TRENDS IN DIETARY COMPOSITION, IMMUNOSTIMULATION, AND NUTRITIONAL DEFICIENCY IN PIEDMONT NORTH CAROLINA AND VIRGINIA, AD 800–1710

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Anthropology.

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ABSTRACT

Sophia C. Dent: Trends in dietary composition, immunostimulation, and nutritional deficiency in Piedmont North Carolina and Virginia, AD 800–1710
(Under the direction of Dale L. Hutchinson)

In this dissertation, I ask how European colonialism affected the nutrition of Siouan communities from Piedmont North Carolina and Virginia, AD 800–1710. I combine dietary stable isotope analysis of dental calculus, paleopathological analysis of immunostimulation and nutrition, and epidemiological analysis using datasets that contain serum nutrition and immune system measurements to reconstruct the nutrition of past Siouan people. My results show that the stable isotope values of paired dental calculus and bone biofractions are correlated, which identifies calculus as an alternative for bone in dietary reconstruction. Calculus stable isotope values from past Siouan people suggest variation among individuals, particularly in their degree of maize consumption. River drainage affiliation and temporal period affiliation both capture some of the variation in dietary composition among the individuals.

Siouan people who consumed more maize or had periodontal disease were more likely to have sphenoid lesions, which are likely associated with chewing muscle hemorrhage stemming from vitamin C deficiency. Vitamin C deficiency increases the risk of periodontal disease, while the chronic immunostimulation of periodontal disease is also expensive for vitamin C. Periodontal disease represents a cyclical relationship between nutrition and infection with ramifications for whole-body health. Vitamin C deficiency was more prevalent among Siouan communities during the late Colonial period (AD 1670–1710) than earlier periods, but neither

maize nor skeletal proxies for immunostimulation were consistently higher in the late Colonial period compared to earlier periods.

I attribute the Colonial period increase in vitamin C deficiency to infectious diseases not observable in the osteological record, Siouan groups altering their subsistence practices in ways that reduced the vitamin C content of food, and/or a population-level shift in peoples' immune system responses and regulation. I conclude that the nutritional ecology of Siouan people did change during the Colonial period, with diet, infectious disease epidemiology, and psychosocial stress all likely playing a role. These findings also have relevance for current concerns in global health: access to adequate and culturally-appropriate nutrition is important to mitigate health disparities during emerging infectious disease spread and dynamic sociopolitical landscapes.

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AIR N_2	Atmospheric nitrogen
CRP	C-reactive protein
C_{spac}	The spacing between the carbon isotope values of carbonate and organic carbon
EA	Elemental analyzer
GCF	Gingival crevicular fluid
IRMS	Isotope ratio mass spectrometry
LEH	Linear enamel hypoplasia
NC	North Carolina
NHANES	The National Health and Nutrition Examination Survey
OLS	Ordinary least squares
PD	Periodontal disease
PNB	Periosteal new bone
RLA	Research Laboratories of Archaeology
VA	Virginia
VNC	Vertebral neural canal
VPBD	Vienna Peedee belemnite
δ	The difference in the sample isotope ratio compared to the standard isotope ratio
‰	Parts per thousand

CHAPTER 1: INTRODUCTION

Nutrition-related disease stemming from both under- and over-nutrition is a pressing global health problem. Present-day nutrition related disease patterns may be partly rooted in historical trajectories of disease that have been reinforced through neocolonial structures and mediated by intergenerational dynamics. Data from past populations is useful to understand how historical processes such as colonialism impacted health and to interpret epidemiological trends that link past to present. In this study, I investigate how stressors associated with colonialism affected the nutritional health of Indigenous communities who lived in the Piedmont region of North Carolina and Virginia from AD 800 to 1710.

Siouan-speaking Indigenous groups from the Piedmont region of North Carolina and Virginia first experienced European colonialism as a heightened risk of raiding and indirect exchange of material culture and pathogens, starting in the early- to mid-17th century. Those exchanges between Siouan groups and European colonizers became direct and sustained following the mid-17th century, and risk of raiding and violence remained high (Davis, 2002; Eastman, 1999; H. T. Ward & Davis, 1999, 2001).

Colonialism also altered the environment in many ways. The geopolitical dimensions of raiding and violence changed as Indigenous groups across the Eastern Woodlands experienced colonialism and Europeans began the commercial trade of enslaved Indians (Eastman, 1999; Ethridge, 2009; Fitts, 2015; Gallay, 2002; H. T. Ward & Davis, 1999). European markets placed high demands on material culture produced by Indigenous communities, such as deerskins, and those demands altered the spatial dimensions of socioeconomic opportunity, increased risk, and

possibly led to anthropogenic resource depletion (Cronon, 1983; Holm, 2002; Lapham, 2005). Furthermore, Europeans also introduced new, sometimes highly virulent infectious diseases to the disease ecology of the Eastern Woodlands (Eastman, 1999; Hutchinson, 2016; Kelton, 2007; H. T. Ward & Davis, 2001).

I am particularly interested in how colonialism affected the nutrition of Siouan communities, as nutrition integrates many aspects of the lived experience, including sociopolitical, ecological, and cultural factors. For example, if a person experienced malnutrition, one potential interpretation is that the nutrients provided by their diet did not meet their physiological needs, whether due to low food availability or diets deficient in one or several nutrients. Low food availability could stem from factors like climate unpredictability and raiding, while foodways practices could affect the nutritional composition of foods.

Another, not mutually exclusive, interpretation of malnutrition is that a person's diet was nutritionally adequate, but infection or disease prevented them from effectively absorbing the nutrients available through food (Brickley, Ives, & Mays, 2020; Scrimshaw, 2003; Thompson & Bentley, 2018). Sociopolitical, ecological, and cultural factors all influence pathogen exposures, the immune response to pathogens, and the cultural response to disease. Nutrition is therefore a useful lens to study how environmental changes associated with colonialism affected the lived experience of past Siouan communities.

I apply nutrition transition theory to frame my questions regarding nutritional change among Piedmont Siouan communities. Nutrition transition theory posits that people are adapted to an expected level of energy, nutrients, and immune stimulation according to the experience of prior generations. A shift in nutrition that deviates from what people are adapted to – due to diet, disease, or other influences that affect the availability of nutrients – can lead to nutrition-related

disease (Burdge et al., 2011; Gluckman & Hanson, 2004; McDade, 2003). If a developing infant's mother experienced low nutrient availability and high infectious disease burden, for example, then the developing infant's metabolic and immune systems may develop in ways that are optimal to survive in an environment with low nutrient availability and high infectious disease exposure (Gluckman & Hanson, 2004; Gowland, 2015; Holt & Jones, 2000; Kuzawa, 2005). However, if the environment they are born into and mature in is instead characterized by high nutrient availability with a low infectious disease burden, then the infant's immune system would develop in a way that is not optimized for the future adult environment. This mismatch between predicted and experienced environments makes that individual more susceptible to disease in adulthood and can possibly affect the metabolic and immune adaptations of their children, as well (Burdge et al., 2011).

Nutrition transition theory is useful to frame ongoing nutrition transitions, such as globalization and the spread of industrial foodways, as well as to conceptualize how, why, and to what effect nutrition changed during past nutrition transitions, such as the agricultural revolution and the industrial revolution (Popkin, 1999). Researchers are actively investigating many different contexts in which nutrition transition may be responsible for the increase in nutrition-related disease (Amuna & Zotor, 2008; Chatzivagia et al., 2019; Piperata et al., 2011; Remis & Robinson, 2014; Weerasekara, Withanachchi, Ginigaddara, & Ploeger, 2018).

Building the body of research that supports nutrition transition theory expands our understanding of the range of expressions and consequences of nutrition transition in groups of diverse cultural, sociopolitical, and ecological backgrounds. I have a similar aim to expand our understanding of nutrition transition, specifically by adding information from past populations. Piedmont Siouan communities experienced displacement and new infectious diseases and forms

of violence as part of colonialism (Davis, 2002; Merrell, 1987; H. T. Ward & Davis, 1999), and may have changed their dietary practices and foodways as a result (Gremillion, 1993a, 1993b; Holm, 1994; Longo, 2018; Mikeska, 2019; Roark, 2020; VanDerwarker, Scarry, & Eastman, 2007). I apply nutrition transition theory to test whether colonial-attendant change affected the nutrition-related disease of Piedmont Siouan communities and hope the findings of this study can bring awareness to the potential nutritional impacts of factors like violence and displacement as they affect groups today.

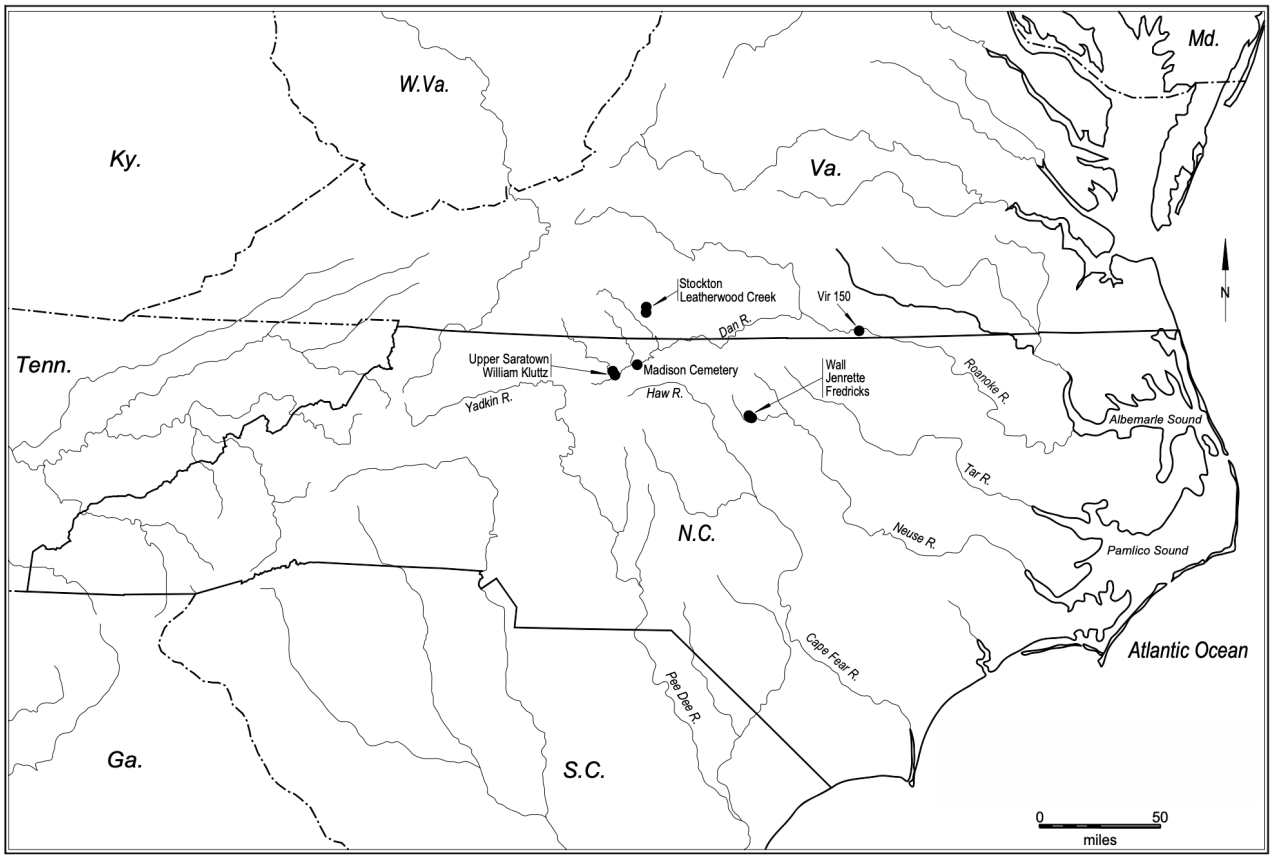
I employ stable isotope analysis, paleopathological analysis, and contemporary nutrition and health datasets to investigate how the nutritional epidemiology of Piedmont Siouan communities who lived from AD 800 to 1710 changed over time. The time span of this study includes four temporal periods – the early Late Woodland period (AD 800–1200), the late Late Woodland period (AD 1200–1620), the early Colonial period (AD 1620–1670), and the late Colonial period (AD 1670–1710). Characteristics of the temporal periods are included in the chronological framework provided in Chapter 2, along with my rationale for using them in my research design. Most of the archaeological sites represent communities from either the Dan River drainage system or the Eno River drainage system, shown in Figure 1.1.

The Sara Indians and their probable ancestors lived within the Dan River drainage from the early Late Woodland through the late Colonial period (Davis, 2002; Davis & Ward, 1991; H. T. Ward & Davis, 1993, 1999; Wilson, 1983). The ancestors of the Shakori, Saxapahaw, Eno, and/or Adshusheer lived at the late Late Woodland Wall site along the Eno River, while the Occaneechi lived at the late Colonial Fredricks site along the Eno River (Davis, 2002, 2016; H. T. Ward & Davis, 1993). People from the river drainages included in this dissertation are dissimilar in ways that may impact interpretations; the Sara and their ancestors are represented

throughout the entire time period of this study, unlike the groups from the Wall and Fredricks sites. Also, the Dan River groups likely have ancestor-descendent continuity, while the Occaneechi moved to the Eno River from the Roanoke River and their relationship with the earlier Wall site occupants is less clear.

Figure 1.1

Map of relevant Piedmont river drainages, courtesy of R.P. Stephen Davis, Jr., Research Laboratories of Archaeology.



Note that the Eno River is indicated by the Wall and Fredricks site and is a tributary of the Neuse River.

Chapters 2, 3, and 4 provide background to the overall project and support the synthesis of the various analyses employed in this research. Chapter 2 provides regional context and specifically focuses on temporal trends in factors that may have affected Piedmont Siouan dietary decisions and disease exposures. Chapter 3 provides an overview of the research

questions of the overall study and the theoretical frameworks I use to interpret my findings. Chapter 4 provides background information on the materials and methods of this study. The results chapters (Chapters 5–8) include additional, specific information on materials and methods. Chapters 5–8 are written as individual papers, but collectively contribute to the overarching questions of whether and why the nutritional epidemiology of Piedmont Siouan communities changed after the onset of colonialism.

Chapter 5 (Paper 1) tests whether dental calculus is an effective proxy for bone in dietary reconstruction through stable isotope analysis. Direct dietary analysis is useful to reconstruct what individual people ate out of the available resources; however, stable isotope analysis converts analytical materials from a solid to a gas and is therefore a destructive analytical technique (Ambrose & Krigbaum, 2003; T. Brown & Brown, 2011). Descendant communities of past populations often find the destruction of primary biomaterials such as bones and teeth ethically problematic. For example, destructive analysis of primary biomaterials violates North American Indigenous groups' stipulations regarding the treatment of their ancestors (Larsen & Walker, 2005; Scott & Poulson, 2012).

Dental calculus provides a potential solution to this ethical dilemma. It is a secondary biomaterial composed largely of bacteria that accumulates on the human skeleton but can be removed without harming the underlying teeth (Scott & Poulson, 2012). The descendant communities of the Piedmont Siouan groups have given permission for dental calculus to be used in destructive chemical analyses for this dissertation. Chapter 5 presents a method to prepare dental calculus for stable isotope analysis and ordinary least squares regression formulae to approximate bone stable isotope values from dental calculus.

Chapter 6 (Paper 2) employs the methods and regression formulae presented in Chapter 5 to analyze the dietary composition of Piedmont Siouan people. I use principal components analysis to evaluate patterns among the diets of people that I sampled for dental calculus and compare the isotopic results to existing archaeological information on Piedmont Siouan foodways. Carbon isotope values, which likely correspond to differences in maize consumption, explained the majority of the variation in the data. The diets of most sampled Siouan people are characterized by C_4 energy and mixed C_3-C_4 protein, which represents a high contribution of maize and a mix of terrestrial and aquatic protein. Temporal period and river drainage association both appear to explain some of the dietary differences among the Siouan individuals whose diets were measured.

Chapter 7 (Paper 3) tests the relationship between lesions of nutritional deficiency and other variables that potentially contribute to nutrition, including dietary composition and skeletal proxies of immunostimulation (stimulation of an immune response). My findings indicate that more people from the late Colonial period had sphenoid lesions (likely indicative of vitamin C deficiency) than people from the Late Woodland period. Logistic and ordinary least squares regression models identified that high $\delta^{13}C_{carbonate}$ (whole diet carbon) was associated with sphenoid lesions, as were lesions associated with periodontal disease and episodic growth disruption (linear enamel hypoplasia). However, neither high $\delta^{13}C_{carbonate}$ nor skeletal indicators of periodontal disease were consistently more prevalent in the late Colonial period than the earlier periods, and linear enamel hypoplasia prevalence was consistent across all temporal periods.

This led to the question: If none of the variables associated with vitamin C deficiency were more prevalent in the Colonial period, then why were people from the Colonial period more

likely to experience vitamin C deficiency? Missing covariates, such as infectious diseases and foodways practices likely contributed to the risk of vitamin C deficiency. I also draw from ecological immunology to suggest an alternative interpretation to missing covariates – it is possible that the immune response to stressors, not the prevalence of stressors themselves, may have changed over time. People from the Colonial period may have had a more severe immune response (higher magnitude, longer duration) to immunostimulation, which would have been more expensive for the body's nutritional stores. Early-life disease exposure, intergenerational shifts in nutrition and disease ecology, and compounding sources of stress are all potential explanations for a change in immune response severity (Crespo, Klaes, Switala, & DeWitte, 2017; Long & Nanthakumar, 2004; McDade, 2003, 2005; Sheldon & Verhulst, 1996).

In Chapter 8 (Paper 4), I use data from the National Health and Nutrition Examination Survey (NHANES) collected during 2003 and 2004 to further examine some of the patterns identified in the Piedmont Siouan skeletal sample. Logistic and ordinary least squares regression models, as well as causal mediation analysis, indicate that periodontal disease is associated with lower serum vitamin C levels and that the immune response likely mediates the relationship between infection and vitamin C (Elste, Troesch, Eggersdorfer, & Weber, 2017; Nishida et al., 2000; Nualart et al., 2002; Nuzzi, Lokuta, & Huttenlocher, 2007; Padayatty & Levine, 2016; Washko, Wang, & Levine, 1993). The associations among the NHANES data therefore support that periodontal disease and vitamin C deficiency co-occur in past and present, and reaffirm that it is important to consider both diet and the immune system in the study of nutrition.

I also test the theory from ecological immunology that compounding sources of stress can affect the immune response and thus create differences among people's susceptibility to disease and damaging effects of disease. In particular, I test the relationship between food insecurity and

periodontal disease, and identify that food insecure people were more likely to have periodontal disease than food secure people, even after controlling for frequency of dental visits, household income, micronutrient levels, and whether they smoked or not. Food insecurity is also significantly associated with lower levels of C-reactive protein (a protein that signifies active inflammation) as well as lower levels of neutrophils, which are cells that are recruited to sites of infection to help clear the infection and mitigate tissue damage. This indicates that food insecurity may alter the immune response. Overall, I suggest that psychosocial stress associated with food insecurity makes periodontal disease more likely, and that periodontal disease stimulates a sustained immune reaction, which is expensive for the body's finite micronutrient stores. Though more research is needed, these findings move us closer to understanding why people have variable immune responses and how this impacts their nutrition.

In Chapter 9, my conclusion chapter, I synthesize the information from the results papers to interpret changes in evidence of Piedmont Siouan nutritional deficiency over time. I conclude that the nutritional ecology of Piedmont Siouan groups changed from the Late Woodland to the Colonial period, likely due to a complex combination of factors that include dietary and epidemiological factors. Missing covariates also contribute to the change in nutrition, as well. Some of these may be related to colonialism, such as psychosocial stress from new patterns of socioeconomic inequality, raiding and violence, or from factors like climate unpredictability, compounding the challenges of an already dynamic sociopolitical landscape.

I identify the main contributions of my dissertation as follows. In Chapter 5, I experimentally measure bone-calculus isotopic spacing, which improves the use of dental calculus for dietary stable isotope analysis. In Chapter 6, I add direct dietary information to the existing literature on Piedmont Siouan foodways. The stable isotope data provides new evidence

of plant and animal use for some sites that lack analyzed plant remains or that have potentially skewed compositions of faunal remains due to collection methods (VanDerwarker, 2001). In Chapter 7, I identify that some skeletal lesions associated with immunostimulation (such as periodontal disease) correlate with nutritional deficiencies. And in Chapter 8, I conclude that psychosocial stress associated with food insecurity may dysregulate the immune response, leading to an inappropriately high or low immune response to a stressor and likely affecting micronutrient needs as a result. The work presented in this dissertation raises additional questions and exciting opportunities for future research, which are provided in Chapter 9.

CHAPTER 2: THE CONTEXT OF PIEDMONT SIOUAN GROUPS' SUBSISTENCE ACTIVITIES AND NUTRITION OUTCOMES (AD 800–1710)

The temporal span of this study provides a window into the lived experience of Siouan people from the Late Woodland through the Colonial periods in Piedmont North Carolina and southern Virginia. I use a chronological framework that separates the Late Woodland period into early and late components (AD 800–1200 and 1200–1620) and separates the Colonial period into early and late components (AD 1620–1670 and 1670–1710), as well. I use these categories to capture important differences in Siouan lifeways related to my main research questions while also maximizing the within-group sample size.

Differences in subsistence systems and dietary composition, disease ecology, and psychosocial stress are the aspects of Piedmont Siouan lifeways most relevant to my research questions. I provide an overview of temporal trends in subsistence, disease ecology, and factors that may have affected psychosocial stress in the below paragraphs to justify my chronological framework, and further detail is provided in the ensuing sections of this chapter. Figure 2.1 shows a timeline of the events relevant to nutritional epidemiology that occurred during the time period of this study

Dietary composition and settlement population size likely changed across the Late Woodland period, as Siouan groups lived in larger groups and intensified maize agriculture during the latter half of the Late Woodland period (H. T. Ward & Davis, 1999, 2001). Droughts may have affected subsistence practices and dietary composition during the middle Late Woodland period (AD 1000–1300) and the late Colonial period (D. W. Stahle, Cleaveland, &

Hehr, 1988). And, the Little Ice Age, a climate event characterized by lower temperatures, especially in the north Atlantic region, potentially impacted subsistence practices during the end of the Late Woodland period (Fitzgerald, 2001; Ljungqvist, 2018).

Disease ecology may have changed during the second half of the Late Woodland period, as larger, less transient populations can support more endemic and possibly virulent pathogens (Ewald, 1994). The most impactful change in disease ecology from the time period of this study, however, likely came from introduced infectious diseases that led to epidemics (Dobyns, 1993; Hutchinson, 2016; Kelton, 2007). While evidence strongly supports that Siouan groups who lived during the late Colonial period experienced epidemics, (Eastman, 1999; H. T. Ward & Davis, 1993, 2001), it is possible that infectious disease impacted Siouan groups in earlier temporal periods, as well. The use of medicinal taxa and practices of rebuilding structures and destroying utilitarian items (perhaps cleansing rituals) increased starting in the terminal Late Woodland into the early Colonial period (Eastman, 1999; Roark, 2020; VanDerwarker et al., 2007), providing potential evidence of Siouan responses to an increased infectious disease burden.

While climate unpredictability may have heightened psychosocial stress, raiding likely did, as well (Birhanu, Ambelu, Berhanu, Tesfaye, & Woldemichael, 2017; Gray et al., 2003; Novak, Geronimus, & Martinez-Cardoso, 2017; Pike, Straight, Oesterle, Hilton, & Lanyasunya, 2010; Pike & Williams, 2006; Stanke, Kerac, Prudhomme, Medlock, & Murray, 2013). Raiding for food caches increased in the latter half of the Late Woodland period (H. T. Ward & Davis, 1993). Raiding took on new form in the late Colonial period, with the added risk of captive taking for the European trade of enslaved Indigenous peoples (Ethridge, 2009; Fitts, 2015; Gallay, 2002; H. T. Ward & Davis, 1999). Other potential sources of increased psychosocial

stress include socioeconomic uncertainty and inequality associated with the deerskin trade (Lapham, 2005; H. T. Ward, 1987; H. T. Ward & Davis, 2001; Waselkov, 1997) and disruption in the continuity and succession of traditional social roles of power due to the deerskin trade, population amalgamation, and death due to epidemic disease (Jaeggi et al., 2021; Merrell, 2009; Van Bortel et al., 2016).

It would be ideal to further stratify the temporal categories into a third Late Woodland category (AD 1450–1620), since the people who lived during this time may have experienced population disruption and amalgamation (H. T. Ward & Davis, 1993), increased their use of medicinal taxa (Roark, 2020), and perhaps diversified their subsistence systems (Longo, 2018; Wilson, 1983). However, the skeletal sample does not include enough people from that time period to separate them into their own comparative group. In some areas of the dissertation, I compress the temporal categories into two categories (Late Woodland and Colonial) rather than four to preserve within-group sample size. When using two categories, Late Woodland encompasses AD 800–1620 and Colonial encompasses 1620–1710.

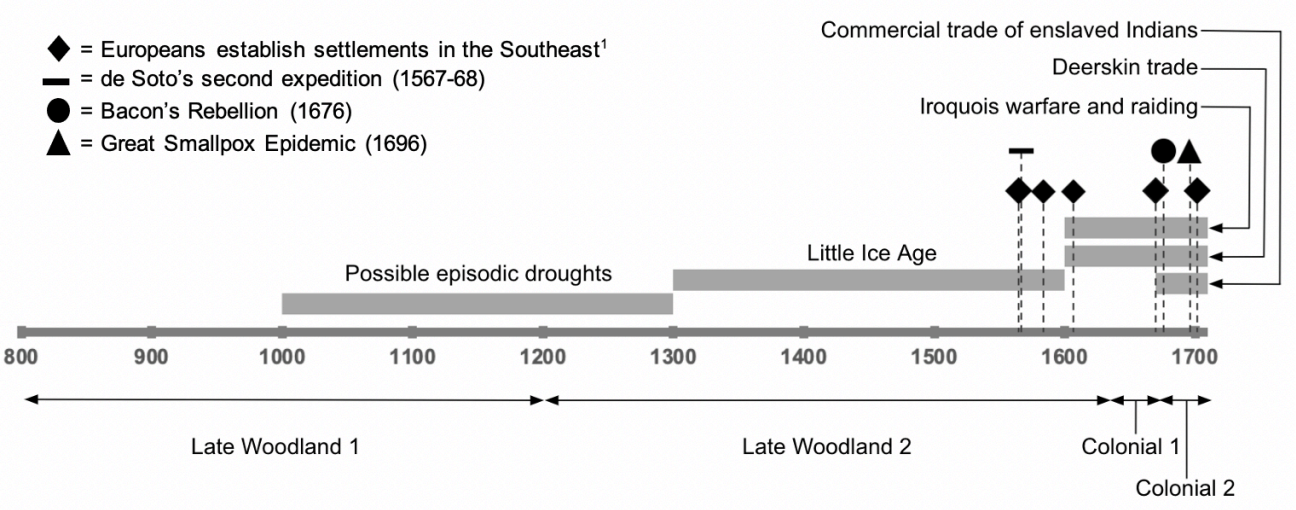
2.1 Geopolitical landscape

Siouan-speaking groups selected the fertile floodplains of the Piedmont river bottoms for their settlement sites during the first half of the Late Woodland period, and began to reside at and return to these sites in more permanent ways during the latter half of the Late Woodland period (Merrell, 1987; H. T. Ward & Davis, 1999, 2001). The Piedmont Siouan groups also increased their surplus food production during the latter half of the Late Woodland period, likely facilitated in part by the congruence between their cultivation techniques, increased settlement permanence, and the productive landscape (H. T. Ward & Davis, 2001). However, stored surpluses may have contributed to a heightened risk of raiding by other groups. Fortifications of Siouan settlements

during the latter half of the Late Woodland period provide evidence that groups did experience threats, possibly related to stored surpluses (H. T. Ward & Davis, 1993).

Figure 2.1

Timeline of events relevant to the nutritional epidemiology of Piedmont Siouan communities, AD 800–1710



[1] Dates of European settlements: 1565 (Spanish at St. Augustine), 1585 (English at Roanoke), 1607 (English at Jamestown), 1670 (English at Charleston), 1702 (French at Mobile). Sources include Ethridge (2009) and H. T. Ward & Davis (1999).

Siouan communities moved across the Piedmont and possibly merged with other groups during the second half of the Late Woodland period. Ceramic analyses from Late Woodland contexts of the Haw and Eno Rivers (AD 1400–1600), for example, indicate that external groups may have moved into the Piedmont and "adapted and possibly incorporated the indigenous population into a new cultural complex" (H. T. Ward & Davis 1993:413). Potential amalgamation was also seen among Dan River communities, which were organized as dispersed households during the early part of the Late Woodland period, and later merged into larger villages. The Siouan groups adapted their social organizations and cultural patterns as part of the ensuing interactions of community movement and amalgamation (H. T. Ward & Davis, 1993). This formation of new cultural complexes and larger, amalgamated communities indicates that

the Piedmont Indigenous groups had experience interacting with and incorporating new groups into their settlements and social interaction spheres. However, the degree of population influx to the Piedmont – as well as the motivations for population movement – became more dynamic in the Colonial period.

Some Colonial period Piedmont Siouan groups aligned their settlement locations and adjusted their intergroup relationships to participate in the trade with Europeans. The Occaneechi, for instance, lived on a main trading path from Fort Henry[1] into the Piedmont, which they leveraged to position themselves as middlemen in the Indigenous–European trade of the mid-1600s (Davis, 2002; Merrell, 2009). Ethnohistoric evidence suggests that the Occaneechi exerted force to control trade, accrue power, and maintain their role as middlemen (Eastman, 1999; H. T. Ward & Davis, 2001). During this time, Piedmont Siouan groups also experienced threats of slave raiding due to the European commercial trade of enslaved native peoples. This commercial slave trade began in the mid- to late-17th century (Ethridge, 2009; Fitts, 2015; Gallay, 2002; H. T. Ward & Davis, 1999).

Fitts (2015:301) emphasizes the violence and sociopolitical disruption caused by the European enslavement and trade of Indigenous peoples: "the Indian slave trade led to demographic instability in southeastern North America as preexisting systems of ritualized hostage taking and captive adoption were co-opted for profit." Some have speculated that the Occaneechi were involved in the raiding, capture, and enslavement of other Indigenous people

[1] English colonists established Fort Henry on the Appomattox River in present-day Petersburg, Virginia so they could expand their trading activity from Tidewater Virginia westward into other areas of Virginia and southward into the Piedmont region of Virginia and North Carolina (H. T. Ward & Davis, 1999).

(Ethridge, 2009; Fitts, 2015), though it is debated whether the archaeological and ethnohistoric evidence support the Occaneechi as involved in captive taking.

Whether threats of raiding and enslavement came from other Piedmont groups or not, there *is* evidence that threats came from Indigenous groups outside of the Piedmont. The Five-Nations Iroquois engaged in warfare and captive-taking, which they increased in scale in the face of geopolitical conflict and epidemics (Davis, 2002; Ethridge, 2009). The effects of Iroquois warfare and attendant slave raiding were felt broadly across eastern North America, including by the Piedmont Siouan groups in the latter half of the 17th century (Ethridge, 2009; Merrell, 1987).

The Occaneechi lost their stronghold as middlemen in the Indigenous–European trade following Bacon's Rebellion[2] in 1676 (H. T. Ward & Davis, 2001). This opened up opportunities for other Piedmont Siouan groups to trade directly with Europeans. Direct trade, threats of slave raiding, and population loss due to epidemics all likely influenced the geopolitical decisions of Piedmont Siouan communities throughout the late-17th and early-18th centuries. At that time, Siouan communities migrated and aggregated with other groups to increase their resilience to sociopolitical and epidemiological threats and gain socioeconomic opportunities (Davis, 2002; Ethridge, 2009; Merrell, 2009; H. T. Ward & Davis, 1993). Indigenous groups migrated out of the Piedmont by 1710 and merged with other tribes in Virginia and South Carolina, which was concurrent with large-scale population movement across southeast North America (Ethridge, 2009; Merrell, 2009).

[2] Nathaniel Bacon led a rebellion of non-elite planters and indentured servants against the Virginia government; members of the rebellion "led indiscriminate attacks on [local] Indians, in open defiance of the governor" (Taylor, 2001:149). These attacks caused local Indigenous communities to leave the area.

2.2 Socioeconomic foci

The mid-to-late Late Woodland tradition of aggregating into densely populated, more permanent villages likely benefitted agricultural surplus production, as people were able to invest more in the landscape (H. T. Ward & Davis, 1993, 2001). Yet as groups began to live in larger settlements, they may have needed to re-negotiate social positions of power, as well as practices of food and material culture production (Merrell, 2009). These negotiations likely led to new cultural practices. Both Dan River and Eno River Siouan groups increasingly engaged in large-scale food production events (possibly feasting) across the Late Woodland period. They also began to engage in pipe smoking more regularly, possibly as a quotidian activity. Siouan groups likely adopted and adapted both feasting and pipe smoking as ways to express social status in a dynamic landscape (Eastman, 1999; Lapham, 2005; H. T. Ward, 1987; H. T. Ward & Davis, 1993, 2001; Waselkov, 1997).

