apatite and collagen, the isotopic composition of dental calculus is derived from distinct chemical and mechanical formation processes and provides a different source for information on the diet of past human populations.

1.4.1 Apatite

Biogenic apatite, also known as hydroxyapatite has a chemical formula of $\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$ and is the mineral portion that makes up the main structure of bones and teeth. The material for the production of bioapatite is derived from the diet as a whole, which includes all of the carbohydrates, fats, proteins, and dietary minerals (Koch et al. 1997: 418). Because teeth are formed in the first years of an organism's life, enamel records isotopic information from that period (Lee-Thorpe 2008: 927). Structural of bone carbonate remodels over a period of time and records an average of the diet from the last several years of lifetime. Bones and dentin are high in organic content at approximately 20% relative to enamel, which is mostly inorganic with roughly 2% of its structure composed of organic material (Koch et al. 1997: 417-418). Many of the constituent parts of bioapatite can be used to acquire oxygen isotope values, but only the carbonate (CO_3) portion can be used to analyze carbon isotopes. CO_3 can substitute for either OH or PO_4 in the chemical formula for hydroxyapatite. The CO_3 is believed to be resistant to diagenetic chemical changes, allowing for diagenetic carbonates to be removed with an acetic acid pretreatment (Kohn and Cerling 2002: 456). The type of bone is important to isotopic studies of fossilized, or partially fossilized bones, as certain types are more susceptible to diagenetic alterations than others. Cancellous bone is more susceptible than cortical bone because of its higher surface area. Enamel is the least susceptible of all due to its low organic content. Isotopic data from archaeological enamel can generally be considered credible unless there is specific reason to doubt the integrity of the sample. As Kohn and Cerling (2002: 458) report there is no evidence for diagenetic alteration of

enamels as far back as the Miocene in age because of the large crystal size of enamel based bioapatites.

1.4.2 Collagen

Traditionally, collagen has been the preferred material for stable isotope studies. In sites with favorable preservation conditions, it can persist in archaeological bones for long periods of time (Ambrose 1990). Collagen has explicit guidelines for C/N ratios, as well as the necessary percent carbon and nitrogen in samples for them to be considered reliable. Modern collagen consists of approximately 43% carbon and 16% nitrogen by weight, which gives it an atomic C/N ratio of ~3.2. According to Ambrose and Norr (1992), samples with C/N between 2.9 - 3.6 are generally considered acceptable. Even as percent carbon and nitrogen drop through time as samples degrade, those with 6.9 and 2.4 percent carbon and nitrogen respectively have yielded viable results (Ambrose and Norr 1992: 403). This predictability is possible because collagen is a protein with a known chemical structure. Because collagen is a conglomeration of amino acids, it can yield information on both the carbon and nitrogen portions of the diet, as opposed to bioapatite, which has no nitrogen portion (Lee-Thorpe 2008). Collagen is produced from dietary proteins consumed. Therefore the stable isotope profile reflects that portion of the diet. Apatite, on the other hand, which provides information on the bulk diet (Kellner and Schoeninger 2007: 1112).

1.4.3 Calculus

Calculus is the result of mineralization of dental plaque, a biogenic film that forms on the surfaces of teeth. Similar to biogenic apatites, the proportion of calculus made up of organic material in fresh calculus is between 15% and 20% by dry weight (Lieverse 1999: 220). Because of the organic component, which contains carbohydrates, lipids, and proteins, as well as taxa from the microbiome, both stable carbon and nitrogen analyses are possible. The inorganic portion of calculus is largely made up of calcium phosphates, which are present in solution in saliva to protect the teeth from consumed acidic materials (Salazar-García et al. 2014: 71). These phosphates include several minerals also present in the bioapatites present in bone, such as brushite, whitlockite, and hydroxyapatite (Poulson et al. 2013: 4577). The composition of calculus is the consequence of a number of local environmental factors, both inside and outside of the oral cavity. These include the composition of an individual's diet and genetic factors controlling the production and composition of saliva (Lieverse 1999). Calculus is assumed to accumulate until it is manually removed. Within the last half-decade, studies have come out both advocating for (Eerkens et al. 2014; Poulson et al. 2013; Scott and Poulson 2012) and against (Salazar-García et al. 2014) the use of dental calculus in stable isotope analysis.

In the pilot study for the method, Scott and Poulson (2012) found that a number of medieval Spanish burials yielded $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values consistent with those previously reported in studies utilizing collagen. Additionally, a sample from an Inuit individual exhibited results consistent with expectations (i.e. heavy $\delta^{15}\text{N}$ values associated with extreme focus on marine based proteins and depleted $\delta^{13}\text{C}$ values indicative of a vegetable diet devoid of C_4 plants), and with values reported from a study of Greenlandic Inuit fingernail samples. Conversely, a study by Poulson et al. (2013) returned

unexpected results. Individuals from seven Precolumbian sites in Chile yielded abnormally high $\delta^{15}\text{N}$ values. The authors indicate that the fixation of nitrogen in organisms living in arid environments through the excretion of nitrogen depleted urea, as indicated by previous studies could partially explain the high $\delta^{15}\text{N}$ values in the ultra-arid coastal sites (Poulson et al. 2013: 4581). They suggest that another possible source of high $\delta^{15}\text{N}$ values may have been the use of seabird guano, specifically as a fertilizer. Rather than representing a weakness of the method, the unexpected results point out its strength at potentially identifying anomalous patterns.

Salazar-García et al. (2014) added collagen based stable isotope analysis to test the accuracy of the results obtained from calculus samples from the same individuals. Their results raised a number of interesting questions, as well as some implications as to the proper scale of analysis for paleodietary studies using data derived from dental calculus. The authors found a much wider range for the $\delta^{13}\text{C}$ values of dental calculus (range=13.9‰) than for either bone or dentin derived collagen (range=2.5‰ and 3.5‰ respectively) (Salazar-García et al. 2014: 73). As this wider range suggests, a number of calculus samples were pronounced outliers. Eerkens et al. (2014) point out a number of reasons for the differences between collagen and calculus derived stable isotope values. According to their findings, calculus tends to be depleted in $\delta^{13}\text{C}$ values, but enriched in $\delta^{15}\text{N}$ compared to collagen (Eerkens et al. 2014: 69). Future studies are necessary to better understand the degree and reliability of this enrichment and depletion.

Carbon isotopes may be depleted because they go through a fractionation process in their incorporation into corporeal tissues, like collagen, or apatite, while a portion of the carbon represented in calculus is derived from unprocessed plant material. The

enrichment of the nitrogen values most likely comes from the bacterial activity in the oral cavity (Eerkens et al. 2014: 69). Additionally, the authors identified intra-individual differences, with different isotopic values between calculus sampled taken from different teeth. Despite these discrepancies, the authors find that the mean isotopic values of calculus and those of collagen and apatite match well. This agrees with the findings of both Scott and Poulson (2012), who found similarities between observed calculus values and expected values based on previous isotopic studies of collagen, and Salazar-García et al. (2014), whose mean isotopic values of calculus and collagen were virtually identical. Thus, calculus based paleodietary studies are most useful at the inter-site level, using mean values of groups to establish changes over space or through time. As a final note, Eerkens and coauthors point out that the outliers in the Salazar-García et al. (2014) study had C/N ratios higher than 12 suggesting possible sample degradation. The inclusion of these samples inflated the width of the range of the calculus samples.

Recently, Price et al. (2018) examined the properties of the organic and mineral fractions of calculus in an early first millennium B.C. Greek colony in modern Turkey. Price et al. (2018: 98) found that in their set of archaeologically-derived calculus samples, the organic fraction was ~3.4%, as opposed to the 15% - 20% noted by Lieveise (1999). Further investigation is required to test whether this finding is consistent across populations, but it represents an important step in understanding the composition of archaeological calculus.

In addition to the organic fraction, Price et al. (2018) use a method developed for isolating structural carbonate from biogenic apatite to separate what they call the “mineral” fraction. In this effort, they found no correlation between the mineral fraction

and bioapatite obtained from the same individuals. While it is not surprising that calculus derived $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values differ from those of collagen given the highly specific source from which collagen derives its carbon and nitrogen, apatite derives its carbon from the bulk diet, so it is interesting that the isotopic signatures of the mineral fraction of calculus did not correspond more closely with the bioapatite values.

On the other hand, if bone apatite represents the fractionated $\delta^{13}\text{C}$ signature of the entire diet, then removal of the organic fraction may have altered the initial signature in a manner not currently understood. They conclude that “Since the latter data ($\delta^{13}\text{C}$ values of bioapatite) are well known to be useful indicators of paleodiet, we infer that isotopic analyses of dental calculus do not, at least at this site, provide useful paleodiet information” (Price et al. 2018: 101). What the lack of correlation between the mineral fraction of calculus and bioapatite may suggest is that the carbon found in each is not derived from the same source. In other words, it is premature to assume that the source of carbon in the mineral fraction of calculus is analogous to the mineral fraction of bone, and therefore premature to reject calculus as a proxy for paleodiet based on the lack of correlation between the two. Without further tests on the correlation of bulk calculus, which may be a better representative of bulk diet, and bioapatite, the question remains open.

For a pilot study on the paleodiet of El Opeño, calculus is an excellent material for three reasons. First, the removal of dental calculus from teeth does not alter the teeth in any way. While sampling procedures for bioapatite and collagen require the removal and destruction of a small amount of bone, individuals sampled for dental calculus remain completely intact. Second, analysis of calculus is less expensive in terms of time and

materials required to carry it out. Third, the question of whether the inhabitants of El Opeño had shifted from low-level food production and exploitation of a diverse diet, to agriculture and a diet focused on C₄ plants is not dependent on picking up fine-grained changes in $\delta^{13}\text{C}$ values that might be obscured by the admittedly wider ranges of values observed between individuals from one site. The use of dental calculus for this study allows for the greatest amount of data to be obtained, with no damage to the osteological remains in the most economical way.

2.1 West Mexico Materials

Calculus from the West Mexico population was sampled at the Dirección de Antropología Física at the Museo Nacional de Antropología in Mexico City. Individuals had to meet three criteria for inclusion in the study: 1) a provenience of Formative period or earlier, 2) a sufficient quantity of calculus to carry out the analysis (approximately 10 mg), and 3) the integrity of the remains would not be compromised by the sample extraction process. These were the same general criteria used for the collection of the other sample populations. Additionally, the use of preservatives such as polyvinyl alcohol (PVA), which are common in older museum collections, was considered as a potential source of carbon contamination but there appeared to be none.

Of note in the West Mexico population was the general lack of calculus for the earliest populations from the region. Fewer than 25% of the individuals who met the first criterion also satisfied the second. Ultimately, 18 samples from 16 individuals were collected from two sites in West Mexico.

Fifteen of the individuals sampled are from the El Opeño collection. The burials from the shaft tombs of El Opeño are generally well preserved. While there were a number of primary burials recovered from the site, all 15 individuals represented in this study were part of nine commingled burials from Tombs 5 and 7. One individual was sampled from the Presa de La Villita collection, which was excavated in the late 1960s ahead of the placement of a large dam in the Lower Balsas Valley on the border between the states of Guerrero and Michoacán. Unlike the burials at El Opeño, which were placed in tombs, the individuals at La Villita were buried in graves. The time span for Presa La Villita is much wider than that of El Opeño, with no known absolute dates for the burials

themselves. There are clear signs of occupations during the Middle and Late Formative period and again during the Postclassic at the La Villita site (Pulido 2003); however, the individual sampled is likely from the Formative occupation given that it was recovered as a primary burial in an extended position, with no metal grave goods. The La Villita population was generally in worse condition than that of El Opeño, and the individual sampled was the only one with a sufficient amount of calculus to extract without completely depleting the material present on the teeth.

2.2 Nevada Stable Isotope Lab Samples

In all, the global population consists of 474 individuals from 14 populations (see Table 2.1) after eliminating replicate analyses of the same individuals. Where replicate analyses occurred, the average of the observations was taken and used as an individual data point. The reasoning behind this action was to prevent double sampling. Where two or more observations on the same individual were taken, the resulting information gives a more precise measurement of an individual, but not a more accurate representation of the population as a whole. All 474 individuals were collected by members of the University of Nevada, Reno anthropology department, or their collaborators and were processed by Dr. Simon Poulson at the Nevada Stable Isotope Lab.

Samples were drawn from around the world, often from museum collections (Figure 2.1). . Several came from medieval-style cemeteries from subarctic to temperate climatic zones. Samples from the tropics came from various environmental contexts. Those from South America are associated with diverse contexts, from arid valleys to

characteristic, high-precipitation tropical conditions. Some of the samples are comprised of multiple sites but were condensed when appropriate. Calculus samples from the Modern sample, previously reported by Dorio (2012), were collected from local residents of Reno, Nevada during routine dental cleanings.

Table 2.1. Populations in the Nevada Stable Isotope Lab set

Population	N	Source
Basque	57	Scott and Poulson 2012
Chile	28	Poulson et al. 2013
Colombia	19	this study
Greenland Inuit	29	this study
Greenland Norse	10	this study
Hohokam	3	this study
Iceland	14	this study
Maya	73	Harvey 2018
Modern	33	Dorio 2012
Palau	8	this study
Peru	36	this study
Portugal	59	this study
UK	89	this study
West Mexico	16	this study



Figure 2.1. Location of samples from the Nevada Stable Isotope Lab set

3.1 Methods

Sampling was conducted with a dental explorer used to scrape calculus deposits from the teeth into small vials, which were used to transport the samples to the Nevada Stable Isotope Lab at the University of Nevada, Reno. Tools were cleaned by rinsing them in clean water and wiping them down with a clean cloth between samples. Samples were taken either from anterior teeth or posterior teeth, where possible. Calculus samples were double rinsed in deionized water and dried overnight at 50° C. Then they were pulverized using a stainless steel, piston style mortar and pestle. The mortar was cleaned with methanol between samples to avoid contamination. Approximately 4 mg of powdered sample from each individual were measured into 4x6 mm tin capsules, along with three empty capsules as controls and 12 capsules were loaded with a standard (acetanilide, C₈H₉NO). The quantity of sample to be used in the analysis was determined through pilot analyses, which gave a preliminary idea as to the carbon and nitrogen concentrations of the samples. When possible, more material was utilized in populations with pilot analyses indicating a higher ratio of carbon to nitrogen. Samples, blanks, and standards were then combusted and analyzed in a Micromass Isoprime stable isotope ratio mass spectrometer.

In addition to the bulk calculus analysis, powdered samples from 11 individuals of the West Mexico population was placed in silver capsules and filled with 50 ml of deionized water before being placed in a glass desiccator with concentrated HCl. As the calculus in the oral cavity is porous and directly exposed to post-depositional processes, the HCl treatment was designed to test for the influence of non-organic carbon (mainly carbonates) on the $\delta^{13}\text{C}$ values of the bulk calculus samples. Other methods for the isolation of the organic fraction of calculus, such as Price et al. (2018), have utilized

intact chunks of calculus to minimize surface area exposure to acid. For the current study, to avoid damage to the organic component of the calculus while maximizing exposure of intrusive carbonates to acid, powdered sample was enclosed in the desiccator, allowing HCl vapor to combine with the water in the silver capsules and gently react with carbonates in the samples. After allowing them to react for 36 hours, the samples were dried and the silver capsules were rolled up and encapsulated in tin capsules and processed as before in the Micromass Isoprime mass spectrometers. The data from the West Mexico population were added to data from four other populations. Summary statistics on the global population, and data analysis were calculated using the GUI RStudio (RStudio Team 2016), with the packages *psych* (Revelle 2018), *dplyr* (Wickham et al. 2017), and *knitr* (Xie 2018). Plots were produced using the package *ggplot2* (Wickham 2016).

4.1 West Mexico Results

Table 4.1 shows the results of stable isotope analysis of the 16 individuals from the West Mexico population. The weight % carbon has a mean value of 2.11% (range = 0.85% - 3.40%) and a mean $\delta^{13}\text{C}$ -14.8‰ (range = -20.0‰ - -10.9‰). Given the extremely low weight % nitrogen in the West Mexico samples (mean = 0.13%, range = 0.04% - 0.40%), it was not possible to derive $\delta^{15}\text{N}$ values of the individuals.

Table 4.1. Individual data from bulk calculus in the West Mexico population

Individual	Site	Weight % Carbon	Weight % Nitrogen	Atomic C/N	$\delta^{13}\text{C}$
PSO 1	El Openo	1.30	0.06	25.50	-12.10
PSO 2	El Openo	1.62	0.07	26.22	-13.41
PSO 3	El Openo	1.13	0.11	12.07	-13.84
PSO 4	El Openo	2.15	0.13	19.10	-16.98
PSO 5	El Openo	0.85	0.04	22.63	-18.80
PSO 6	El Openo	1.30	0.05	31.48	-15.21
PSO 7	El Openo	3.40	0.40	9.86	-15.03
PSO 8	El Openo	1.20	0.10	13.60	-14.92
PSO 9	El Openo	1.60	0.11	16.95	-12.71
PSO 10	El Openo	2.52	0.25	11.69	-13.48
PSO 11	El Openo	2.83	0.21	15.54	-17.07
PSO 12	El Openo	3.29	0.12	32.49	-19.95
PSO 13	El Openo	2.24	0.18	14.91	-10.88
PSO 14	El Openo	2.10	0.10	24.03	-13.47
PSO 15	El Openo	3.15	0.14	27.17	-17.73
PSO 16	La Villita	3.12	0.04	88.67	-11.41

The individual from Presa La Villita was the most suspect of the samples, with a C/N ratio of 88.67. There is little information concerning the conditions of the

burials at La Villita, although their excavation was part of a salvage project carried out ahead of the placement of a dam in the lower Balsas drainage. The El Opeño individuals were selected from the collection resulting from excavations in Tombs 5 and 7 during the 1991 field season of Oliveros (2009). These tombs were intact and displayed no signs of looting or disturbance after they were sealed following their use.

While the seals were intact on the tombs, diagenetic alteration remains a likely explanation for the low levels of carbon and even lower levels of nitrogen in the samples given the assessment of post-depositional flooding events by Oliveros (2009: 36). Diagenesis could reflect either post-depositional additions or subtractions to the dental calculus leading to alteration through the transference of minerals in solution with the precipitation, particularly nitrogen poor, isotopically heavy minerals, such as carbonates.

To correct for the addition of environmental carbonates, when there was a sufficient quantity of dental calculus sample remaining, individuals were reanalyzed after weak acid treatment (see Table 4.2). This acid treatment was meant to isolate the organic fraction of the calculus from the mineral portion because the former is less susceptible to diagenesis. With respect to the West Mexico population, the acid treatment affected the carbon portion of the samples more than the nitrogen. This is expected, as the desired outcome of the treatment process was to remove the inorganic carbon compounds that may have contaminated the samples following deposition.

In this aspect, the outcomes of the treatment were uniform. The weight percent carbon was lowered in all individuals, with an average change of -0.79% . Additionally, acid treatment resulted in lighter $\delta^{13}\text{C}$ values in every sample in the West Mexico population with an average shift of 5.5‰ (average of -13.7‰ in the bulk calculus

Table 4.2. Bulk calculus and organic calculus data by individual for West Mexico

Individual	Weight % C		Weight % N		C/N		$\delta^{13}\text{C}$	
	Bulk	Organic	Bulk	Organic	Bulk	Organic	Bulk	Organic
PSO-01	1.30	0.83	0.06	0.10	25.25	10.07	-12.1	-19.0
PSO-03	1.13	0.75	0.11	0.10	12.07	8.57	-13.8	-18.2
PSO-05	0.85	0.68	0.04	0.05	22.63	16.18	-18.8	-22.1
PSO-06	1.30	1.13	0.05	0.09	31.48	14.48	-15.2	-22.4
PSO-08	1.20	0.94	0.10	0.11	13.60	9.61	-14.9	-18.8
PSO-09	1.60	0.87	0.11	0.10	16.95	10.68	-12.7	-17.5
PSO-10	2.52	1.42	0.25	0.18	11.69	9.30	-13.5	-17.8
PSO-13	2.24	1.07	0.18	0.18	14.91	6.93	-10.9	-15.8
PSO-14	2.10	1.26	0.10	0.13	24.03	11.05	-13.5	-19.5
PSO-16	3.12	0.52	0.04	0.06	88.67	9.62	-11.4	-21.0

samples and -19.2‰ in the organic, acid-treated calculus samples). The effects on the nitrogen portion of the samples were negligible. The percent of nitrogen in the organic samples was higher in most cases (6 samples out of 10), but the overall change in the average percent of nitrogen was .006% lower than in the bulk calculus samples. As a result, the level of nitrogen in the organic calculus samples of the West Mexico individuals was still too low to yield $\delta^{15}\text{N}$ data.

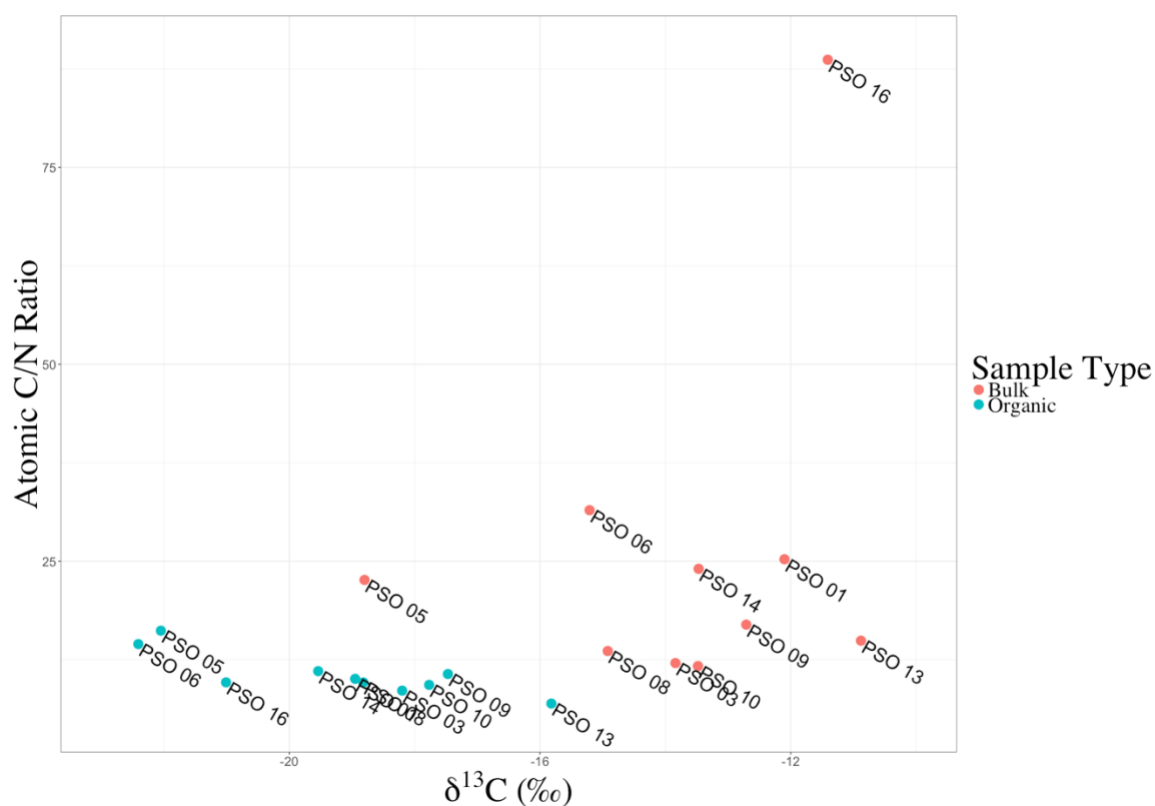


Figure 4.1. Effects of HCl acid treatment on C/N ratio and $\delta^{13}\text{C}$ values by individual

The reduction in the carbon content of the samples resulted in lower atomic C/N ratios in every sample. The greatest shift in atomic C/N ratio was in the individual from La Villita. This individual, with a bulk calculus C/N value of 88.70 was lowered to 9.62 after acid treatment. Two explanations are possible. First, there was an error in

The initial weighing, or notation of the bulk sample (i.e., a mistranscribed digit, or an improperly zeroed scale). Second, the bulk sample from this individual was heavily influenced by nitrogen poor materials, such as carbonates, or a large chunk of nitrogen poor organic material. Whatever may explain that specific example, the overall trend in the samples is towards lower C/N ratios in the organic calculus when compared to the bulk calculus, with an average shift of -15.5 (-8.4 when the La Villita outlier is taken out).

4.2 Nevada Stable Isotope Lab Results

To test whether isotopic differences in diet are retained in stable isotope values of calculus, West Mexico data are compared to those of the other Nevada Stable Isotope Lab samples (see Table 4.3).

Table 4.3. Mean data on bulk calculus by population

Population	N	Weight % Carbon	Weight % Nitrogen	Atomic C/N	C/N SD	Above C/N of 12	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$ SD	$\delta^{15}\text{N}$ N	$\delta^{15}\text{N}$ N/A
Basque	57	4.91	0.77	7.65	1.56	1/57	-21.2	0.98	11.8	0
Chile	28	11.91	1.41	9.55	2.70	4/28	-17.6	1.74	24.0	0
Colombia	19	3.98	0.40	13.87	4.72	11/19	-18.1	1.08	8.2	0
Greenland Inuit	29	7.01	1.16	7.21	0.77	0/29	-18.2	0.88	19.9	0
Greenland Norse	10	7.19	0.82	10.78	4.41	2/10	-23.3	1.49	14.6	0
Hohokam	3	3.20	0.42	9.82	2.60	1/3	-13.0	1.92	7.8	1
Iceland	14	4.37	0.60	8.84	1.75	1/14	-23.0	0.56	11.6	0
Maya	73	3.59	0.32	15.13	9.92	42/73	-16.2	2.59	8.6	53
Modern	33	25.04	5.49	5.37	0.40	0/33	-19.6	1.38	6.9	0
Palau	8	2.76	0.19	18.51	6.15	8/8	-15.4	0.72	11.2	7
Peru	36	6.12	1.12	6.76	1.78	1/36	-14.7	1.76	13.3	20
Portugal	59	3.92	0.34	16.68	13.23	34/59	-18.6	2.07	6.5	21
UK	89	4.28	0.64	8.01	1.16	1/89	-21.6	0.59	12.4	4
West Mexico	16	2.11	0.13	24.49	18.54	14/16	-14.8	2.66		16

4.3 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

4.3.1 Carbon Heavy Group

Figure 4.2 shows a boxplot of the bulk calculus $\delta^{13}\text{C}$ values for the 14 populations tested by the Nevada Stable Isotope Lab. There are some readily discernible patterns that divide the populations along geographic lines. The heaviest mean $\delta^{13}\text{C}$ values are associated with populations located in the New World.

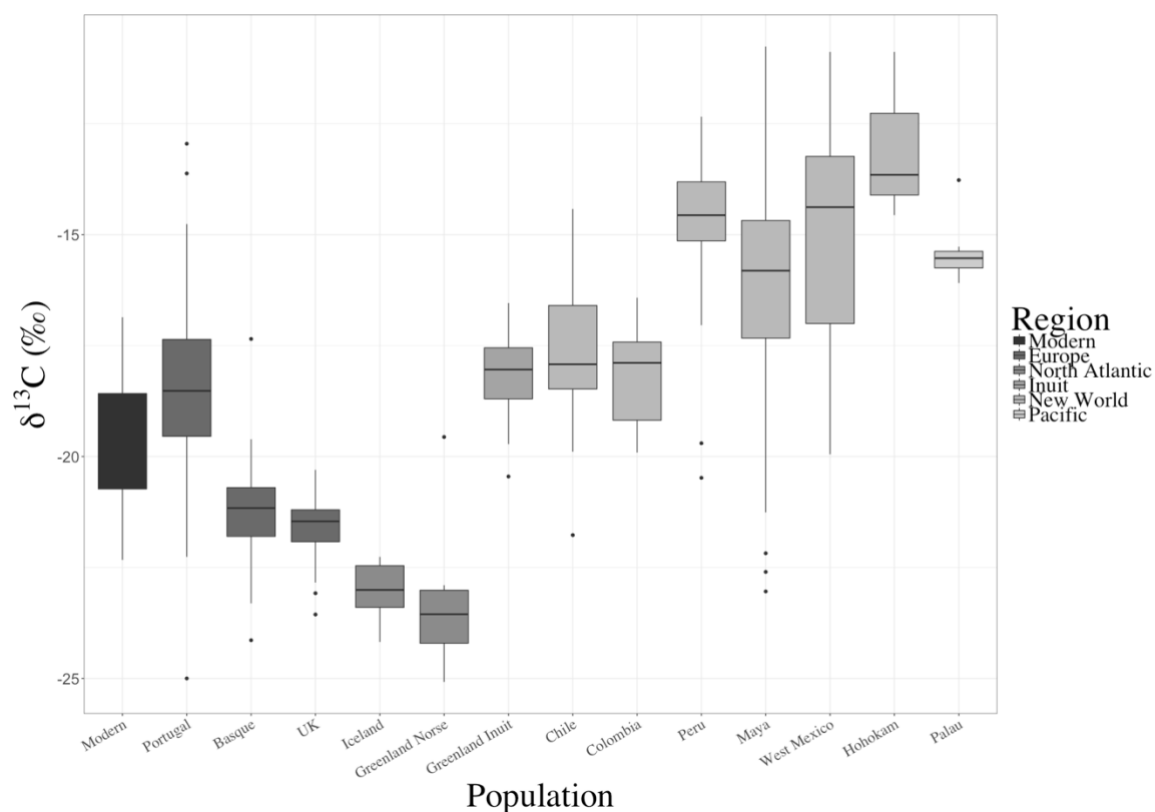


Figure 4.2. $\delta^{13}\text{C}$ values of bulk calculus by population

The Hohokam exhibit the heaviest $\delta^{13}\text{C}$ values at -13.0‰ (Table 5.2). As a maize growing, agricultural society, located in southern Arizona, these results are reasonable; however, the extremely small sample size ($N=3$) should be noted. The individuals from Peru were excavated from Vegachaoq Moqo, a site related to the Middle Horizon dating between AD 600 - 1000 (Balvin Vilcahuaman 2013). This sample is the next heaviest

with a mean $\delta^{13}\text{C}$ value of -14.7‰. Isotopic studies on collagen at the nearby and contemporaneous site of Conchopata yielded average $\delta^{13}\text{C}$ value of -10.7‰ with a range between -13.2‰ and -8.2‰ (Finucane et al. 2006: 1770). While the calculus derived values are slightly lighter, they still reflect a diet high in C_4 plants given the collagen values at Conchopata and the similar calculus values of the Hohokam maize agriculturalists.