Piedmont Siouan groups also developed new (and renegotiated extant) socioeconomic roles in response to the shifting Indigenous–European trading network. For example, young men of the Piedmont Siouan groups may have devised new socioeconomic roles as entrepreneurs in the deerskin trade, through which they gained socioeconomic power with the intensification of hunting for large-scale hide production (Lapham, 2005; H. T. Ward, 1987; H. T. Ward & Davis, 2001; Waselkov, 1997). Siouan people produced more animal hides in response to the increased demand for furs by European markets, and possibly traveled farther and for longer periods of time to increase this production of hides (Holm, 2002; Lapham, 2005).

Research by Mikeska (2019) supports the idea that at least some Siouan groups traveled farther and spent more time to acquire deer. She examined the strontium isotopic ratio of deer tooth enamel from various sites in the Piedmont region, and identified that late Colonial Siouan

groups from the Dan River may have increased their zone of deer exploitation compared to Late Woodland and early Colonial Dan River groups. Eno River groups show the opposite trend across time (larger to smaller deer procurement zone). Choices about time allocation, as well as distance and time away from habitation sites on hunting excursions, provide a potential example of how Indigenous groups shifted their socioeconomic activities to navigate emergent trading networks.

More Siouan people interacted with Europeans when Indigenous-European trading became more direct following Bacon's Rebellion in 1676. Direct trade increased the likelihood of pathogen exchange. Some researchers (Dobyns, 1993; Smith, 1987) suggest that new infectious diseases such as smallpox may have started to spread among southeastern Indian communities as early as the mid-16th century, following European arrival to and movement through the Southeast. Ethnohistoric and archaeological evidence from the Piedmont region support that high-mortality epidemics probably did affect Piedmont Siouan communities by the time of the Great Smallpox Epidemic in 1696 and possibly earlier (during the first half of the colonial period) for Dan River site occupants, though not as early as the mid-16th century (Eastman, 1999; Kelton, 2007; H. T. Ward & Davis, 1993, 2001).

The increased morbidity and mortality brought by these new diseases may have created uncertainty about the continuity and succession of traditional social and political roles of power in an already dynamic social landscape (Merrell, 2009). It may have also influenced Siouan groups' choices regarding ritual scheduling and settlement location. It is possible that disrupted group sizes affected subsistence activities, as well (Eastman, 1999).

2.3 Ecological factors

Socioeconomic activities and geopolitical strategies of Piedmont Siouan groups were intricately and bidirectionally tied to local ecology. The productivity and predictability of ecological zones affected communities' choices and opportunities regarding surplus production and resource extraction. On the other hand, human activities affected the balance of local species and functioning of the ecosystem. The productive river bottoms of the Piedmont were conducive to the mixed subsistence strategy of the Siouan-speaking groups, as well as the population expansion and agricultural intensification seen in the second half of the Late Woodland period (Merrell, 1987; H. T. Ward & Davis, 1999). The ways in which humans interacted with, managed, and worked parts of their landscape (for example, clearing some areas for burning, maintaining thicket in others) affected soil composition and shaped the habitats of other species (Cronon, 1983; Eastman, 1999; A. F. Graham, 2018; Hammett, 2000).

The agricultural products of maize, beans, and squash continued to be dietary mainstays through the Late Woodland period to the Colonial period; however, zooarchaeological and archaeobotanical research suggests that the Piedmont Siouan groups (especially those from the Dan River) may have expanded their subsistence base during the second half of the Late Woodland period to include a more diverse set of plant and animal resources (Gremillion, 1989; Holm, 1987; Longo, 2018; Wilson, 1983).

It is possible that climate change intersected with other factors to influence this dietary expansion. Climate reconstructions using tree ring data demonstrate interannual variation in temperature and moisture for the entire temporal span of the present study (AD 800–1710)[3] (D.

[3] The tree ring data was generated from cypress trees in Cape Fear (southeastern North Carolina) and provide a record of moisture and temperature for the growing period of cypress trees spanning AD 372 to 1985 (D. W. Stahle et al., 1988).

W. Stahle et al., 1988). However, the researchers who generated the tree ring data identified two "significant climate anomalies" in the climate history of North Carolina, one of which was a period of droughts that likely affected the Piedmont from AD 1000 to 1300 (D. W. Stahle et al., 1988).

Considering the history of interannual climate variation in Piedmont North Carolina, Siouan groups likely had strategies to maintain resilience despite aspects of unpredictability in their environments. They may have broadened their repertoire of subsistence activities to mitigate the risk of lower crop yields, which is a practice that would have generated higher species diversity in the archaeological record (Crumley, 1994; Wilson, 1983). They also possibly engaged in other strategies and preparations, including moving settlement sites, intensifying in warfare and/or raiding, and shifting their schedule of ritual activity (Crumley, 1994; Leslie & McCabe, 2013; Ljungqvist, 2018; VanDerwarker et al., 2007; Winterhalder, 1990).

The other significant climate anomaly identified through tree ring data was a period of wet, cool conditions that emerged in AD 1300–1600, representing the start of the Little Ice Age, and lasting into the 19th century (Eastman, 1999; Fitzgerald, 2001; Holm, 1994; Ljungqvist, 2018). North American Indigenous groups likely experienced ecological effects due to the Little Ice Age, particularly those who lived at higher latitudes, such as the Huron (Fitzgerald, 2001). Areas in northwest Europe experienced shorter growing seasons (by approximately five weeks) in the 16th century, and Fitzgerald (2001) suggested that the Huron may have experienced similar challenges to their subsistence scheduling (Grove, 1988). Moreover, the Huron's maize crops were particularly affected, because the colder temperatures of the Little Ice Age pushed the zone of productive maize cultivation southward, excluding the Hurons' settlements from its parameters. Indirect effects of northern groups' reduced crop yields (population movement,

warfare, raiding) rippled throughout the Eastern Woodlands (Blanton, 2004; Trigger, 1976), and climate reconstructions indicate that areas of North Carolina did experience severe droughts during the first half of the 18th century (Roark, 2020; D. K. Stahle, Burnette, & Stahle, 2013). Piedmont Siouan groups may have therefore sustained both direct and indirect effects of climate unpredictability during the Colonial period.

Just as ecological changes influenced geopolitical and socioeconomic strategies, human responses to unpredictable environments in turn affected local ecology. The mid-17th century deerskin trade exemplifies how the activities of Piedmont Siouan groups may have altered local ecology. The rapid intensification of deer hunting and attendant extraction of deer during the colonial period may have affected local deer populations. Holm (2002) compared the composition of faunal remains in different temporal assemblages from Siouan Dan River settlement sites, and identified a relative decrease in deer biomass from the first to the second half of the 17th century, which she attributed to a decrease in local deer population sizes, possibly due to higher exploitation of deer, and/or a delocalization of deer hunting activity.

Population changes in any one species, which could result from either over-hunting or delocalized hunting, has wide-reaching effects for local ecology. Other species are prone to shift in population size or habitat zone in a way that cascades through the whole food chain, and people may choose to alter their strategies of landscape management to emphasize the procurement of other resources (Hutchinson, 2016).

2.4 Infectious disease ecology

Infectious diseases are caused by a microorganism (virus, bacterium, helminth, fungus, or protozoan) that stimulates an immune response. People can be exposed to infectious diseases

through person-to-person transmission, vectors (such as insects), or contact with environmental reservoirs of the pathogen (such as rotting wood) (Wiley & Allen, 2017).

Virulence describes the severity of the effects of a disease-causing agent. Low population density generally does not support highly virulent pathogens that require direct transmission. This is because the pathogen needs to be transmitted between humans to survive, which requires that a proportion of the human population remains able to spread the disease. Highly virulent diseases cause severe debilitation (reducing the likelihood of human-to-human spread), and do not leave enough unaffected people capable of transmission in communities with low population densities (Ewald, 1994; Wiley & Allen, 2017).[4] While the larger population sizes of Siouan groups who lived during the second half of the Late Woodland period, especially those from the Dan River, may have supported an increase in infectious diseases via more opportunities for their direct transmission and more potential hosts, population sizes in the Piedmont were probably never large enough to support the evolution of high virulence in pathogens native to the Piedmont disease ecology (Barrett, Kuzawa, McDade, & Armelagos, 1998; M. N. Cohen & Armelagos, 1984; Ewald, 1994). However, the disease ecology of the Piedmont changed once multiple Piedmont Siouan groups began to trade directly with Europeans (rather than indirectly through Indigenous trading networks).

People involved in direct trade introduced pathogens such as the smallpox virus that had evolved to be highly virulent in densely populated urban centers. These urban centers had large enough populations (and enough population movement) to sustain the spread of the pathogen

[4] Some vector-borne diseases can circumvent this problem of population density, because they do not require the affected person to be mobile – the vector spreads the pathogen in lieu of the infected person (Ewald, 1994). Mosquitoes, for example, spread the plasmodia that cause malaria. However, there still needs to be enough people within a vector's habitat range to support the spread of a disease, and the local ecology must also provide ample habitats for the vector.

(Barrett et al., 1998; Duggan et al., 2016). When smallpox was introduced to Piedmont Siouan communities, its evolutionary trajectory towards high virulence caused severe morbidity for Siouan people. Moreover, the Piedmont Siouan groups had no prior exposure to and thus no immune defense against newly-introduced pathogens (Crosby, 1976; Kelton, 2004). This mismatch between evolved and introduced ecologies of smallpox, in conjunction with the unprimed immune systems of the people newly introduced to the virus, led to severe epidemics among Indigenous communities (Dobyns, 1993; Kelton, 2007).

2.5 Relevance of geopolitical, socioeconomic, ecological, and infectious disease ecology factors for Siouan subsistence and nutritional epidemiology

Table 2.1 provides a summary of several important temporal trends seen over time in the Piedmont and how those trends provide useful context to interpret changes in Siouan dietary composition and nutritional paleopathology. The factors included in Table 2.1 likely intersected with each other to influence how Siouan groups made their subsistence decisions, as well as where, when, and how often they came into contact with pathogens or other exposures that can cause disease, whether infectious or noncommunicable.

Noncommunicable diseases also involve an immune response, but are not caused by the transmission of a microorganism between people. Noncommunicable disease types include injury, genetic anomalies, prolonged psychosocial or biochemical stress, and autoimmune reactions (McElroy & Townsend, 2009). For example, the Indigenous-European trade may have contributed to immune reactions not only through introduced pathogens, but also through increased likelihood of people experiencing psychosocial stress. The trade and interaction with colonial forces increased socioeconomic opportunities for some, while furthering disempowerment for others. A landscape of stratification and inequality can lead to differential psychosocial stress and possibly immune system stimulation by extension (Kemeny &

Schedlowski, 2007; Sapolsky, 2018). The physiological connection between psychosocial stress and immune system stimulation is explored further in Chapter 8.

Table 2.1

Factors that May Have Influenced Siouan Groups' Dietary Decisions and Disease Exposures

Aspects of Siouan communities' lived experience	Temporal trends	Potential dietary and epidemiological outcomes
Population density	Groups increased their population sizes from the early to late Late Woodland period (group amalgamation) Population size decreased in the late Colonial period	Larger group sizes may have facilitated the production and storage of surplus food Group amalgamation may have increased the sharing of pathogens and created uncertainty in succession of sociopolitical roles
Raiding	Groups began to fortify their settlement sites during the Late Woodland period, suggesting that they experienced threats of raiding Raiding associated with captive-taking and enslavement increased in the Colonial period	The possibility of frequent site movement may have prompted groups to engage less in food production activities that require long temporal relationships with the landscape Threats of raiding and violence may have increased psychosocial stress
Deerskin trade	Early and late Colonial groups engaged in the deerskin trade. Some groups (like the Occaneechi) acted in a more direct role as middlemen in the trade Siouan groups began to trade directly with Europeans (rather than through middlemen) in the late Colonial period	Some members of the group may have reduced the time they spent on other food production activities to increase deerskin production Direct trade likely increased exposure to pathogens Over-exploitation of deer may have shifted local species composition and disease ecology Inter- or intra-group social stratification and inequality possibly increased psychosocial stress

Climate unpredictability	Episodic droughts occurred during both the Late Woodland and Colonial periods	Groups likely practiced a diversity of food production activities to avoid resource stress
	The Little Ice Age began during the end of the Late Woodland period and lasted through the Colonial period	Crop losses elsewhere in the Eastern woodlands may have increased the likelihood of intergroup raiding and thus threats to stored foods

2.6 Summary

Overall, subsistence scheduling, dietary choices, and disease exposures were complex and multifaceted during the time period of this study. Threats of raiding, merging of diverse ideas and experiences, shifting impetuses for increased production of food crops or animal hides, ritual scheduling, and dynamic socioeconomic roles are all examples of factors that may have influenced the dietary decision-making of Piedmont Siouan groups, as well as their immunological exposures, both of which converge to affect nutritional health.

CHAPTER 3: RESEARCH QUESTIONS AND LITERATURE REVIEW

The overarching research question of this study asks: Did the proportion of people affected by nutritional deficiency change in the Colonial period compared to the earlier Late Woodland period? Nutrition stems from more than just dietary composition, and multiple theoretical frameworks are therefore useful to study nutrition. In this dissertation, I use oral focal infection theory to consider how physiological responses such as an immune response can affect a person's susceptibility to nutrition-related disease, and ecological immunology to evaluate how multiple factors alter the magnitude and duration of the immune response.

I situate these frameworks within the context of colonialism to account for the effects of shifting spatial and social distributions of opportunity and risk on past communities' dietary choices and nutritional health. Table 3.1 provides a research design summary. It includes the research questions that guide this study, as well as the theoretical frameworks, materials, methods, and measures used to evaluate the research questions.

3.1 Nutrition transition in colonial context

Nutrition transition is a population-level shift in the proportion of people affected by nutrition-related disease and is often attributed to how such factors as globalization of food markets, climate change, and adoption of new food production systems (e.g. the transition to agriculture) affect food access, food availability, and other structural risk factors of disease. Nutrition transitions have multiple demographic and epidemiological impacts, including effects on fertility rates, the incidence of chronic metabolic disease, and infant morbidity and mortality (Delisle, 2008; O'Dea & Piers, 2002; Popkin, 2002).

Table 3.1

Guide to the Research Questions and the Theoretical Frameworks, Samples, Measures, and Methods used to Address Them.

	Research question	Theoretical framework(s)	Sample, measures, and methods	Relevant chapters
Overarching research question	Did the proportion of people affected by nutritional deficiency change in the Colonial period compared to the earlier Late Woodland period?	Nutrition transition Biocultural origins of health disparities in imperialist systems	Skeletal remains of past Siouan people from Piedmont NC and VA spanning AD 800 to 1710 Skeletal analysis with a focus on lesions of nutritional deficiency	Chapter 7
First supporting research question	Does stable isotope analysis of dental calculus yield dietary information?	Dietary interpretation from stable isotope ratios of biological materials (reviewed in Chapter 4)	Bone and dental calculus samples from multiple time periods, geographical regions, and sociocultural backgrounds Stable isotope analysis	Chapter 5
	Does direct dietary information from Piedmont Siouan individuals agree with existing archaeological and ethnohistoric information about Piedmont Siouan foodways?	Dietary interpretation from stable isotope ratios of biological materials Political ecology	Stable isotope ratios from Piedmont Siouan people Stable isotope ratios of local plant and animal samples (interpretive baseline)	Chapter 6, 7

	Is there an association between skeletal evidence of nutritional deficiency and dietary composition?	Creative resilience of Indigenous groups to colonial-attendant environmental change	Remains of past Siouan people from Piedmont NC and VA spanning AD 800 to 1710 Skeletal analysis with a focus on lesions of nutritional deficiency	
Second supporting research question	Is there an association between skeletal evidence of immunostimulation and nutritional deficiency?	Oral focal infection theory Ecological immunology	Remains of past Siouan people from Piedmont NC and VA spanning AD 800 to 1710 Skeletal analysis with a focus on lesions of infection and inflammation	Chapter 7
	What affects the magnitude of the immune response to physiological stress?	Oral focal infection theory Ecological immunology	Participants in NHANES 2003-2004 Causal mediation analysis	Chapter 8

Raschke and Cheema (2008:662) studied the effects of a nutrition transition in Kenya, Uganda, and Tanzania. They argue that the nutrition transition there, most recently characterized by globalization of food markets, is part of a historical trajectory stemming from colonialism. They emphasize: “It is imperative that greater efforts be directed towards exposing the colonial and neocolonial forces which have undermined food security and health status in East Africa. Heightened awareness of these forces is essential for proposing genuine solutions to the nutrition

transition and related non-communicable disease epidemics throughout this region and, indeed, worldwide."

Though Raschke and Cheema write about different regions, cultures, and colonial systems than what Piedmont Siouan people experienced, the colonial factors they refer to as the earliest origins of a nutrition transition have parallels to the colonial system in eastern North America. Raschke and Cheema discuss how colonists enslaved Indigenous peoples, seized productive land, greatly disrupted local ecology, extracted certain resources for international markets, and complicated and sometimes disempowered women's socioeconomic roles in trade (Cronon, 1983; Eastman, 1999; Gallay, 2002; Lapham, 2005; Merrell, 2009; Waselkov, 1997). Neocolonial processes such as continued displacement, discriminatory policies that affect food security and socioeconomic inequality, and attempts at religious conversion and forced assimilation create continued barriers to health and wellbeing.

I agree that nutrition transition theory should consider historical trajectories. Historical trauma affects health (Conching & Thayer, 2019), as do neocolonial structures. And, our phenotypes are influenced by trajectories that possibly span multiple generations. A child's pathways for growth, immune maturation, metabolism, and endocrinology are optimized according to experiences during early development, much of which occur in utero. The pathways are therefore influenced by the mother's interaction with her environment (Barker, 1998). During dynamic periods of environmental change (such as with nutrition transition and colonialism), developmental optimization for previous generations' experienced resources is not actually optimal for the child in later life, and may result in increased risk of disease and severe effects from disease (Burdge et al., 2011; Gluckman & Hanson, 2006).

It is useful to examine nutrition in the past to best understand how historical trajectories in nutrition and nutrition-related disease are part of ongoing nutrition transitions. Estimating nutritional changes and nutritional epidemiology in the past provides a baseline that we can use to interpret how nutrition and nutrition-related disease have changed over time. The main goal of this dissertation is to examine if, how, and why nutrition-related disease prevalence changed due to factors associated with colonialism.

Since I argue the importance of adding historical information and deep time perspectives to nutrition transition research, I use the terms "colonial" and "colonialism" throughout this dissertation to frame the "new, shared social terrain" (Silliman, 2005:62) of Indigenous-European interaction as a long process that extends into the present (Gosden, 2004). The period I refer to as "early Colonial" was likely characterized by only indirect trading relationships between Siouan groups and Europeans, rather than the direct, sustained interactions that the terms "colonization" and "colonialism" sometimes convey (Waselkov & Paul, 1980). Because this dissertation investigates nutrition, however, I use "colonial" to encompass the whole time period during which the effect of European colonists may have influenced processes relevant to nutrition, even if those processes occurred prior to direct interactions. For Piedmont Siouan groups, this time period is 1620–1710.

During this time, Piedmont Siouan groups may have altered their subsistence practices to accommodate the increased demand for deerskins or as an adaptation to more frequent settlement relocation (Gremillion, 1987, 1989). Siouan groups experienced new infectious diseases, and may have changed their ritual scheduling to promote healing (Eastman, 1999; Roark, 2020; VanDerwarker et al., 2007). And, individuals possibly renegotiated their social identities as groups amalgamated, group members devised new social roles to participate as entrepreneurs in

the deerskin trade, and epidemics caused heightened disruption in succession of social roles (Eastman, 1999; Lapham, 2005; Lightfoot, Martinez, & Schiff, 2010). However, colonial interactions and processes were by no means uniform, as Siouan groups involved in colonial interactions had different group sizes, cultural practices, social structures, and ecological knowledge that affected their intentions and actions as part of the shared socioeconomic terrain with Europeans. Therefore, while I do examine temporal trends in nutrition-related disease, I also examine differences among the cultural groups who lived within different river drainages, where possible.

To best appreciate the multiple influences on nutrition-related disease, I consider the effects of both diet and physiology (specifically, the interaction between the immune system and micronutrient metabolism) on nutrition. Immunostimulation, also known as an immune system reaction, is expensive for the body to initiate and sustain in terms of physiological resources, of which micronutrients are an important category (Calder, 2013; Carr & Maggini, 2017). Immune system reactions are difficult to study osteologically, as they often involve soft tissues that do not preserve in skeletal remains. Oral focal infection theory suggests that diseases of the mouth have systemic impacts due to the immune system response to dental disease; therefore, skeletal evidence of dental disease provides a potential window into immunostimulation (Han & Wang, 2013; Offenbacher & Beck, 2014).

3.2 Oral focal infection theory

Oral focal infection theory particularly emphasizes the whole-body effects of periodontal disease, which starts as a bacterial infection of the tissues surrounding teeth (Hajishengallis, 2014). Periodontal disease progresses as disease-causing bacteria thrive and reproduce instead of being cleared by the immune response, which stimulates a continued immune response by the

host that damages the periodontal soft tissues and eventually the alveolar bone surrounding teeth (Graves, 2008; Hajishengallis, Moutsopoulos, Hajishengallis, & Chavakis, 2016; Takayanagi, 2005). While periodontal pathogens stimulate localized production of inflammatory mediators, such as cytokines and interleukins, this local immune response can become systemic through two suggested pathways.

Bacterial translocation is one suggested pathway – the bacteria and their byproducts may enter the bloodstream through even minor agitations to the inflamed tissues, allowing the bacteria to disperse and cause infections elsewhere in the body (Han & Wang, 2013; Scannapieco, 2013). Immune dysregulation is the other purported pathway – chronic immunostimulation can interfere with the immune system's regulatory mechanisms and the balance between anti- and pro-inflammatory pathways (Hajishengallis, 2015).

Immune dysregulation could cause the body to experience an inappropriate response to other physiological stressors, such as exposure to viruses, psychosocial stress, and wound healing (Crespo et al., 2017; Lamster & Ahlo, 2007; Seymour, Ford, Cullinan, Leishman, & Yamazaki, 2007). Dental diseases, particularly periodontal disease, are a strong indicator of immunostimulation that can be studied in skeletal remains (DeWitte & Bekvalac, 2011; DeWitte & Stojanowski, 2015).

3.3 Ecological Immunology

The immune response to immunostimulation varies from person to person, and ecological immunology suggests that multiple contextual factors shape the magnitude and duration of a person's immune response (McDade, 2003, 2005). Trade-offs during the development of the immune system, the inherent plasticity of the immune system during adult life, and the compounding immunological stressors are all factors that influence the immune response

(Abrams & Miller, 2011; Gowland, 2015; Long & Nanthakumar, 2004). Trade-offs during immunological development (which occur during early life) are shaped by cues signaled by an infant's mother regarding her environmental exposures, as well as exposures the infant themselves experiences during early life. These could be related to available nutritional resources and/or the burden of infectious disease in the local disease ecology.

The immune system and other physiological pathways such as musculoskeletal growth and reproduction compete for the body's finite resources, and the developing infant may experience tradeoffs in development of one or multiple of these systems if their developing environment signals resource stress (Burdge et al., 2011; Gluckman, Hanson, & Beedle, 2007; McDade, 2003; Wells, 2010). For example, a developing infant may invest less in specific, energetically-expensive immunological pathways to favor investment in general immunological pathways such as those involved in the inflammatory response (McDade, 2003). Such tradeoffs can affect the regulation and magnitude of the immune response to certain stressors later in life.

Additionally, the immune system retains some degree of plasticity even after it has fully developed, and it can thus make trade-offs in its function if the body is in a low-resource state. This is so the body can conserve and divert resources to other physiological processes that require resources, such as psychosocial stress, reproduction, and musculoskeletal maintenance (Long & Nanthakumar, 2004; McDade, 2005). For example, psychosocial stress suppresses the pro-inflammatory and specific antibody responses of the immune system, while promoting the anti-inflammatory response (Akcali, Huck, Tenenbaum, Davideau, & Buduneli, 2013; Glaser & Kiecolt-Glaser, 2005; Kemeny & Schedlowski, 2007; Rosania, Low, McCormick, & Rosania, 2009), and ecological immunology suggests that the body may do this to prevent the immune

system from initiating an expensive response when there are competing demands on the body's resources (McDade, 2005).

The theory of ecological immunology also suggests that sources of immunostimulation can intersect to dysregulate the immune response and therefore cause a person to experience an inappropropriately high or low response to a stressor (Crespo et al., 2017; Seymour et al., 2007). This idea of intersecting and compounding sources of immunostimulation draws from the concept of allostatic load, which refers to the degree and duration of stress experienced by a person and suggests that prolonged stress exposure can lead to wear and tear on the body that negatively affects health (McEwen, 1998; Sapolsky, 2018; Wiley & Allen, 2017). A high allostatic load, which involves chronic immunostimulation, can be damaging to a person's tissues and organs and dysregulate the activity of a person's immune system (Crespo et al., 2017; A. Graham, Allen, & Read, 2005).

To provide an example of allostatic load in the context of ecological immunology, we can consider how a person may experience musculoskeletal injury and psychosocial stress at the same time. The process of healing from injury involves a sustained immune response (Einhorn & Gerstenfeld, 2015), as does psychosocial stress, which can result from threats of raiding, climate unpredictability, food insecurity, and socioeconomic inequality (among other stressors) (Gowda, Hadley, & Aiello, 2012; Kemeny & Schedlowski, 2007; McEwen, 1998; Pike & Williams, 2006). Prolonged immunostimulation caused by high allostatic load (such as the concurrent experience of wound healing and psychosocial stress) shapes disease risk and severity and is therefore important to consider in the reconstruction of population epidemiology (Crespo et al., 2017; A. Graham et al., 2005). Additionally, multiple vitamins and minerals are critical to the functioning of the immune system, and someone with a prolonged and dysregulated immune

response may have nutritional requirements that exceed their dietary intake, leading to nutrient deficiencies (Calder, 2013; Scrimshaw, 2003).

3.4 Summary

Historical trajectories may influence patterns of nutrition-related disease in current global health. Factors involved in colonialism such as socioeconomic and dietary change, increased risk of raiding and violence, altered disease ecologies, and dynamic social roles and landscapes of power likely affected nutrition and nutrition-related disease. Those changes to nutrition may be part of a trajectory that has been reinforced through colonial and neocolonial structures to influence present-day nutrition and nutrition-related disease (Raschke & Cheema, 2008; Ulijaszek, Mann, & Elton, 2012). Characterizing nutrition-related disease in the past, and how it changed with colonialism, provides an important baseline to begin to merge past and present information in the study of nutrition transitions. To do that, we need information about diet as well as immune system stimulation and function, given the synergism between nutrition and infection (Scrimshaw, 2003).

Stable isotope analysis is useful to reconstruct diets, and the relationship between isotopic composition of diets and tissues is reviewed in Chapter 4. The immune system stimulation and function of past people are not as easy to study. I suggest that oral phenotypes such as periodontal disease are a useful proxy for immune stimulation, guided by oral focal infection theory. However, there is biocultural variation in the immune response. For example, someone who experienced stressors in early life, or has chronic immunostimulation from psychosocial stress and/or inflammatory disease may have a dysregulated immune response. Therefore, I use ecological immunology, which highlights how biocultural factors may affect immune system

regulation and dysregulation to interpret my findings regarding the relationship between immunostimulation and nutrition.

CHAPTER 4: MATERIALS, MEASURES, AND METHODS

The nutritional epidemiology of past Piedmont Siouan communities from AD 800 to 1710 is my primary research focus, and the skeletal remains of past Siouan people are therefore the main research sample presented in this study. I also analyze several research samples in addition to the Siouan Piedmont skeletal sample in order to pilot my dietary reconstruction methods and refine my interpretation of how various skeletal lesions are connected by physiological systems. Table 4.1 includes information on the skeletal sample of Piedmont Siouan populations, the sample used to pilot dietary reconstruction of dental calculus, and the contemporary sample used to investigate physiological pathways.

Table 4.1

Summary of Research Populations

Sample set	No. of individuals	Relevant Chapter(s)
Past Siouan populations	145	
Dan River populations	101	6, 7
Eno River populations	17	
Roanoke River populations	27	
Dental calculus isotopic study	62	5
NHANES PD-nutrition study†	1853	8
Interpretive baseline samples	88	6

† National Health and Nutritional Examination Survey (NHANES), periodontal disease (PD)

4.1 Siouan people from Piedmont North Carolina and Virginia, AD 800–1710

I use the following time periods in this study: the Late Woodland period, split into early and late (AD 800–1400 and AD 1400–1620, respectively), and the Colonial period, also split into early and late (AD 1620–1670 and 1670–1710, respectively). Chapter 2 reviewed

geopolitical, sociocultural, ecological, and epidemiological trends that occurred during these respective periods.

Dan River Drainage

Siouan groups from the early Late Woodland period transitioned from living in dispersed settlements to the larger, more populous settlements surrounded by palisades that were characteristic of the second half of the Late Woodland period (H. T. Ward & Davis, 1999). Maize was a significant dietary element of Late Woodland groups from the Dan River, especially those who lived during the second half of the period (Gremillion, 1989; H. T. Ward & Davis, 1999).

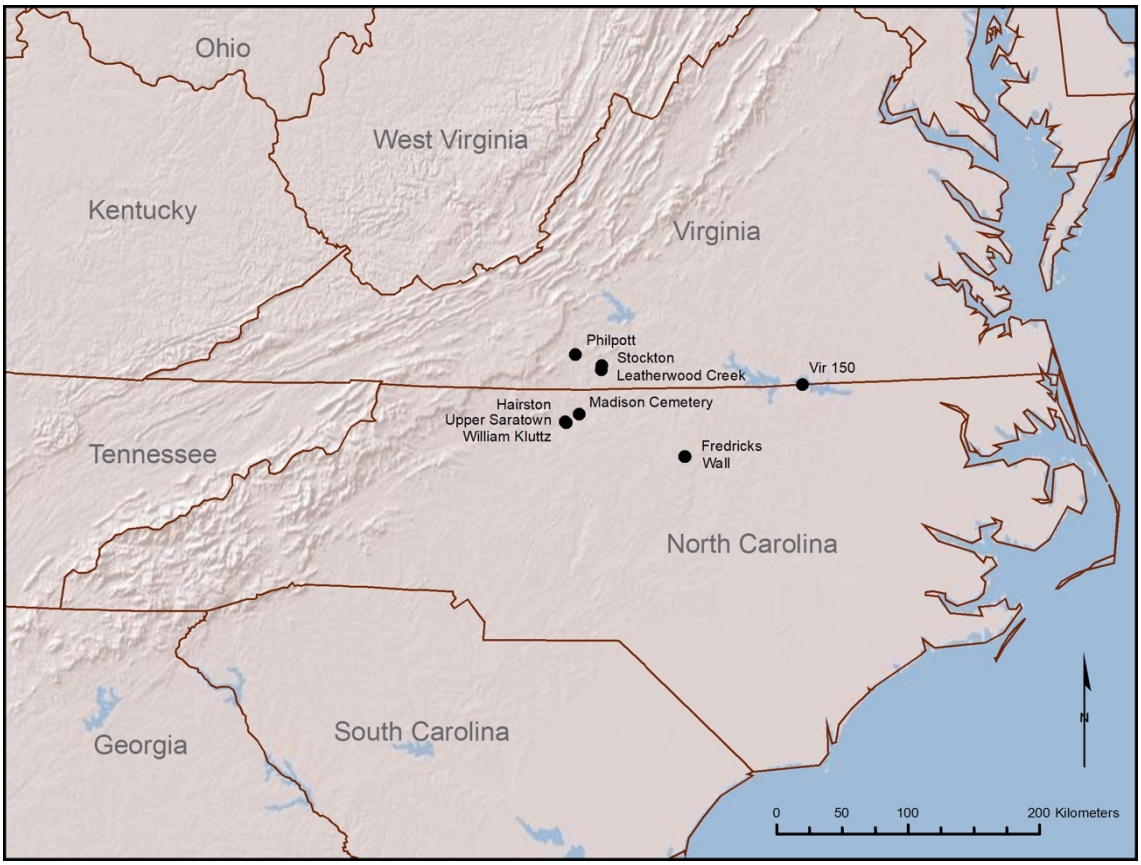
Archaeological information suggests little change in settlement or subsistence patterns through the second half of the Late Woodland into the early Colonial period; however, settlement patterns transitioned from dense, compact settlements to dispersed households towards the end of the late Colonial period (Gremillion, 1989; H. T. Ward & Davis, 1999). Information on the burials from the Dan River drainage sites is presented below. According to the initial skeletal inventory by Pat Lambert, 17.5% of the individuals from the Dan River drainage sites were completely or nearly completely preserved, 25.4% were partially preserved, and 57.1% were very incompletely preserved (Davis, Lambert, Steponaitis, Larsen, & Ward, 1998). Individuals represented by complete or nearly complete skeletons have at least 75% of their individual skeletal elements present and observable. By contrast, individuals represented by partial skeletons have 25-75% and individuals represented by very incomplete skeletons have less than 25% of their skeletal elements preserved.