The $\delta^{13}\text{C}$ values (mean = -14.8‰) for West Mexico are most like those of the Peru and Hohokam samples, indicating that the values at El Opeño align with known maize agriculturalists, and C_4 plants likely comprised a large proportion of the diet at the site. The Palau and Maya populations could also be included in this group with mean $\delta^{13}\text{C}$ values of -15.4‰ and -16.2‰ respectively.

4.3.2 Carbon Light Group

On the other side of the carbon spectrum, there is a clear grouping between the two European populations of the North Atlantic. The Icelandic and Greenlandic Norse samples have $\delta^{13}\text{C}$ values lower than -20. These medieval groups date between the 10th and 15th centuries A.D. The Greenlanders and Icelanders were pastoralists, mixing limited C_3 crops with marine and terrestrial meat resources.

Previous studies on the $\delta^{13}\text{C}$ of the Icelandic diet (Sveinbjörnsdóttir et al. 2010) show collagen $\delta^{13}\text{C}$ values range from -20.1‰ to -16.4‰ (mean = -19.13‰); Nelson et al. (2012) found a similar range of -18.9‰ to -14.1‰ (mean = -16.0‰) for collagen from the Greenlandic Norse populations. These studies focused on the potential for $\delta^{13}\text{C}$ values to identify differences in the protein component of the diet, specifically whether it was

possible to quantify the proportion of the diet made up of marine based proteins, such as fish and seals, in relation to terrestrial proteins, like caribou and domesticated sheep. As noted by Chisholm et al. (1992) in their study British Colombian native Americans, heavier $\delta^{13}\text{C}$ values indicated a greater reliance on marine resources. Sveinbjörnsdóttir et al. (2010: 694) found that, according to collagen $\delta^{13}\text{C}$ values, the Icelandic samples in their study overlapped with those of previous studies on the Greenlandic Norse (Arneborg et al. 1999), while exhibiting a lighter mean $\delta^{13}\text{C}$ value. This trend is reversed in the calculus derived data of the current study, although the values are practically identical (-23.3‰ mean for the Greenlandic Norse and -23.0‰ for the Icelanders). Still the calculus results preserve the greater range of the values in the Greenlandic Norse ($\sigma = 1.49$) than in the Icelanders ($\sigma = .56$), as demonstrated in previous studies (Sveinbjörnsdóttir et al. 2010).

The Basque and United Kingdom (UK) samples form a European group (in which Portugal is included because of its geographic proximity to the other two) that resembles, to a great extent, the North Atlantic group. At -21.6‰ and -21.2‰, the mean $\delta^{13}\text{C}$ values for the UK and Basque populations cluster with slightly lighter values than those of the North Atlantic group. While the time span for the Basque population ranges from the 12th to the 19th century AD (Scott and Poulson 2012: 1389), based on the heavy mean $\delta^{13}\text{C}$ value, greater access to C_4 plants that accompanied Spanish exploration of the New World presumably did not have a drastic impact on the diet. The Portugal sample is an outlier in several respects. In terms of context, it is the oldest population, with some of the individuals dating to the European Neolithic. Regarding $\delta^{13}\text{C}$ values, their range is much higher ($\sigma = 2.07$) than almost every population, with the exception of West

Mexico ($\sigma = 2.66$).

4.3.3 Carbon Intermediate, Nitrogen Heavy Group

The groups in the center of the distribution of $\delta^{13}\text{C}$ values reflect more than one dietary pattern. While values between the two extremes of C_3 and C_4 primacy as dietary staples could indicate populations whose diets consisted of a mix of C_3 and C_4 plants, marine protein resources affect the $\delta^{13}\text{C}$ values of collagen. The most securely interpretable sample from this group is the Greenlandic Inuit population, which demonstrates this phenomenon. With a mean $\delta^{13}\text{C}$ value of -18.2‰ , the Inuit fall almost exactly between mean values represented in this study. Colombia, Chile, Portugal and the Modern populations all group with the Inuit population in the middle to some extent. Differences in $\delta^{15}\text{N}$ values are subtler than those of the $\delta^{13}\text{C}$ values (Figure 4.3). When the $\delta^{15}\text{N}$ is added to the $\delta^{13}\text{C}$ data, the samples with $\delta^{13}\text{C}$ values between the two extremes form two additional groups (Figure 4.4).

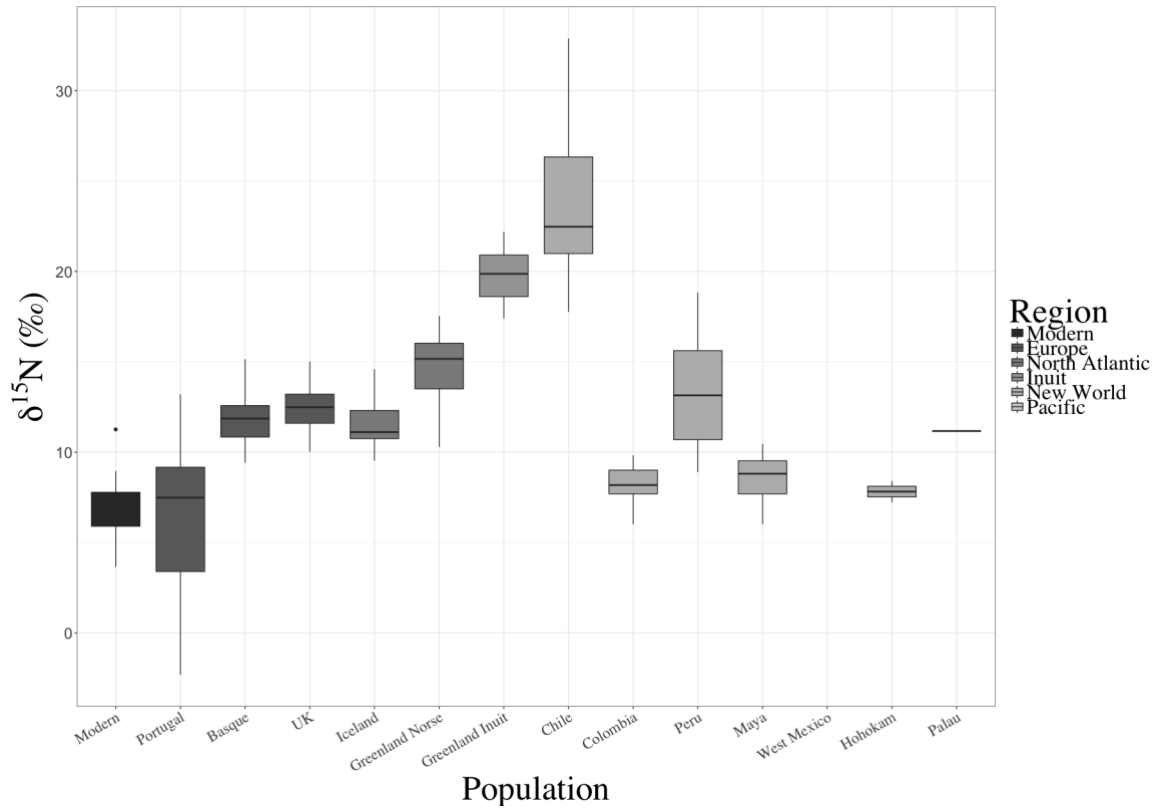


Figure 4.3. $\delta^{15}\text{N}$ values of bulk calculus by population

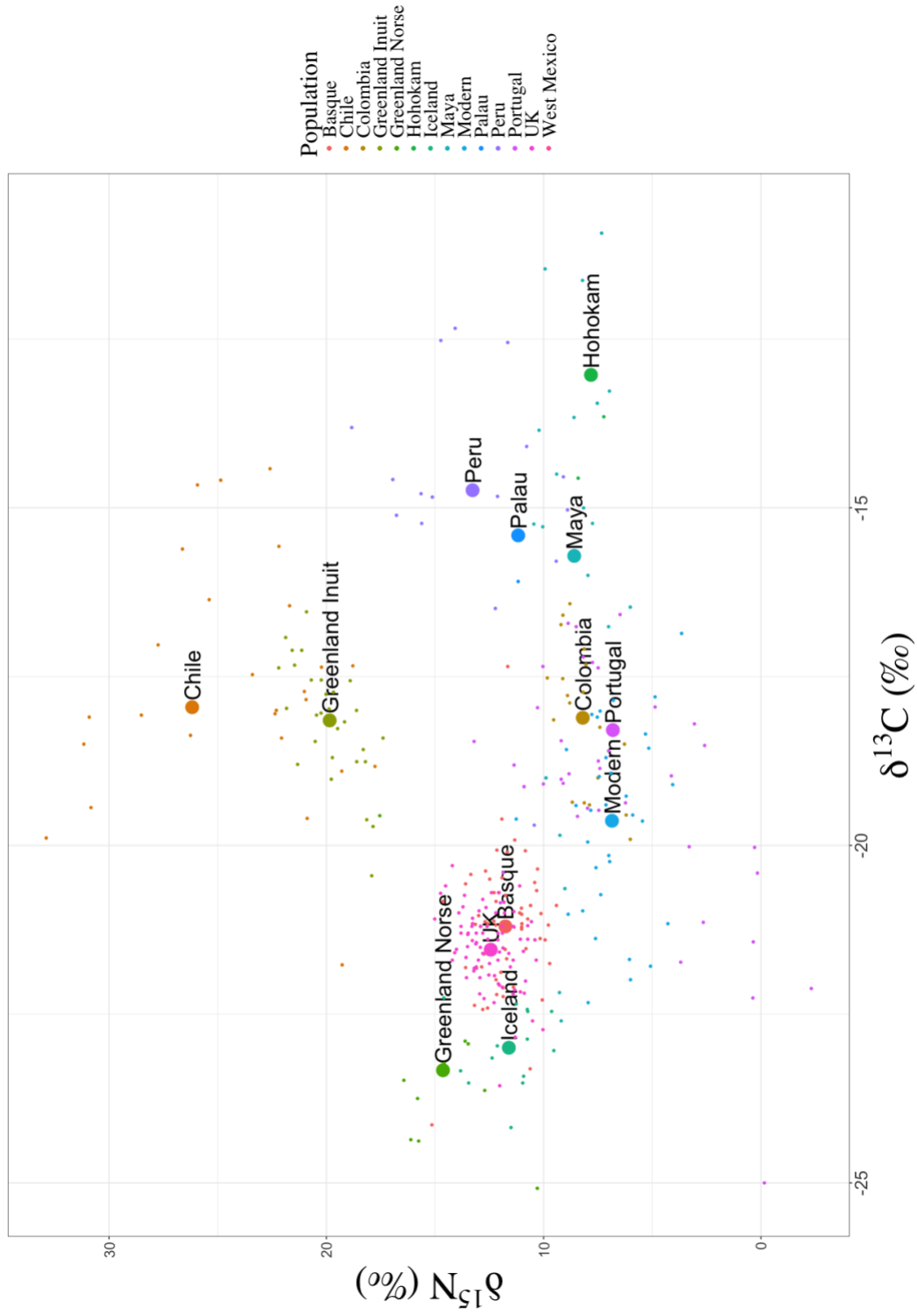


Figure 4.4. Scatter plot of all bulk calculus samples with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with markers at population means

The Inuit and Chilean populations form a group with intermediate $\delta^{13}\text{C}$ values and high $\delta^{15}\text{N}$ values. These two populations have the heaviest isotopic nitrogen values of the 14 populations, supporting the idea that marine protein consumption is responsible for $\delta^{13}\text{C}$ values lighter than those evident in the proposed C_3 group. Poulson et al. (2013) proposed an alternative explanation for the relatively light $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ values. The temporal distribution of the Chile population is wide, including sites dating from 2300 B.C. to A.D. 1476 (Poulson et al. 2013: 4578). The earlier sites most likely did not consume C_4 plants in large quantities, but maize played a significant role in the diet of the later sites. Islands covered by thick deposits of guano off the Chilean and Peruvian coasts were tapped for fertilizer because of the extremely high nitrogen content of guano (Poulson et al. 2013: 4585). While there is no archaeological evidence for pre-Columbian use of guano as a fertilizer, modern cultivation experiments with guano demonstrate significantly heavier $\delta^{15}\text{N}$ values for in the resulting plant tissue, elevated as much as +44.7‰ (Szpak et al. 2012: Table 3). Where there is an approximately 3‰ enrichment of $\delta^{15}\text{N}$ from one trophic level to the next (Schwarcz and Schoeninger 1991: 299), an enrichment of 45‰ in nitrogen is a jump of almost fifteen trophic levels.

4.3.4 Carbon Intermediate, Nitrogen Light Group

Three samples are neither heavily skewed towards the light C_3 signatures of North Atlantic and European groups nor the presumed maize eating populations from the New World. First, they are separated from the Inuit and Chilean populations by their low $\delta^{15}\text{N}$ values. The central position of the Modern population on the x axis is unsurprising given the ready access to both C_3 and C_4 plants. Hair and fingernail samples from the same

individuals were ~2‰ heavier on average than the corresponding calculus samples, indicating a diet with mixed C₃ and C₄ components (Dorio 2012: 69). The Colombia sample likely derived calories from both C₃ and C₄ plant sources and does not exploit high trophic level protein sources. Portugal, as noted, is anomalous in several ways. Two individuals exhibited negative δ¹⁵N values and four more individuals have values < 1. The explanation for these low values is unclear. Colombia, given its location on the x-axis around -18.2‰, near that of Chile (-17.6‰), suggests this population may have had a mixed subsistence economy, with C₄ plants providing some input into the diet, rather than the heavier δ¹³C resulting from marine protein, as the mean δ¹⁵N value for the population is among the lowest in the global sample (+8.2‰).