Leatherwood Creek (Vir196)

The Leatherwood Creek site (44Hr1) is located in the Smith River drainage system, which is a tributary of the Dan River (Davis, Lambert, et al., 1998; Davis & Ward, 1991; Gravely, 1983) (Figure 4.1). Avocational archaeologists excavated the site in 1968 and 1969. Material culture from the site suggests that it was occupied during the Late Woodland period (AD 1000–1450) (Davis, Lambert, et al., 1998; H. T. Ward & Davis, 1999). Radiocarbon dates suggest there may have been two separate Late Woodland occupations: AD 1235–1280 and AD 1405–1450 (Gallivan, 1997).

Figure 4.1

Map of relevant Siouan archaeological sites (courtesy of R.P. Stephen Davis Jr., Research Labs of Archaeology)



The Leatherwood Creek site was abandoned by the end of the Late Woodland period, but ceramic evidence suggests that some groups of the Late Woodland period Dan River settlements, like those living at Leatherwood Creek, may have amalgamated into the larger communities more characteristic of the latter half of the Late Woodland period (Davis & Ward, 1991; Eastman, 1999; Wilson, 1983). People from other areas across the Piedmont may have also joined these communities, as ceramic assemblages from terminal Late Woodland and Colonial period Dan River settlements show possible cultural influence from groups who lived in the area of the Catawba River (Davis & Ward, 1991; Wilson, 1983). Therefore, I interpret temporal trends among the Dan River skeletal populations according to the hypothesis that people who lived at the Leatherwood Creek site and the Stockton and Philpott sites (described below) may have been ancestral communities of the Colonial period Dan River communities.

Excavations at Leatherwood Creek revealed nine human burials, and Richard Gravely (one of the avocational archaeologists who excavated Leatherwood Creek) donated the skeletal remains and associated funerary objects from these burials to the Research Labs of Archaeology (RLA), along with excavation notes from the site (Davis, Lambert, et al., 1998; Davis & Ward, 1991; Gravely, 1983; H. T. Ward & Davis, 1999).

Stockton (Vir231)

The Stockton site is located close to the Leatherwood Creek site, also in the Smith River drainage system (Davis, Lambert, et al., 1998) (Figure 4.1). Avocational archaeologists excavated the site in 1969 and1970. The material culture evidence from the site suggests that people lived at the Stockton site during the Dan River phase of the Late Woodland period (AD 1000–1450) (Davis, Eastman, Maher, & Gravely, 1997; Davis, Lambert, et al., 1998; H. T. Ward & Davis, 1999). The Stockton site is one of several circular, palisaded settlements built during

the Late Woodland period in the Dan River drainage (Davis & Ward, 1991; H. T. Ward & Davis, 1999). The circular organization of the Stockton site differs from the Leatherwood Creek site, which was composed of linearly organized, dispersed structures (Gravely, 1983; H. T. Ward & Davis, 1993, 1999).

People who lived at Late Woodland sites in the Dan River drainage may have amalgamated into larger communities along with groups from other areas of the Piedmont later in the Late Woodland period. See the above section on Leatherwood Creek for more information on the possible sociocultural affiliation among groups who lived in the Dan River drainage system during different temporal periods. Richard Gravely, one of the excavators of the Stockton site, donated the skeletal remains from 24 human burials excavated at Stockton to the RLA. He also donated funerary objects from some of the burials and excavation records from the site (Davis, Lambert, et al., 1998).

Philpott (Vir199)

The Philpott site (44Hr4) is located on the Smith River northwest of the Leatherwood Creek and Stockton sites (Figure 4.1). Avocational archaeologists excavated the site in 1964–1965, 1974–1975, and 1985 (Davis, Eastman, Maher, & Gravely, 1998; Davis, Lambert, et al., 1998). Artifacts recovered from the site suggest that people sporadically occupied the Philpott site between 8000 BC and AD 1200, after which they built a large, palisaded village that they occupied during the Late Woodland period (AD 1200–1400). Excavations also revealed evidence of another occupation at the Philpott site, dated AD 1620–1710 (Davis, Eastman, et al., 1998). While multiple Late Woodland sites were located along the Smith River, most sites from the Colonial period were located on the Dan River, thus making the Philpott site an interesting point

of inquiry into the population movement and aggregation of the periods postdating the Late Woodland period (Davis, Eastman, et al., 1998).

Ceramics found at the Philpott site from the Late Woodland component suggest that the people who lived at the Philpott site shared sociocultural affiliation with the residents of Leatherwood Creek and Stockton, and may have all been ancestral to the Sara who lived at later Dan River sites (Davis, Eastman, et al., 1998; Davis & Ward, 1991). Excavations revealed 21 burials dating to AD 1300–1400 and two burials associated with the AD 1620–1710 component (Davis, Eastman, et al., 1998; Davis, Lambert, et al., 1998).

Hairston (Sk1)

The Hairston site (31Sk1, also known as Early Upper Saratown) is located on the Dan River near where it meets with Town Fork Creek, in the same vicinity as the Upper Saratown site (Wilson, 1983) (Figure 4.1). The site was systematically excavated by the RLA in 1981, though relic hunters had already looted the site in the previous decades, causing damage to the archaeological record and human skeletal remains (H. T. Ward & Davis, 1999).

Siouan groups built and used the site during the latter half of the Late Woodland period (AD 1450–1620). Ethnohistorians note that members of the Sara tribe lived in the Dan River drainage system in the 17th and early 18th centuries, in an area closely correlated with the location of the Upper Saratown archaeological site (Davis and Ward, 1999; Wilson, 1983). The proximity of the Hairston site to the Upper Saratown site, and the material culture similarities between the two, suggest that the people of the Hairston site are affiliated with and possibly ancestral to the Sara (Wilson, 1983).

RLA excavations in 1981 revealed six human burials that had not been looted. Davis, Lambert, et al. (1998:42) note the likelihood of more burials that did not preserve well:

"Wilson's excavation also recovered human bone fragments from general excavation contexts. In addition to these remains, several human bone fragments were collected in 1974 while mapping the site and making a controlled surface collection. These remains likely represent the contents of burials that were disturbed by prior looting." Gravely excavated six burials at the site in the late 1960s, and donated the skeletal remains and associated funerary objects from those burials to the RLA (Davis, Lambert, et al., 1998), some of which are possibly associated with the first half of the Colonial period.

Upper Saratown (Sk1a)

The Upper Saratown site (31Sk1a) is a large village surrounded by palisades located adjacent to the Hairston site on the Dan River (Figure 4.1). Relic hunters discovered and looted the site in the late 1960s through early 1970s, until the RLA commenced a ten-year archaeological project (1972-1981) to salvage and recover information from the archaeological features (H. T. Ward & Davis, 1999). Upper Saratown is a multicomponent site that has features and burials associated with the early–middle Late Woodland phase (AD 800–1450), and also includes features and burials associated with the Colonial period – both early and late (AD 1620–1670 and AD 1670–1710, respectively) (Eastman, 1999).

Ethnohistoric and material culture evidence indicate that the Sara were the occupants of Upper Saratown during the 17th and early 18th centuries (Davis & Ward, 1991). It is probable that earlier occupants of the Upper Saratown site were ancestral to the Sara, as evidenced by similarities in ceramic assemblages, as well as regional trends in site amalgamation (H. T. Ward & Davis, 1993). RLA excavations revealed 112 burials. Many of the burials had been previously disturbed by looters, which compromised the preservation of skeletal remains and mortuary evidence (Davis, Lambert, et al., 1998).

Madison Cemetery (Rk6)

Madison Cemetery (31Rk6) is located on the Dan River near its confluence with the Mayo River (Figure 4.1). Avocational archaeologists excavated the Madison Cemetery site in 1966–1967. The archaeologists referred to this village site as a cemetery because of the high number and density of burials excavated (Eastman, 1999; Gravely, 1969; H. T. Ward & Davis, 1999). Many of the graves contained glass beads and copper ornaments, which were produced by Europeans and acquired through trade (H. T. Ward & Davis, 1999). The prevalence and types of archaeological material culture suggest that the Madison Cemetery site dates to the Colonial period, AD 1670–1710 (Eastman, 1999; Gravely, 1969; H. T. Ward & Davis, 1999).

The people from the Madison Cemetery site are likely associated with the Sara tribe (Davis, Lambert, et al., 1998; H. T. Ward & Davis, 1999). Original excavations revealed 130 burials at the Madison Cemetery site, and Gravely donated the skeletal remains from 45 of those burials (which are mostly represented by teeth) to the RLA, along with funerary objects and excavation records (Davis, Lambert, et al., 1998).

William Kluttz (Sk6)

The William Kluttz site (31Sk6) is located very close to the Hairston and Upper Saratown sites, all on the Dan River near where it meets Town Fork Creek (Figure 4.1). These three sites – William Kluttz, Hairston, and Upper Saratown – were extensively looted in the late 1960s and early 1970s, which damaged at least 45-50 burial pits at the William Kluttz site (Davis, Lambert, et al., 1998). Members of the RLA salvaged and mapped visible archaeological information when they were first informed of the damage to the site, and formally excavated the site in 1988.

The William Kluttz site had both an early-middle Late Woodland component, as well as a Colonial period component (Eastman, 1999). The Colonial period village site was probably

occupied for a short period of time during the temporal window of AD 1690 to 1710 (H. T. Ward & Davis, 1999). Ward and Davis (1993) suggest that the William Kluttz site may represent the terminal occupation of the Sara in the Dan River drainage, after which the Sara moved southward and eventually merged with the Catawba (Davis & Ward, 1991). The people who lived at the William Kluttz site may have been more socioculturally diverse than earlier Dan River drainage communities, as the variation in stylistic and functional profiles of ceramic artifacts from the William Kluttz site led Ward and Davis (1999:250) to postulate that "fragments of ethnically diverse Siouan groups may have merged with the Sara at the William Kluttz site to form a dispersed refuge community."

The RLA obtained some of the human skeletal remains from the people who looted the William Kluttz site and RLA members surface collected exposed human bone that had been disturbed by plowing and by looters. Twelve burials were also excavated during the 1988 field season, and many more burial pits were identified but not excavated (Davis, Lambert, et al., 1998; H. T. Ward & Davis, 1993). A large proportion of the burials from the William Kluttz site consist of juvenile remains. Ward and Davis (1993) suggest that these burials were tightly clustered and shallowly-interred, possibly made over a short period of time. There was no evidence of traditional mortuary practices accompanying their interment. Some adult burials, however, were placed away from the juvenile cemetery and displayed traditional burial structure and mortuary process (H. T. Ward & Davis, 2001).

Rk8 and the Sharp Site (Rk12)

Rk8 (31Rk8) and the Sharp site (31Rk12) were both salvage excavations of sites on the Dan River following Hurricane Agnes in 1972. A single burial, possibly from the Late Woodland

period (AD 1000–1450) was exposed at Rk8, and three Dan River phase burials were exposed at the Sharp site (31Rk12) (Davis, Lambert, et al., 1998).

Eno River Drainage

People who lived within the Eno River and nearby Haw River drainage systems changed their community structures in somewhat similar ways as groups from the Dan River drainage system; namely, they shifted from dispersed households to more compact villages encircled by palisades during the latter half of the Late Woodland period (H. T. Ward & Davis, 1999). However, Siouan groups who lived within the Eno River drainage system never resided in as large or populated of settlements as Siouan groups from the Dan River, nor did they continuously occupy sites for as long as some of the Dan River sites were occupied. For example, approximately 100 to 150 people lived at the Wall Site (terminal Late Woodland) for several decades (Davis & Ward, 1991; Petherick, 1987; H. T. Ward & Davis, 1999).

This is in contrast to the larger population density at the Upper Saratown site (estimated at 200 to 250 residents) on the Dan River, which includes archaeological features from the Late Woodland period through the Colonial period (Eastman, 1999). Information on the burials from Eno River drainage sites is presented below. According to Lambert's analysis, 27.8% of the individuals from the Eno River drainage sites were completely or nearly completely preserved, 55.6% were partially preserved, and 16.7% were very incompletely preserved (Davis, Lambert, et al., 1998).

Wall (Or11)

The Wall site (31Or11) is a village on the Eno River that contained numerous circular houses surrounded by a palisade (Davis & Ward, 1991; Petherick, 1987; H. T. Ward & Davis, 1999) (Figure 4.1). It is associated with the Late Woodland period (AD 1400–1600) (Davis,

2016; H. T. Ward & Davis, 1999). Joffre Coe, a UNC-Chapel Hill archaeologist, first excavated the site in 1938, followed by excavations led by Robert Wauchope in 1940–1941. The RLA revisited and resumed excavations at the Wall site in 1983, and the site is a current location of RLA field schools (Davis, Lambert, et al., 1998; H. T. Ward & Davis, 1999).

Ceramic similarities between the pottery assemblage at Wall and the assemblages at sites associated with the Shakori and Saxapahaw suggest that Wall was likely built and occupied by ancestors of the Shakori, Saxapahaw, Eno, and/or Adshusheer (Davis, 2002, 2016). One burial was excavated during the 1938 field season, and four more during the 1940–1941 excavations. Three additional burials were excavated during the 1983–1984 excavations (Davis, Lambert, et al., 1998).

Fredricks (Or231)

The Fredricks site (31Or231) is located on the Eno River near the Wall site (Figure 4.1). Members of the RLA excavated the Fredricks site between 1983 and 1986 (Davis & Ward, 1991; Dickens, Ward, & Davis, 1987; H. T. Ward & Davis, 1999). Excavations revealed many European artifacts at the Fredricks site, indicating that the group who resided at Fredricks likely engaged in direct trade with Europeans (H. T. Ward & Davis, 1999). The material culture assemblage of the Fredricks site, in conjunction with ethnohistoric reports, suggests that the Occaneechi tribe constructed and lived at the Fredricks site for less than one decade during the period of AD 1680–1710 (Davis, 2002; H. T. Ward & Davis, 1999).

Twenty-six burials were associated with the Occaneechi occupation of the Fredricks site, 13 of which were excavated and removed during the 1983–1985 field seasons (Driscoll, Davis, & Ward, 2001). The burials at the Fredricks site included many European-produced grave goods, which were likely acquired through trade. Additionally, the style of grave diverged from earlier

periods – the Occaneechi at the Fredricks site built "straight-sided graves dug with metal tools" rather than the shaft-and-chamber burial pits used by earlier groups living along the Eno River (H. T. Ward & Davis, 1999:242).

Roanoke River Drainage: Vir150

Vir150 (44Mc645) is located in the Roanoke River drainage (Figure 4.1). It was excavated by UNC-Chapel Hill archaeologists in 1962 as part of the archaeological survey for Virginia Power and Light Company's Gaston reservoir (Davis, Lambert, et al., 1998). The material culture from the site suggests that people occupied Vir150 during the Late Woodland period (AD 1000–1400). However, the sociocultural affiliation of the people who lived at Vir150 is unclear, and more analysis is needed to refine our understanding of who lived at the site and when (Davis, Lambert, et al., 1998; VanDerwarker, 2001). Excavations at Vir150 revealed 29 human burials. According to Pat Lambert's analysis, 53.85% of the burials from Vir150 were completely or nearly completely preserved, 34.62% were partially preserved, and 11.54% were very incompletely preserved (Davis, Lambert, et al., 1998).

Previous research on Piedmont Siouan skeletal sample

S. Homes Hogue and Patricia Lambert, both RLA affiliates, investigated the disease and trauma patterns, biodistance, and dietary trace element composition of the Siouan Piedmont populations. Hogue (1988) collected data on people from the Wall, Hairston, Upper Saratown, Fredricks, and Shannon sites. The Shannon site (44My8) is a Late Woodland site (AD 1450–1525) in the New River drainage system that is possibly associated with the Tutelo Indians (Benthall, 1969; Hogue, 1988).

Hogue used inductively coupled argon plasma emission spectrometry to measure the skeletal content of the trace elements magnesium, zinc, copper, and vanadium from rib and

femur samples of Siouan Piedmont individuals. Her results, which she acknowledged were complicated by a low sample size for the Eno River groups and the potential of soil contamination in trace element analysis, indicated that people from the Fredricks site had the lowest skeletal magnesium concentrations, and that people from the Upper Saratown site had the highest zinc concentrations. She interpreted these results to suggest that the Occaneechi at the Fredricks site may have consumed less maize than other groups (since maize provides magnesium), and the Sara at Upper Saratown may have consumed more animal protein (which contains zinc) than other, particularly earlier, groups. However, controlled diet studies have indicated that the magnesium content of bone is not a reliable indicator of diet. The body's regulation of zinc, along with the susceptibility of zinc to diagenetic alteration, also positions zinc as an unreliable indicator of diet (Ezzo, 1994; Klepinger, 1990).

Hogue also analyzed the prevalence of porous cranial lesions, periodontal disease, osteitis, carious lesions, and linear enamel hypoplasia (LEH)[5]. She calculated the percent of the population affected by each condition and identified that people from the Fredricks site had the highest percentage of cribra orbitalia (porous lesions of the eye orbits), osteitis, LEH, and carious lesions compared to the other sites. Fredricks individuals had the second highest frequency of porotic hyperostosis (porous lesions of the parietal bones and sometimes the occipital bone) and periodontal disease, following Wall and Shannon site individuals, respectively. People from Upper Saratown had the lowest or second lowest frequency of all conditions she analyzed.

[5] I detail most of these measures in the ensuing "skeletal analysis" section. Hogue (1988:187) defined osteitis as "an inflammation of the bone caused by trauma, infection, or disease," which likely corresponds to the periosteal new bone lesions that I observed. Linear enamel hypoplasia is a manifestation of growth disruption that affects enamel deposition and can be observed on teeth.

Lambert analyzed all of the skeletal populations outlined in the above "Siouan People from Piedmont North Carolina and Virginia" section, which she identifies as "northern Piedmont populations" in her work. She used osteological and paleopathological analyses to measure the frequency of carious lesions, cribra orbitalia, LEH, and periosteal lesions. Her results indicate that the frequency of carious molars among northern Piedmont groups declined from the Late Woodland to the Colonial period, as did the frequency of people with periosteal new bone lesions on their tibiae. She also identified that more people had LEH in the Colonial period than the Late Woodland period. However, the percent of people affected by cribra orbitalia showed little change over time (Lambert, 2000).

4.2 Research population for dental calculus isotopic study (Chapter 5)

Multiple biomaterials are useful for reconstructing diet from past and modern populations – bone, tooth enamel, hair, and nails are all formed from metabolic byproducts of diet and can be analyzed via stable isotope analysis to measure a person's dietary composition (Ambrose, 1993; Reitsema, 2013; Schoeninger, 2011). Scott and Poulson (2012) first identified the potential of dental calculus as a biomaterial for stable isotope analysis. Dental calculus is formed when the glycoprotein and bacterial plaque that coats tooth surfaces becomes mineralized (Lieverse, 1999). Both the glycoproteins and the minerals are dietary in origin (Chapter 5; Marsh & Martin, 2009). Dental calculus may also contain plant remains such as starch granules, phytoliths, and macrobotanicals, as well as proteins and other chemical compounds indicative of diet, cultural practices (e.g. working fibers with teeth), and possibly medicinal plant consumption (Buckley, Usai, Jakob, Radini, & Hardy, 2014; Charlton et al., 2019; Hendy et al., 2018; Radini, Nikita, Buckley, Copeland, & Hardy, 2017).

Dental calculus can be removed and analyzed without harming the underlying skeleton (Scott & Poulson, 2012). I evaluate the association between dental calculus and bone isotopic ratios in Chapter 5 (Paper 1) to ascertain whether dental calculus can be used as a proxy for bone in stable isotope analysis. Testing the association between calculus and bone required a skeletal sample from which both bone and calculus samples could be measured, and I aimed to include people from a diversity of time periods and geographic regions. A diverse research population strengthens the interpretation that any calculus-bone correlations are the result of human metabolism and physiology, rather than the result of specific cultural practices of food preparation, or an artificial signature from the soil chemistry of specific burial contexts.

Not everyone in a population has sufficient dental calculus for stable isotope analysis; therefore, using dental calculus as the primary biomaterial for dietary reconstruction through stable isotope analysis skews the sample towards older adults with more calculus appreciation. There is some literature regarding the effect of diet on dental calculus formation, but no clear consensus. An *in vitro* study identified that carbohydrate and oil consumption increased calculus formation, while protein consumption decreased calculus formation (Hidaka & Oishi, 2007). A review by Lieverse (1999) suggested an opposite effect – that protein consumption affects oral salivary pH to promote calculus formation. Hydration status, saturation of the saliva with calcium phosphate, bacterial biofilm effects, and endocrinology all affect biofilm formation and calculus mineralization (Lieverse, 1999; Tawfig et al., 2017).

Therefore, it is theoretically possible that the use of dental calculus for dietary reconstruction also limits our window of population diets to only those who consumed diets that promote dental calculus formation. However, diet is only one factor of many that influences calculus formation. More research is necessary. Chapter 5 includes more specific details on the

groups whose dietary isotopic ratios I use to test the association between calculus and bone isotopic ratios.

4.3 Research population for study linking periodontal disease to nutrition and the immune system (Chapter 8)

Oral focal infection theory is one of the guiding frameworks of my research. It posits that infections of the mouth have the potential to lead to systemic infection and inflammation (see Chapter 3). I am particularly interested in using oral focal infection theory to consider how infections of the mouth may affect nutrition. The immune system is the theoretical link between oral infections and nutrition, as an immune system reaction (immunostimulation) is expensive for many of the body's resources, including nutrition (Scrimshaw, Taylor, & Gordon, 1968; Tomkins, 2003). However, immune system function is most directly measured through soft tissue, which is not accessible in skeletal remains. Datasets that contain nutrition, immune system, and periodontal disease information from living humans are therefore an important complement to data from past populations, as they allow us to test correlations among multiple systems of the body, including those not preserved in skeletal remains.

I used the National Health and Nutrition Examination Survey (NHANES) 2003–2004 dataset for my research in Chapter 8, as it contains information on periodontal disease, which is a main skeletal phenotype of interest in this study and for oral focal infection theory in general. The study presented in Chapter 8 used a subset of the NHANES 2003–2004 participants: participants were included if they had measurements for all of the phenotypes of interest, but were excluded if they reported that they smoke cigarettes, as cigarette smoking both exacerbates periodontal disease and reduces the body's levels of vitamin C and is therefore a significant confounding factor (Barbour et al., 1997; Nishida et al., 2000; Pamuk et al., 1994).

4.4 Floral and faunal interpretive baseline samples from Piedmont North Carolina (Chapter 6)

Dietary reconstruction through stable isotope analysis is improved by comparing the isotopic ratios of food sources to the isotopic ratios of human biomaterials such as bones, teeth, and – in the case of my research – dental calculus. Food sustains, builds, and maintains the human body, and the chemical composition of foods is reflected in the chemical composition of biological tissues. As a result, we are able to approximate dietary composition from the stable carbon and nitrogen isotope ratios of the body's tissues (Schwarcz & Schoeninger, 1991). Dietary approximation from biomaterials is improved by a specific understanding of the isotopic ratios of foods available to past people, because foods vary in isotopic composition based on multiple factors, many of which relate to ecosystem dynamics (Ambrose, 1991, 1993; Katzenberg & Weber, 1999; Tieszen, Senyimba, Imbamba, & Troughton, 1979). It is therefore useful to measure the isotopic values of food samples local to a person's area of subsistence.

I sampled local plants and animals from the north-central North Carolina Piedmont for the study presented in Chapter 6 (Paper 2) to improve the dietary interpretation of past Piedmont Siouan people. Though I sampled modern plants and animals as well as archaeological animal bones, I prioritized modern samples to minimize destructive sampling of archaeological collections. Archaeological fauna were included to check for congruence of isotopic ratios across modern and archaeological contexts, as anthropogenic landscape changes can alter modern isotopic ratios compared to their earlier analogues in the archaeological record (Eerkens, Mackie, & Bartelink, 2013; Schoeninger, 2010). Appendix 2 contains information about the plant and animal samples used to generate an interpretive baseline with which to compare human dietary isotopic measurements.

4.5 Key Measures and Methods

I combine multiple methods to reconstruct the nutrition of past Siouan communities. These methods include: skeletal analysis with a focus on lesions of nutritional deficiency and immunostimulation, dietary reconstruction using stable isotope analysis, and data from living populations to test pathways linking skeletal lesions to nutrition and biocultural context. I use logistic and linear regression models, causal mediation analysis, and exploratory data analysis to test relationships among my data. The following section provides a brief overview of the key measures and methods that I used in my research, and more specific information on the variables and methods can be found in Chapters 5 through 8.

Skeletal analysis

Nutrition, infection, and inflammation are all processes that disrupt bone metabolism and cause skeletal lesions. Information on the pattern and location of skeletal lesions is useful to reconstruct physiological processes. I use terminology throughout the dissertation to refer to different parts of the bones and teeth that may be affected by lesions. Table 4.2 provides a guide to this terminology. It is important to note that most of the biological elements described below play a role in the immune system. Berger et al. (2021) provides a review of skeletal immunology.

Table 4.2

Brief Guide to Skeletal and Dental Biology Based on White et al. (2012) and Hillson (1997)

Biological element	Description	Function
Periosteum	Thin tissue that covers bone surfaces	Nourishes bone, anchors muscles to bone, contains bone-forming cells
Spongy bone	Aka trabecular bone; lightweight, porous bone found on the interior of bones	Biomechanical support, mineral reservoir, supports blood formation (hematopoiesis; production of red and white blood cells)

Cortical bone	Dense bone on the external surfaces of bones Is first deposited as woven bone (characterized by disorganized structural proteins) and remodeled into lamellar bone (structural proteins are uniformly organized and thus mechanically stronger)	Protects bone, maintains body structure and weight bearing, mineral reservoir In long bones, houses and protects the yellow marrow, which stores fat and contains mesenchymal stem cells
Tooth enamel	Hard, hyper-mineralized tissue on the exposed surfaces of teeth	Protects teeth, facilitates chewing, source of stored minerals
Cementum of tooth root	Hard tissue that covers the tooth roots	Protects tooth roots, anchors the periodontal ligament
Dentine	Mineralized tissue that underlies the cementum and enamel and encases the pulp and root chamber.	Reinforces enamel, provides some defense again wear and carious lesion destruction, protects the pulp and root from infection.
Pulp chamber and canal(s)	Soft tissue housed within the tooth	Contains nerves and blood vessels to protect and maintain tooth health
Periodontal ligament	Connective the tooth roots to the surrounding alveolar bone	Anchors the tooth, provides nutrients to the alveolar bone and cementum
Gingiva	Aka gums; the soft tissue that surrounds teeth and overlies the alveolar bone	Protect teeth and bone against mechanical stress and microorganisms. Also protect roots, alveolar bone, and dental pulp from infection.
Alveolar bone	Bone surrounding tooth roots	Holds teeth in place, helps mitigate mechanical stress of tooth use
Osteoblasts	Bone forming cells	Form bone to support skeletal growth and maintenance
Osteoclasts	Bone resorbing cells	Remove bone to support skeletal growth and maintenance

Lesions of nutritional deficiency

I examined cranial bones for lesions indicative of nutritional deficiency. Porosity and vascularization are the lesion types most implicated in nutritional deficiency, but I also recorded lesions types possibly indicative of remodeled nutritional lesions, including abnormal thickening of the cranial bones, and pitting of the cortical surface of cranial bones (Wilczak, 2011). Porosity of cranial bones results from several processes, including hematopoietic marrow expansion, subperiosteal hematomas, vascular pathology, and disrupted bone mineralization and deposition. Micronutrient deficiencies may contribute to each of these processes. During hematopoietic expansion, the body can shift bone metabolism to increase the ratio of spongy-to-cortical bone, and the spongy bone can even perforate the cortical bone, leading to porosity (Ortner, 2003; Walker, Bathurst, Richman, Gjerdrum, & Andrushko, 2009). Expansion of the spongy bone is known as porotic hyperostosis if it affects the parietal and occipital bones, and cribra orbitalia if it affects the eye orbits (Brickley & Ives, 2008; Waldron, 2009; Walker et al., 2009; Wilczak, 2011).

Both iron and vitamin B_{12} have been suggested to cause porous lesions due to marrow expansion in hematopoietic areas (Brickley & Ives, 2008; Ortner, 2003; Walker et al., 2009), though this is debated. Walker et al. (2009) and Waldron (2009) argued that iron deficiency anemia *prevents* bony expansion for marrow hypertrophy and that cranial lesions could only be caused by vitamin B_{12} deficiency, genetic anemias, and red blood cell diseases like malaria (Gowland & Western, 2012; McIlvaine, 2015). Rothschild (2020) argues that lesions on the eye orbits are vascular, not porous, in nature and therefore cannot be caused by anemia but rather vascular disorders.

Like marrow expansion, hemorrhage can be another cause of cranial lesions. Hemorrhage of the cranial soft tissue leads to inflammation of the periosteum covering cranial bones – the periosteum is a vascularized tissue that is adhered to bone and promotes bone maintenance (Klaus, 2014b; Waldron, 2009). Periosteal inflammation can disrupt healthy bone metabolism. Vitamin C deficiency can cause hemorrhage and attendant periosteal inflammation, and especially affects the regions of the cranium where chewing muscles attach, including the eye orbits, mandibular ramus, zygomatic bones, and parietal bones. The eye orbits are another cranial region that is commonly affected (Brickley & Ives, 2008; Ortner, 2003). Ortner and Ericksen (1997) suggest that hemorrhage-induced porosity of the greater wing of the sphenoid bone is particularly indicative of vitamin C deficiency, though Waldron (2009) suggests that more clinical evidence is needed to confidently link sphenoid porosity with hemorrhage resulting from vitamin C deficiency.

Cranial porosity can also result from the deposition of poorly mineralized bone, which occurs during a disruption in bone mineralization pathways and is associated with vitamin D deficiency and calcium deficiency. Dietary minerals, biomechanical influences, and hormone pathways all maintain healthy bone mineralization. If any of these influences are disrupted, the body may deposit poorly mineralized bone, which manifests on the cranium as porous or pathologically thin bone (Brickley & Ives, 2008; Reginato & Coquia, 2003). For example, pregnancy and lactation both significantly but temporarily affect bone metabolism, stimulating high bone turnover and negative bone balance during certain stages (Brickley & Ives, 2008; Kovacs & Kronenberg, 2013; Møller et al., 2013), though the relationship between reproductive endocrinology and bone metabolism is moderated by the number of pregnancies, spacing of pregnancies, and length of breastfeeding. Agarwal's (2012) review of this relationship suggests

that women who had more children and extended breastfeeding had higher bone mineral density than women with fewer children and shorter breastfeeding. The frontal bone, parietal bones, and temporal bones are the most common cranial sites of poorly mineralized bone (Brickley & Ives, 2008).

Deficiencies in individual micronutrients are difficult to distinguish in skeletal remains due to two main factors: the interrelationships between micronutrients in metabolism and physiology, and the process of skeletal remodeling. Certain micronutrients, such as dietary iron and vitamin C, play complementary roles in metabolism and enhance the other's absorption, while others have antagonistic roles that decrease the other's absorption, such as calcium and phosphorous (Higdon, 2000, 2001). Remodeling occludes skeletal lesions over time and makes it difficult to identify lesions that occurred earlier in life.

When a person recovers from a micronutrient deficiency and their cranial bones begin to heal, the skeletal remodeling process transforms the original lesion. For example, healing and remodeled lesions originally caused by hematopoietic expansion, hemorrhage, and hypomineralization may all present as porosity and are very difficult to differentiate in the absence of radiographic examination (Brickley & Ives, 2008; Walker et al., 2009). To best pursue differential diagnosis of cranial lesions, I recorded multiple variables on the extent and pattern of cranial lesions and examined patterns in lesion location. Skeletal manifestations of processes such as hemorrhage and cranial bone thinning are useful evidence of physiological stress.

Lesions of infection and inflammation

The skeleton is integrated with the immune system, and both local and systemic immune reactions are recorded on the skeleton (Berger et al., 2021; Klaus, 2014a; Walsh et al., 2006).

Infection or trauma to the tissues surrounding bone, such as the periosteum, stimulates an inflammatory reaction and thus disrupts the normal balance of bone loss and bone deposition (Ragsdale, 1993; Weston, 2008). For example, an injury to the tibia may cause a hematoma (bruise) that damages the periosteum and separates it from the cortical bone. Mechanically, this separation between the periosteum and the underlying cortical bone reduces the blood supply to the bone, causing the bone to become necrotic, which increases osteoclastic activity to remove the damaged bone. Bone deposition via osteoblastic activity then resumes once the blood supply is restored (Jaffe, 1972; Weston, 2008).