4.4 Atomic C/N Ratio

Eerkens et al. (2014: 70) suggest that calculus samples should have an atomic C/N ratio of 12 or under if they are to be used in stable isotope analysis. Results including all samples are reported, followed by observations on individual samples falling below the suggested threshold of 12. Although both weight % carbon and weight % nitrogen were depleted in the West Mexico samples compared to other populations, they were more depleted of nitrogen than they were of carbon. This is in accord with the high atomic carbon to nitrogen (C/N) ratio, which is the highest of all global populations (mean = 24.49, range = 9.86 – 88.67), with 14 of 16 individuals exhibiting C/N ratios higher than 12. Unsurprisingly, the lowest C/N values come from the Modern sample with a mean C/N ratio of 5.37. Modern calculus C/N ratios come close to approximating those of proteins. Dorio (2012: 59) proposes that the anomalously low C/N ratios were the result

of high concentrations of biofilm remaining in the calculus samples. The biofilm found on the surfaces of teeth, known as plaque, consists of bacteria, fungi, and additional microorganisms, as well as their byproducts, along with proteins, amino acids, and carbohydrates (Hillson 1996: 255). The low C/N ratios of the Modern samples indicates dead bacteria and other organic materials contributed more to the observed carbon and nitrogen concentrations than is the case in archaeological calculus samples. As proteins and the simpler amino acids are nitrogenous by nature, they exhibit C/N ratios in line with those of collagen, the protein in bone that is the standard biomaterial for stable isotope analysis in paleodietary studies.

In the total sample, 120 of the 474 individuals have C/N ratios over 12. Of the 14 populations processed at the Nevada Stable Isotope Lab, nine have means below the C/N ratio of 12. While the geographic patterns apparent in the $\delta^{13}\text{C}$ results are not as strong in the atomic C/N ratios, it appears likely that geography plays some role (Figure 4.5). When the samples are grouped latitudinally as opposed to regionally (i.e., inside or outside the tropics), the pattern makes more sense. Both the Basque and UK populations fall well below the criterion of 12 with mean values of 7.65 and 8.01 respectively; only one individual from each sample falls above the acceptable threshold. The mean C/N ratios for the Icelandic and the Greenlandic Norse samples also fall well below the proposed standard of 12 indicating good preservation of the samples.

It is unknown to what extent post-depositional processes cause diagenetic alteration to fully mineralized dental calculus. Presumably, time since deposition and post-depositional environment play key roles in the degradation of organic compounds in calculus, thereby impacting C/N ratio; however, no systematic studies of diagenesis in

dental calculus have been undertaken to date.

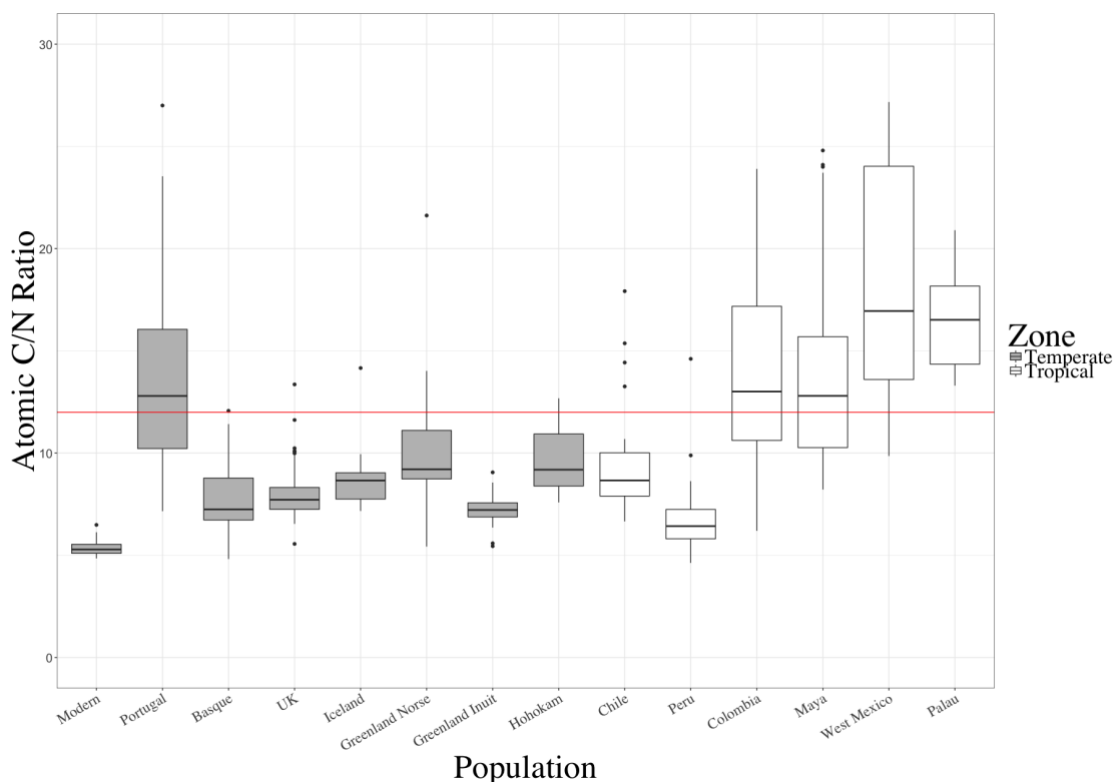


Figure 4.5. Atomic C/N ratio of bulk calculus by region. The line represents the benchmark of 12

Of the nine populations with mean C/N values below 12, only the populations from Peru and Chile are in the tropics. The hypothesis of Poulson et al. (2013) on the consumption of plants in contact with nitrogen rich materials could explain the low C/N ratios of both the Chile and Peru samples, as both locations were known historically for their use and export of fertilizer (Poulson et al. 2013: 4583). The alternative explanation for the low atomic C/N ratios in these two populations is that, although they are both located within the tropic zone, they are both from arid or semi-arid valleys on the Pacific coast of South America.

Portugal is the only sample outside the tropics that has a mean C/N ratio over 12 (mean = 16.68, with 34 of 58 individuals over 12 and the widest range of C/N values

of any population). There is obviously an issue with the integrity of the Portuguese calculus samples, as this was the only population that had individuals with negative $\delta^{15}\text{N}$ values. Explanation of these results clearly requires attention. Of the two individuals with negative $\delta^{15}\text{N}$ values, one of them has a C/N ratio above the acceptable threshold with a value of 27, but the C/N ratio of the other is within acceptable parameters at 8.12. While simply a hypothesis at this point, the most parsimonious explanation for the high mean C/N value for the Portugal population is the extreme age of the samples.

4.5 Correlation

Correlation tests serve as one avenue of investigation into the effects of post-depositional alteration on the integrity of bulk calculus samples. The assumptions behind these correlation tests are that higher C/N ratios indicate a higher level of diagenetic change through the addition of carbonate rich materials, and that a strong correlation between C/N ratio and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values may indicate a dependence of the latter variables on the former. In other words, the question is: are $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values reflecting post-depositional alteration rather than dietary signatures?

Table 4.4 shows the results of these tests. When all samples are considered regardless of C/N ratio, there is a slight negative correlation ($r = -0.16$, $p < 0.01$) between $\delta^{15}\text{N}$ and atomic C/N ratio, while the correlation between $\delta^{13}\text{C}$ and C/N is positive ($r = 0.29$, $p < 0.01$, see Figures 4.6a and 4.6b). After eliminating the samples with atomic C/N values greater than 12, both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ correlations to C/N ratio flatten and the resulting p-values indicate that the correlation is not significant. Recognizing that the Modern

population with its anomalously low C/N values may influence the correlation, another set of correlations was performed on archaeological calculus, excluding the Modern population. The result is a slightly negative correlation between $\delta^{15}\text{N}$ and C/N values ($r = -0.19$, $p < 0.01$, Figure 4.7a). There is no significant correlation between the $\delta^{13}\text{C}$ values and C/N in archaeological bulk calculus samples where the C/N ratio is ≤ 12 (Figure 4.7b).

Table 4.4. Correlation tests on bulk calculus

Test	r	r ²	p-value
All Individuals $\delta^{15}\text{N}$ to C/N (Fig. 4.6a)	-0.16	0.03	<0.01
All Individuals $\delta^{13}\text{C}$ to C/N (Fig. 4.6b)	0.29	0.09	<0.01
Individuals ≤ 12 $\delta^{15}\text{N}$ to C/N	0.04	<0.01	0.44
Individuals ≤ 12 $\delta^{13}\text{C}$ to C/N	-0.01	<0.01	0.91
Archaeological Samples $\delta^{15}\text{N}$ to C/N	-0.30	0.09	<0.01
Archaeological Samples $\delta^{13}\text{C}$ to C/N	0.29	0.08	<0.01
Archaeological Samples ≤ 12 $\delta^{15}\text{N}$ to C/N (Fig. 4.7a)	-0.19	0.04	<0.01
Archaeological Samples ≤ 12 $\delta^{13}\text{C}$ to C/N (Fig. 4.7b)	-0.03	<0.01	0.64

* Archaeological samples include all populations except Modern

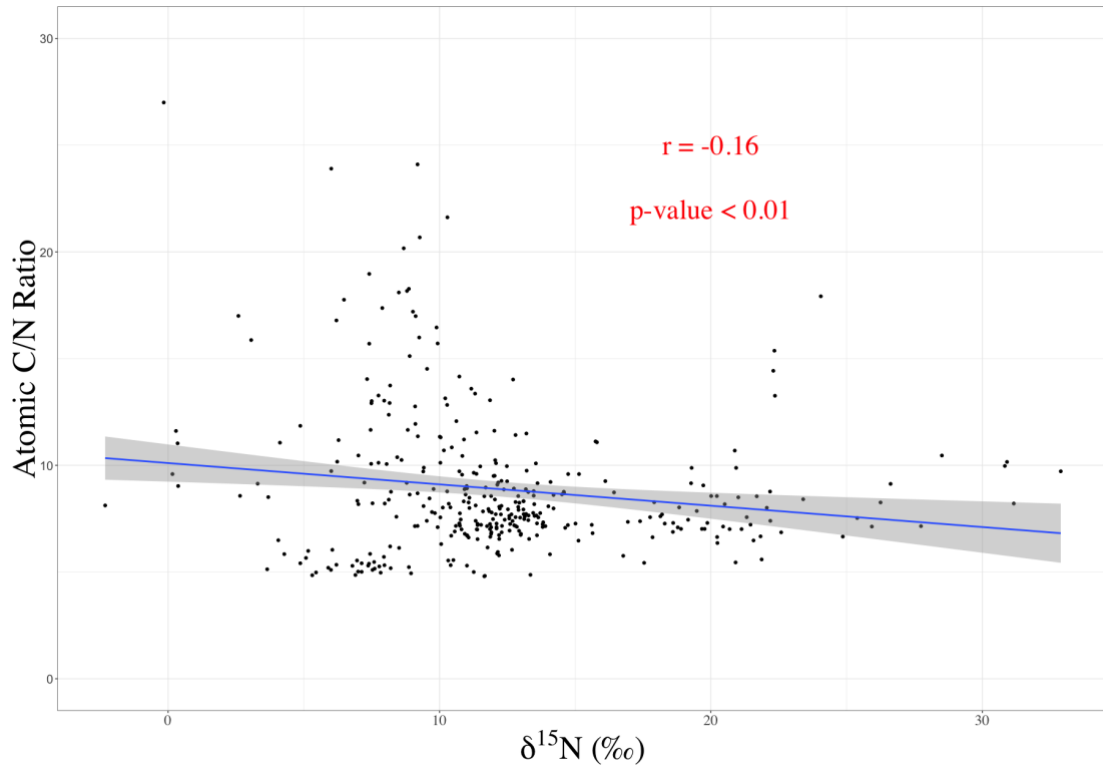


Figure 4.6a. Linear regression between atomic C/N ratio and $\delta^{15}\text{N}$ values for all samples

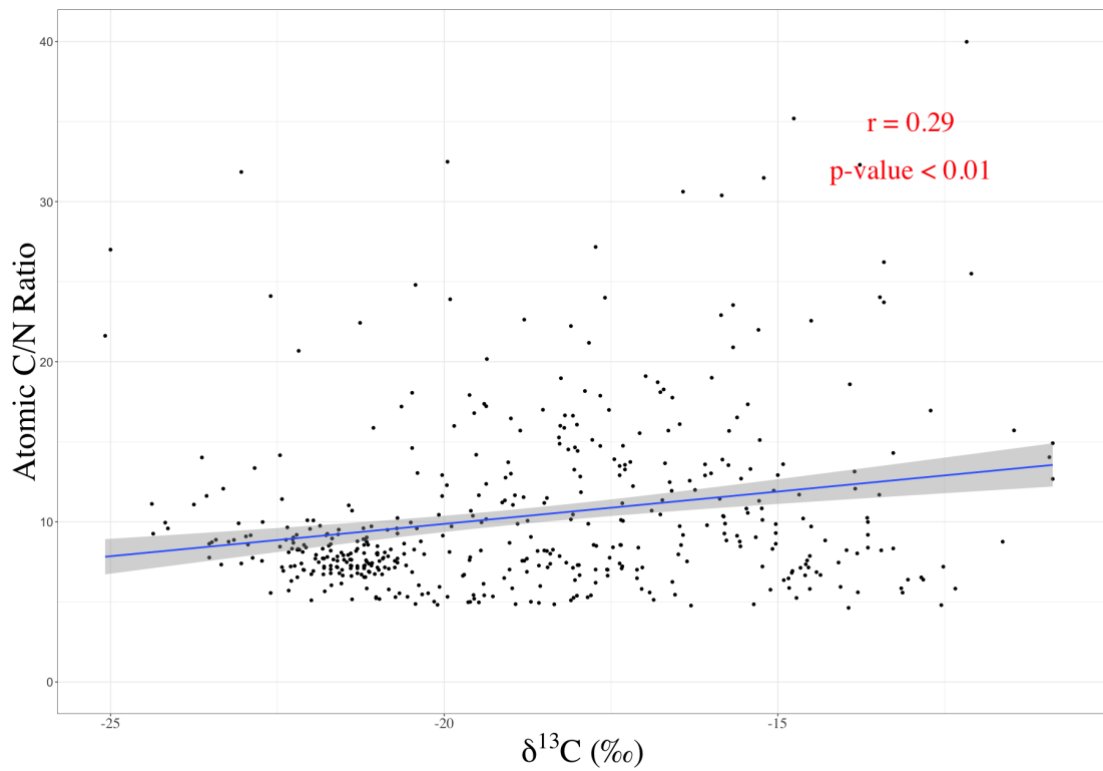


Figure 4.6b. Linear regression between atomic C/N ratio and $\delta^{13}\text{C}$ values for all samples

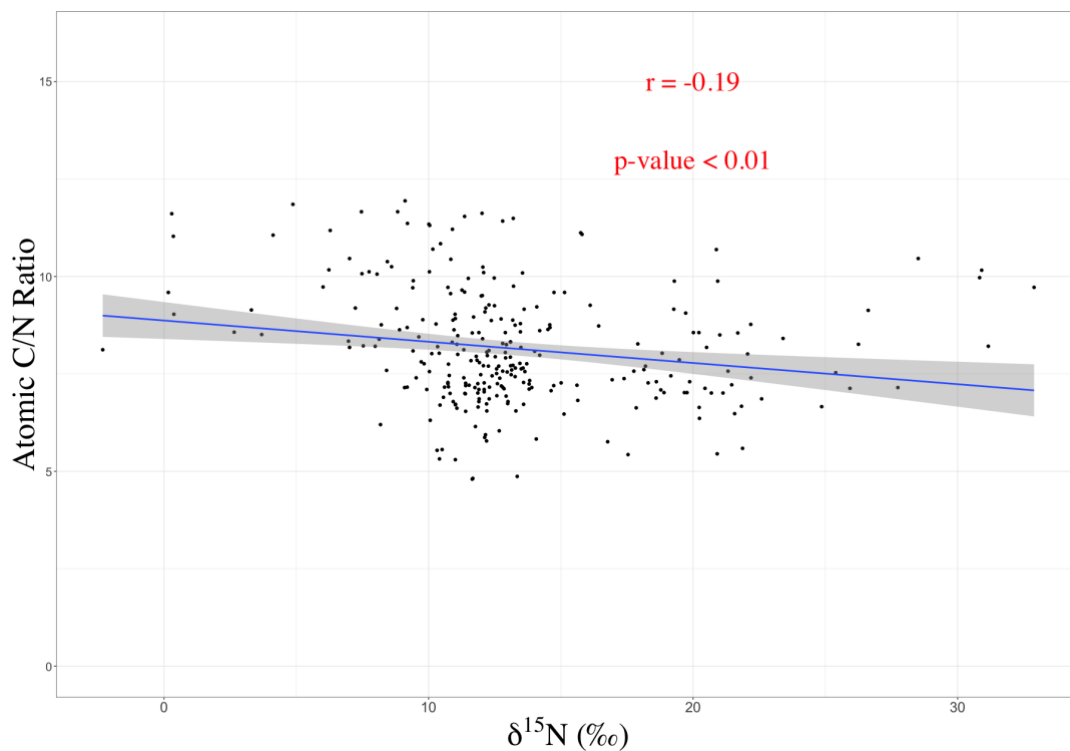


Figure 4.7a. Linear regression between atomic C/N ratio and $\delta^{15}\text{N}$ values for all samples with $\text{C/N} \leq 12$, excluding Modern

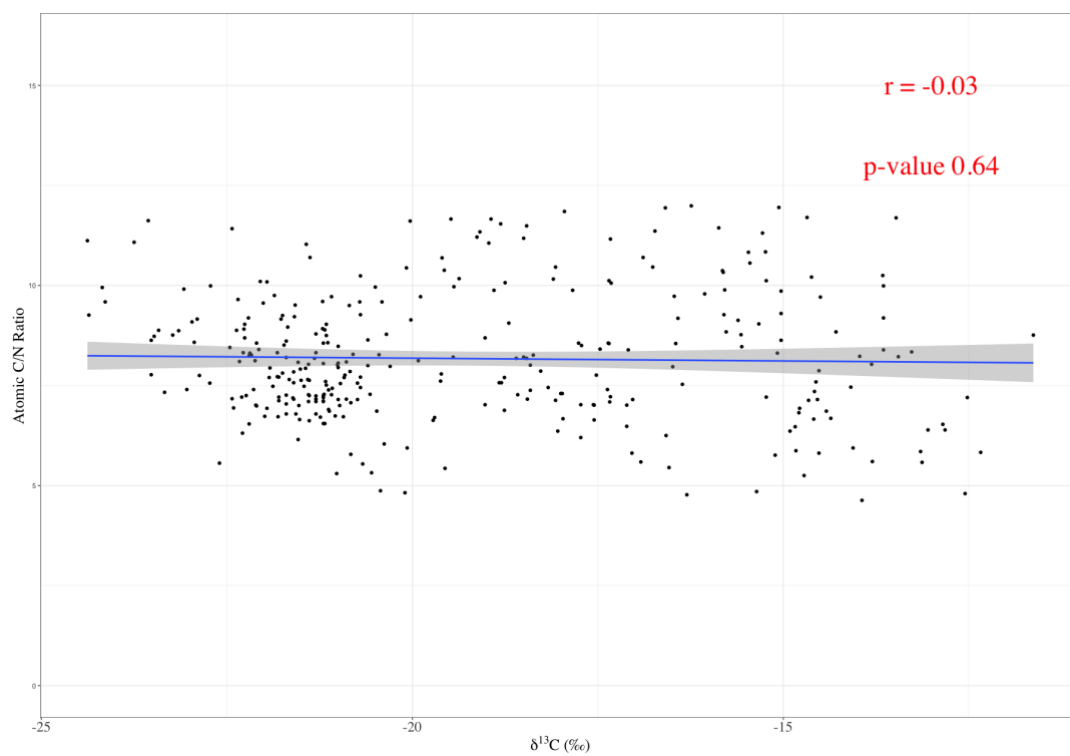


Figure 4.7b Linear regression between atomic C/N ratio and $\delta^{13}\text{C}$ values for all samples with $\text{C/N} \leq 12$, excluding Modern

As diagenesis is driven by site specific conditions, correlation tests were performed on the population level, where the number of individuals was greater than 25. Table 4.6 shows the results of correlation tests on the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and C/N ratio in eight of the 14 populations from the Nevada Stable Isotope Lab set.

Table 4.5. Population based correlations to C/N ratio in bulk calculus

Population	N	$\delta^{13}\text{C}$ r	p-value	$\delta^{15}\text{N}$ r	p-value
Basque	57	-0.24	0.07	-0.09	0.52
Chile	28	-0.42	0.03	-0.01	0.94
Inuit	29	-0.51	<0.01	-0.33	0.08
Maya	73	<0.01	0.97	0.42	0.06
Modern	33	-0.20	0.27	-0.30	0.09
Peru	36	-0.49	<0.01	-0.25	0.36
Portugal	59	0.61	<0.01	-0.08	0.65
Portugal ≤ 12	25	0.34	0.10	0.22	0.29
UK	89	-0.34	<0.01	0.09	0.41

$\delta^{13}\text{C}$ values tend to be more highly correlated with C/N values and have lower p-values than the $\delta^{15}\text{N}$ values. The Maya population is of note, as the pattern observed in the other populations (i.e., higher correlation between $\delta^{13}\text{C}$ and C/N ratio with lower p-values, and lower correlation between $\delta^{15}\text{N}$ and C/N ratio with higher p-values).

The correlations between $\delta^{13}\text{C}$ values and C/N ratio are all negative, with the exception of the Maya and Portuguese samples. This result is opposite the expectation that higher C/N ratio indicates diagenetic addition of carbon, where the most likely contaminant is isotopically heavy carbonate material. Instead, this suggests that in most populations in the Nevada Stable Isotope Lab set, higher C/N ratios are correlated with lighter $\delta^{13}\text{C}$ values, indicating a lack of influence of post-depositional added carbonate.

The positive correlation between $\delta^{13}\text{C}$ and C/N ratio in the Portugal population provides a further indication that diagenesis has occurred in those samples in a meaningful way. When Portuguese samples with a C/N ratio > 12 are excluded from the analysis, correlation between $\delta^{13}\text{C}$ and C/N ratio drops. This reduction suggests that correlation between C/N ratio and $\delta^{13}\text{C}$ values can be a useful indicator of the level of diagenetic contamination of bulk calculus samples.

4.6 Acid Treatment

In addition to performing acid treatment on individuals from the West Mexico population, samples from six additional populations were subjected to acid treatment. As with the West Mexico population, individuals from the Hohokam, Iceland, Inuit, Maya, Palau and UK populations were powdered, measured into silver cups, and exposed to HCl vapor. The results obtained from the acid treatment of the other four populations exhibited identical effects to those observed in the West Mexico population (i.e., lower weight % C, lighter $\delta^{13}\text{C}$ values, and lower C/N ratios Table 4.6).

Table 4.6. Bulk and organic calculus data

Population	N	Weight %		$\delta^{13}\text{C}$		C/N	
		C Bulk	C Organic	Bulk	Organic	Bulk	Organic
Greenland Inuit	20	6.84	6.31	-18.1	-19.0	7.33	6.59
Hohokam	3	3.19	2.22	-13.0	-16.3	9.82	6.82
Iceland	3	4.07	3.57	-23.3	-24.9	8.03	6.90
Maya	17	3.90	2.71	-17.1	-18.9	13.94	9.11
Palau	5	2.76	1.42	-15.2	-21.7	19.24	9.13
UK	58	4.29	3.73	-21.4	-23.3	7.53	6.38
West Mexico	10	1.74	0.95	-13.7	-19.2	26.13	10.65

Figure 4.8 demonstrates the reduction in the weight percent of carbon in the organic fraction as opposed to the corresponding samples of bulk calculus, while leaving the weight percent nitrogen largely unaltered. Boxplots (Figures 4.9 a and b) show these effects on the mean weight percentages by population. Again, the shift towards lower weight percentage of carbon is universal across populations, whereas the effects on weight percent nitrogen vary, although the shifts are so small as to be negligible. This observation demonstrates that the nitrogen content of the sample is concentrated in the organic fraction of the calculus.

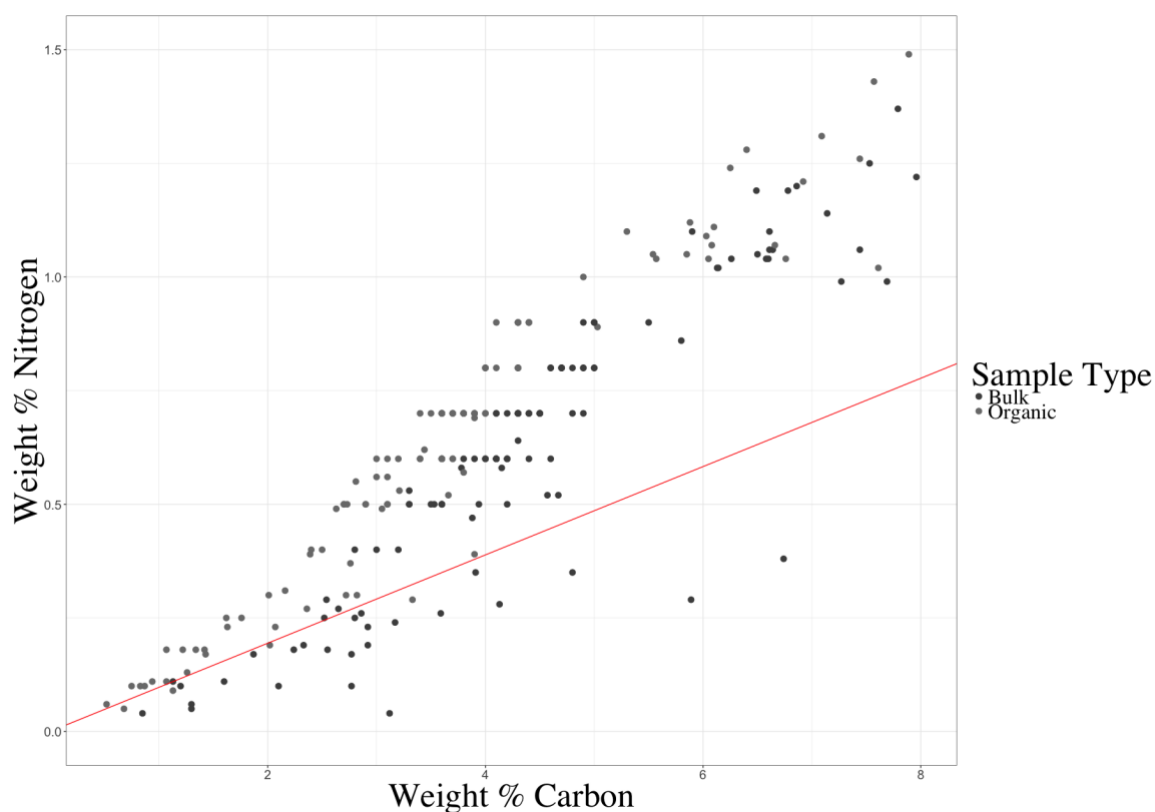


Figure 4.8. Effects of HCl acid treatment on weight % carbon and weight % nitrogen. Red line represents C/N ratio of 12