If the injury and bone damage stimulate an immune response (which is likely), the body recruits inflammatory mediators to the site of the injury. Inflammatory mediators increase both bone gain and bone loss through different pathways: they dilate blood vessels, which reduces oxygen to the bone and increases bone loss, but they also cause blood vessels to become leaky, which excretes proteins that osteoblasts use to produce new bone (Klaus, 2014a; Roberts & Manchester, 2007; Weston, 2008). Bone is not just a passive target of the immune system, but instead communicates with and feeds back into the immune system. For example, osteoblasts amplify the immune response and local inflammation (Berger et al., 2021; Varoga et al., 2009). Other influences, including additional sources of immunostimulation in the body, hormone profiles, and nutritional status, intersect with the bone-immune nexus to alter the ratio of bone removal vs. bone deposition during an inflammatory reaction (Berger et al., 2021; Klaus, 2014a; Takayanagi, 2005; Walsh et al., 2006; Weston, 2008).

Newly deposited bone, whether on top of the surface of existing cortical bone or filling the void of damaged cortical bone, is at first woven (poorly organized, porous bone) and over time becomes remodeled into lamellar bone (uniformly organized, with its edges integrated into

the surface of the existing cortical bone) (Ortner, 2003; Weston, 2008; Wilczak & Jones, 2011). The visible differences in the textures of woven new bone and remodeled healing bone are useful to distinguish whether the lesion was active or healing at the time the person died. Periosteal new bone is one of the phenotypes that I use to examine extra-oral skeletal inflammatory reactions in Chapter 7 (Paper 3).

Periodontal disease observation in skeletal remains

Periodontal disease is a bacterial- and inflammation-associated disease that occurs when the immune response to chronic bacterial infection causes destruction of the alveolar bone surrounding teeth (Hajishengallis, 2015; Hajishengallis & Lambris, 2012; Jenkinson, 2011; Lamont, Koo, & Hajishengallis, 2018). It is clinically diagnosed using measurements of the degree of bone loss and the damage to soft tissues surrounding the teeth, including the periodontal ligament and the gingiva (gums) (Chapter 8).

The skeletal involvement of periodontal disease suggests that it should be identifiable in the osteological record, and bioarchaeologists have used various measurements to identify periodontal disease in past populations, including degree of calculus deposition (Wasterlain, Cunha, & Hillson, 2011), depth of alveolar resorption (DeWitte & Bekvalac, 2011; Kingsmill, 1991; Lavigne & Molto, 1995; Tuggle & Watson, 2019), texture of the alveolar bone (DeWitte & Bekvalac, 2011; Hillson, 2000; Kerr, 1988; Tomczyk, Turska-Szybka, Zalewska, & Olczak-Kowalczyk, 2017; Tuggle & Watson, 2019; Wasterlain et al., 2011), and microbial analysis of pathogens commonly implicated in periodontal disease (Adler et al., 2013; Warinner, Speller, & Collins, 2015). However, there is not a standard method to identify periodontal disease in skeletal remains, nor is there a clear threshold to demarcate disease vs. pre-disease in a way that corresponds to clinical assessment in living populations.

Periodontal disease may also affect systemic immune function and is indeed linked to other diseases, many of which involve dysregulated inflammation (Chaffee & Weston, 2010; Genco, Grossi, Ho, Nishimura, & Murayama, 2005; Kaur, White, & Bartold, 2013; Scannapieco, Bush, & Paju, 2003). DeWitte and Bekvalac (2011) investigate the relationship between periodontal disease and immune function in the past by testing the association between periodontal disease and periosteal new bone. Crespo et al. (2017) also test this relationship by investigating how the magnitude of inflammatory products released by human cells upon exposure to *Porphyromonas gingivalis* (a common pathogen implicated in periodontal disease) changes if the cells are first exposed to the bacteria that cause tuberculosis and leprosy.

In Chapters 7 and 8, I test the relationship between periodontal disease and nutritional deficiency, as micronutrients affect periodontal disease progression and support immune system function (Hujoel & Lingström, 2017; Ritchie & Kinane, 2003; Scrimshaw et al., 1968; Tomkins, 2003). Additional studies that improve periodontal disease assessment in past populations and test the relationship between periodontal disease and other skeletal phenotypes will advance the interpretation of the systemic involvement of periodontal disease in past and present.

Stable isotope analysis

My research employs carbon and nitrogen isotopic analysis of dental calculus as a potential proxy of bone for direct dietary reconstruction. The protein and mineral phases of both bone and dental calculus contain carbon and nitrogen. Their stable isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$) are controlled by those of the diet (Ambrose, 1993; Ambrose & Norr, 1993). It is possible to ascertain an individual's dietary composition by comparing skeletal isotope ratios to those of food categories that have consistent differences in isotopic composition (e.g. different trophic levels of protein).

Measurement of stable isotopes

There are three naturally occurring carbon isotopes: ^{12}C, ^{13}C, and ^{14}C. Of these, ^{12}C and ^{13}C are stable (they do not decompose into different isotopes or elements over time). The majority of carbon atoms in existence are ^{12}C (98.93%) and ^{13}C (1.07%) (T. Brown & Brown, 2011; Ehleringer & Rundel, 1989). The natural abundance of radiocarbon (^{14}C) is too low (one part per trillion or less) to detect with instruments for stable isotope analysis. The heavier carbon isotope, ^{13}C, decreases and increases in concentration in food chains through a process known as fractionation. Fractionation occurs during certain biochemical reactions (e.g., photosynthesis or metabolism) and results in the preferential uptake or excretion of one isotope over another. The lighter carbon isotope, ^{12}C, is preferentially excreted and respired (as breath CO_2) when a consumer metabolizes food and breaks down the dietary elements in food to build and maintain its bodily tissues, so most tissues become enriched in ^{13}C (Ambrose & Norr, 1993; DeNiro & Epstein, 1978).

The ratio of heavy-to-light isotopes in biological tissues and in food is measured through comparison to a standard using the following formula:

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

In this formula, R represents the ratio of the heavier to lighter isotope of the element of interest, X represents the heavier isotope of the element of interest, and delta (δ) represents the difference in the sample isotope ratio compared to standard isotope ratio. The unit for delta is permil (‰), because the difference in ratios between sample and standard is measured in parts per thousand (Ambrose, 1990; Schwarcz & Schoeninger, 1991).

The reference standard for carbon isotopes is the Vienna Peedee belemnite (VPBD) fossil carbonate; all samples and additional standards are reported as ‰ deviation from VPBD

(Coplen, 1995). There are two naturally occurring isotopes of nitrogen: ^{14}N and ^{15}N, both of which are stable. The former comprises 99.64% of all nitrogen atoms, and ^{15}N comprises the remaining 0.36%. The reference standard is atmospheric nitrogen (AIR N_2) (Coplen, 1995; Mariotti, 1983).

Stable isotopes in food chains

Stable isotopes enter the food chain through primary producers, such as plants and algae. Terrestrial plants assimilate atmospheric carbon dioxide (CO_2) and fix it into organic compounds through photosynthesis. Aquatic plants assimilate dissolved CO_2 (Pingram, Collier, Hamilton, David, & Hicks, 2012), and they uptake nitrogen through atmospheric fixation (atmospheric nitrogen) or assimilate nitrogen found in soil and water (Finlay & Kendall, 2007). Plants that build their tissues using carbon from the atmosphere follow one of three photosynthetic pathways: C_3, C_4, and Crassulacean acid metabolism (CAM).

All plants, no matter which pathway they use for photosynthesis, preferentially uptake ^{12}C during photosynthesis, thereby enriching the $^{12}C/^{13}C$ ratio slightly over the ratio in atmospheric CO_2 (A. J. Ward, 2005). Plants that follow the C_3 pathway also have a second fractionation step, as they prefer to convert CO_2 molecules containing ^{12}C into glucose, which further enriches the lighter isotope in the tissues of C_3 plants. This results in a $\delta^{13}C$ value of approximately -24 to -36‰ for C_3 plants, as ^{13}C is depleted compared to ^{12}C.

Plants that follow the C_4 pathway do not have a second fractionation step, but they instead convert all the available CO_2 (after the initial uptake step common to all photosynthetic plants, which prefers ^{12}C) into glucose. This results in a $\delta^{13}C$ of approximately -12.5‰ for C_4 plants; ^{13}C is still depleted compared to ^{12}C, yet less so than in C_3 plants (Bender, 1968; M. Brown & Ortner, 2011; O'Leary, 1988). Plants that follow the C_3 pathway include wheat, barley,

rice, root crops, legumes, and vegetables. Plants that follow the C_4 pathway include maize, sorghum, and millet, and sugar cane (Ambrose & Norr, 1993). CAM plants can vary photosynthetic process according to environmental conditions, resulting in a $\delta^{13}C$ that encompasses the range of C_3 and C_4 plants (-10 to -30‰). CAM plants include cacti, agaves, and bromeliads such as pineapple (Ambrose & Norr, 1993; Griffiths, 1992)

Nitrogen isotopes, like carbon isotopes, also undergo fractionation within food webs. Nitrogen-fixing soil bacteria and endosymbiotic N-fixing bacteria associated with plants preferentially assimilate ^{14}N (Ambrose, 1991; Yoneyama et al., 1986). The majority of plants that have N-fixing symbiotic bacteria are leguminous plants, for which the main source of nitrogen is atmospheric nitrogen. The $\delta^{15}N$ for nitrogen-fixing plants is thus very low – averaging approximately 1‰ (Tykot, 2004).

Other plants take up N from the soil in the form of nitrate, ammonium, and ammonia, all of which have higher $^{15}N/^{14}N$ than their soil organic matter, mineral, and groundwater substrates (Ambrose, 1991; Cui, Lamade, Fourel, & Tcherkez, 2020). Non-nitrogen-fixing plants have a higher $^{15}N/^{14}N$, with an average $\delta^{15}N$ of approximately 3‰ (T. D. Price & Burton, 2010). Animal tissues retain ^{15}N and preferentially excrete ^{14}N (Ambrose, 1991; C. Delwiche & Steyn, 1970; P. Delwiche, Zinke, Johnson, & Virginia, 1979). As a result, $\delta^{15}N$ increases with each trophic level of the food web by approximately 3-4‰. See Chapter 6 for a further discussion on isotopic ecology, particularly the isotopic enrichment that occurs with each ascending step of the food chain.

Routing of stable isotopes to biological tissues

The body synthesizes collagen (the major structural protein found in bone) from amino acids in dietary proteins. Collagen is built from essential, non-essential, and conditionally essential proteins. Essential amino acids must be supplied by the diet and are routed directly from the protein portion of the diet. Non-essential amino acids do not need to be supplied directly by the diet, but can be synthesized by the organism using multiple dietary macronutrients. Non-essential amino acids therefore represent isotopes supplied by the whole diet, not just protein (Ambrose, 1993). However, some non-essential amino acids are considered conditionally essential because growth and recovery from illness and injury are impaired when the diet does not supply adequate amounts of the conditionally essential amino acids (Fernandes, Nadeau, & Grootes, 2012; Jim, Jones, Ambrose, & Evershed, 2006; Reeds, 2000).

Essential and conditionally essential amino acids comprise ~60% of the carbon atoms in collagen. Collagen thus contains more carbon atoms from the protein portion of the diet rather than the whole diet. During collagen formation, both ^{13}C and ^{15}N are enriched relative to diet, resulting in collagen $\delta^{13}C$ that is ~5‰ higher and $\delta^{15}N$ that is ~2.5 to 4‰ higher than consumed dietary sources (Ambrose, 1993; Ambrose, Butler, Hanson, Hunter-Anderson, & Krueger, 1998; T. Brown & Brown, 2011; DeNiro & Epstein, 1978; France & Owsley, 2013; Jim et al., 2006).

While collagen $\delta^{13}C$ derives from dietary protein with some influence from non-protein dietary sources, the mineral portion of bone, enamel, and likely calculus derives from all dietary macronutrient sources. Skeletal, enamel, and calculus mineral contains calcium phosphate called bioapatite that contains small percentages of structural and adsorbed carbonate (CO_3) (Lee-Thorpe & van der Merwe, 1991). Dissolved metabolic CO_2 and HCO_3 from energy metabolism are incorporated into bioapatite as carbonate. The carbon in carbonate comes from all dietary

macronutrient sources (carbohydrates, lipids, and proteins), and there is no bias towards one particular class of macronutrient like there is in collagen formation (Ambrose & Norr, 1993).

Processes involved in bioapatite synthesis result in carbonate $\delta^{13}C$ that is ~9.4% higher than that of dietary sources (Ambrose, 1993; Krueger & Sullivan, 1984; Lee-Thorpe, Sealy, & van der Merwe, 1989; Tieszen & Fagre, 1993). It is necessary to analyze the isotopic composition of both collagen and carbonate for dietary reconstruction, because the difference between $\delta^{13}C$ of carbonate and of collagen provides an interpretation of the difference in isotopic composition of whole diet and dietary protein (Ambrose & Norr, 1993; Hedges, 2003). Chapter 5 (Paper 1) outlines how the formation of dental calculus shares common pathways with the formation of bone.

Contemporary health datasets as a complement to skeletal research

All NHANES methods are available online through the Centers for Disease Control, and I summarize the data collection methods of the phenotypes of interest (periodontal disease, immune system, nutrition) in Chapter 8 (Paper 4).

Statistical analyses

Several statistical analyses are used throughout this study, including logistic and ordinary least squares regression (Chapters 5, 7, and 8), causal mediation analysis (Chapter 8), and principal components analysis (Chapter 6). I provide a brief overview of regression modeling below, and details about causal mediation analysis and principal components analysis can be found in the relevant chapters. All statistical analyses for this study are performed using R statistical software (R Core Team 2013).

Logistic and linear regression

Regression models test the association between a dependent (outcome, y) variable and an independent (explanatory, x) variable. Ordinary least squares (OLS) regression is used when both the dependent and the independent variables are continuous, and logistic regression is used when the dependent variable is categorical or binomial (Imai, 2018). The beta-coefficient and standard error of the regression model are indicators of the association between x and y. The beta-coefficient is an estimate of the magnitude of change in y for every one-unit change in x, and the standard error is an estimate of the uncertainty of the beta-coefficient (Imai, 2018).

Covariates, or additional independent variables, can be added to the model if there are likely to be confounding variables in a relationship. For example, age is a confounding variable in the relationship between temporal period (x) and periodontal disease (y); this is because periodontal disease is much more likely to affect older individuals than younger individuals. Regression models also estimate the p-value of the association; when the p-value is low ($p < 0.05$), the association between x and y can be interpreted as a significant association. The minimum sample size for regression models is debated: some suggest a minimum sample size of 50 individuals (VanVoorhis & Morgan, 2007), while others suggest a minimum sample size as low as 10 individuals (Peduzzi, Concato, Kemper, Holford, & Feinstein, 1996). I set 30 as my minimum sample size for regression analyses and increased the minimum sample size by 10 individuals for every added covariate.

CHAPTER 5 (PAPER 1): PREDICTING BONE $\delta^{13}C$ AND $\delta^{15}N$ FROM DENTAL CALCULUS USING BIOFRACTION PURIFICATION AND REGRESSION MODELS

5.1 Introduction

Skeletal tissues such as bone and enamel are well-established analytical materials for paleodietary reconstruction through stable isotope analysis (Ambrose, 1990, 1993; Ambrose & Norr, 1993; DeNiro & Epstein, 1978, 1981; Lee-Thorpe et al., 1989; Schoeninger, DeNiro, & Tauber, 1983; Schwarcz & Schoeninger, 1991). However, the use of primary biomaterials such as bones and teeth for destructive techniques is ethically problematic. Dental calculus is a secondary biomaterial (accumulates on, but is not integral to, the human skeleton) and may therefore provide an ethical alternative to obtaining paleodietary information (Scott & Poulson, 2012).

Dental calculus is a mineralized biofilm that accumulates on dental surfaces and is therefore not a primary biomaterial. Though not a primary biomaterial, it nonetheless provides direct biocultural information about past populations. It is formed when the pellicle – a glycoprotein protective coating on the surface of teeth – is colonized by oral bacteria to create dental plaque. Minerals from oral fluids such as saliva precipitate into the plaque matrix, trapping the bacteria, their byproducts, and other debris into a strongly adhered deposit on dental surfaces (Greene, Kuba, & Irish, 2005; Jepsen, Deschner, Braun, Schwarz, & Eberhard, 2011; Levine, 2010; Lieverse, 1999).

The organic and mineral constituents of dental calculus have multiple analytical benefits. Genetic sequencing of dental calculus reveals the composition of the oral microbiome and its

relationship to disease in past individuals (Adler et al., 2013; De La Fuente, Flores, & Moraga, 2013; Warinner, Rodrigues, Vyas, Trachsel, & Shved, 2014; Warinner et al., 2015; Weyrich, Dobney, & Cooper, 2015). Starch grains, phytoliths, proteins, and other compounds and debris trapped in the dental calculus matrix provide evidence of individuals' diet and behaviors (Bergström, 1999; Charlton et al., 2019; Eerkens et al., 2018; Hardy et al., 2009; Hendy et al., 2018; Henry, Brooks, & Piperno, 2011; Henry & Piperno, 2008; Radini et al., 2017; Radini et al., 2019; Tromp & Dudgeon, 2015). And, the stable isotope ($\delta^{13}C$ and $\delta^{15}N$) composition of dental calculus may yield paleodietary information. However, previous studies provide equivocal evidence regarding the comparability of dental calculus to bone and tooth and stable isotope ratios (Eerkens et al., 2014; S. D. Price, Keenleyside, & Schwarcz, 2018; Salazar-García, Richards, Nehlich, & Henry, 2014; Scott & Poulson, 2012).

In this paper, we present new methods for the stable isotope analysis of dental calculus that improve the correlation between dental calculus and bone isotopic composition. We developed methods that purify the organic and mineral biofractions of dental calculus for separate isotopic analysis of each phase, and we apply ordinary least squares (OLS) regression models to test the comparability between the organic and carbonate phases of paired bone and dental calculus samples. We sampled the dental calculus of people from multiple geographic regions and temporal periods who had different subsistence practices. Diversifying the sample in this way provided data that span a larger range of available carbon and nitrogen isotopic ratios, which improves our regression models.

Previous research on the stable isotope analysis of dental calculus

Scott and Poulson (2012) measured calculus $\delta^{13}C$ and $\delta^{15}N$ and compared them to published bone $\delta^{13}C$ and $\delta^{15}N$ values from the same region. They were the first to introduce calculus as a potential material for paleodietary reconstruction via stable isotope analysis. Eerkens et al. (2014) analyzed paired bone and calculus samples from the same individuals and found that calculus isotopic ratios are positively correlated to bone below a calculus atomic carbon-to-nitrogen (C:N) ratio of 12. Salazar-García et al. (2014) also analyzed paired bone-calculus samples and found large variances in $\delta^{13}C$, $\delta^{15}N$, %C, %N, and C:N of calculus compared to collagen, as well as low R^2 in linear regressions between calculus and collagen isotopic ratios. They concluded that isotopic compositions of calculus and collagen are not comparable.

The above-described studies analyzed bulk dental calculus, with the organic and mineral components combined. Price et al. (2018) separated dental calculus into its organic and mineral biofractions using chemical pre-treatments. They then analyzed the isotopic correlations between paired bone and calculus biofraction isotope ratios. The authors did not find a significant correlation between organic calculus and bone collagen $\delta^{13}C$ or calculus and bone carbonate $\delta^{13}C$. While they did find a correlation between calculus and bone $\delta^{15}N$, the correlation between the $\delta^{15}N$ and C:N of their study samples raised concerns of diagenetic contamination of $\delta^{15}N$. Overall, they did not identify dental calculus organic $\delta^{13}C$ and $\delta^{15}N$ values as reliable proxies for bone values.

We also chemically separate calculus organic matter and mineral in our study. The main methodological differences between ours and previous studies include: the addition of a rinse step (to pH neutral) of the demineralized calculus organic biofraction, grinding the calculus

sample designated for mineral purification using an agate mortar and pestle, using unbuffered acetic acid (rather than buffered) for removal of diagenetic carbonates in mineral purification (Balasse, Ambrose, Smith, & Price, 2002), and using OLS regression models to predict bone $\delta^{13}C$ and $\delta^{15}N$ from calculus measurements. Our findings indicate that the isotopic measurements of dental calculus and bone are significantly associated. As Price et al. (2018) noted, separating the organic and mineral biofractions of calculus are necessary to compare calculus to bone collagen or bone carbonate, as well as to make dietary interpretations from calculus carbon isotopic ratios. The following notes elaborate on the importance of organic and mineral biofraction separation:

1. Apatite from bones and teeth has a different diet-tissue enrichment than collagen – bone and tooth apatite carbonate $\delta^{13}C$ is enriched by 9-10‰ relative to diet in non-ruminants, while collagen is enriched by ~5‰ when dietary proteins and non-proteins have similar $\delta^{13}C$ (Ambrose & Norr, 1993). Dietary reconstruction from bone is therefore accurate when apatite and collagen are analyzed separately. While the amount of enrichment (or depletion) of calculus $\delta^{13}C$ and $\delta^{15}N$ relative to diet has not yet been experimentally determined, it is likely that calculus carbonate and organic $\delta^{13}C$ differ in their diet-tissue enrichment because calculus formation shares similar pathways to bone formation. For example, carbonates are always enriched by ~9.5‰ at ~37°C relative to the dissolved CO_2 and HCO_3 from which they are formed (Emrich, Ehhalt, & Vogel, 1970). Controlled diet experiments with rodents (Ambrose & Norr, 1993; Tieszen & Fagre, 1993) demonstrate that this ~9.5% fractionation factor applies to both biogenic and geogenic carbonates, and thus also likely to calculus carbonate.
2. The proportions of carbonate and organic carbon in calculus (and archaeological bone) may vary; if so, then the isotopic composition of whole calculus reflects the proportions of carbonate to organic matter rather than diet. Therefore, calculus biofractions should be separated so the varying ratios of carbonate to organic carbon do not affect the comparability of dietary interpretation from different sampled individuals. This is especially important given that carbonate and organic carbon may reflect different portions of the diet. Protein and non-protein components of the diet may represent different $\delta^{13}C$ (marine versus terrestrial and C_3- vs. C_4-based foods), and analyzing the carbonate and organic carbon separately can therefore increase the resolution of dietary interpretation.
3. Experimental controlled diet studies have not been performed to measure the influence of diet on calculus isotopic composition, and the contribution of different dietary macromolecules to calculus organic $\delta^{13}C$ remains unclear. The composition of calculus organic matter is more heterogenous that than of bone collagen, and contains plant

microfossils, fungi, microbial DNA, and other organic molecules (Buckley et al., 2014; Charlier et al., 2010; Hendy et al., 2018; Lieverse, 1999; Mann et al., 2018; Radini et al., 2017; Warinner et al., 2015). Archaeological specimens may also include slight soil organic contamination (Mann et al., 2018; Warinner et al., 2014). Isolating and analyzing the organic biofraction of dental calculus will help us better understand the isotopic composition (e.g. weight percent carbon and carbon-to-nitrogen atomic ratio) and how it varies between individuals and across contexts.

4. Isolating the calculus carbonate biofraction will help us better understand the variation in calculus carbonate content, as calculus carbonate may reflect the carbon isotopic composition of diet-derived metabolic carbon, as well as unknown contributions from oral microbiome metabolism, atmospheric CO_2, and diagenetic soil and groundwater carbonates (in archaeological specimens).

Overall, purifying and analyzing the mineral and organic biofractions of calculus separately improve our understanding of calculus composition and variability and are necessary steps for accurate dietary interpretation. Due to differences in metabolism and routing, bone carbonate reflects whole diet, while collagen reflects mainly dietary protein (Ambrose & Norr, 1993). Whether similar routing of dietary protein to consumer protein and total diet carbon to consumer carbonate occurs in calculus formation is unknown. In this paper, we separate the biofractions to test a) whether calculus isotopic composition reflects endogenous or contaminated sources, and b) if the stable isotope compositions of calculus and bone biofractions are correlated, which can indicate whether calculus formation pathways may be similar to those of bone.

5.2 Materials and Methods

Fifty-six samples from archaeological contexts and nine from modern contexts were included in this analysis (Table 1). Paired bone and calculus were analyzed for all groups except those from the Late Woodland North Carolina Piedmont. For the anatomical study skeletons, third molar dentine – rather than bone – was compared to corresponding calculus samples in order to minimize destructive analysis of the skeleton.

Table 5.1

Sources of Dental Calculus Samples

Group Description by Temporal Period and Geographic Region	Sample Size	Dates Represented
Late Woodland North Carolina (NC) Coastal	18	AD 800–1650
Late Woodland–Mississippian Illinois Floodplain	9	AD 1200–1300
Modern Anatomical	9	Modern
Historic cemetery, NC Piedmont	3	AD 1800–1900
Late Woodland–Colonial NC-Virginia Piedmont	22	AD 1000–1700
Early Horizon–Early Intermediate Bolivia Lake shore	4	800 BC–AD 200

Bone phase separation

Bone collagen was extracted following Ambrose (1990), with modifications described in Hu et al. (2006). An additional lipid removal step (petroleum ether) was used for the modern dentine samples (Jim, Ambrose, & Evershed, 2004). Bone collagen isotopic measurements from the Late Woodland North Carolina coastal group were previously reported by Hutchinson (2002). Bone carbonate samples were purified following Ambrose and Norr (1993) and Balasse et al. (2002). Carbonate isotopic measurements from the Late Woodland North Carolina coastal group and the Bolivian lake shore groups were previously reported in Hutchinson (2002) and Juengst et al. (in press), respectively.

Calculus biofraction separation

Calculus organic biofraction

Using a sterilized scalpel, 8-15mg of calculus was flaked from teeth, broken into small pieces (not exceeding ~9mm^2), weighed, and added to an acid-washed, annealed (540°C) pre-weighed 5 ml beaker. The beaker (plus the sample) was reweighed to calculate sample weight. Approximately 3ml of 0.2M HCl was added to the beaker containing the sample. The purpose of this step is to dissolve the mineral phase of calculus, including carbonate. Twelve hours after acidification, the sample was inspected under the microscope. Demineralized calculus pieces are

more translucent and pliable, have neutral buoyancy, and have no signs of effervescence in HCl. Any calculus pieces that had demineralized after twelve hours were transferred to a labeled, weighed, annealed 9 mm diameter Pyrex glass culture tube using an acid-washed, annealed glass Pasteur pipette fitted with a latex bulb.

After demineralized pieces were transferred, if the beaker still contained a portion of the sample that was not yet demineralized, it was replenished with 0.2M HCl to maintain 3 ml volume. The process of decanting demineralized pieces and refreshing the acid in the beaker was repeated every 12 hours until all pieces were demineralized and transferred to the culture tube – a 48–60 hour process for the samples in this study. After complete demineralization and transfer, the glass culture tube containing the demineralized pieces was capped with aluminum foil and centrifuged. After centrifugation, the supernatant was decanted using an annealed Pasteur pipette and discarded. The demineralized calculus pellet was suspended in distilled H_2O (dH_2O) and the tube capped.

The demineralized calculus was then rinsed to neutrality with dH_2O four times, following this centrifugation and rinse process. After the fourth rinse, the sample was pipette-decanted of as much of the rinse supernatant as possible, then capped with aluminum foil, frozen, and lyophilized (freeze-dried). The dried tube was reweighed in order to calculate insoluble organic matter concentration as weight percent of the whole dry sample before demineralization.

Calculus mineral biofraction

Approximately 5mg of calculus was used for purification of the calculus mineral matrix. Following Ambrose and Norr (1993) and Balasse et al. (2002), the samples were finely crushed in an agate mortar and pestle and added to pre-weighed 1.5 ml microcentrifuge tubes, then reweighed to determine sample weight. The tubes were filled to the 1.5 ml line with 2.63%

NaOCl (sodium hypochlorite), made by dilution of Clorox bleach that has a concentration of 8.25% NaOCl with 2.14 parts water to one-part NaOCl, and subsequently vortexed. After ~12 hours of treatment, the tubes were centrifuged and the Clorox supernatant decanted and discarded using a micropipette. This step is designed to remove organic matter that could impede the removal of diagenetic carbonate.

The sample pellets were rinsed in their tubes four times with dH_2O, with centrifugation and pipette-decanting between rinses. After the final rinse, 0.1M acetic acid was added to the tubes at a volume of 0.1 ml per 1 mg of calculus starting weight. After four hours of treatment, the tubes were centrifuged, the acetic acid supernatant was decanted and discarded using a pipette, and the purified mineral matrix pellet rinsed four times with dH_2O. After the last rinse, the samples were frozen and lyophilized. This acid treatment is designed to remove absorbed diagenetic and weakly bound adsorbed carbonates.

Calculus Isotope Ratio Mass Spectrometry (IRMS) analysis

Organic calculus and bone collagen samples were analyzed using a Carlo-Erba NC 2500 Elemental Analyzer (EA) with a Conflo II interface coupled to a Finnigan MAT 252 Isotope Ratio Mass Spectrometer (IRMS) or with the same EA attached to a Conflo IV interface coupled to Delta V Advantage IRMS. Approximately 400-500 µg of demineralized calculus organic matter was added to tin combustion capsules and capsules were folded and compressed to form a small pellet, then reweighed before analysis. USGS 40 and USGS 41 isotopic reference materials certified by the National Institutes of Science and Technology were used for two-point instrument calibration because they bracket the range of natural abundance of most organic matter. These standards were also used within sample sets as isotopic correction standards.

Amino acids thiourea, serine, and methionine were used as internal working standards to monitor analysis performance during machine operation, as these purified amino acids span a wide range of isotopic compositions, stoichiometric carbon and nitrogen concentrations, and different atomic C:N ratios. They are used to monitor accuracy and precision of analyses of elemental and isotopic composition over a wide range of organic material compositions, which is particularly important for a composite material such as calculus organic matter, in contrast to purified proteins such as skeletal collagen and hair keratin.

Calculus mineral phase samples were analyzed using a Finnigan Kiel III Carbonate automated carbonate reaction and cryogenic distillation device coupled with a Finnigan MAT 252 IRMS. Approximately 550-650 μg of the mineral calculus biofraction was added to glass reaction tubes for analysis. NBS 18 and NBS 19 were used for two-point instrument calibration and as within-run isotopic correction standards. Sample isotopic values were corrected, when necessary, using a linear regression equation. Information on sample replicates is provided in Appendix 1.

OLS Linear Regression Models

We assessed the relationship between calculus and bone isotopic values by applying a linear regression model using the ordinary least squares (OLS) method. We used measured calculus isotopic ratios as the predictor variable and measured bone isotopic ratios from the same individual as the outcome variable. The OLS method fits a line to the data in a way that minimizes the aggregate difference (also known as the residual) between the predicted and observed outcome variable (Imai, 2018). We tested an OLS regression model with group random intercepts to evaluate whether the use of samples from different temporal and geographic groups affected the association between observed and predicted isotopic measurements. Using group

random intercepts generates an overall regression line for the combined data, and measures how much the intercept of a group within that data differs from the overall intercept of the regression line (Gerstman, 2014).

We found consistent results with both OLS models – the model that includes group random intercepts, and the model without random intercepts. The consistent results between these models indicates that the regression between calculus and bone isotopic ratios were similar for all individuals across the various groups included in this study. We therefore opted for the more straightforward OLS model (without group random intercepts) to generate regression models from our data.

5.3 Results and Interpretation

Results of organic biofraction isotopic analyses

Table 2 includes descriptive statistics for the weight percent carbon and nitrogen, atomic C:N ratio and percent recovery of all organic biofraction calculus samples. Results from each individual sample are presented in Appendix 1.

Table 5.2

Descriptive Statistics for Organic Biofraction Calculus Samples, n = 53

	Wt % Carbon	Wt % Nitrogen	Atomic C:N	% Recovery
Average	42.82	8.54	6.04	6.37
± st dev	9.72	2.13	0.92	2.87
Median	45.88	8.96	5.68	6.05
IQR	40.62–49.24	7.90–10.10	5.49–6.31	4.74–6.85
Min	12.21	1.84	4.44	2.38
Max	54.12	11.52	9.30	20.37

Figures 5.1–5.3 show that there are no consistent associations between organic $\delta^{13}C$ and either percent recovery of the organic biofraction, weight percent carbon, or atomic C:N. This indicates that organic $\delta^{13}C$ of the samples in this study reflect biogenic origins, not

contamination from soil or other postmortem sources. For example, if the samples with the highest atomic C:N ratio had the lowest $\delta^{13}C$, then this could indicate C_3-rich humic acid contaminants (Ambrose, 1990), but this was not the case for the samples analyzed in this analysis. There are also no clear effects of percent recovery on weight percent carbon or atomic C:N (Figures 5.4 and 5.5, respectively), especially if the outliers are taken out of consideration. The outliers for percent recovery, identified using the 1.5*IQR method, are the samples with greater than 10.02% recovery. The lack of correlation between percent recovery and C:N suggests that our organic biofraction purification method successfully removed carbonates.

Figures 5.6–5.8 show that $\delta^{15}N$ is not affected by percent recovery, weight percent nitrogen, or atomic C:N. Like $\delta^{13}C$, the $\delta^{15}N$ of the calculus organic biofraction reflects biogenic sources rather than contamination. Percent yield does not apparently affect weight percent nitrogen (Figure 5.9), especially if the percent recovery outliers mentioned above are removed.

Though the outliers for percent yield fall within the $\delta^{13}C$ and $\delta^{15}N$ ranges of other sampled individuals from the same group (individuals from the North Carolina–Virginia (NC-VA) Piedmont), they are outliers for several measurements of sample composition, including weight percent carbon, atomic C:N, and weight percent nitrogen. Samples that are outliers for multiple sample composition measurements should be critically evaluated. In the case of our study, the outliers from the NC-VA Piedmont were not part of the samples included in the regression analyses, because corresponding bone samples were not sampled from individuals from the NC-VA Piedmont. Further research is needed to understand what can cause low weight percent carbon and nitrogen in the organic biofraction of calculus.

Figure 5.1

$\delta^{13}C$ of the organic biofraction, compared to the percent recovery of the organic biofraction purification

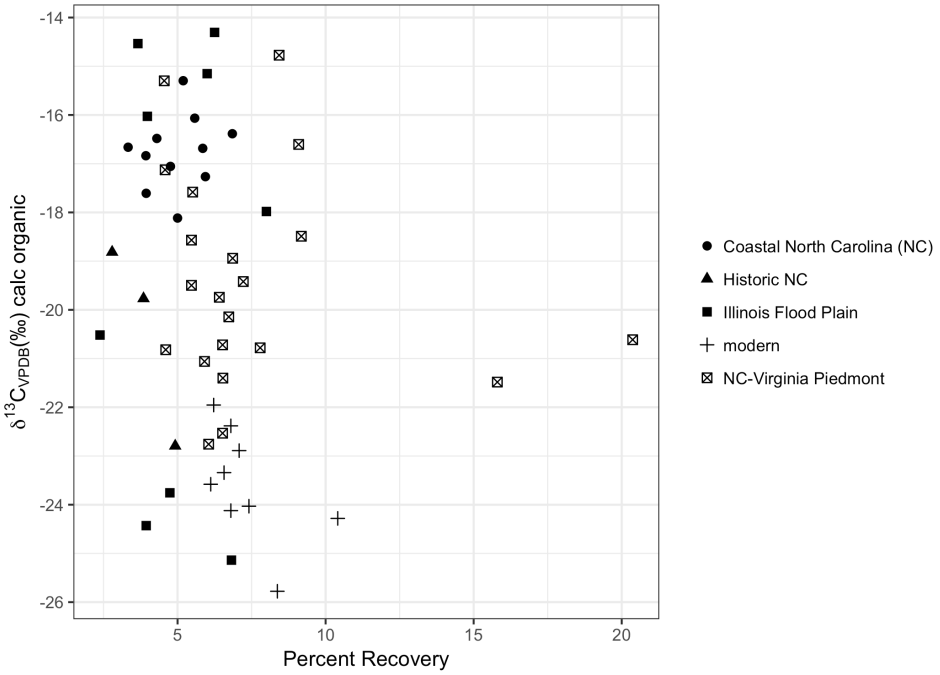


Figure 5.2

$\delta^{13}C$ of the organic biofraction compared to weight percent carbon

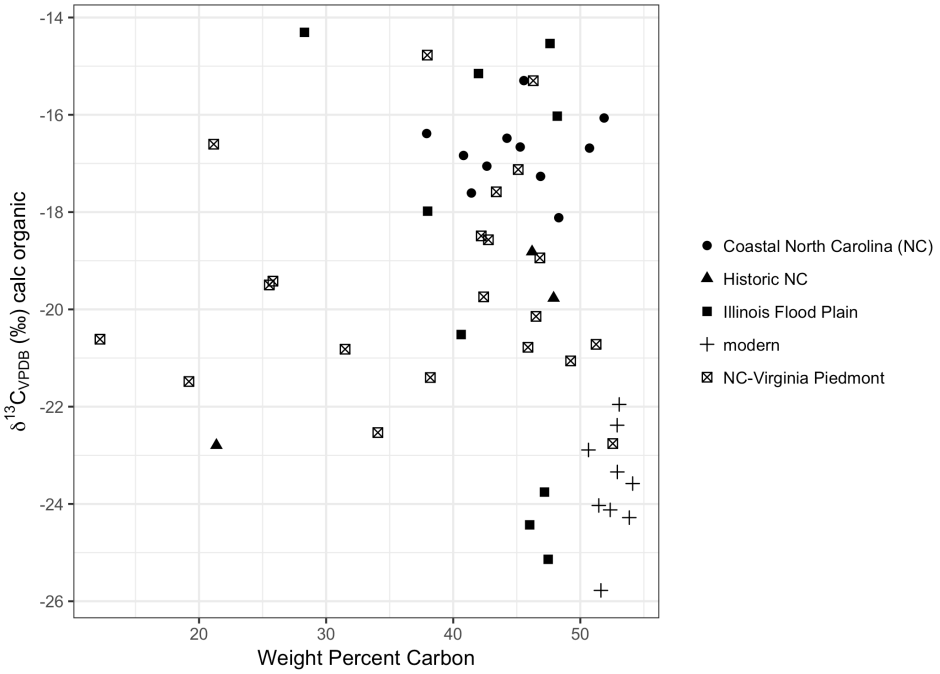


Figure 5.3

$\delta^{13}C$ of the organic biofraction compared to atomic C:N ratio

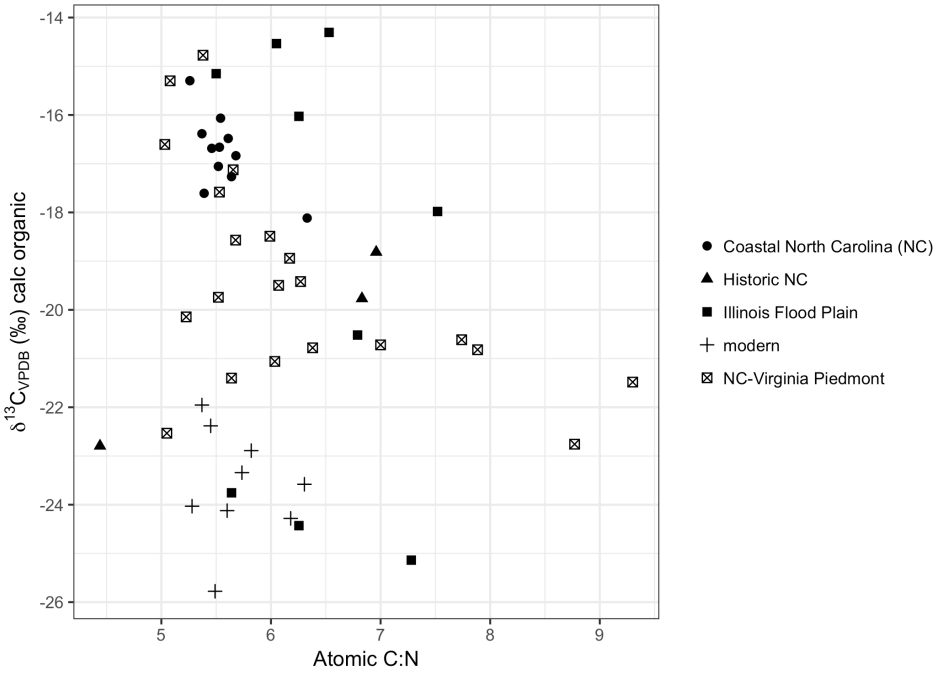


Figure 5.4

Weight percent carbon of the organic biofraction samples compared to percent recovery of the organic biofraction purification

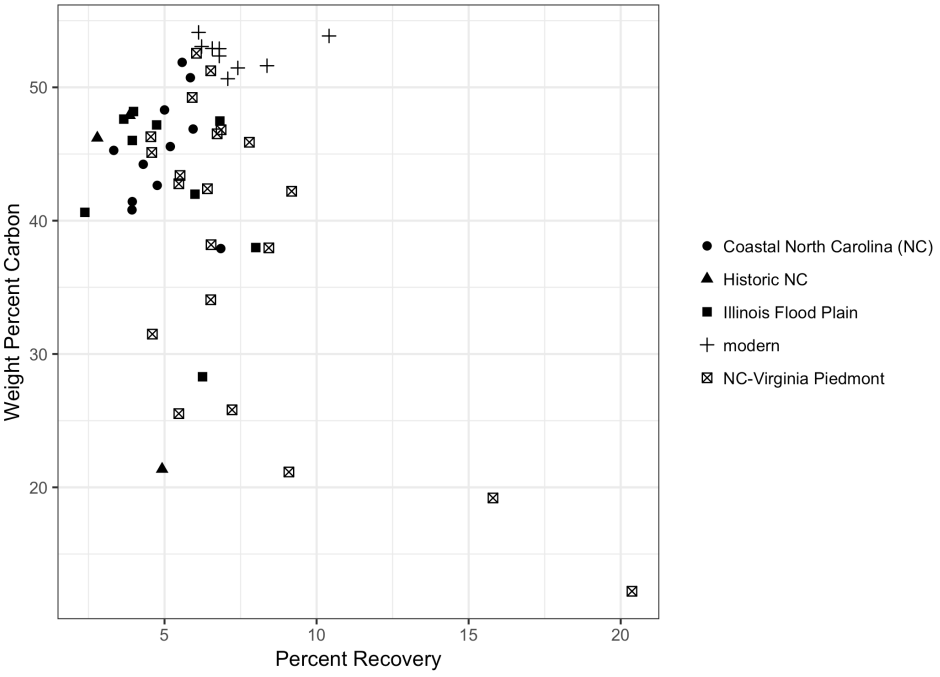


Figure 5.5

Atomic C:N ratio of the organic biofraction samples compared to percent recovery of the organic biofraction purification

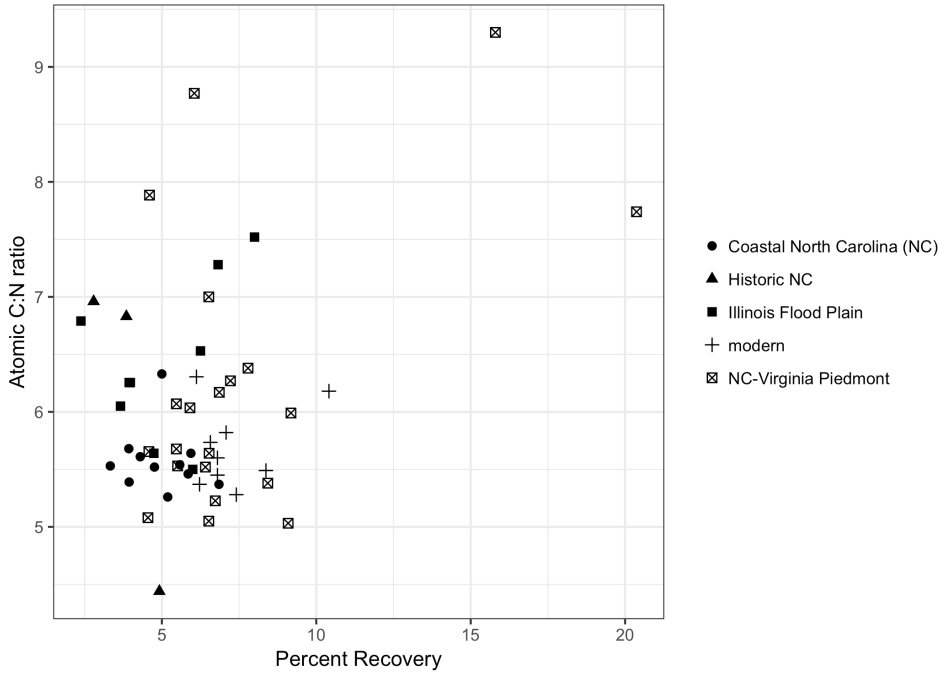


Figure 5.6

$\delta^{15}N$ of the organic biofraction samples compared to percent recovery of the organic biofraction purification

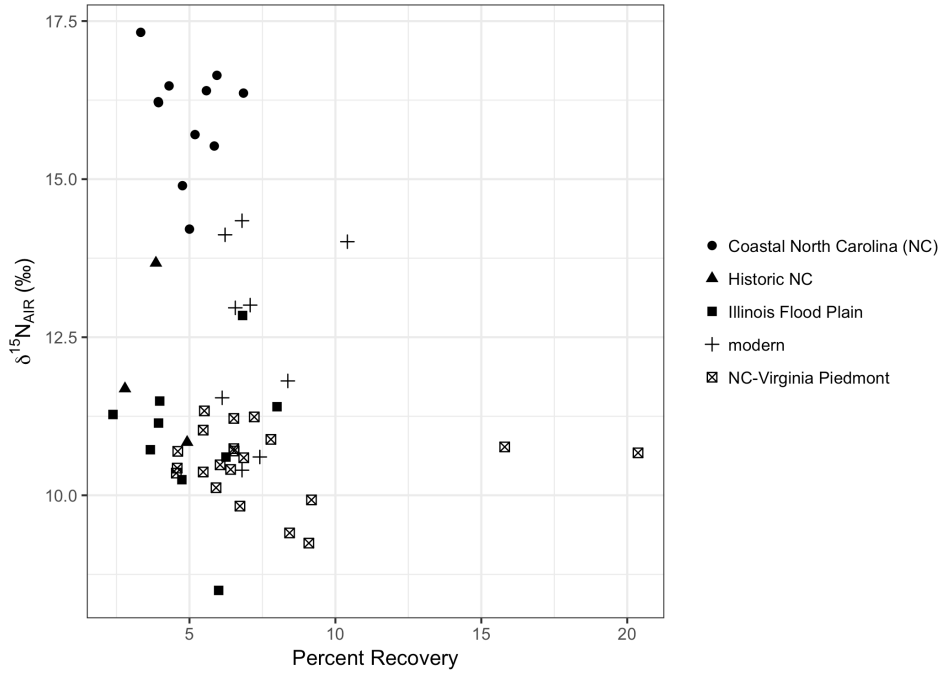


Figure 5.7

$\delta^{15}N$ of the organic biofraction samples compared to weight percent nitrogen

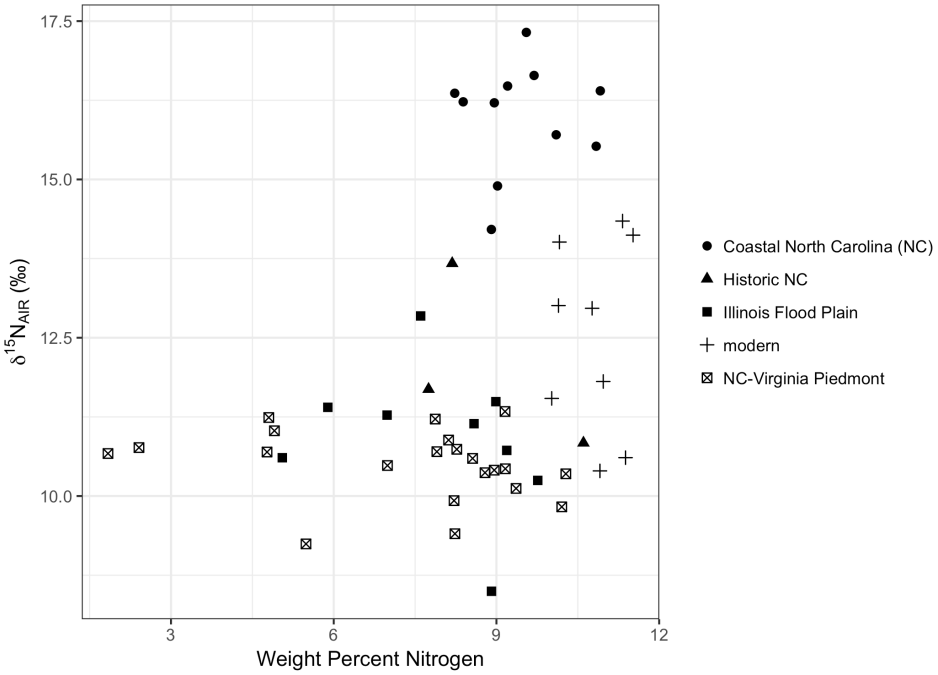


Figure 5.8

$\delta^{15}N$ of the organic biofraction samples compared to atomic C:N

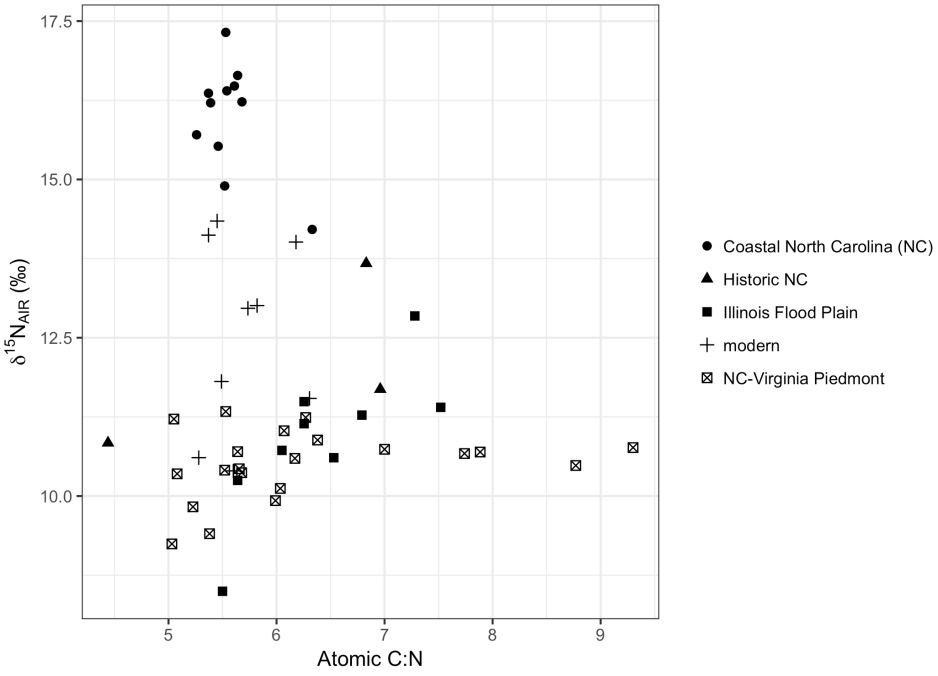
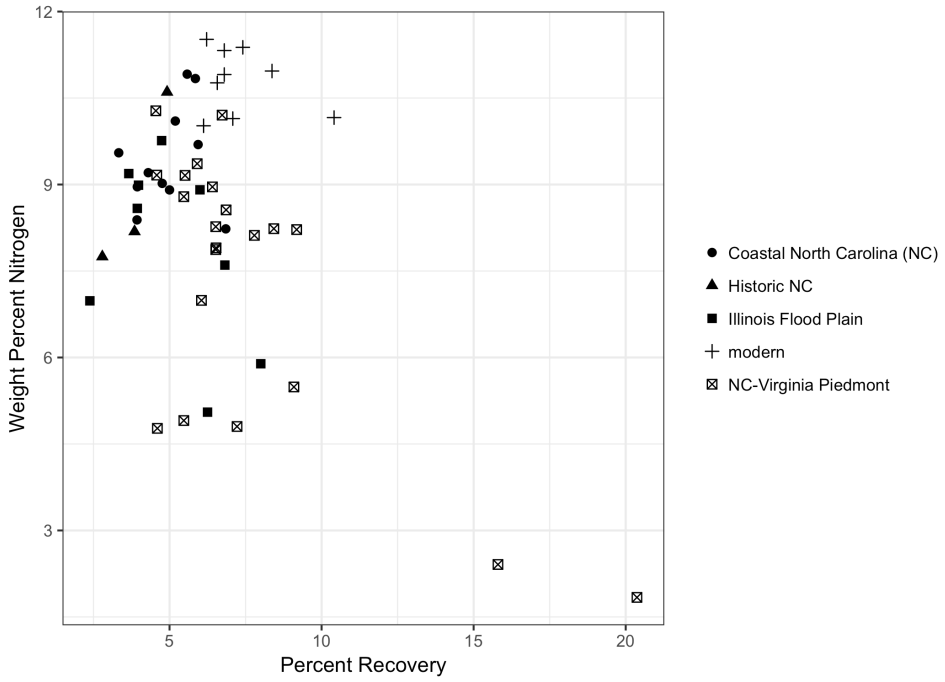


Figure 5.9

Weight percent nitrogen of the organic biofraction samples compared to percent recovery of the organic biofraction purification



Results of mineral biofraction isotopic analyses

Table 5.3 includes descriptive statistics for the mineral biofraction samples, specifically weight percent carbon and percent recovery of the mineral biofraction purification. Results from each individual sample are presented in Appendix 1.

Figures 5.10 and 5.11 show that neither calculus carbonate $\delta^{13}C$ and percent recovery nor carbonate $\delta^{13}C$ and weight percent carbon are correlated. The lack of correlation suggests that dental calculus carbonate $\delta^{13}C$ reflects biogenic sources. There is one outlier in figure 5.11 (identified using the 1.5*IQR method) that has high weight percent carbon and high carbonate $\delta^{13}C$. We suggest that samples with aberrantly high weight percent carbon should be removed from interpretation. The outlier does not have a high percent recovery compared to the other

samples (Figure 5.12), which demonstrates the importance of analyzing multiple indicators of sample composition and preservation.

Table 5.3

Descriptive Statistics for Mineral Biofraction Calculus Samples, n = 52

	Wt % Carbon	% Recovery
Average	0.61	70.28
± st dev	0.51	8.14
Median	0.54	71.43
IQR	0.47–0.66	64.46–75.60
Min	0.19	36.59
Max	4.11	82.69

Figure 5.10

$\delta^{13}C$ of the mineral biofraction compared to percent recovery of the mineral biofraction purification

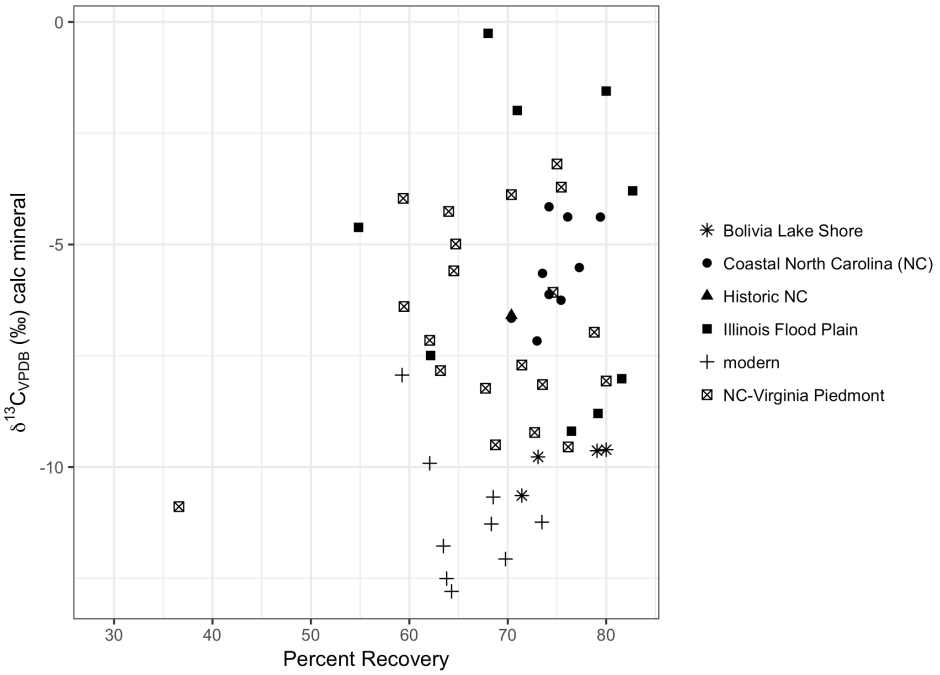


Figure 5.11

$\delta^{13}C$ of the mineral biofraction compared to weight percent carbon

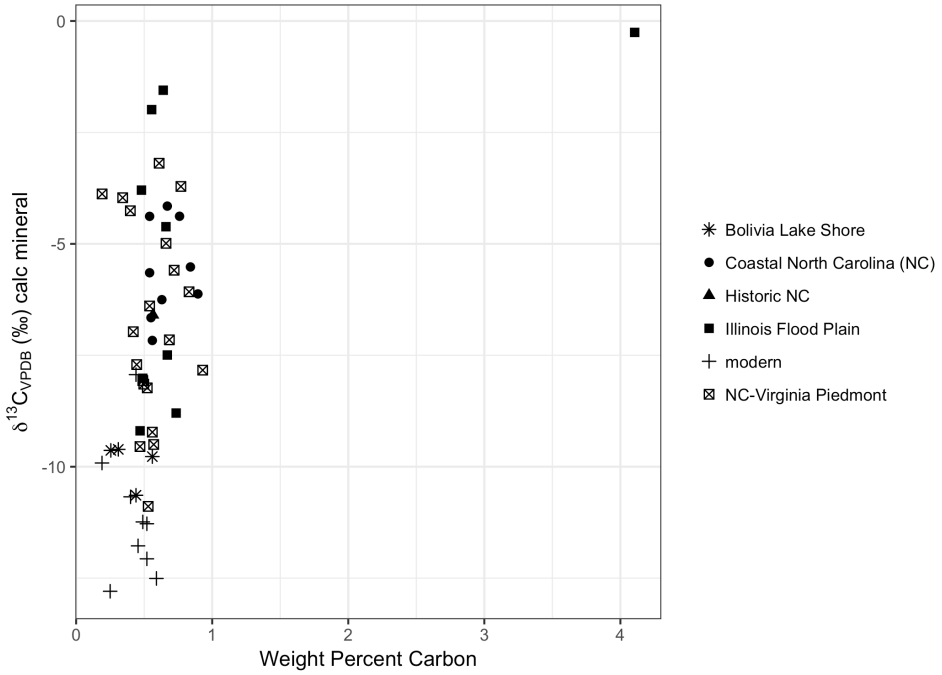
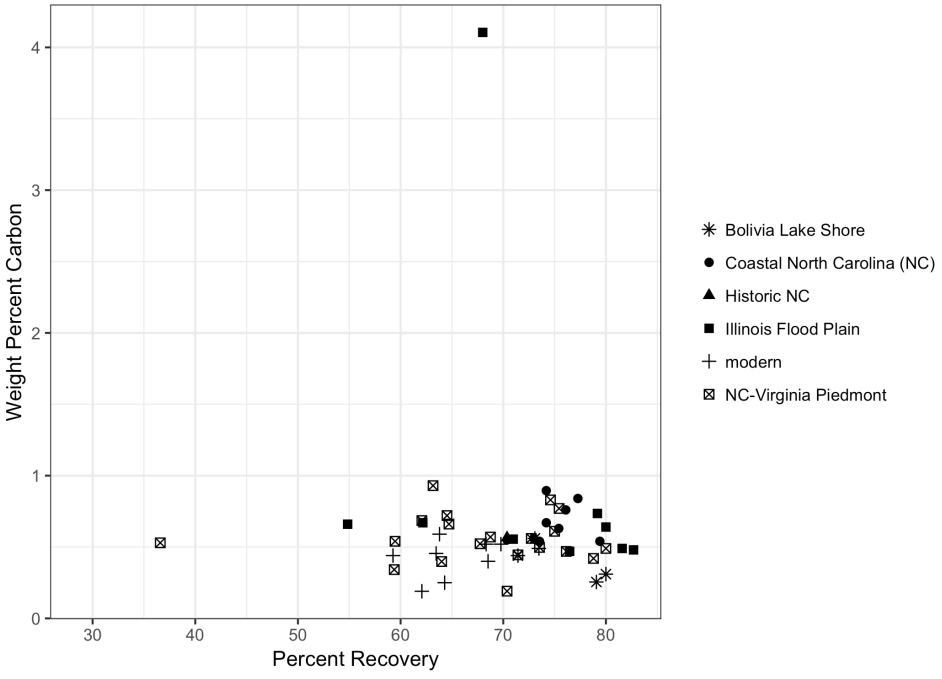


Figure 5.12

Weight percent carbon of the mineral biofraction compared to percent recovery of the mineral biofraction purification



Results of the OLS regression models

The results from the OLS regression models that assess the correlation between calculus and bone isotopic ratios are presented in Table 5.4 and Figures 5.13-5.15. Results include the beta-coefficient (the slope of the regression line), with the standard error of the beta-coefficient in parentheses. We also included the adjusted R^2 value, which represents the proportion of the variance in the outcome variable that is explained by the model. The beta-coefficients between paired calculus and bone $\delta^{13}C_{carbonate}$, $\delta^{13}C_{organic}$, and $\delta^{15}N$ all have p-values that are significant at the level of alpha < 0.001.