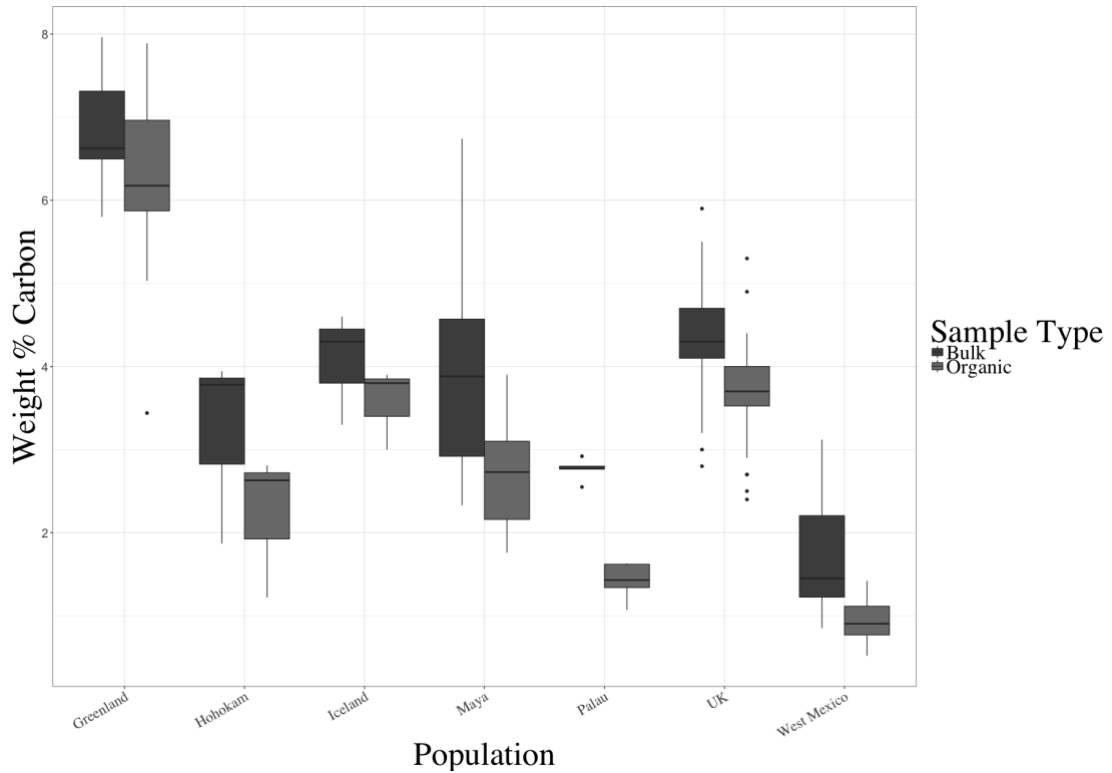


Figure 4.9a. Change in weight % carbon by population

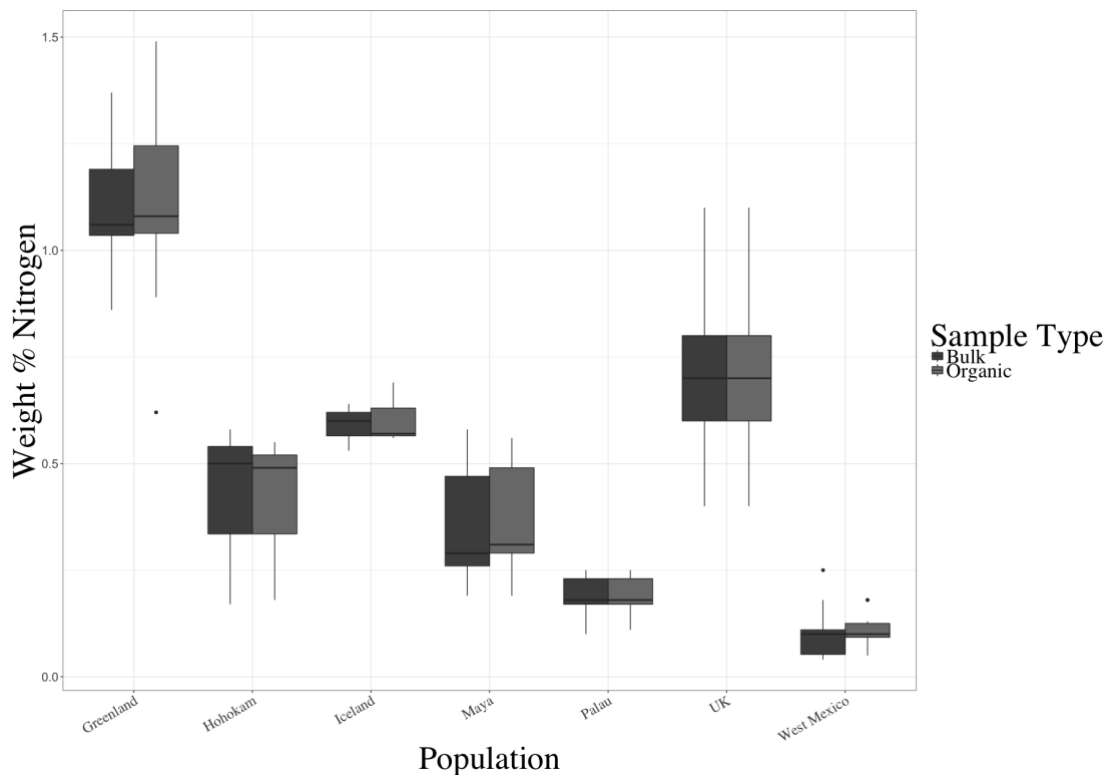


Figure 4.9b. Change in weight % nitrogen by population

Figure 4.10 shows the shift towards lighter $\delta^{13}\text{C}$ and lower atomic C/N values in the organic fraction versus bulk calculus. These effects are universal across populations as well, as demonstrated by Figures 4.11 a and b. Unfortunately, even with lower C/N ratios, few acid-treated samples yielded $\delta^{15}\text{N}$ results with the exception of those samples from the UK population. Acid-treated samples from the Maya population failed to yield $\delta^{15}\text{N}$ data where such values were derived from bulk calculus samples from the same individual. This indicates a minimum quantity of nitrogen is necessary to estimate $\delta^{15}\text{N}$ compositions. Using the median weight percent nitrogen (.61%) from the global population as an arbitrary value, bulk calculus samples with nitrogen content greater than or equal to the median yield $\delta^{15}\text{N}$ values 91% of the time (224/245), as opposed to those below the median, which only yield $\delta^{15}\text{N}$ values 56% of the time (128/229). Variable concentrations of nitrogen in calculus should be taken into account when planning a sampling program, as samples with lower nitrogen concentrations require a greater amount of material to yield data on $\delta^{15}\text{N}$.

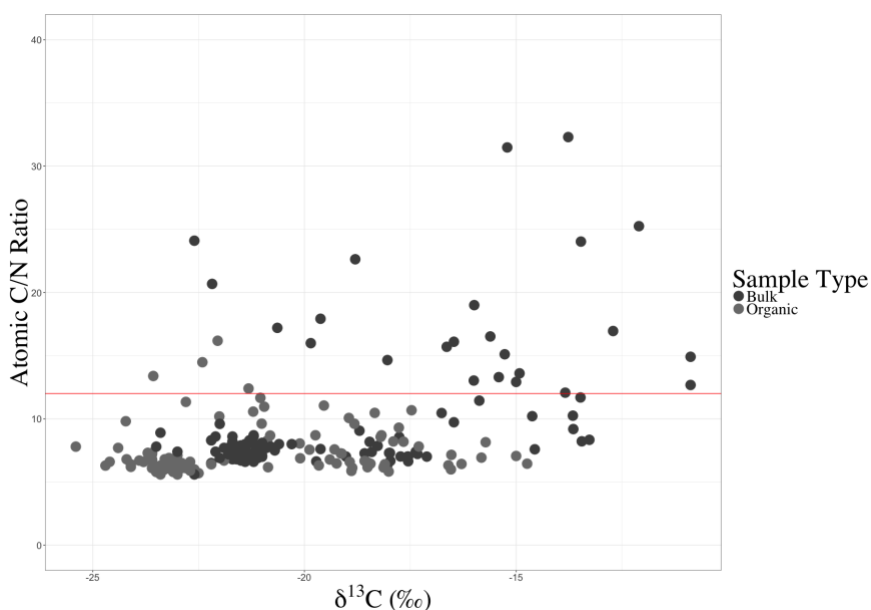


Figure 4.10. Effects of HCl acid treatment on C/N ratio and $\delta^{13}\text{C}$ values

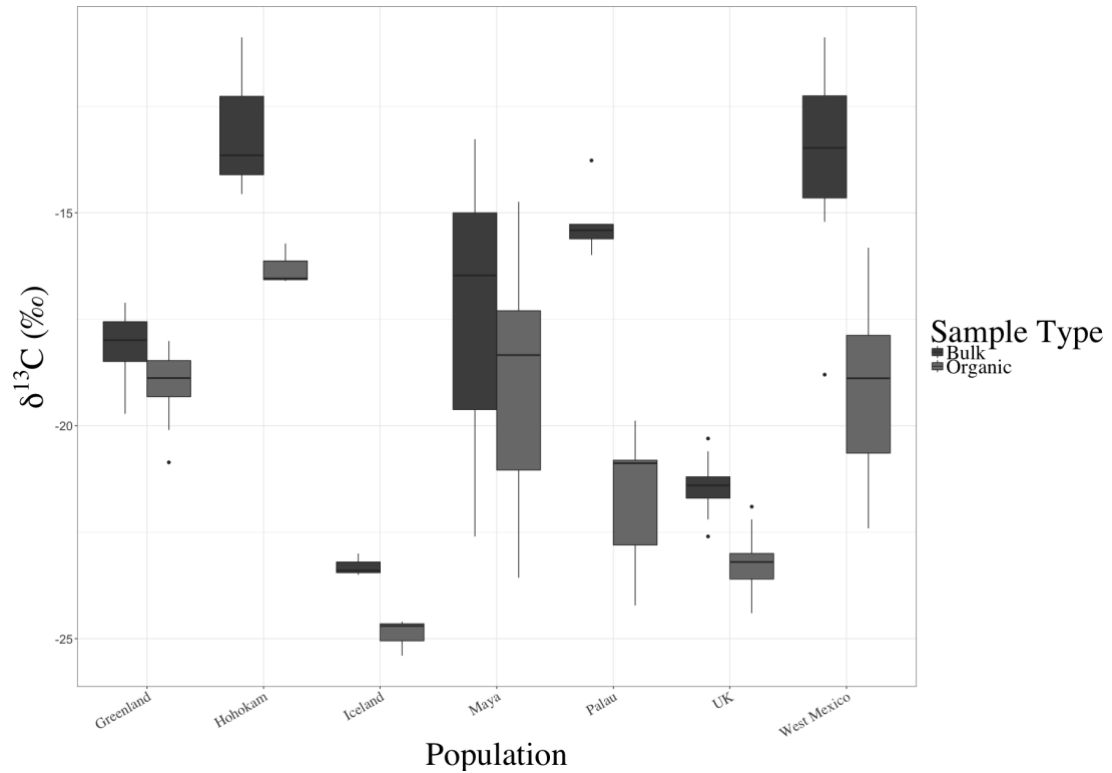


Figure 4.11a. Change in $\delta^{13}\text{C}$ values by population

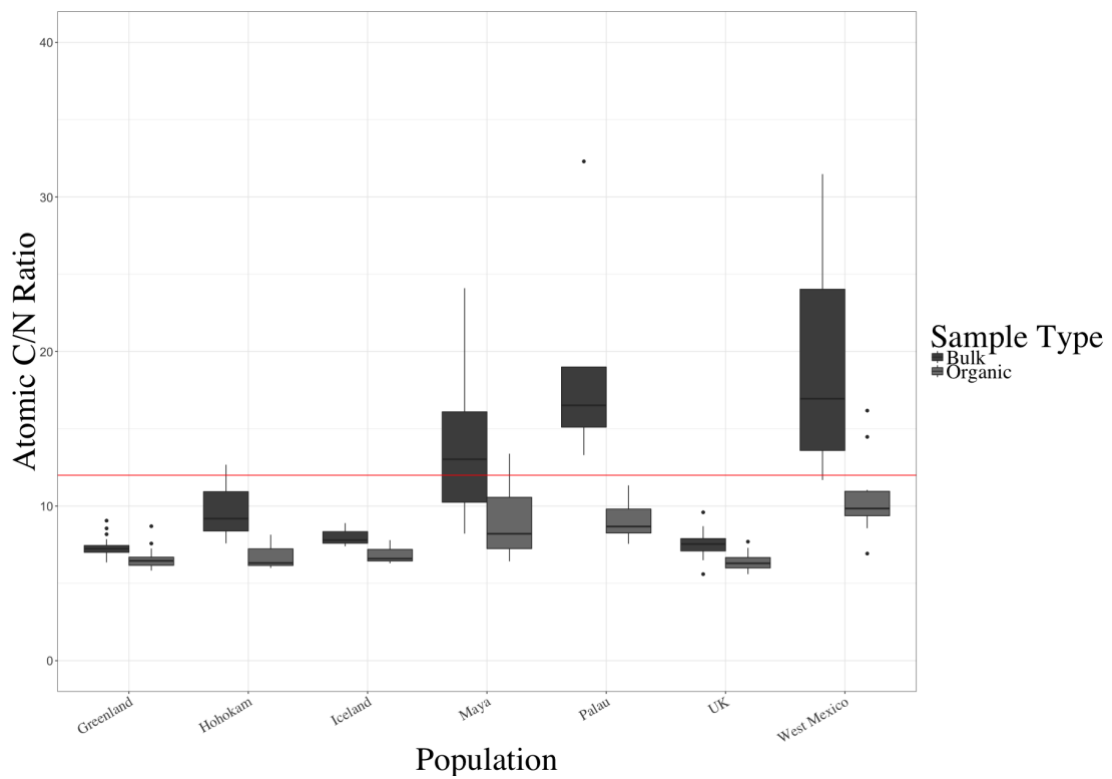


Figure 4.11b. Change in C/N ratio by population

Table 4.7 shows the results of correlation analysis between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and C/N ratios obtained from the acid-treated Inuit and UK samples and the bulk calculus samples of the same individuals. Because one of the outcomes of acid treatment is lighter $\delta^{13}\text{C}$ values, the correlations in the bulk samples, which were already negative, are more pronounced in the acid-treated samples. The UK individuals were the only acid-treated samples to yield $\delta^{15}\text{N}$ data on enough individuals to allow for statistical analysis. These findings will be discussed in greater detail below.

Table 4.7. Correlation with C/N ratio in paired bulk and organic samples

Population	N	Sample	$\delta^{13}\text{C}$ r	p-value	$\delta^{15}\text{N}$ r	p-value
Inuit	20	Bulk	-0.15	0.53	NA	NA
	20	Organic	-0.36	0.11	-0.06	0.8
UK	58	Bulk	0.08	0.53	0.12	0.35
	58	Organic	-0.37	<0.01	<0.01	0.98

4.7 C/N Ratio \leq 12

Figure 4.11 shows that, when individuals with C/N ratios $>$ 12 are eliminated, the regional patterns stay largely the same. The cluster that represents the European and North Atlantic populations, as well as the group that represents the Chilean and Inuit populations are unchanged as few individuals from these large populations had C/N ratios over 12. Peru, Colombia, and Maya samples all shift towards the right of the plot, although the mean $\delta^{13}\text{C}$ value for the Colombia and Maya populations is more pronounced (+0.5‰ shift). The aberrant Portuguese sample shifted to the lighter side of the x axis (-1.4‰ shift).