The results suggest that:

- For every one-unit (1‰) increase in the $\delta^{13}C$ of calculus carbonate, there is a 1.15‰ increase in the $\delta^{13}C$ of bone carbonate
- For every 1‰ increase in the $\delta^{13}C$ of calculus organic material, there is a 0.97‰ increase in the $\delta^{13}C$ of bone collagen
- For every 1‰ increase in the $\delta^{15}N$ of calculus, there is a 0.85‰ increase in bone collagen $\delta^{15}N$

Table 5.4

Results from OLS Linear Regression Models

	$\delta^{13}C$ bone carbonate	$\delta^{13}C$ bone collagen	$\delta^{15}N$ bone	$\delta^{13}C$ calculus carbonate	C:N calculus
$\delta^{13}C$ calculus carbonate	1.15*** (0.10) $r^2 = 0.83$				
$\delta^{13}C$ calculus organic		0.97*** (0.11) $r^2 = 0.76$		0.82*** (0.09) $r^2 = 0.70$	-0.68 (0.47) $r^2 = 0.02$
$\delta^{15}N$ calculus			0.85*** (0.09) $r^2 = 0.77$		-0.49 (0.35) $r^2 = 0.02$

*** indicates that the p-values of the beta-coefficients are significant at alpha < 0.001

Figure 5.13

Calculus carbonate $\delta^{13}C$ vs. bone carbonate $\delta^{13}C$. Solid line represents the regression line, dotted lines represent the 95% confidence interval

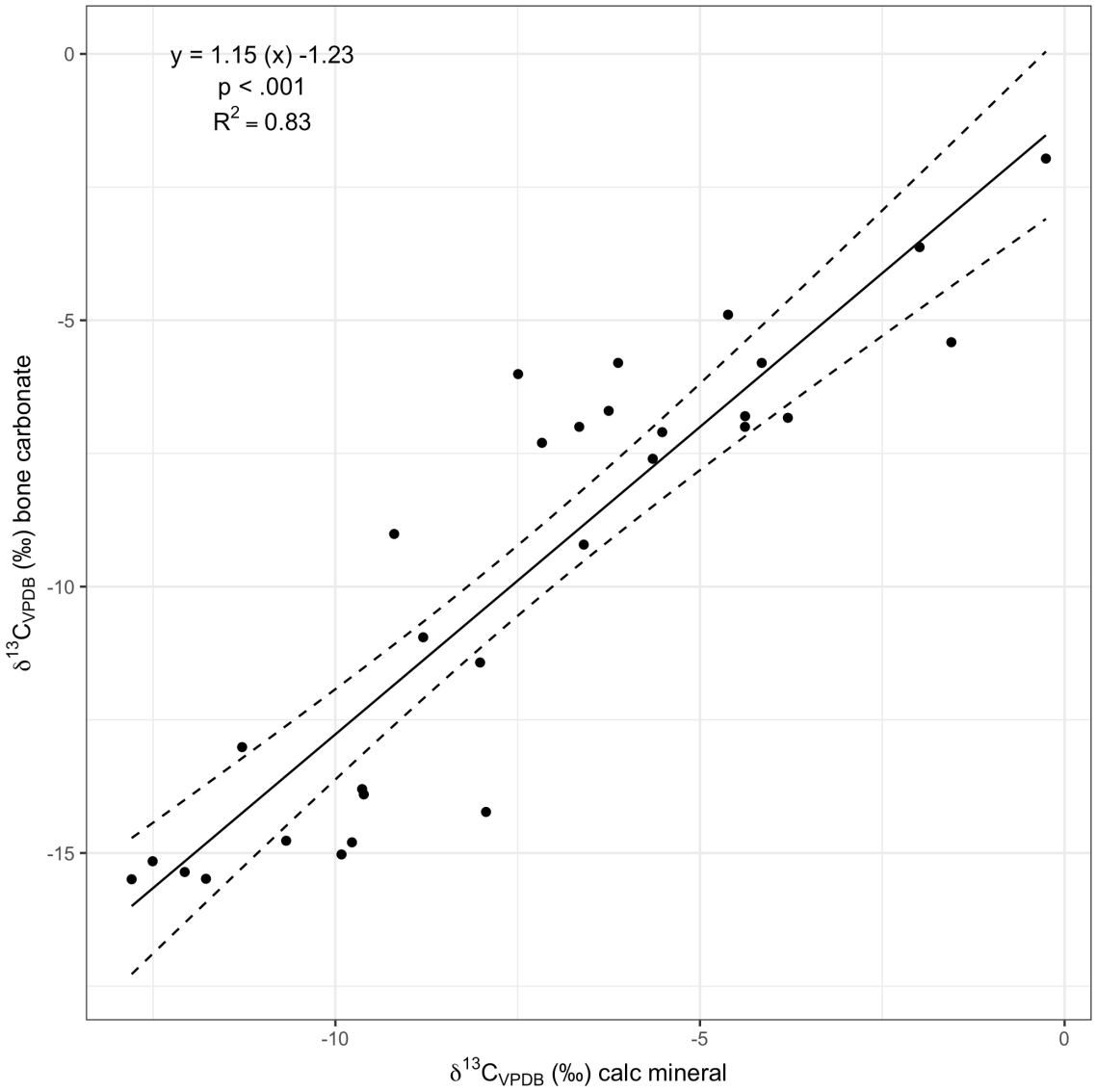


Figure 5.14

Calculus organic $\delta^{13}C$ vs. bone collagen $\delta^{13}C$. Solid line represents the regression line, dotted lines represent the 95% confidence interval

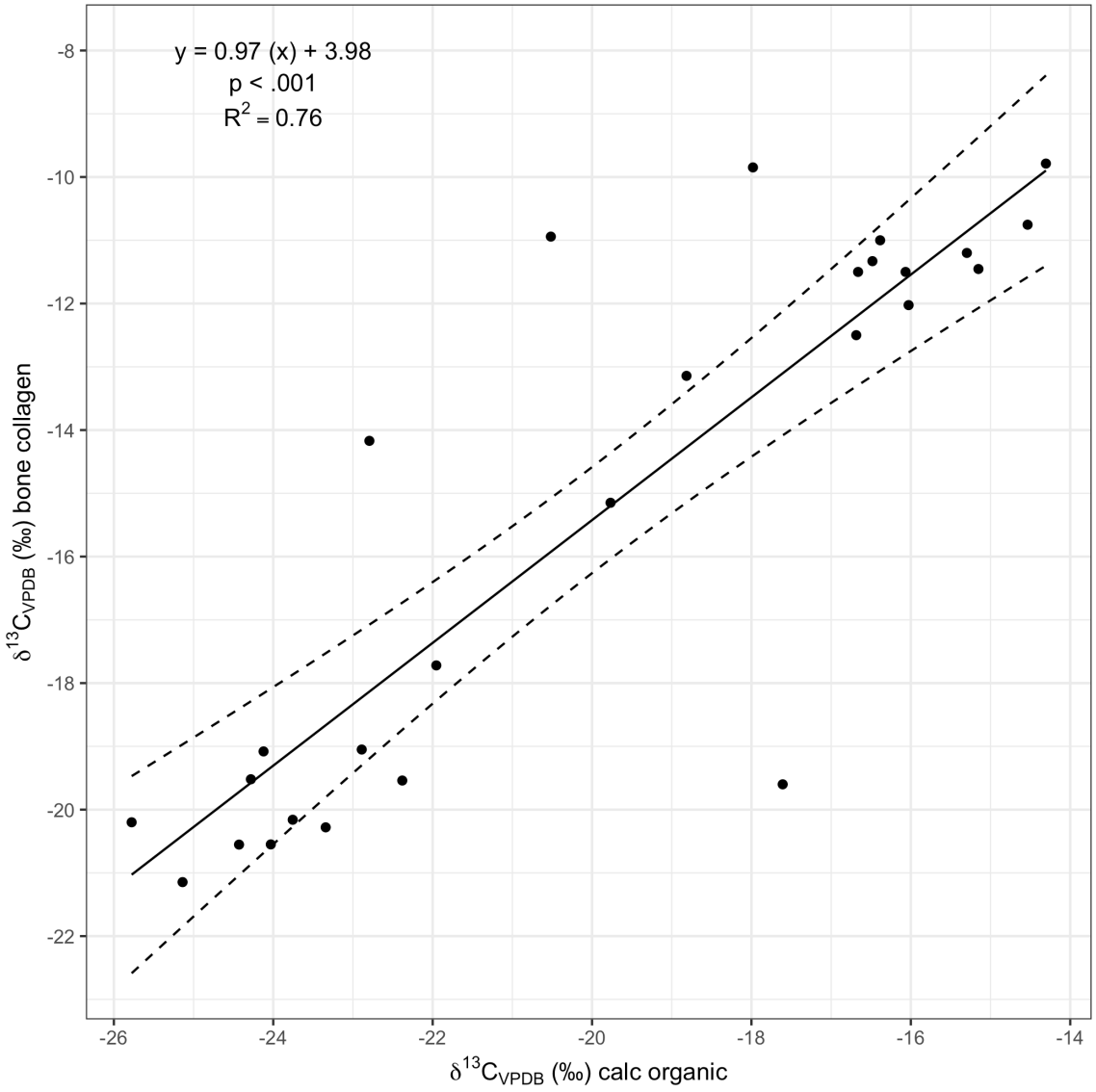
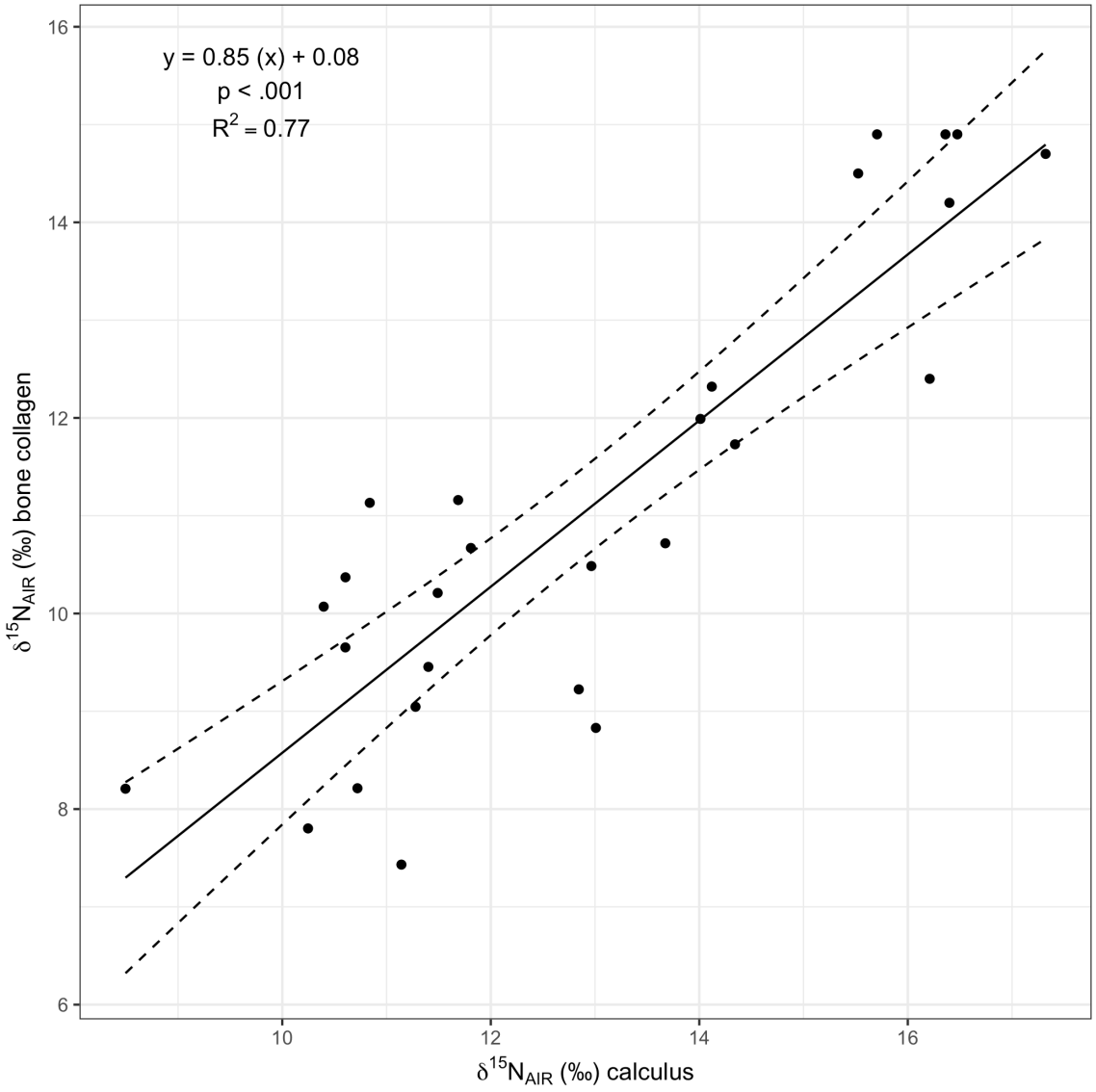


Figure 5.15

Calculus $\delta^{15}N$ vs. bone collagen $\delta^{15}N$. Solid line represents the regression line, dotted lines represent 95% confidence interval



5.4 Discussion

The results of our study suggest that dental calculus and bone have correlated, but not directly equivalent, isotopic ratios. The correlation indicates that dental calculus and bone structural components may derive from similar chemical pools in the body. The spacing between calculus and bone isotopic ratios is likely a result of different biochemical compositions of the two materials, as well as effects of the metabolism of bacteria present in dental plaque. In the

following sections, we describe possible physiological processes that connect bone and calculus formation to similar biochemical pools. We also discuss the possible factors that create the calculus-bone $\delta^{13}C$ and $\delta^{15}N$ spacings.

Interpreting carbonate $\delta^{13}C$ of dental calculus

Figure 5.16

Pathways linking dietary intake to calculus and bone carbonate $\delta^{13}C$. GCF: gingival crevicular fluid

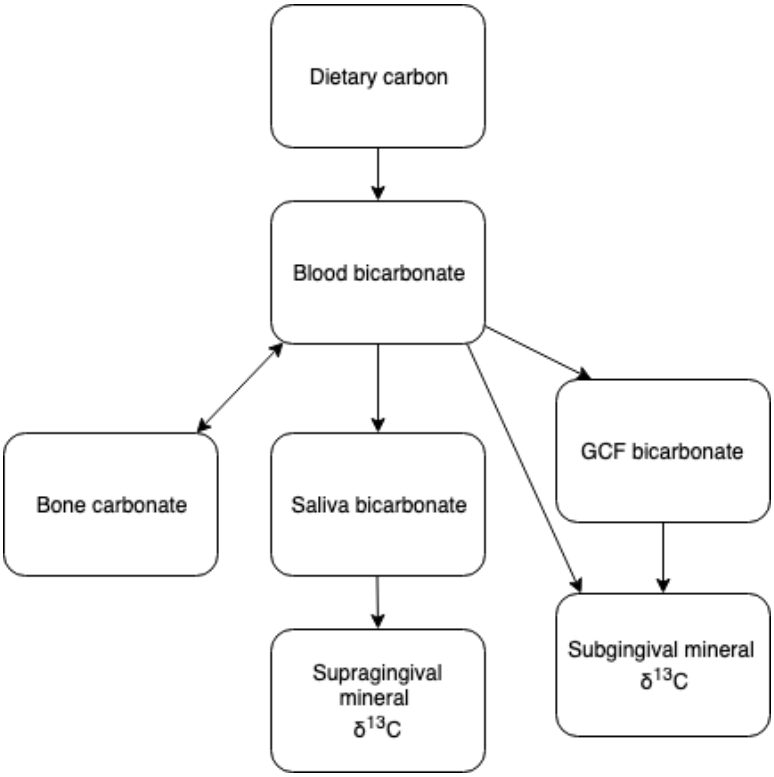


Figure 5.16 suggests a possible pathway that links bone and calculus carbonates to a common source. Bone structural carbonates are derived from blood bicarbonate, which is a product of the metabolism of all dietary carbon sources (Ambrose & Norr, 1993; Schoeller et al., 1984). Salivary glands take up blood bicarbonate, which forms part of the salivary bicarbonate content (Jones & Lefeuvre, 1989; Sand, 1951). Salivary bicarbonate likely precipitates into the

calculus matrix, thus making up part of the mineral biofraction that we isolate and analyze for stable light isotope analysis of calculus. Saliva provides molecules for supragingival calculus, which accumulates on tooth enamel above the gum line. By contrast, gingival crevicular fluid (GCF) provides molecules for subgingival calculus, which accumulates on tooth surfaces below the gum line (Humphrey & Williamson, 2001).

Gingival crevicular fluid has a higher bicarbonate content than saliva and is also in exchange with blood bicarbonate (Bickel, Munoz, & Giovannini, 1985). As subgingival calculus accumulates, it irritates the gums and causes them to pull further away from the root, creating a periodontal pocket. Irritated gums and periodontal pockets are susceptible to bleeding, thus contributing blood bicarbonate directly to the subgingival calculus substrate.

The correlation between calculus and bone carbonate is a result of the biochemical exchange between blood and oral fluids. The $\delta^{13}C$ of dental calculus carbonate is, on average, $2.36 \pm 1.81‰$ more ^{13}C-enriched than bone carbonate $\delta^{13}C$. Several factors could contribute to this enrichment. Not all salivary and GCF bicarbonate is from blood bicarbonate (Sand, 1951). Factors involved in localized bicarbonate production could create isotopic differences between the carbonate $^{13}C/^{12}C$ ratios of blood and oral substrates (Jones & Lefeuvre, 1989). Bacterial-mediated factors, including bacterial bicarbonate metabolism, and atmospheric CO_2, which has a pre-industrial $\delta^{13}C$ of approximately -6.5‰, could also contribute to the high $\delta^{13}C$ of calculus carbonate (Keeling et al., 2017; Marino & McElroy, 1991; Moulton-Barrett, Triadafilopoulos, Michener, & Gologorsky, 1993; Zhang et al., 2009). It is also possible that localized bone breakdown (associated with periodontal disease) could contribute to higher calculus carbonate $\delta^{13}C$, as bone carbonate is ^{13}C-enriched over that of dietary carbon and blood bicarbonate and

may be available through GCF, depending on the permeability of local vasculature (Ambrose & Norr, 1993; Lamster & Ahlo, 2007).

Interpreting organic $\delta^{13}C$ of dental calculus

Figure 5.17

Pathways linking dietary intake to calculus organic and bone collagen $\delta^{13}C$ and $\delta^{15}N$

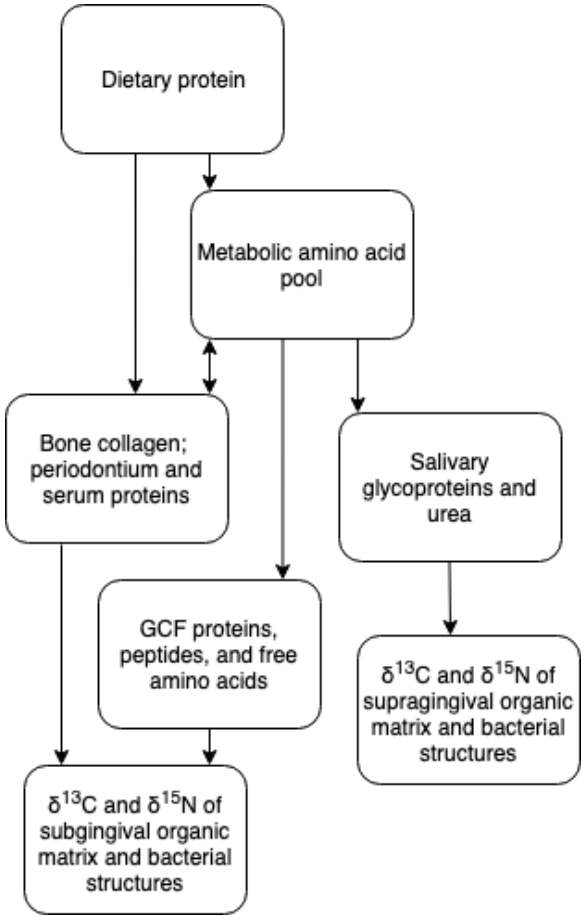


Figure 5.17 outlines how dietary protein may influence both calculus and bone isotopic composition. Bone collagen is sourced from dietary amino acids – routed directly from dietary amino acids or synthesized from the body amino acid pool and its precursors (Chisholm, 1989; Jim et al., 2006). The amino acid pool is generated by neosynthesis of non-essential amino acids, breakdown of dietary proteins and turnover of body tissues (Harvey & Ferrier, 2011). In addition

to being used to build collagen, the amino acid pool also supplies part of the protein, peptide, and amino acid composition of saliva and GCF. Like the mineral biofraction of calculus, the organic calculus biofraction (plaque matrix and bacteria) is largely formed and maintained by components of saliva and GCF. While fermentable carbohydrates can provide an external source of nutrients for plaque bacteria, salivary and GCF carbon sources have a larger influence on calculus organic $\delta^{13}C$. This is supported by the persistence and growth of oral bacteria through periods of human fasting or even intubation (Marsh & Martin, 2009).

Calculus organic $\delta^{13}C$ values are $4.57 \pm 2.08‰$ less ^{13}C-enriched than bone collagen, likely due to these differences between collagen biosynthesis and dental plaque formation. Collagen biosynthesis involves isotopic fractionation of the individual amino acids (Hare, Fogel, Stafford Jr, Mitchell, & Hoering, 1991) and preferential excretion of ^{12}C, leading to ^{13}C-enriched isotopic ratios of collagen compared to diet. Whether this fractionation occurs in plaque matrix protein formation is unknown, though an absence of diet-protein isotopic enrichment could lead to a lower organic $\delta^{13}C$ of calculus. The presence of lipids, bacterial waste products, and other organic debris in the calculus matrix could also lead to this calculus-collagen spacing, as our method for organic biofraction purification did not involve a filtration or a lipid removal step (Ambrose & Norr, 1993; S. D. Price et al., 2018).

Interpreting organic $\delta^{15}N$ of dental calculus

Bone collagen $\delta^{15}N$ is both directly and indirectly sourced from dietary proteins (Ambrose & Norr, 1993; Jim et al., 2006), and the pathways that link dietary protein to dental plaque formation and protein content may be similar to those shown in Figure 5.17. Proteins preserved in dental calculus are likely derived from diet, mediated by metabolism.

Calculus is ^{15}N-enriched compared to collagen, with an average calculus-collagen spacing of 1.87 ± 1.19‰. Bacterial metabolism and pathological processes, such as the biochemical changes in gingival crevicular fluid associated with periodontal disease, are possible reasons why calculus $\delta^{15}N$ is higher than that of collagen. Some oral bacteria prefer peptides over free amino acids (Carlsson, 1997). Bacteria also consume urea, a ^{15}N-depleted waste product of tissue formation (Nascimento, Gordan, Garvan, Browngardt, & Burne, 2009). Both could alter the $^{15}N/^{14}N$ isotopic ratio compared to collagen. Additionally, as subgingival calculus accumulates and periodontal pockets enlarge, molecules from host tissues (periodontal tissues, blood, and inflammatory mediators) are increasingly available in GCF (Lamster & Ahlo, 2007). In this case, the plaque matrix may incorporate enriched ^{15}N from human tissue breakdown. The microbial community is one trophic level above its food source; oral bacteria should have a higher $\delta^{15}N$ than the human host, because part of their N is derived from human tissues.

The C:N ratio of dental calculus is higher (more carbon) than that of the pellicle, bacteria, and plaque proteins, likely due to the inclusion of bacterial-derived extracellular polysaccharides and lipids, both of which contribute carbon but not nitrogen (Murty et al., 1985; Parker & Creamer, 1971).

Dietary interpretation from dental calculus

The significant correlation between calculus and bone biofractions, as well as the predictable spacing between calculus and bone, indicates that the isotopic composition of both calculus and bone derive from a similar endogenous source – likely diet. We apply the OLS regression models used to compare calculus and bone (Table 4) to generate equations that can be used to predict bone $\delta^{13}C$ and $\delta^{15}N$ values from calculus:

predicted bone carbonate $\delta^{13}C = 1.15*(\text{calculus carbonate } \delta^{13}C) - 1.23$

predicted bone collagen $\delta^{13}C = 0.97*(\text{calculus organic } \delta^{13}C) + 3.98$

predicted bone collagen $\delta^{15}N = 0.85*(\text{calculus } \delta^{15}N) + 0.08$

Residual plots (Figures 5.18-5.20) display the difference between actual and predicted bone isotopic ratios and are therefore useful to address how accurately the OLS equations predict bone $\delta^{13}C$ and $\delta^{15}N$ from calculus isotopic measurements. Most bone carbonate $\delta^{13}C$ predictions fall within ±3‰ of the measured values (Figure 5.18). Most predicted values for collagen $\delta^{13}C$ also fall within ±3‰ of the measured values, though there are some values with a greater predicted-measured difference (Figure 5.19). Of the four residuals that exceed ±3‰, two were predicted from calculus samples with aberrant C:N (outside of the average range). All predicted bone collagen $\delta^{15}N$ values fall within ±3‰ of the measured $\delta^{15}N$ values (Figure 5.20).

Figure 5.18

Residuals from using calculus carbonate $\delta^{13}C$ values to predict bone carbonate $\delta^{13}C$ values

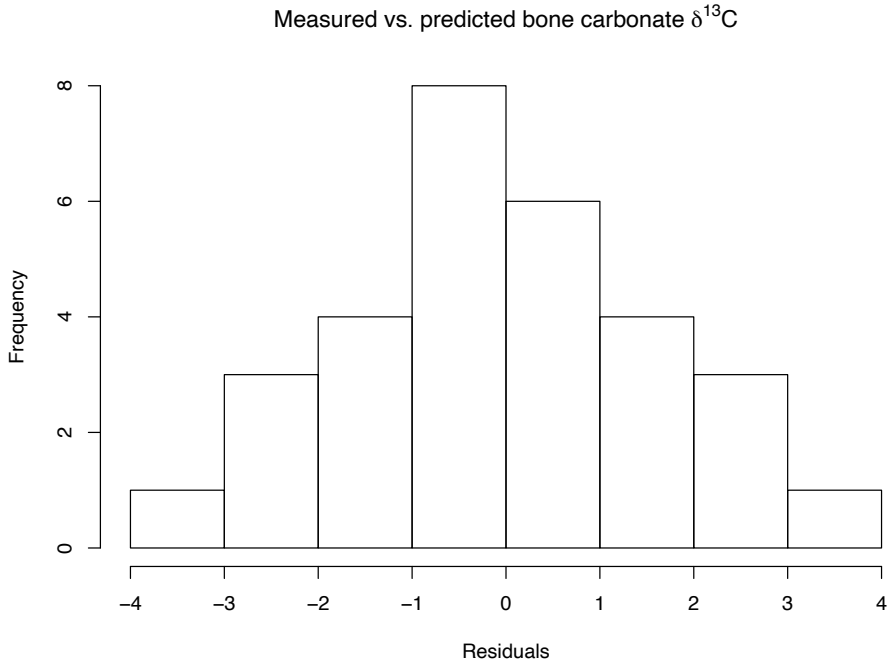


Figure 5.19

Residuals from using calculus organic $\delta^{13}C$ values to predict bone collagen $\delta^{13}C$ values

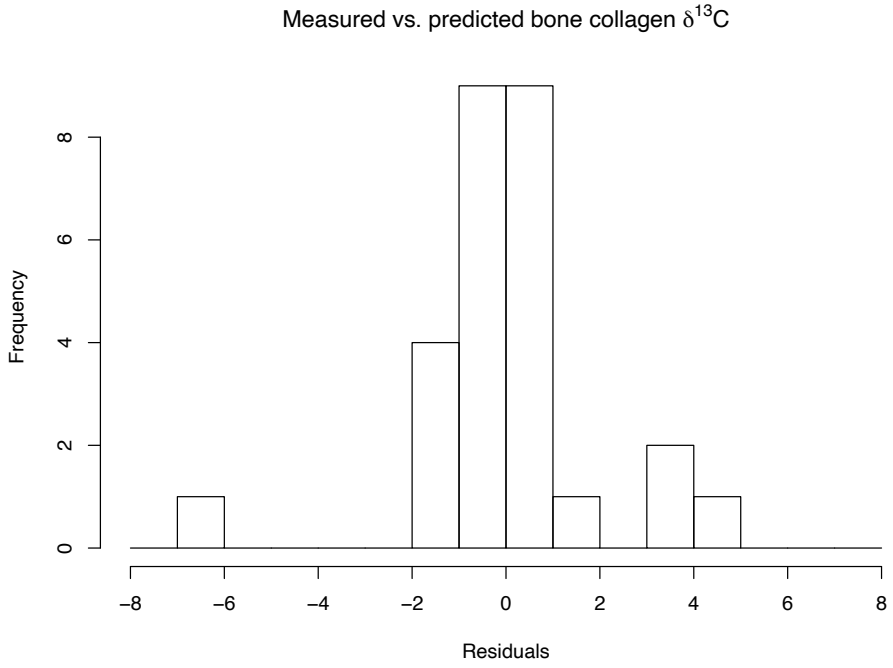
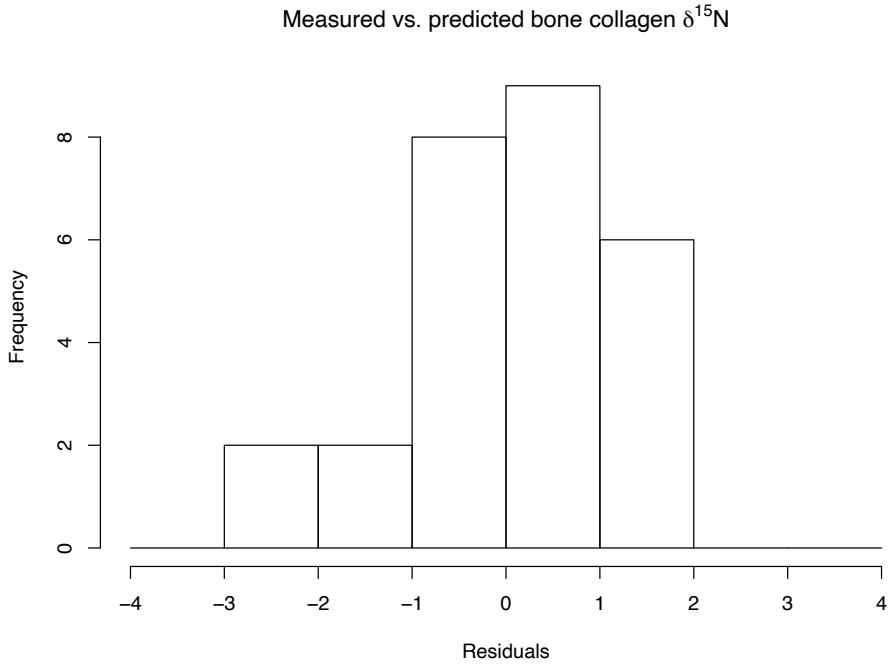


Figure 5.20

Residuals from using calculus $\delta^{15}N$ values to predict bone collagen $\delta^{15}N$ values



To further employ the models, five individuals were randomly selected for dietary interpretation. Table 5.5 shows the measured bone $\delta^{15}C_{carbonate}$, $\delta^{13}C_{organic}$, and $\delta^{15}N$ values compared to calculus measurements that have been regression-corrected to approximate bone values.

Table 5.5

Applying the OLS Regression Equations to Predict Bone $\delta^{13}C$ and $\delta^{15}N$

Individual	carbonate $\delta^{13}C$		collagen $\delta^{13}C$		$\delta^{15}N$	
	measured	predicted	measured	predicted	measured	predicted
Individual 1, Illinois Flood Plain	-2.00	-1.53	-9.79	-9.90	9.65	9.09
Individual 2, Modern Anatomical	-15.03	-12.68	-19.54	-17.73	11.73	12.26
Individual 3, Modern Anatomical	-15.61	-14.82	-20.55	-19.33	10.37	9.09
Individual 4, Illinois Flood Plain	-11.40	-10.48	-20.55	-19.72	7.45	9.54
Individual 5, NC Coastal	-6.80	-6.29	-11.5	-11.61	14.20	14.01

Of the 15 predicted-measured observations in Table 5.5, ten of the residuals are less than 1.0‰ and 13 are less than 2.0‰. The largest residual in Table 5.5 is a 2.35‰ difference in carbonate $\delta^{13}C$ (Individual 2). Several factors may influence the larger predicted-measured differences, including: physiological differences between calculus and bone formation, differences in the temporal resolution of calculus versus bone, pathological conditions, and our use of different skeletal elements for bone-calculus comparison. As a whole, skeletal elements are comparable intra-individually; however, dietary change during the life course can lead to

variation across elements. In a sample size of ten skeletons, Fahy et al. (2017) found a maximum collagen $\delta^{13}C$ difference of -1.58‰ in the occipital-pelvis values of one individual and a maximum $\delta^{15}N$ difference of 3.0‰ occipital-pelvis in another.

Overall, the regression models indicate that calculus isotopic composition is useful for dietary reconstruction at the individual level. Further analysis with additional samples will refine both the regression equations, as well as parameters that help assess sample composition, including weight percent carbon and C:N atomic ratio. Calculus isotopic ratios are also useful for dietary interpretation at the population level. The populations in the study practiced multiple subsistence strategies, and the $\delta^{13}C$ and $\delta^{15}N$ ranges of both calculus and bone capture these dietary differences (Figures 5.21-5.23). Isotopic ratios from both bone and calculus indicate that NC coastal populations consumed marine protein (high $\delta^{15}N$, low organic carbon $\delta^{13}C$, and high carbonate $\delta^{13}C$) (Figures 5.21-5.23). The population from the Illinois Flood Plain temporally spans the period before and during the intensification of maize consumption, and both calculus and bone carbonate and organic carbon $\delta^{13}C$ capture this C_3–C_4 transition (Figures 5.21 and 5.22).

Figure 5.21

Range of calculus carbonate $\delta^{13}C$ compared to bone carbonate, by group

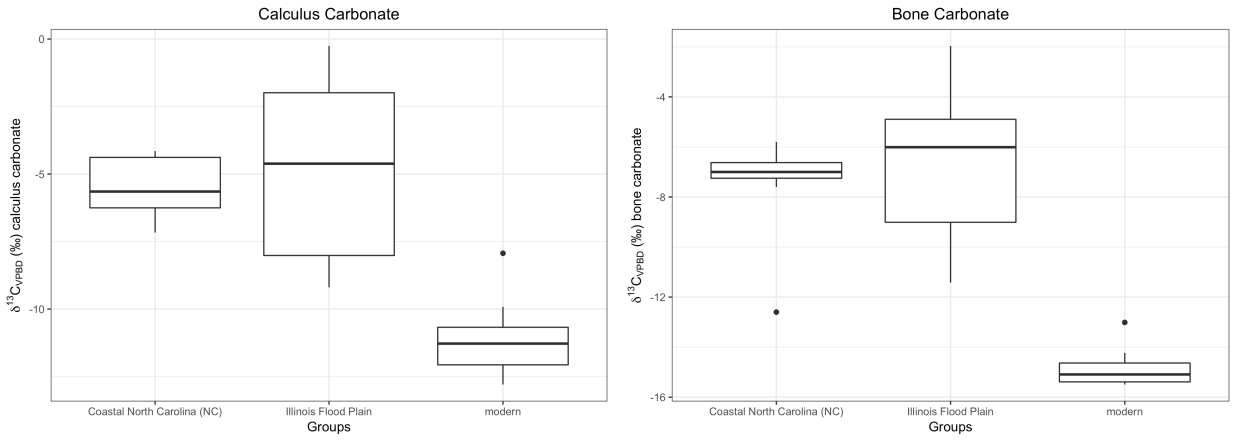


Figure 5.22

Range of calculus organic carbon $\delta^{13}C$ compared to bone collagen, by group

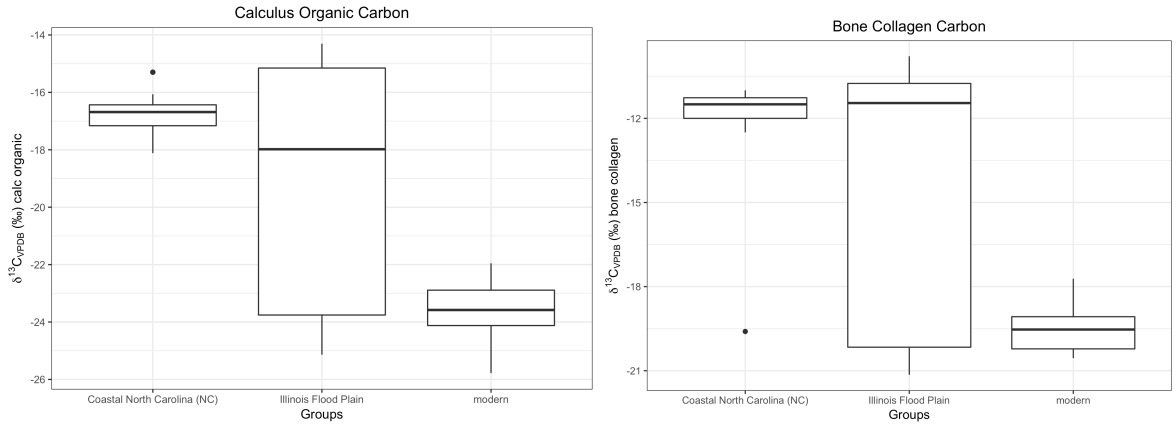
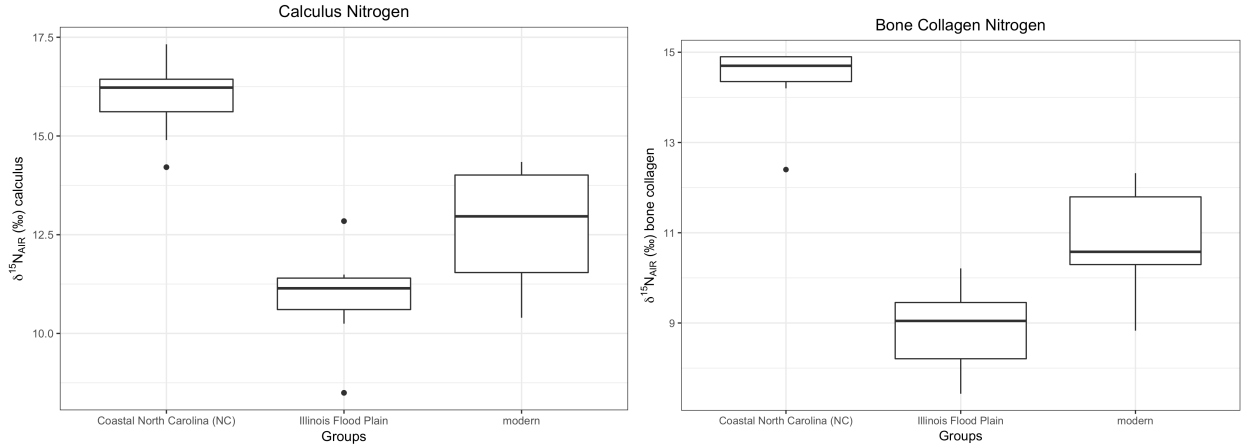


Figure 5.23

Range of calculus $\delta^{15}N$ compared to bone collagen, by group



Limitations and other considerations of stable isotope analysis of dental calculus

One probable source of intra-individual variation in calculus relates to the different exposures in oral environments. On the same tooth, supra- and subgingival calculus are maintained by saliva and GCF, respectively, which have distinct chemical compositions and thus foster different types of bacteria. Additionally, calculus deposits on different teeth within the dental arcade have varying proximities to multiple salivary glands, as well as different exposure to food and occupational debris within the mouth. As a result, isotopic composition may differ

between supra- and subgingival calculus, as well as calculus deposits on different teeth (Hayashizaki et al., 2008). However, it is difficult to distinguish supra- and subgingival deposits on skeletal remains (Lieverse, 1999), and it is often necessary to combine multiple deposits to meet the requisite analytical mass for calculus stable isotope analysis.