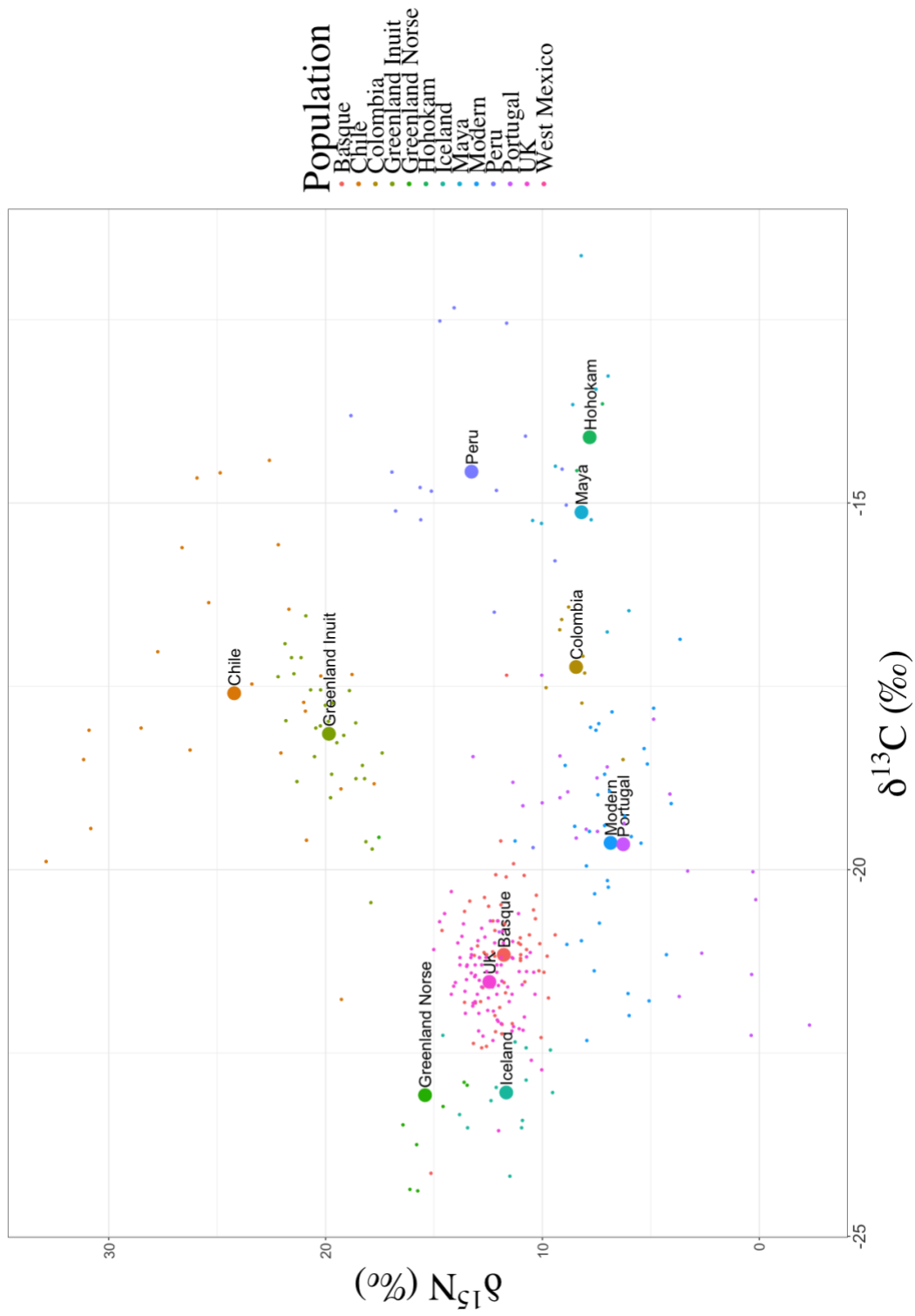


Figure 4.12. Scatter plot of all bulk calculus samples with C/N ratios ≤ 12 with markers at population means

Figure 4.12 demonstrates the trend in $\delta^{13}\text{C}$ values to narrower ranges and heavier means for samples with $\text{C/N} \leq 12$, except in the case of Portugal. As with the acid treatment, the effects of discarding samples with C/N ratios > 12 on the $\delta^{15}\text{N}$ values were not uniform. However, the exclusion of samples with high C/N ratios left the $\delta^{15}\text{N}$ values largely unchanged. The mean values of the 12 populations with $\delta^{15}\text{N}$ data did not change more than 1‰ in either direction. The maximum change in the negative direction is seen for Portugal (-0.5‰), while the Greenlandic Norse show the greatest shift in mean $\delta^{15}\text{N}$ value (+0.8‰, see Figure 4.13). Mean values in weight percent carbon and weight percent nitrogen were similarly unpatterned (Table 4.8). These results suggest carbon and nitrogen concentrations are largely independent of C/N ratio. Furthermore, when taken with the correlation tests mentioned above, $\delta^{13}\text{C}$ values are more drastically impacted by C/N ratios > 12 than $\delta^{15}\text{N}$ values.

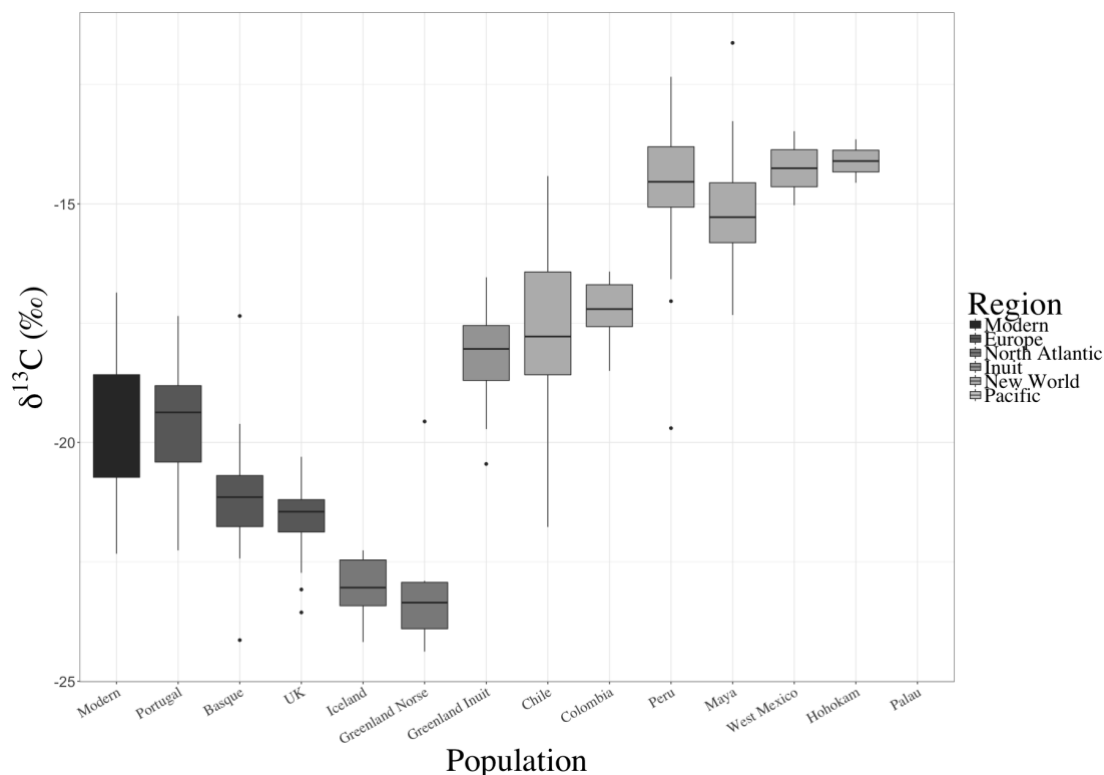


Figure 4.13. $\delta^{13}\text{C}$ values by population for samples with C/N ratios ≤ 12

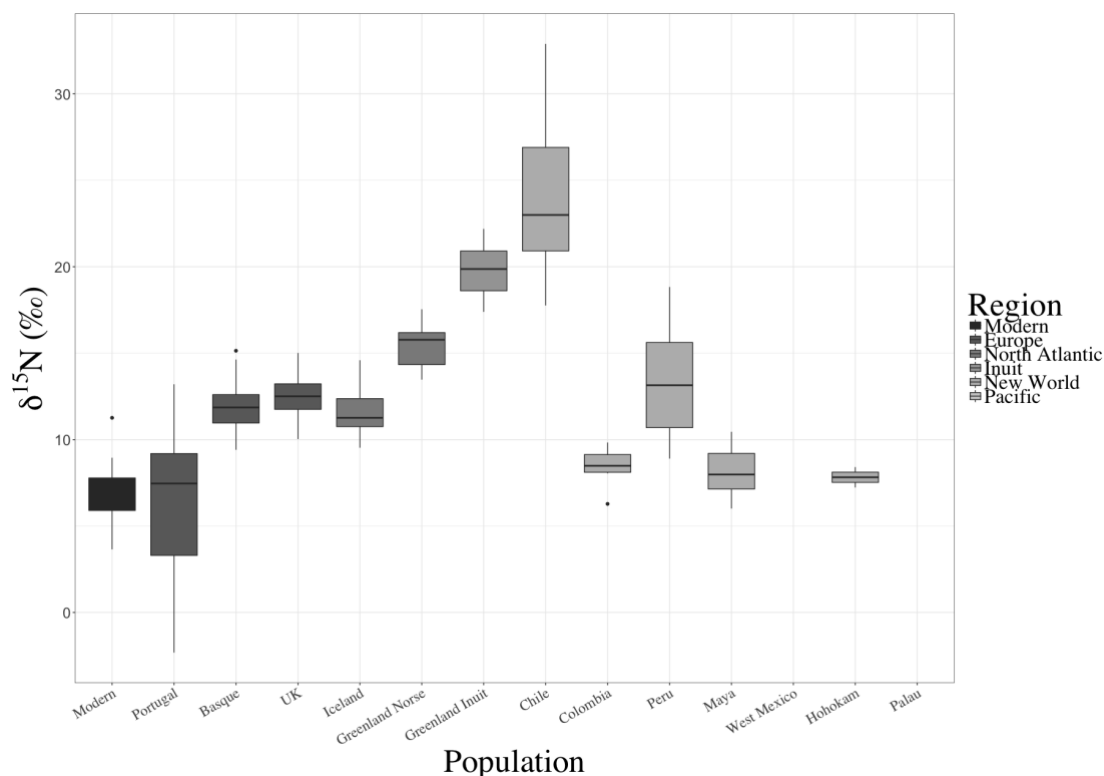


Figure 4.13. $\delta^{15}\text{N}$ values by population for samples with C/N ratios ≤ 12

Table 4.8 demonstrates that acid treatment of samples with bulk calculus C/N ratios ≤ 12 has the same outcomes as it does on all bulk calculus samples. In other words, $\delta^{13}\text{C}$ values in acid-treated samples are not comparable to those obtained from bulk calculus. Mean $\delta^{13}\text{C}$ values from the New World all become heavier when samples with C/N > 12 are excluded from analysis, shifting them closer to a C₄ derived signature, whereas acid treatment universally shifts mean $\delta^{13}\text{C}$ values to the lighter side of the scale. Without controlled experiments investigating the offset of acid-treated calculus samples from bulk calculus samples and, ultimately, from a known diet, it is not possible to speak to which of these mean $\delta^{13}\text{C}$ values are closer to the actual dietary signature of these populations.

Table 4.8. Mean data in samples with $C/N \leq 12$ and the associated organic samples

Sample Fraction	Population	N	$\delta^{13}C$	wt % C	$\delta^{15}N$	wt % N	Atomic C/N
Bulk with $C/N \leq 12$	Hohokam	2	-14.1	3.86	7.8	0.54	8.39
	Iceland	3	-23.3	4.07	11.3	0.59	8.03
	Inuit	20	-18.1	6.84	19.8	1.09	7.33
	Maya	7	-14.9	3.71	NA	0.45	9.81
	UK	58	-21.4	4.29	12.2	0.68	7.53
	West Mexico	1	-13.5	2.52	NA	0.25	11.69
Organic	Hohokam	2	-16.6	2.72	8.8	0.52	6.16
	Iceland	3	-24.9	3.57	NA	0.61	6.90
	Inuit	20	-19.0	6.31	NA	1.12	6.59
	Maya	7	-16.7	2.88	NA	0.47	7.19
	UK	58	-23.3	3.73	12.3	0.69	6.38
	West Mexico	1	-17.8	1.42	NA	0.18	9.30

5.1 Other Calculus-Based Isotopic Studies

Since the study by Poulson et al. (2013) on calculus in Chile, three studies have been published using dental calculus for stable isotope analysis. Salazar-García et al. (2014) looked at paired samples of bulk calculus, and bone and dentin collagen from a Medieval cemetery in Spain. Their analyses found the range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in calculus to be much greater than those in the corresponding collagen samples. They also found $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of calculus were poorly correlated to corresponding collagen samples from the same individuals (2014:74). The authors concluded that isotopic data from bulk calculus cannot be treated as analogous to those obtained from collagen. This is expected as the carbon represented in collagen is derived primarily from the protein portion of the diet (Kellner and Schoeninger 2007: 1112) and there is no reason to assume that calculus formation is constrained by the same biological processes. With respect to their wide range of $\delta^{13}\text{C}$ values in a single sample (Salazar-García et al. 2014), the range of $\delta^{13}\text{C}$ values for all 474 individuals analyzed in this study is 14.3‰ and no single sample has a range greater than the Maya at 12.3‰. When individuals with C/N ratios over 12 are excluded, the Peru population exhibits the widest range at 7.4‰. In the Spanish sample of Salazar-García et al. (2014), the mean $\delta^{13}\text{C}$ value shifts from -15.5‰ to -16.5‰ when samples are sorted based on the $\text{C/N} \leq 12$ benchmark. This new mean value is identical to the mean value the authors reported for bone collagen (Salazar-García et al. 2014: Table 1).

Eerkens et al. (2014) conducted a paired analysis on additional archaeological populations from sites in California and the Sudan. The authors made several observations on their own data and commented on the study of Salazar-García et al.

(2014). Regarding their own samples, they observed that bulk calculus $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ correlated more closely with collagen derived values than with $\delta^{13}\text{C}$ values of bone derived apatites (2014:67). They also note a trend that $\delta^{13}\text{C}$ values tend to be lighter, while $\delta^{15}\text{N}$ values tend to be heavier in bulk calculus compared to collagen derived values. Finally, the authors point out that correlations between calculus derived values and bone derived values gets weaker when atomic C/N ratios go above 12. The authors conclude that the resolution of isotopic analysis of bulk calculus is strongest at the inter-site level (Eerkens et al. 2014: 69). This point is borne out in the current study, where regional groupings become apparent when population means are plotted on the same scatter plot.