Calculus is not available among all members of a population, and is therefore not useful for dietary questions that involve age-specific sampling. Edentulous people, such as infants and older adults, as well as children whose teeth are relatively new to their dental arcade, do not have appreciable calculus deposits. Individuals with high salivary and GCF pH and mineral saturation are more likely to have calculus deposits. This varies according to age, hormones, and possibly proportion of carbohydrates and protein in the diet (Caso et al., 2000; Roberts-Harry & Clerehugh, 2000).

The temporal resolution of calculus deposits is also unclear, as there is inter-individual variation in calculus accumulation rate. Calculus accumulation may be episodic and therefore introduce variation to dietary interpretations. Seasonal changes in diet could create pH and chemical differences in oral substrates and thus calculus accumulation. Physiological changes such as pregnancy, which alters oral pH and hormone profiles, may also create temporary interruptions in calculus accumulation (Laine et al., 1988). Dietary isotopes from these periods of life would therefore be less represented in calculus deposits.

5.5 Conclusion

The main research question that guided this paper is: Are mineral and organic biofractions of dental calculus associated with the carbonate and collagen biofractions of bone? Our results show a statistically-significant association for $\delta^{13}C_{carbonate}$, $\delta^{13}C_{organic}$, and $\delta^{15}N$ of calculus and bone, which suggests that dental calculus is a reliable biomaterial for dietary

reconstruction through stable isotope analysis and a promising alternative to primary biomaterials for destructive analysis. This research has two main contributions: 1) the methods introduced in this paper successfully separate calculus into its mineral and organic biofractions, and 2) regression equations can be used to predict bone $\delta^{13}C$ and $\delta^{15}N$ from calculus isotopic measurements.

Chemical separation of the biofractions of dental calculus is necessary to use calculus as a proxy for bone in dietary stable isotope analysis, and the lack of correlation between calculus $\delta^{13}C$ and $\delta^{15}N$ to weight percent carbon, weight percent nitrogen, C:N atomic ratio, and percent recovery indicate that the isotopic composition of calculus is endogenous and probably linked to diet.

Both calculus and bone $\delta^{13}C$ and $\delta^{15}N$ values differentiate the groups represented in this study, which have distinct dietary profiles. At the individual level, using equations from OLS regression models facilitates prediction of bone $\delta^{13}C$ and $\delta^{15}N$ from calculus $\delta^{13}C$ and $\delta^{15}N$. Many predicted bone $\delta^{13}C$ and $\delta^{15}N$ values are similar to measured values and would thus yield the same dietary interpretations. Larger predicted-measured values are likely a result of differences in the temporal resolution of calculus compared to bone as well as pathological processes, both of which are productive areas for future exploration.

CHAPTER 6 (PAPER 2): THE DIETARY COMPOSITION OF PIEDMONT SIOUAN GROUPS (AD 800–1710)

6.1 Introduction

This paper examines whether Siouan Indian communities from the Piedmont region of north-central North Carolina and southern Virginia adapted their dietary strategies (and thus changed their dietary composition) to navigate the geopolitical, socioeconomic, ecological, and epidemiological changes influenced by European colonialism. To address this question, I analyze the diets of Siouan people who lived during the Late Woodland period through the Colonial period (AD 800–1710) using dietary reconstruction with stable isotopes of dental calculus, and a diverse range of modern species in the Piedmont food web. I also compare resultant trends to the existing archaeological evidence of Piedmont Siouan foodways.

Siouan communities exchanged material culture items, pathogens, and foods with European groups, first indirectly through native trade networks (Merrell, 1987; H. T. Ward & Davis, 2001), then directly following a period of middlemen-mediated exchange in the early-17th century (Davis, 2002; H. T. Ward & Davis, 1993, 1999). Siouan groups experienced threats of violence, captive-taking, and enslavement that changed in scale and intensity due to European colonialism and commercial trade of enslaved native peoples (Ethridge, 2009; Gallay, 2002; Merrell, 2009; H. T. Ward & Davis, 2001). Previous studies have suggested that Siouan groups navigated these colonial-attendant changes by shifting their socioeconomic foci to create new roles in the emerging trade in animal hides, amalgamating with other groups, and altering the

schedule of their ritual activity (Gremillion, 1989; Lapham, 2005; VanDerwarker et al., 2007; H. T. Ward, 1987; H. T. Ward & Davis, 2001; Waselkov, 1997).

Questions remain, however, regarding if, how, and why Siouan groups altered their foodways during colonialism. Archaeobotanical and zooarchaeological research indicates an overall trend of stability and continuity in Piedmont Siouan dietary composition spanning the Late Woodland through the Colonial period (Gremillion, 1989; Holm, 1994; Melton, 2014; Roark, 2020; VanDerwarker et al., 2007), though the researchers did note some temporal and geospatial differences in foodways composition (Gremillion, 1989; Holm, 1994; Longo, 2018; Roark, 2020; Wilson, 1983). I test how direct dietary evidence through stable isotope analysis compares to the evidence provided by the archaeological evidence of food remains and ethnohistoric information. Direct dietary evidence complements the existing data sources because it tests what people were eating out of the available resources. It also gives us a window into the more quotidian diet, whereas archaeological features may represent a shorter period of time or even a single event that is potentially not representative of a person's usual diet (VanDerwarker et al., 2007).

6.2 Materials and Methods

Dental calculus samples

Dental calculus is a mineralized bacterial deposit that accumulates on teeth and can be removed and analyzed without harming the skeleton (Scott & Poulson, 2012). Dental calculus starts forming as a plaque that is the product of bacterial growth, and the growth substrate is assumed to be human tissues in the oral environment (saliva, gingival crevicular fluid, epithelial cells) with a small contribution from food residues (Marsh & Martin, 2009). The dental plaque transforms into dental calculus when it becomes mineralized from salivary and gingival

crevicular fluid minerals. Dental calculus was sampled from 23 individuals. Two criteria were used to identify individuals to sample: quantity of dental calculus present on an individual's dental arcade, and temporal and river drainage affiliation. I aimed to sample individuals from different temporal periods as well as river drainage association to best examine dietary patterns across time and among sociocultural groups. Table 6.1 shows the list of sampled individuals and contextual information including age, sex, temporal context, and river drainage association.

Table 6.1

Piedmont Siouan Individuals Sampled for Dietary Reconstruction

Individual	Age	Sex	Temporal Period	River Drainage
Or11 Bu.1	45-60	Male	Late Woodland 2	Eno
Or11 Bu.3	35-45	Male	Late Woodland 2	Eno
Or231 Bu.3	40-50	Male	Colonial 2	Eno
Or231 Bu.4	20-28	Male	Colonial 2	Eno
Or231 Bu.5	35-50	Male	Colonial 2	Eno
Or231 Bu. 6	30-44	Female	Colonial 2	Eno
Rk6 Bu.97	> 21	UnID	Colonial 2	Dan
Rk6 Bu.BC	> 21	UnID	Colonial 1-2	Dan
Sk1 Bu.6	> 21	UnID	Indet.	Dan
Sk1a Bu.4	25-30	Male	Late Woodland 1	Dan
Sk1a Bu.2	20-25	Male	Colonial 2	Dan
Sk1a Bu.62	20-40	Male	Colonial 2	Dan
Sk1a Bu.74	35-45	Male	Colonial 2	Dan
Vir150 Bu.12	20-30	Female	Late Woodland 1-2	Roanoke
Vir150 Bu.14	40-49	Female	Late Woodland 1-2	Roanoke
Vir150 Bu.19	35-45	Male	Late Woodland 1-2	Roanoke
Vir150 Bu.23	20-25	UnID	Late Woodland 1-2	Roanoke
Vir150 Bu.25	30-39	Male	Late Woodland 1-2	Roanoke
Vir150 Bu.27	> 21	UnID	Late Woodland 1-2	Roanoke
Vir150 Bu.4	40-55	Female	Late Woodland 1-2	Roanoke
Vir231 Bu.14	35-44	Female	Late Woodland 2	Dan
Vir231 Bu.5	18-24	Female	Late Woodland 2	Dan
Vir231 Bu.6	23-29	Female	Late Woodland 2	Dan

Late Woodland 1 (AD 800–1200), Late Woodland 2 (AD 1200–1620), Colonial 1 (AD 1620–1670), Colonial 2 (AD 1670–1710)

Scott and Poulson (2012) first identified the potential of dental calculus to yield dietary information through stable isotope analysis. Eerkens et al. (2014), Salazar-Garcia et al. (2014),

and Price et al. (2018) subsequently made improvements to the analysis of dental calculus, including direct comparison of dental calculus and bone from the same individuals, and chemical separation of calculus into its organic and mineral biofractions. This paper follows methods for calculus purification, analysis, and regression-correction outlined in Chapter 5 (Paper 1).

Interpretive baseline samples

I compared the dietary isotopic composition of Piedmont Siouan people's diets to an interpretive baseline of food isotopic ratios. To generate this baseline, I sampled plant and animal taxa likely consumed by Piedmont people, and used ethnohistoric and archaeological information as sampling guides (Eastman, 1999; Gremillion, 1993a; Holm, 2002; VanDerwarker et al., 2007; H. T. Ward & Davis, 1999; Wilson, 1983). This baseline includes faunal remains from the archaeological record of the Piedmont sites, supplemented with modern samples from ecological zones near the location of Siouan archaeological sites. I added modern faunal samples to minimize the destructive sampling of archaeological collections. Modern plants from the locations of archaeological sites were also included in the baseline, but I did not sample archaeological plant remains in order to further preserve archaeological collections. Sample material and isotopic values are provided in Appendix 2.

Modern plant samples and animal flesh samples were triple-washed via sonication in distilled, deionized water, frozen, and freeze-dried. The samples were not defatted. Archaeological faunal bone samples were chemically separated into their collagen and bioapatite fractions; collagen was extracted following Ambrose (1990) and bioapatite was purified following Balasse et al. (2002).

Isotope ratio mass spectrometry of dental calculus and interpretive baseline samples

The Illinois State Geological Survey Stable Isotope Lab analyzed the isotopic composition of all samples. The carbon and nitrogen isotope ratios of all organic samples, including modern plant and animal samples, faunal bone collagen, and dental calculus, were measured using a Carlo-Erba NC 2500 Elemental Analyzer (EA) with either a Conflo II interface coupled to a Finnigan MAT 252 IRMS or a Conflo IV interface coupled to a Delta V Advantage IRMS. A Kiel Carbonate Device III coupled to a Finnigan MAT 252 IRMS was used to measure the carbon isotope ratios of the carbonate in the mineral biofraction of dental calculus samples.

For organic samples, USGS 40 and USGS 41 were used for two-point machine calibration and as within run isotopic correction standards. The amino acids thiourea, serine, and methionine were used as internal working standards to monitor analysis accuracy and instrument drift during analysis of a sequence comprising 14 standards and 32 samples, 3 of which are sample replicates. For carbonate samples, NBS 18 and NBS 19 standards were used to monitor accuracy and precision of analysis and correction of sample $\delta^{13}C$ and $\delta^{18}O$ values. Corrections are made only if the set of standards in a run deviate systematically in one direction from their certified values. All full runs on the Kiel device include three NBS 18 and three NBS 19 standards, and 38 carbonate samples, three of which are sample replicates. Samples with replicates are indicated in Table 6.2.

Conversion and discrimination factors of sample $\delta^{13}C$ and $\delta^{15}N$

The ratio of heavy-to-light isotopes in biological tissues and in food is measured through comparison to a standard using the following formula:

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

In this formula, R represents the ratio of the isotope of interest to the most abundant isotope (often heavy isotope/light isotope), X represents the isotope of interest, and delta (δ) represents the difference in sample isotope ratio compared to standard isotope ratio. The unit for delta is permil (‰), as the difference from sample to standard is measured in parts per thousand (Ambrose, 1990; Schwarcz & Schoeninger, 1991).

Regression equations were used to approximate bone-equivalent isotopic ratios from measured calculus carbon and nitrogen isotope ratios, and those regression equations are provided in Chapter 5 (Paper 1). Both raw and bone-approximated calculus isotope ratios are included in Table 6.2. Human isotope ratios were also directly compared to the interpretive baseline of local food sources. Direct comparison between human and food isotope ratios requires the use of a diet-to-tissue correction factor to adjust for the isotopic offset that occurs during human metabolism and thus make human and food isotopic ratios directly comparable (Ambrose, 1991, 1993, 2000; Ambrose et al., 1998; Ambrose & Norr, 1993; Bocherens & Drucker, 2003; DeNiro & Epstein, 1978, 1981). Diet-to-tissue correction factors include:

$$\delta^{15}N_{diet} = \delta^{15}N_{bone\ approx.} - 3.5‰$$
$$\text{organic } \delta^{13}C_{diet} = \text{organic } \delta^{13}C_{bone\ approx.} - 5.1‰$$
$$\text{mineral } \delta^{13}C_{diet} = \text{mineral } \delta^{13}C_{bone\ approx.} - 9.4‰$$

The $\delta^{13}C$ and $\delta^{15}N$ of archaeological faunal bone samples must also be adjusted to make them comparable with modern faunal (soft tissue) samples. The following conversion factor approximates muscle $\delta^{13}C$ from collagen $\delta^{13}C$ (DeNiro & Epstein, 1978, 1981):

$$\delta^{13}C_{muscle} = \delta^{13}C_{collagen} - 3‰$$

A final correction factor is used to adjust the $\delta^{13}C$ of modern food samples to make them comparable to archaeological analogues. Carbon isotope ratios differ between modern and

archaeological food samples because of the effect of large-scale combustion of fossil fuels on atmospheric carbon isotopic ratios, which skewed carbon ratios of industrial-era foodwebs compared to preindustrial food webs. Keeling's (2017) correction factor for temporal differences in atmospheric $\delta^{13}C$ is:

$$\delta^{13}C_{preindustrial} = \delta^{13}C_{modern} + 2‰$$

6.3 Results and Interpretations

Dental calculus stable isotope results

Table 6.2 includes the raw calculus $\delta^{15}N$, organic $\delta^{13}C$, and carbonate $\delta^{13}C$ from Piedmont Siouan individuals. Calculus $\delta^{15}N$ is correlated with bone $\delta^{15}N$, which represents the protein portion of the diet (Ambrose, 2000; DeNiro & Epstein, 1981). Calculus organic $\delta^{13}C$ is correlated with bone $\delta^{13}C_{collagen}$, which mostly reflects the carbon from the protein portion of the diet, with some influence from other dietary macronutrients (Ambrose & Norr, 1993; Jim et al., 2006). Our method of calculus organic biofraction purification does not involve ultrafiltration or a lipid removal step; therefore, it is a diverse composite material compared to purified bone collagen, which has a uniform, well-established composition (Ambrose, 1990). Calculus $\delta^{13}C_{carbonate}$ is correlated with bone $\delta^{13}C_{carbonate}$, which represents carbon from all dietary macronutrients (Ambrose & Norr, 1993; Jim et al., 2006). See also Chapter 4 and Chapter 5 of this dissertation for more information on isotope routing from diet to tissues, and the comparability of calculus to bone.

In addition to the raw calculus values, Table 6.2 also includes calculus $\delta^{15}N$, $\delta^{15}C_{organic}$, and $\delta^{15}C_{carbonate}$ that have been converted using regression equations to account for the experimentally-derived isotopic differences between calculus and bone. This was done because diet-tissue isotopic spacing factors have been experimentally established for bone but not

calculus. Therefore, I converted the calculus $\delta^{15}N$ and $\delta^{13}C$ to best approximate bone, so the values could be compared to food categories as in Figure 6.3. Regression equations used to estimate bone values from calculus $\delta^{15}N$ and $\delta^{13}C$ are provided below and explained in detail in Chapter 5.

$$\text{predicted bone collagen } \delta^{15}N = 0.84*(\text{calculus } \delta^{15}N) + 0.23$$

$$\text{predicted bone collagen } \delta^{13}C = 0.98*(\text{calculus organic } \delta^{13}C) + 4.06$$

$$\text{predicted bone carbonate } \delta^{13}C = 1.19*(\text{calculus carbonate } \delta^{13}C) - 0.96$$

A carbon spacing measurement (C_{spac}) is also included in Table 6.2. C_{spac} is useful to estimate the isotopic profile of the protein carbon compared to the whole diet carbon (Ambrose & Norr, 1993). C_{spac} was calculated as $\Delta^{13}C_{\text{calculus carbonate – calculus organic}}$ using bone-approximated rather than raw calculus $\delta^{13}C$ so that it could be compared to interpretive ranges established through experimental work that used bone $\delta^{13}C$ (Ambrose & Norr, 1993).

Figure 6.1 shows calculus $\delta^{15}N$ vs. $\delta^{13}C_{carbonate}$, both converted using regression equations to approximate bone values. This plot is a good representation of whole diet. Figure 6.2 shows calculus $\delta^{15}N$ vs. $\delta^{13}C_{organic}$, which were also both converted using regression equations to approximate bone values. Figure 6.3 shows regression-converted calculus $\delta^{15}N$ vs. regression-converted $\Delta^{13}C_{\text{calculus carbonate – calculus organic}}$, which is useful to examine whether a person's dietary energy and protein share a carbon source (e.g. C_3 protein and C_3 energy), or if they diverge. Tables 6.3 and 6.4 show the descriptive statistics for $\delta^{15}N$, $\delta^{13}C_{organic}$, $\delta^{13}C_{carbonate}$, and $\Delta^{13}C_{\text{carbonate – organic}}$ by temporal period and river drainage. The values shown in both tables are regression-converted to approximate bone values. Values from Rk6 individuals are excluded from the descriptive statistics because of poor quality control metrics (Table 6.5)

Table 6.2

Raw and Bone-Approximated (Denoted as -B) Calculus Isotopic Ratios

Individual†	$\delta^{15}N$	$\delta^{15}N$-B	$\delta^{13}C_{organic}$	$\delta^{13}C_{organic}$-B	$\delta^{13}C_{carbonate}$	$\delta^{13}C_{carbonate}$-B	C_{spac}
Or11.1	10.4	8.9	-20.3	-15.7	-7.8	-10.3	5.4
Or11.3	10.7	9.2	-20.7	-16.1	-7.6	-9.9	6.2
Or231.3	10.6	9.1	-18.9	-14.4	-6.1	-8.2	6.2
Or231.4	10.9	9.3	-20.8	-16.2	-9.2	-11.9	4.3
Or231.5	11.0	9.5	-19.5	-14.9	-5.6	-7.7	7.3
Or231.6	10.7	9.1	-20.6	-16.0	-6.6	-8.8	7.2
Rk6.97	10.7	9.2	-20.6	-16.0	-5.0	-7.0	9.0
Rk6.BC	11.2	9.6	-17.8	-13.3	-2.9	-4.6	8.8
Sk1.6	10.4	8.9	-15.3	-10.9	-3.7	-5.5	5.3
Sk1a.2	10.0	8.6	-21.1	-16.5	-5.8	-7.9	8.6
Sk1a.4	11.2	9.6	-19.4	-14.9	-4.3	-6.1	8.7
Sk1a.62	9.7	8.3	-21.9	-17.3	-7.3	-9.6	7.6
Sk1a.74	10.5	9.0	-18.3	-13.8	-6.3	-8.6	5.2
Vir150.4	10.5	9.0	-20.3	-15.8	-4.0	-5.9	9.9
Vir150.12	10.4	8.9	-19.7	-15.2	-7.2	-9.5	5.7
Vir150.14	11.2	9.6	-22.5	-17.9	-10.9	-13.8	4.1
Vir150.19	12.3	10.5	-17.1	-12.7	-8.1	-10.6	2.0
Vir150.23	9.9	8.5	-18.5	-14.0	-8.2	-10.7	3.2
Vir150.25	9.8	8.4	-20.9	-16.3	-9.5	-12.2	4.1
Vir150.27	10.7	9.2	-21.4	-16.8	-9.5	-12.2	4.5
Vir231.5	9.8	8.4	-17.9	-13.4	-3.9	-5.7	7.7
Vir231.6	9.4	8.1	-14.8	-10.4	-5.0	-7.0	3.3
Vir231.14	9.3	8.0	-15.6	-11.1	-3.2	-4.9	6.2

†the number after the decimal indicates burial number, so Or11.1 is Or11 Burial 1.

Figure 6.1

Bone-approximated dental calculus $\delta^{15}N$ vs. bone-approximated dental calculus $\delta^{13}C_{carbonate}$

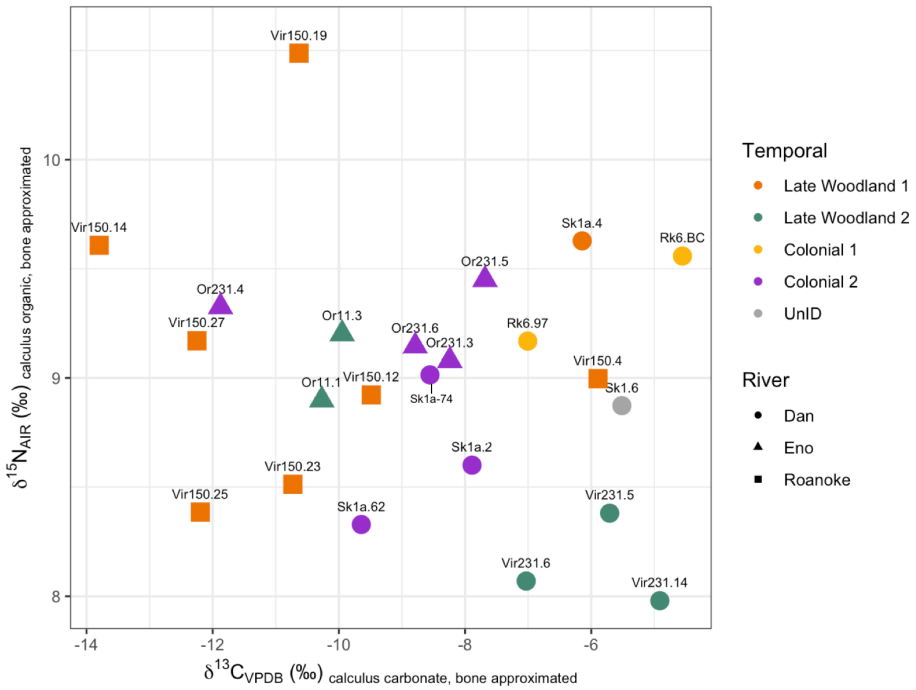


Figure 6.2

Bone-approximated dental calculus $\delta^{15}N$ vs. bone-approximated dental calculus $\delta^{13}C_{organic}$

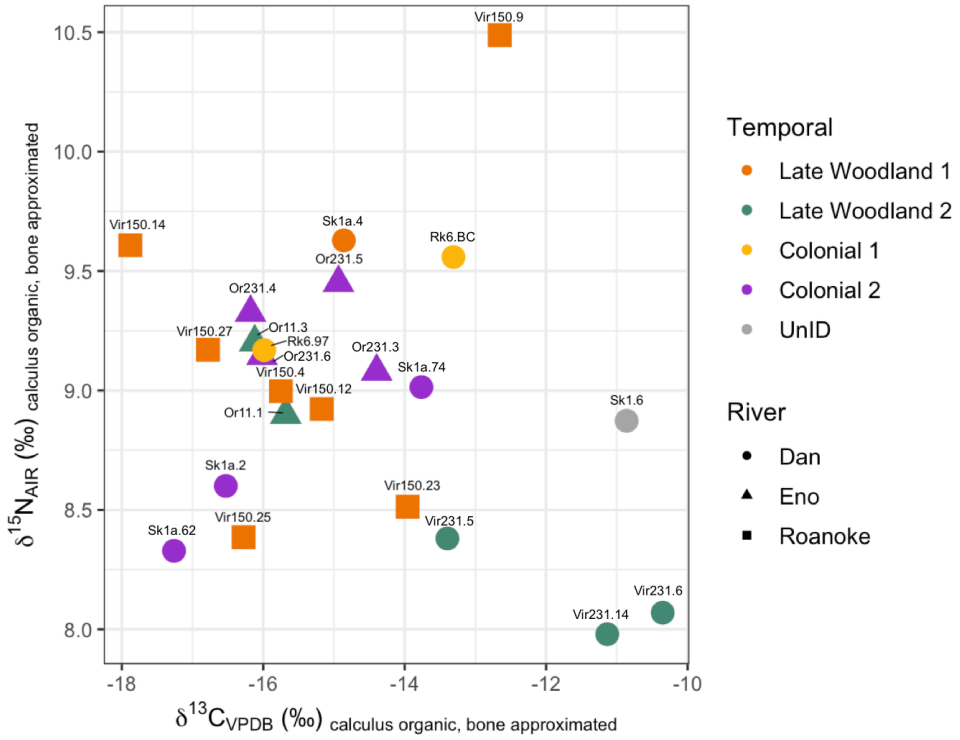


Figure 6.3

Bone-approximated $\delta^{15}N$ vs. bone-approximated $\Delta^{13}C_{calculus\ carbonate\ -\ calculus\ organic}$

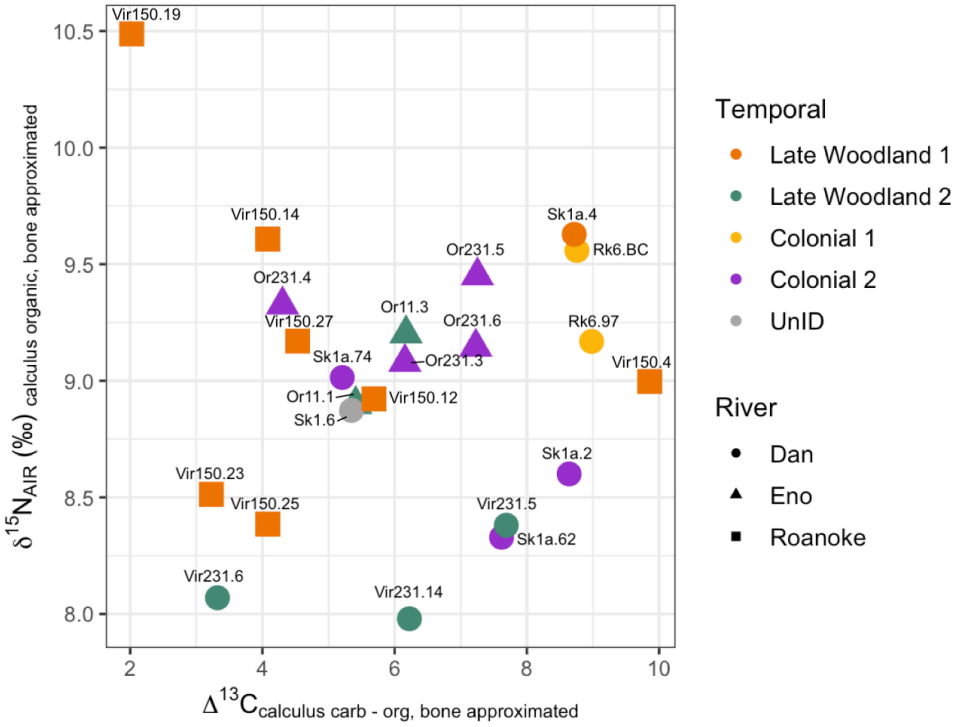


Table 6.3

Descriptive statistics for isotopic measurements, by temporal period (values are regression-converted to approximate bone values)

		Late Woodland (AD 800–1620)	Colonial (AD 1620–1710)
$\delta^{15}N$	Mean (sd)	8.9 (0.7)	9.0 (0.4)
	Median (IQR)	8.9 (0.8)	9.1 (0.4)
$\delta^{13}C_{organic}$	Mean (sd)	-14.6 (2.2)	-15.6 (1.3)
	Median (IQR)	-15.2 (2.7)	-16.0 (1.7)
$\delta^{13}C_{carbonate}$	Mean (sd)	-9.2 (2.9)	-9.0 (1.4)
	Median (IQR)	-9.9 (4.6)	-8.6 (1.2)
$\Delta^{13}C_{carb\text{-}org}$	Mean (sd)	5.5 (2.3)	6.6 (1.5)
	Median (IQR)	5.4 (2.1)	7.2 (1.8)

Table 6.4

Descriptive Statistics for Isotopic Measurements, by River Drainage (Values are Regression-Converted to Approximate Bone Values)

		Dan River	Eno River	Roanoke River
$\delta^{15}N$	Mean (sd)	8.6 (0.5)	9.2 (0.2)	9.2 (0.7)
	Median (IQR)	8.5 (0.6)	9.2 (0.2)	9.0 (0.7)
$\delta^{13}C_{organic}$	Mean (sd)	-13.5 (2.6)	-15.6 (0.7)	-15.5 (1.8)
	Median (IQR)	-13.6 (4.2)	-15.8 (1.0)	-15.8 (2.0)
$\delta^{13}C_{carbonate}$	Mean (sd)	-6.9 (1.7)	-9.5 (1.5)	-10.7 (2.5)
	Median (IQR)	-6.6 (2.4)	-9.4 (1.8)	-10.7 (2.2)
$\Delta^{13}C_{carb\text{-}org}$	Mean (sd)	6.6 (1.9)	6.1 (1.1)	4.8 (2.5)
	Median (IQR)	6.9 (2.6)	6.2 (1.4)	4.1 (1.5)

Table 6.5 includes quality control indicators that are used to assess collagen preservation and may also be useful to assess calculus preservation, though experimental work is required to evaluate quality control indicators for calculus. The atomic ratio of carbon to nitrogen (C:N) is a particularly useful quality control indicator for collagen (Ambrose, 1990), and the pilot work in Chapter 5 (Paper 1) identified that calculus samples from multiple geographic regions and temporal contexts fell within a tight C:N range of 6.01 ± 0.92, marking C:N as a potential indicator of calculus organic matter preservation. Three indicators are used to evaluate the reliability of calculus measurements: atomic C:N ratio, VM28, and VM44.

VM28 and VM44 are voltage measurements of the major ion beam on the mass spectrometer; sample N_2 has a mass/charge ratio of 28 and CO_2 has a mass/charge ratio of 44. If the voltage produced by a sample is below a minimum threshold, it means there is not enough measurable N and C in the sample. This could reflect either a very small sample weight, or a sample with low percent N and/or C. In the latter case, very low C and N indicates that the sample is largely inorganic. Low sample weight is a common issue with dental calculus because of the limited size of the calculus deposits on teeth. Low sample weights may increase the likelihood of low voltages, less reliable measurements, and therefore some variation in sample

$\delta^{15}N$ and $\delta^{13}C$. However, samples that have normal weights but low voltages may have low weight percent carbon and nitrogen and may therefore be largely inorganic with only traces of organic soil contaminants and/or calculus organic material. Table 6.5 shows that samples with low weight percent C and N also have high C:N which we also see in collagen samples that have lost most of their original C and N.