Price et al. (2018) found a strong positive correlation between the $\delta^{15}\text{N}$ values in the organic fraction of calculus and the C/N ratio in their population. The authors took this correlation as an indication of diagenetic change to the calculus. A similar test on the organic calculus derived from the UK population did not yield the same results. The correlation between $\delta^{15}\text{N}$ values and C/N ratios was < 0.01 , with a p-value of 0.98. The UK population has a low mean C/N ratio and only one individual with a C/N ratio greater than 12, indicating good preservation at the sites from which the sample were derived. Given these indications of good preservation, the results of the correlation test may indicate lack of post-depositional contamination as suggested by Price et al. (2018). In any case they reinforce the point that these correlations probably vary from site to site and should be investigated in further detail.

Figure 5.1 shows the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the studies discussed above, plotted along with the global samples in this study. The findings of Price et al. (2018) on

the sample from Apollonia Pontica fits well with the regional group designated as “Europe.” It is important to note that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from Apollonia differ in that they are organic fraction calculus values and based on the observations on the acid-treated samples discussed above, the bulk calculus values of the population would be somewhat heavier than those plotted in Figure 6.1. This shift would bring them even closer to the values of the Basque and UK populations.

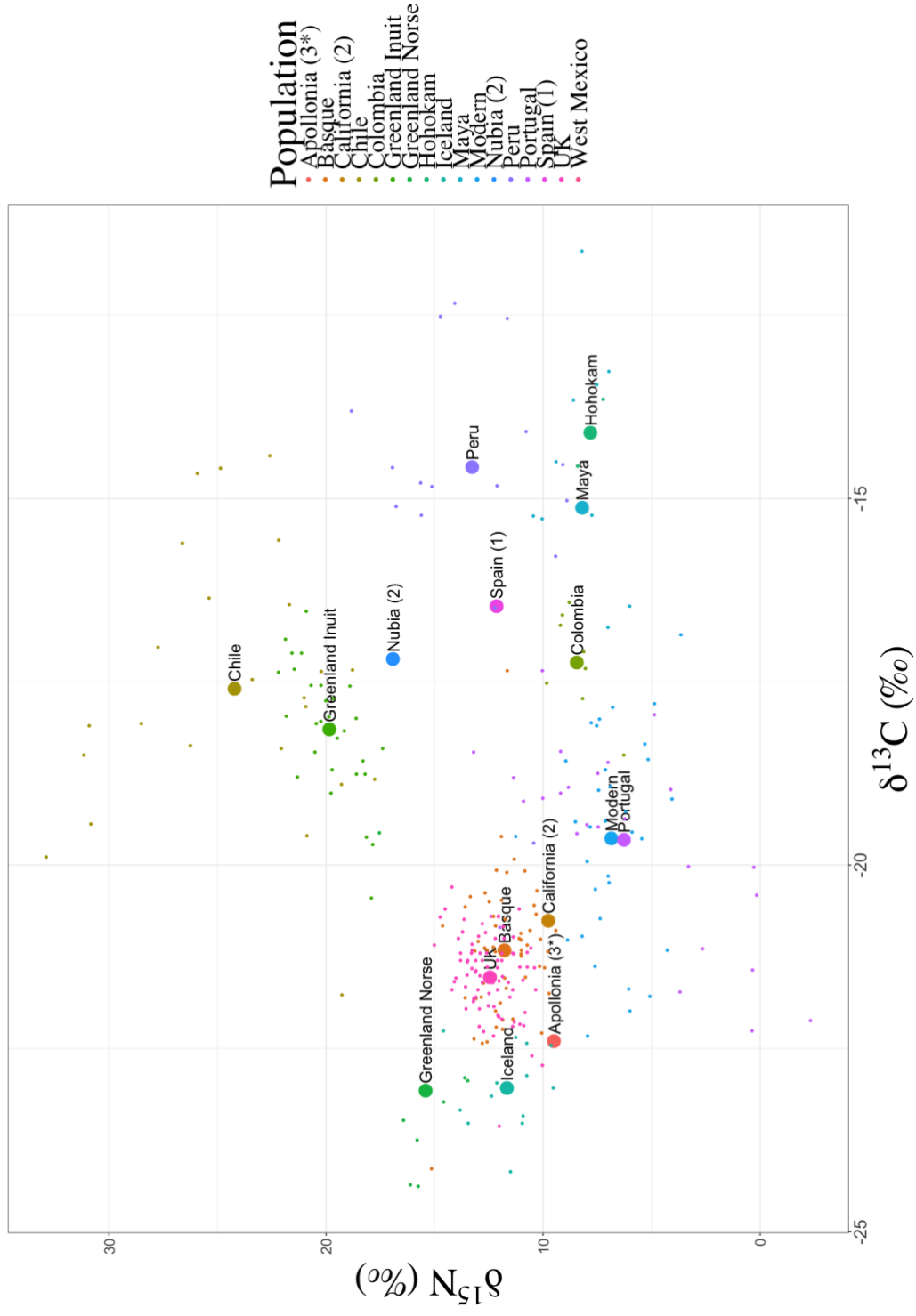


Figure 4.12. Scatter plot of mean values of Nevada Stable Isotope Lab populations with other samples from the literature. All mean values represent samples with C/N ratios ≤ 12 . (1) Salazar-García et al. 2014; (2) Eerkens et al. 2014; (3) Price et al. 2018

The mean values from the sites in California, discussed by Eerkens et al. (2014), fall close to this same group. Based on the observations made in this study, both California and Apollonia conform with populations that do not derive a significant portion of their diet from C₄ plants and do not exploit high-trophic-level animal taxa. The Nubia sample clusters with populations that have been designated as the “carbon intermediate, nitrogen heavy” group. In terms of $\delta^{13}\text{C}$ values, Nubia falls towards the heavier side of the x-axis. Eerkens et al. (2014: 65) identify millet and sorghum as probable contributors to the diet of this population, both of which are C₄ plants. Additionally, the site’s location in the middle of an island suggest that freshwater food sources may have played a prominent role in the diet of individuals in this sample. Alternatively, nitrogen-rich alluvial sediments used as fertilizer may have influenced the $\delta^{15}\text{N}$ values of the population as that observed by Poulson et al. (2014) in the Chile population.

Figure 6.1 substantiates the interpretation of Salazar-García et al. (2014: 73) of the diet from their site in Spain, which they based on collagen derived stable isotope values, that it likely consisted of a mix of C₃ and C₄ plants and that high trophic protein did not play a significant role.

5.2 Composition and Properties of Dental Calculus

Dental calculus is composed of residues introduced into the oral cavity, bacteria, and the byproducts resulting from their interaction. There are many things that may be introduced into the oral cavity that become part of the calculus matrix. Cellulose, starch, phytoliths, hair, and chitin are some of the materials that can be incorporated into a human calculus deposit. The isotopic signature of these materials reflects the diet of the

individual to a variable degree. There is a great deal of variation even within the individual, as noted by Eerkens et al. (2014). On top of the variability in materials incorporated into calculus, there are the more than 700 species of bacteria that make up the oral microbiome (Aas et al. 2002: 5721) and these taxa vary with the oral health of an individual. These species produce several byproducts, and their activity involves isotopic fractionation in the processing of the materials available in the oral cavity.

Whereas the amount of isotopic fractionation between diet and biomaterials is relatively well understood in bioapatites and collagen, analogous studies establishing the offset from diet to dental calculus are lacking. It is not expected that dental calculus is an analogue to either biogenic material. The question is what is being measured by mass spectrometry of dental calculus?

Discussion of the composition of dental calculus is usually framed around an organic fraction and an inorganic fraction. The organic fraction consists of compounds like lipids and proteins, bacteria, metabolic byproducts of the organisms present in the oral cavity, and various residues of both plant and animal materials introduced into the mouth. The composition of the organic fraction is incredibly complex. For example, cellulose ($C_6H_{10}O_5$) is found in large quantities in insoluble plant fibers that can be incorporated into the calculus matrix (e.g. Blatt et al. 2011), but it is also a common metabolic byproduct of various bacteria. Cellulose biosynthesis has been identified in several bacterial taxa, including members of the genus *Gluconacetobacter*, which are commonly found on fresh and rotten fruit, as well as *Escherichia coli* and various species of *Salmonella* (Römling 2002: 205). It is unclear as to what role these and other cellulose synthesizing bacteria play in the normal biome of the oral cavity, but they are

microorganisms humans are routinely exposed to. Thus, the organic fraction of calculus may be partially made up of cellulose, which, in the case of plant-based cellulose, directly reflects the isotopic profile of a part of the plant portion of the material being introduced to the mouth. In the case of bacterial synthesized cellulose, it has gone through an additional layer of fractionation and may be offset from the signature of the dietary carbon.

Researchers have demonstrated the durability of starch granules in the calculus matrix (e.g. Hardy et al. 2009). With an identical chemical formula, starch granules in the matrix return an isotopic profile identical to that of the represented plant components of the diet. Unlike apatite, or collagen, this isotopic signature reflects a dietary profile “unfiltered” by the physiological processes of the body. Other plant micro-remains include phytoliths. The durability of these siliceous bodies makes them useful in paleobotanical studies. Fox et al. (1996) established their durability in the calculus matrix. Strictly speaking, phytoliths are silica (SiO_2) and would not affect $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of calculus samples; however, Hodson (2016) has demonstrated that phytoliths, depending on the species and part of the plant from which they originate, can contain up to 4% “occluded” carbon. Nitrogen, on the other hand, is found in very low concentrations (~0.06%), indicating phytolith derived nitrogen represents little input into the bulk calculus isotopic profile (Hodson 2016: 65-66). Of relevance is the depletion of $\delta^{13}\text{C}$ values in phytoliths relative to plant tissue. This phenomenon has been shown to be more pronounced in C_4 plants than C_3 plants and results in $\delta^{13}\text{C}$ values 10‰ - 14‰ lighter than those of other materials from the same plant (Hodson 2016: 67), effectively giving a C_3 signature to C_4 derived carbon. Hayashizaki et al. (2008: 173) found highly variable silicon content, from 20 to

2500 ppm, in their study on the chemical composition of dental calculus. They attributed this variation to diets high in rice consumption in their sample population, suggesting this input would be difficult to define without similar spectrographic analyses.

Of note is that archaeological calculus has an inorganic fraction that is formed during some portion of the lifetime of the organism and another potential inorganic fraction that is intrusive into the calculus matrix after death. The relationship between these two sources of inorganic carbon is poorly understood. In experiments on modern calculus, Baumhammers and Rohrbaugh (1970) found that for chunks weighing approximately 4.5 mg, a period of 24 hours of soaking in solutions of crystal violet, methylene blue, basic fuchsin, and glycine was sufficient to completely penetrate the calculus. Furthermore, the same period of soaking in a solution of endotoxin from *Salmonella enteritidis* was sufficient for partial penetration (Baumhammers and Rohrbaugh 1970: 40). These findings suggest dental calculus may be particularly susceptible to diagenetic alteration by exposure to materials in solution, which is borne out by the high C/N ratios in non-arid sites in the tropic zone.

6.1 Conclusions: El Opeño

Traditional views of the Formative period in Mesoamerica highlighted the reliance on maize as a staple crop. Recognition that many regions in Mesoamerica were inhabited by low-level food producers during the Archaic period (Kennett 2012) has blurred the boundaries between the Archaic period and the following Formative period. Recent research has demonstrated that regions such as the Gulf Coast and the Soconusco were not as reliant on maize until the beginning of the Middle Formative around 1000 B.C. (Rosenswig et al. 2015; VanDerwarker and Krueger 2012). An alternative perspective from the state of Oaxaca suggests that maize use was consistently important from the Early Formative on (Hepp et al. 2017).

Based on the similarities between $\delta^{13}\text{C}$ values of the individuals from El Opeño and those of the Hohokam and the Middle Horizon Peruvian samples, maize already played a key role in the diet of the site during the period of utilization of the tombs around 1300 B.C. This study further highlights the correspondence between the presence of grater bowl and heavy maize consumption that was noted by both Rosenswig et al. (2015: 99) and Hepp et al. (2012: 710). Given the similarities in ceramic styles and figurine forms between El Opeño, sites of the Capacha tradition in Colima, and Tlatilco in the state of Mexico, further analysis should be done to assess the role of maize in these contemporaneous and culturally linked sites. Future paleodietary analysis should involve paired collagen and calculus samples when possible to refine the accuracy of the method given the high C/N ratios exhibited by the El Opeño samples.

6.2 Conclusions: Calculus Based Paleodietary Analysis

First, this study demonstrates that differences in diet between world samples are preserved in the stable isotopic profile of bulk dental calculus. While they do not show exact correspondence with the isotopic signatures of bone collagen or biogenic apatite (Eerkens et al. 2014; Salazar-García 2014), dental calculus derived isotopic profiles distinguish C₃ consuming populations from C₄ consuming populations and identify the exploitation of high trophic level organisms. As Eerkens et al. (2014) suggested, given the wide range of delta values in calculus derived isotopes, these trends are more visible at the inter-site level. The lack of correlation between $\delta^{13}\text{C}$ values of dental calculus and those of bioapatite is perplexing, as it suggests that calculus derived $\delta^{13}\text{C}$ values do not record bulk diet exactly. This observation highlights the importance of establishing a model for the incorporation of source material into dental calculus in future research. Experiments using organisms with known diets may be a fruitful avenue of investigation such as those carried out by DeNiro and Schoeninger (1983).

Second, $\delta^{13}\text{C}$ values are more highly correlated with C/N ratios when the C/N ratio is higher than 12. However, care must be taken in equating low C/N ratios with accurate results without heed of what is being analyzed. The treatment of West Mexico samples with dilute HCl universally lowered C/N ratios, but it also resulted in lighter $\delta^{13}\text{C}$ values. This effect was universal across all populations subjected to acid treatment. Eliminating the samples with C/N over 12, on the other hand, shifted the mean $\delta^{13}\text{C}$ value to heavier values in the West Mexico population. Given the material record at El Opeño, this heavier value is probably the more parsimonious of the two.

Finally, the point made by Price et al. (2018:102) that organic and mineral components should be analyzed separately is well taken, but analysis of both of these

portions should be reported with bulk calculus data as well to create a better understanding of how all of the parts relate to one another. Further work is necessary to determine the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from dental calculus, those from dietary material, and those from other biomaterials. However, the reduced cost in time and materials, and the low impact left on remains by sampling make dental calculus an attractive alternative or supplement to analysis of traditional biomaterials. At this time the resolution offered by dental calculus in paleodietary studies is admittedly coarse compared to stable isotope values derived from proteinaceous biomaterials. Dental calculus as a material will never replace collagen or bioapatite altogether as a proxy for paleodietary behavior. Rather, it offers a separate avenue of investigation that can help identify areas on which to focus study and complement other proxies, ultimately improving our understanding of ancient dietary behavior.

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