Low VM28 and 44 measurements are shaded in Table 6.5, along with C:N values that exceed two standard deviations from the mean of 6.01 ± 0.92. Samples with low VM28 and 44 that also have low weight percent C and/or N were excluded from further analyses presented in this paper. This includes individuals Rk6.BC and Rk6.97. Individual Sk1a Burial 2 has a high C:N value but acceptable wt%C and wt%N and is included in the below analyses; however, the isotopic ratios of this person's diet should be interpreted with caution.

Interpretive notes

Calculus accumulation is influenced by age, sex, diet, nutrition, behavior, and comorbidities (Bergström, 1999; Lieverse, 1999; Yaussey and DeWitte, 2019). Our current method for stable isotope analysis of dental calculus samples requires an analytical mass of 8-20 mg (Chapter 5), which is an appreciable deposit of dental calculus that is found more commonly in older adults who have gingival and/or periodontal inflammation (Glick, 2014; Lieverse, 1999; Tuggle and Watson, 2019). Calculus samples are therefore not available from every individual in the population and overall represent older adults. The use of calculus for dietary reconstruction does not provide as full of a window into interindividual dietary variation as bone samples. However, stable isotope ratios of dental calculus provide direct dietary information in situations where it would otherwise be inaccessible. There were no clear differences in calculus isotope

ratios among sex and age groups for this study, which suggests that these life history factors do not skew our results.

Table 6.5

Indicators of Calculus Preservation

	Organic biofraction					Mineral biofraction	
Individual	Wt% N	VM28	Wt% C	VM44	C:N	Wt% C	VM44
Or11.1	9.2	1.4	45.2	3.0	5.5	0.9	2.6
Or11.3	8.3	1.4	51.2	3.8	7.0	0.5	1.7
Or231.3	8.6	1.3	46.8	3.1	6.2	0.8	2.1
Or231.4	8.1	1.2	45.9	3.0	6.4	0.6	1.7
Or231.5	4.9	0.7	25.5	1.5	6.1	0.7	2.8
Or231.6	1.2	0.3	12.2	0.9	7.7	0.8	2.7
Rk6.97	0.7	0.1	5.3	0.4	8.9	0.2	0.8
Rk6.BC	0.4	0.2	8.9	0.7	7.4	0.3	1.1
Sk1.6	10.3	1.6	46.3	3.2	5.1	0.8	2.2
Sk1a.4	4.8	0.6	25.8	1.4	6.3	0.4	1.7
Sk1a.2	3.7	1.7	30.6	8.6	8.4	0.9	2.6
Sk1a.62	2.4	0.3	19.2	1.4	7.7	0.8	4.6
Sk1a.74	8.8	1.1	42.1	2.1	5.6	0.6	2.3
Vir150.12	9.0	1.3	42.4	2.6	5.5	0.7	2.3
Vir150.14	7.9	1.0	34.1	1.8	5.1	0.5	2.1
Vir150.19	9.2	1.0	42.8	2.3	5.4	0.5	1.9
Vir150.23	8.2	1.2	42.2	3.1	6.0	0.5	1.9
Vir150.25	9.3	1.4	49.1	3.3	5.9	0.6	1.9
Vir150.27	7.9	1.3	38.2	3.2	5.6	0.5	1.9
Vir150.4	5.9	0.8	35.5	2.4	7.1	0.3	1.4
Vir231.14	9.2	1.5	43.5	3.1	5.3	0.6	1.9
Vir231.5	2.7	0.3	14.0	0.7	6.1	0.2	1.1
Vir231.6	8.2	1.4	38.0	9.4	5.4	0.5	1.5

Isotopic ecology of Piedmont North Carolina and Virginia

The carbon and nitrogen isotopic ratios of modern and archaeological plant and animal samples that were collected in the Piedmont provide an interpretive baseline with which to compare human isotopic ratios. Table 6.6 includes isotopic summary statistics of categories of sampled plants and animals. The $\delta^{15}N$, $\delta^{13}C$ and scientific name of each sampled specimen can be found in Appendix 2. Figure 6.4 shows the spatial relationships of these categories in a $\delta^{15}N$

vs. $\delta^{13}C$ biplot, along with the isotopic ratios of Siouan people. The rationale for why these food categories differ in isotopic ratios is explained below.

Table 6.6

Summary Statistics of Food Categories used for the Piedmont Dietary Interpretive Baseline

	Sample size	$\delta^{13}C$ (mean ± std dev)	$\delta^{15}N$ (mean ± std dev)
Maize	3	-9.20 ± 0.10	0.5 ± 0.54
Nuts	6	-27.95 ± 1.85	0.87 ± 0.76
C_3 greens	9	-27.59 ± 1.46	2.85 ± 3.09
Terrestrial herbivores	9	-24.29 ± 1.31	4.51 ± 1.76
Terrestrial omnivores	14	-23.38 ± 1.90	4.44 ± 3.42
Reptiles	7	-22.81 ± 0.86	8.65 ± 1.31
Archaeological Fish	4	-17.66 ± 1.64	8.35 ± 0.61
Modern Small Fish	6	-22.75 ± 1.37	10.36 ± 1.44
Modern Largemouth Bass	4	-27.24 ± 0.32	14.61 ± 0.83

See Appendix 2 for individual data points from the plant and animal samples

Plant foods: Carbon isotopic profiles C_3 vs. C_4 plants

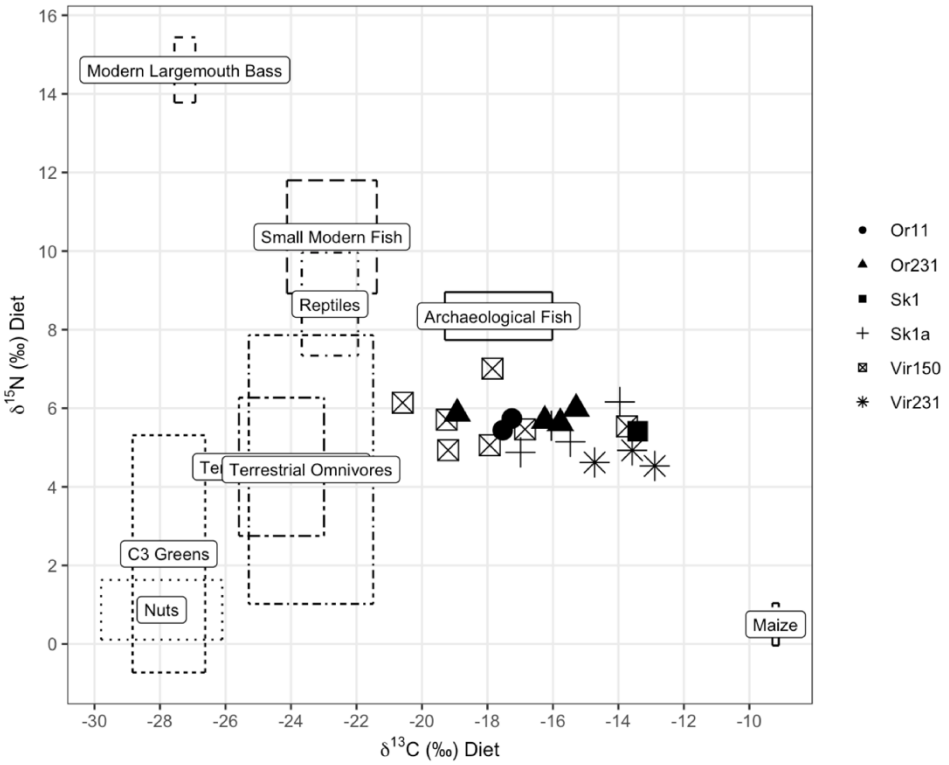
Plants are categorized as C_3 or C_4 plants based on their photosynthetic pathway. Both C_3 and C_4 plants use atmospheric carbon dioxide (CO_2) as their carbon source, but the C_4 photosynthetic pathway assimilates (fixes) more of the heavier carbon isotope (^{13}C) and thus has a less negative $\delta^{13}C$ than C_3 plants (T. Brown & Brown, 2011; Marshall, Brooks, & Lajtha, 2007). Ethnohistoric information and archaeobotanical data indicate that maize (*Zea mays*) was the main C_4 plant food likely consumed by Piedmont Siouan people (Gremillion, 1989; Melton, 2014; VanDerwarker et al., 2007). Other C_4 plants that grow naturally in the study region such as amaranth and purslane (*Amaranthus sp.* and *Portulaca oleracea*, respectively) were not cultivated or stored like maize and therefore had a minor dietary contribution by comparison (Fritz, 1990). The $\delta^{13}C$ of maize from the Piedmont region, is –9.20 ± 0.10‰.

Piedmont Siouan groups likely consumed the following C_3 plants: nuts and seeds, leafy greens, tubers, beans, and fruits (Gremillion, 1989; VanDerwarker et al., 2007). The average

$\delta^{13}C$ of sampled C_3 greens from the Piedmont ranges is -27.59 ± 1.46‰. Carbon isotope values are useful to understand the influence of C_3 vs. C_4 plants on Piedmont Siouan diets.

Figure 6.4

$\delta^{15}N$ vs. $\delta^{13}C_{carbonate}$ of dental calculus, regression-converted to approximate bone values and corrected to dietary values using diet-to-tissue correction factors



Boxes represent one standard deviation. The terrestrial herbivores label is partially obscured by the terrestrial omnivores label and indicates the smaller of the two overlapping ranges.

Protein sources: Trophic-level shifts in carbon and nitrogen

With each ascending step in the food chain, there is an isotopic offset of approximately +1-2‰ for $\delta^{13}C$ and +3-5‰ for $\delta^{15}N$ due to metabolic processes (Bocherens & Drucker, 2003). For example, if a carnivore feeds on an herbivore with $\delta^{15}N$ tissue values of 7‰, the carnivore will convert the herbivore's tissues into its own collagen and muscle protein, which will express a $\delta^{15}N$ value of 10-12‰ (Katzenberg, Herring, & Saunders, 1996; Steele & Daniel, 1978). This

step-wise enrichment is shown between Piedmont C_3 greens, terrestrial herbivores, and terrestrial omnivores in Figure 6.4 and Table 6.6.

Habitat differentiation: Carbon isotopic profiles differ by terrestrial and freshwater habitats

The data from the Piedmont potentially represent two isotopically distinct habitat types: terrestrial woodland and freshwater river system. Each has different isotopic dynamics, which shape the isotope ratios of the plants and animals within those habitats. The terrestrial woodland food web carbon isotope composition is highly influenced by the C_3 plants that make up its base. The average $\delta^{13}C$ of terrestrial herbivores, terrestrial omnivores, and reptiles is -24.3 ± 1.3‰, -23.4 ± 1.9‰, and -22.8 ± 0.9‰, respectively. Some species from terrestrial woodland habitats adjacent to human fields may have consumed C_4 plants as well as C_3 plants, including turkeys, raccoons, and deer. One archaeological raccoon (*Procyon lotor*) sample had a high $\delta^{13}C$ (-19.3‰) compared to other terrestrial omnivores; however, none of the archaeological turkey (*Meleagris gallopavo*) or deer (*Odocoileus virginianus*) samples indicated mixed C_3-C_4 diets.

There is a discrepancy in the isotopic ratios between modern and archaeological fish samples (Figure 6.3, Appendix 2). The average $\delta^{13}C$ of freshwater fish archaeological samples from the Dan River is -17.7 ± 1.6‰, which is consistent with Schoeninger and DeNiro's (1984) reported range of -12.0 to -25.0‰ for the $\delta^{13}C$ of riverine fish. However, the average $\delta^{13}C$ of small freshwater fish from the Dan and Eno Rivers is -22.8 ± 1.4‰, which is on the high end of Schoeninger and DeNiro's range. Additionally, the modern largemouth bass (*Micropterus salmoides*) samples from the Dan River exceed Schoeninger and DeNiro's range, with an average $\delta^{13}C$ of -27.2 ± 0.3‰. Interestingly, the modern and archaeological largemouth bass samples, all from the Dan River, differ by 9.6‰ in $\delta^{13}C$ and 6.3‰ in $\delta^{15}N$.

Ecosystem change likely explains the $\delta^{13}C$ and $\delta^{15}N$ difference between modern and archaeological fish. Chemical runoff, especially from agriculture, could influence the modern isotopic composition of the Dan River and create an isotopic difference between samples from modern and archaeological contexts. It is possible but unlikely that the archaeological fish samples are contaminated, because they indicate good collagen preservation (the samples have an average C:N of 3.3). Furthermore, the archaeological fish $\delta^{13}C$ is consistent with the expected carbon isotopic ratios from river systems with floodplains, which tend to have a higher contribution of ^{13}C-enriched benthic algae (Finlay & Kendall, 2007). For the purposes of dietary analysis in the rest of this paper, I consider freshwater fish as a possible source of ^{13}C-enriched protein, though I aim to test more of the Piedmont aquatic foodweb in future studies to better understand the range of variation.

Several animals may consume resources from both terrestrial and aquatic habitats. Raccoons, for example, may eat fish and shellfish as well as terrestrial resources, resulting in a carbon isotopic profile between the carbon ranges of terrestrial and riverine foodwebs. It is unclear if the high $\delta^{13}C$ of the raccoon indicated above derives from the dietary addition of C_4 plants or riverine protein.

Interpretation of human diets

Generally, individuals with bone-approximated calculus $\delta^{13}C_{carbonate}$ higher than -8‰ have significant C_4 energy contribution to their diets (likely maize) (Ambrose, Buikstra, & Krueger, 2003; Emerson, Hedman, Simon, Fort, & Witt, 2020). Most Piedmont individuals, and especially those from the Dan River, consumed significant C_4 energy. Others likely consumed mixed C_3-C_4 energy (Figure 6.1). Individuals with bone-approximated calculus $\delta^{13}C_{organic}$ higher than -13‰ have significant C_4 contribution to the protein portion of their diet, perhaps from

maize and/or aquatic protein (Figure 6.2, 6.4). Most Piedmont individuals consumed mixed C_3-C_4 protein, with some individuals (Sk1.6, Vir231.14, and Vir231.6) showing $\delta^{13}C_{organic}$ heavily influenced by C_4 protein (Figure 6.2). Individuals with bone-approximated calculus $\Delta^{13}C_{carbonate - organic} > 4.5$ have dietary protein that is more influenced by C_3 and dietary energy that is more influenced by C_4 (Ambrose & Norr, 1993; Pechenkina, Ambrose, Xiaolin, & Benfer, 2005). Most Piedmont individuals either follow this pattern (mixed C_3-C_4 protein, C_4 energy), though some have dietary energy less ^{13}C-enriched than dietary protein (energy heavily influenced by C_3, protein more influenced by C_4 than energy). These individuals are mostly from Vir150, along with individuals Or231.4 and Vir231.6.

6.4 Discussion

Previous researchers emphasize the overall similarities of archaeobotanical and zooarchaeological assemblages recovered from archaeological sites associated with Piedmont Siouan communities (Gremillion, 1989; Holm, 1994; Roark, 2020). Maize was an important cultigen to the Piedmont Siouan communities, and nuts (especially acorn and hickory nuts) were a highly utilized gathered food resource. Beans, fleshy fruits, squash, and starchy and oily seeds were also commonly included in the archaeobotanical record of Piedmont sites (Gremillion, 1989, 1993a; Melton, 2014; Roark, 2020; VanDerwarker et al., 2007).

Mammals were the main animal resource used by Piedmont Siouan groups, with white-tailed deer especially prevalent in faunal assemblages. The remains of birds (primarily passenger pigeon and turkey), fish, and reptiles (mainly box turtle) were also ubiquitous in the zooarchaeological assemblages of Piedmont sites (Holm, 1994, 2002; Longo, 2018; VanDerwarker, 2001). While dietary composition appears similar among Piedmont sites,

researchers have noted some potential differences in foodways assemblages among the river drainages and across time.

Dietary $\delta^{15}N$ and $\delta^{13}C$ of Piedmont Siouan individuals agrees with the paleoethnobotanical data that maize was an important plant resource. Most of the individuals have high carbonate $\delta^{13}C$, suggesting that maize was a large part of their diets (Figures 6.1, 6.4), though there is variability in maize consumption across the sampled individuals. There is also some variability in $\delta^{15}N$ across the sampled individuals, likely relating to the contribution of fish and reptiles to the diet (Figures 6.2, 6.4). This variability will be considered in the context of previous literature on Piedmont Siouan foodways to ask whether the direct dietary data supports the archaeological and ethnohistoric information on Piedmont foodways.

Previous research on the foodways of Piedmont Siouan communities

The following review will summarize main trends in the archaeobotanical and zooarchaeological data that nuance Piedmont foodways. It should be noted that while many of the archaeological studies from the Piedmont region include data from archaeological sites within the Haw River basin, the following review does not include data from the Haw River, only the Eno and Dan River basins, since I did not examine any people from the Haw River archaeological sites as part of my dissertation research.

Differences in archaeobotanial and archaeofaunal remains among the river drainages of the study region

Roark (2020) collated previously published paleoethnobotanical data from the Piedmont region and added recently collected, unpublished data to investigate spatial and temporal differences in plant remains. She applied a correspondence analysis to the data, which indicated that archaeobotanical assemblages from Dan River sites included more maize remains on average than sites within the Eno River drainage. By contrast, assemblages from Eno River sites

contained more acorn than those from Dan River sites (Gremillion, 1993b; Roark, 2020). Roark (2020) concludes that this division between maize and acorn that statistically separates the Dan and Eno River sites is more pronounced than temporal trends in the plant data.

Longo (2018) collated archaeofaunal data from Piedmont sites, and produced heatmaps of the relative abundance of different faunal taxa. The maps show a hotspot of bird remains in the northwest part of the study region, at the Stockton (Vir231) and Leatherwood Creek (Vir196) sites, which are two Late Woodland sites on the Smith River (a tributary of the Dan River). Passenger pigeon was commonly recovered from the Stockton archaeofaunal assemblage, while turkey was more prevalent at Leatherwood Creek (Longo, 2018; VanDerwarker, 2001).

Longo's geospatial analysis also identified a hotspot for reptiles in the central Piedmont, encompassing the Wall (31Or11) and Fredricks (31Or231) sites. Both the Dan and Eno River drainages mapped as cool spots for fish remains compared to the Haw River drainage, the only region of Longo's heatmap that showed a hotspot for fish remains. However, as Vanderwarker (2001) noted, the recovery of fish remains may be biased by the method of recovery (e.g. screening versus collection by hand), as well as by taphonomy, as fish bones can be thin and subject to taphonomic breakdown. While both the Dan and Eno River basins were cool spots for fish remains compared to the Haw River basin, Holm (1994) suggested that communities who lived at the Hairston (31Sk1) and Upper Saratown (31Sk1a) sites within the Dan River drainage used more aquatic resources than communities who lived at the Wall and Fredricks sites on the Eno River.

Differences in food remains among the river drainages may be a result of both ecological factors as well as settlement sizes. Longo (2018:102) noted that the "ecological patchiness of faunal resources and highly localized ecological make-up of each village played a part in

variation of subsistence practices." For example, the larger Dan River may have supported a higher concentration and diversity of fish species, according to Holm (1994). Holm also attributed the greater use of aquatic resources by Dan River communities to their larger settlement and population sizes, as a broader, more diverse resource base may have been helpful to secure food for more people.

Roark also pointed to settlement size to explain the division between maize and acorn that separated the Dan River and Eno River sites. Maize and acorn have "similar nutritional and culinary roles"; however, acorns require time-consuming processing to make them suitable for consumption, and maize may have therefore been a more efficient substitute for larger communities (Roark, 2020:86).

Temporal differences in archaeobotanical and archaeofaunal remains

Many researchers also tested whether there was a change over time in dietary composition, and were particularly interested in whether foodways changed with the onset of colonialism (Gremillion, 1989; Holm, 1994, 2002; Longo, 2018; Melton, 2014; Mikeska, 2019; Roark, 2020; VanDerwarker, Marcoux, & Hollenbach, 2013; VanDerwarker et al., 2007). Some temporal trends were seen across the entire region of study, such as an increase in beans after their introduction to the Piedmont following the terminal Late Woodland period (AD 1450–1620), and a decrease in plants of the Eastern Agricultural Complex between the latter half of the Late Woodland period and the Colonial period. There is also evidence for a decrease in the use of acorn over time. This trend, though apparently region-wide, is only statistically significant for the Dan River drainage sites (Gremillion, 1987, 1989; Roark, 2020).

Regarding region-wide trends in the faunal remains, there were fewer deer remains in later as opposed to earlier contexts, and there is some evidence that a similar decrease was seen

in shellfish remains. Holm (1993:312) observed relatively few shellfish remains in Colonial period component of the William Kluttz site (31Sk6): "the low frequency of shell remains, while contrasting sharply with Lower Saratown (31Rk1) [a middle Late Woodland period site from the Dan River, AD 1000–1450] and the Jenrette site (31Or231a) [an early-middle Colonial site from the Dan River, AD 1650–1680], is similar to that seen at the Fredricks site and may indicate a deemphasis of shellfish utilization among piedmont Siouans by the turn of the 18th century." Note that neither the Lower Saratown nor the Jenrette site are outlined in Chapter 4, because I did not examine any individuals from those sites.

Other temporal trends are unique to the different river drainages. Roark (2020) noted that maize cupules increased over time in archaeological sites from the Dan River (though maize kernels did not show a significant increase). Archaeobotanists interpret maize kernels as evidence of maize consumption, while they interpret cupules as evidence of burning cobs. She noted an opposite trend for the Eno River sites. The late Colonial period Fredricks site (AD 1680–1710) included fewer maize cupules *and* kernels than the Late Woodland Wall site (AD 1500–1600). The Dan River archaeofaunal assemblages show a decrease in deer over time, consistent with region-wide trends, but show a concomitant increase in other fur-bearing mammals, a trend not seen in the Eno River assemblages (Holm, 1987, 1994, 2002; Longo, 2018).

Researchers attribute temporal changes in foodways remains to various factors, including colonial-attendant disease and socioeconomic change, as well as ecological factors such as climate events. Holm (2002) suggests that the decrease in deer, and increase in other fur-bearing mammals seen at early and especially late Colonial period Dan River sites, is the result of the

deerskin trade – the high demand for pelts coupled with the potential overexploitation of deer in the region.

Mikeska's (2019) strontium analysis of deer enamel from multiple Siouan sites within the Piedmont may also support a decrease in deer availability over time, particularly in the Dan River drainage. All deer that she sampled from the Late Woodland Hairston site had strontium ratios local to the Dan River drainage, but 5/6 (83%) of the sampled deer from early Colonial period Upper Saratown component and 9/14 (64%) of the sampled deer from the late Colonial period Upper Saratown component had local ratios. This suggests that people who lived at Dan River sites expanded their deer procurement zone over time. All sampled deer from Eno River sites had strontium ratios local to the Eno River drainage, by contrast to the Dan River sites.

Archaeobotanists also interpret change over time in animal remains as possibly associated with colonial-attendant and ecological change. Roark (2020:74) interprets the region-wide decrease in acorns as a result of "labor constraints, reduced mobility, or changing preferences," all possibly exacerbated by becoming part of a colonial system. Gremillion (1987) applied optimal foraging theory to interpret the decrease in acorns within a cost-benefit framework. Perhaps people spent less time procuring acorns, she suggested, in order to devote more time to procuring and processing deerskins for the fur trade.

Of the decrease in maize seen in Eno River sites over time, Roark points to the possibility that droughts coeval with the Fredricks site occupation reduced maize yields (Roark, 2020; D. K. Stahle et al., 2013). Disease, pests, and conflict among tribes are alternative explanations that she raises, as well. Multiple explanations should be considered given that maize did not apparently decrease over time in Dan River archaeobotanical assemblages. Feature context should also be considered, as not all food remains indicate consumption; instead, as VanDerwarker et al.

(2007:44) emphasize, some food remains "are the result of intentional destruction related to renewable ceremonialism, perhaps associated with a mourning ritual." Cultural events, and potential changes in their frequency and scheduling, may also affect the quantity and diversity of food remains preserved in archaeological contexts.

Comparison of stable isotope data of Piedmont Siouan diets to previous research

I apply principal components analysis (PCA) using the R statistical software princomp function to test whether the isotopic data supports the paleoethnobotanical and zooarchaeological data. Specifically, I tested whether the isotope data support changes in dietary composition over time and among the river drainages of the study region. PCA can simultaneously visualize the three isotopic proxies of Piedmont Siouan diets ($\delta^{13}C_{carbonate}$, $\delta^{13}C_{organic}$, and $\delta^{15}N$) in a way that captures the maximum amount of variation in the data. Ellipses can be added to the data to examine differences across time (Figure 6.5) and between the river drainages (Figure 6.6). The Roanoke River samples from Vir150 are excluded from the PCA sample since there are no plant samples from Vir150 and the main reason I apply PCA to the isotope data is to compare the dietary isotopic trends to previous researchers' interpretations about trends in archaeological plant and animal data. PCA plots and data that include Vir150 can be found in Appendix 3.

The first principal component (PC1) accounted for 69% of the variance in the data, while the second and third principal components (PC2, PC3) accounted for 22 and 9%, respectively (Table 6.7). PC1 is positively correlated with both organic and carbonate $\delta^{13}C$, indicating that they tend to vary together. Individuals with high PC1 scores have high organic and carbonate $\delta^{13}C$. PC2 is negatively correlated with $\delta^{15}N$. Individuals with high PC2 scores have low $\delta^{15}N$. The third principal component is negatively correlated with organic $\delta^{13}C$ and positively correlated with carbonate $\delta^{13}C$. This suggests that for some individuals, organic and carbonate

$\delta^{13}C$ are inversely associated – people with low organic $\delta^{13}C$ have high carbonate $\delta^{13}C$. But remember that this only accounts for 9% of the variation in the data. While most (69%) of the dietary isotopic variability among the sampled individuals is best explained by how organic and carbonate $\delta^{13}C$ vary together, there is some deviation from that pattern. The individuals with the highest PC3 scores have the lowest organic $\delta^{13}C$ and the highest mineral $\delta^{13}C$ (Figure 6.7).

Table 6.7

Proportion of variance explained by principal components, and rotation of axes with each component

	PC1	PC2	PC3
Standard deviation	1.44	0.82	0.51
Proportion of variance	0.69	0.22	0.09
Cumulative proportion	0.69	0.91	1.00
$\delta^{15}N$	0.49	-0.86	-0.13
$\delta^{13}C$ mineral	0.60	-0.44	0.66
$\delta^{13}C$ organic	0.63	-0.24	-0.74

Figure 6.5

Principal components plot, people differentiated by temporal period

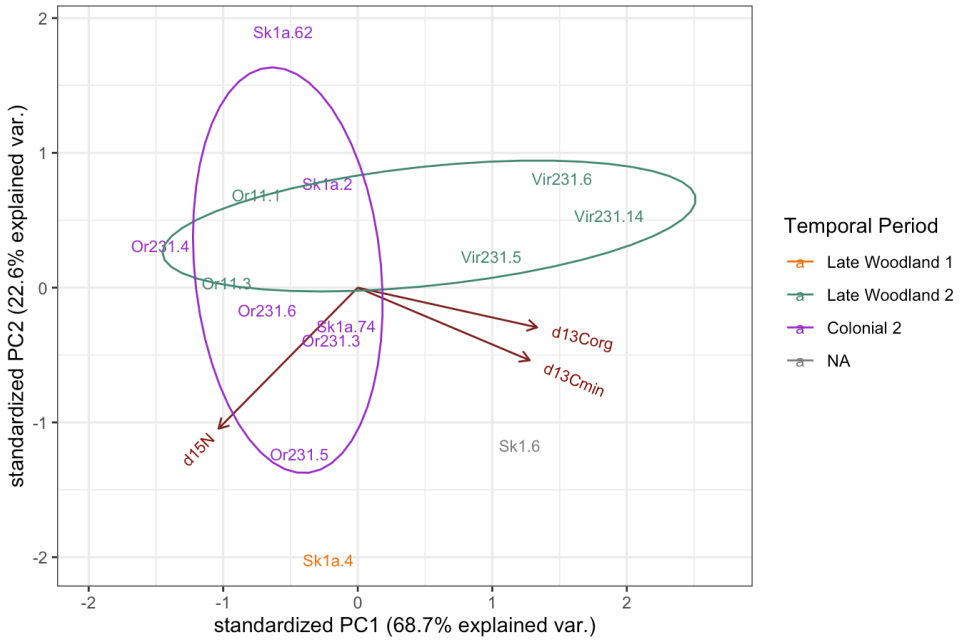


Figure 6.6

Principal components plot, people differentiated by river drainage

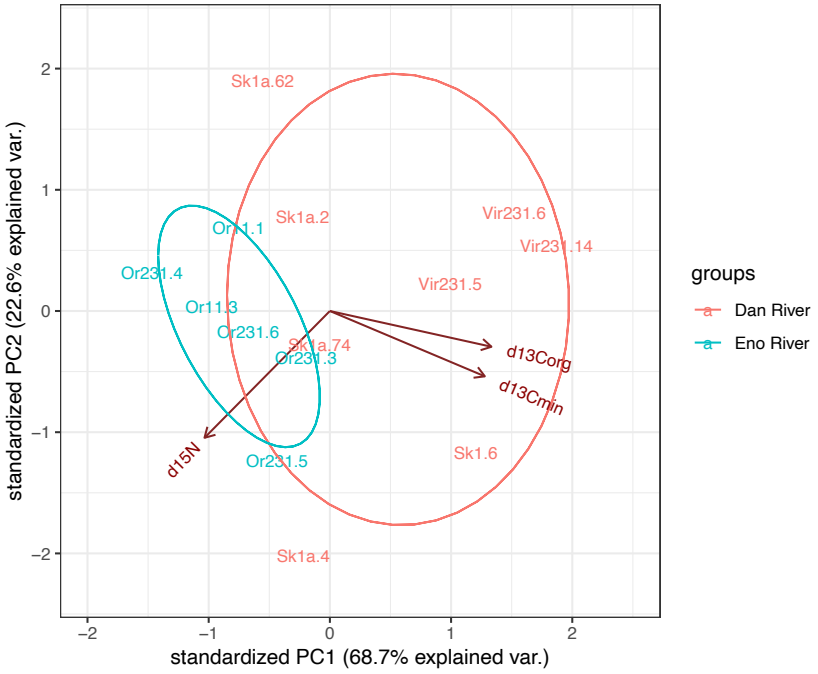
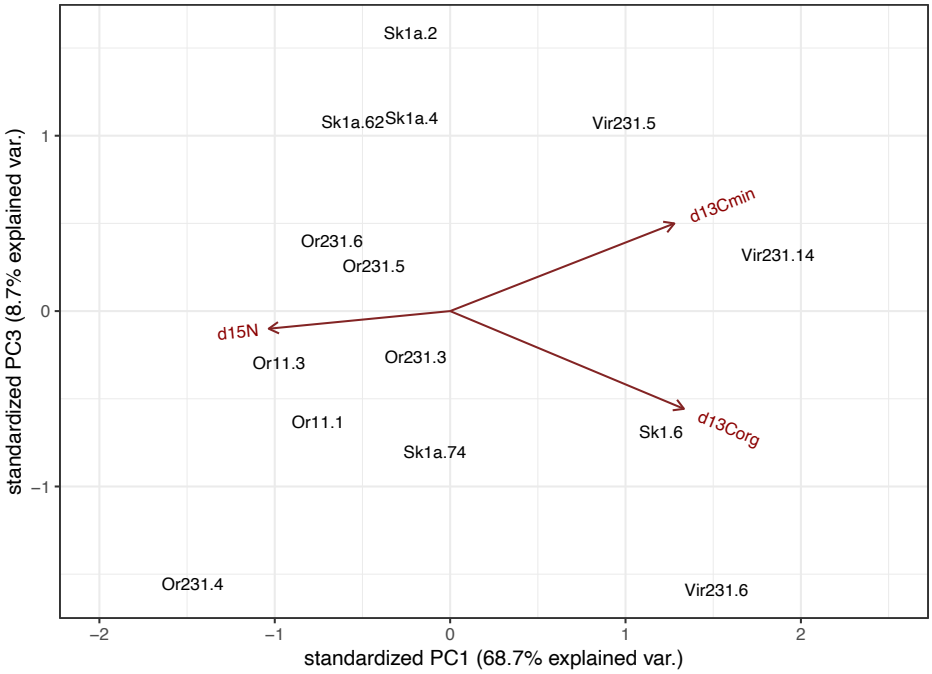


Figure 6.7

Principal components plot, PC3 vs. PC1



Figures 6.5 and 6.6 help visualize wither the dietary stable isotope data support trends in the archaeobotanical and zooarchaeological data. Roark (2020) noted that Dan River sites had more maize remains than Eno River sites. It does appear that people from the Dan River may have consumed more maize than people from the Eno River, as they have higher PC1 scores. Roark (2020) also noted that maize remains increased over time in the Dan River plant assemblages and decreased over time in the Eno River assemblages.

These trends are not apparent in the isotope data. Late Woodland Dan River individuals from Stockton (Vir231) and Hairston (Sk1) have higher PC1 scores than Dan River Colonial period individuals from Upper Saratown (Sk1a) (one individual from Upper Saratown – Sk1a.4 – is from the Late Woodland period) (Figure 6.6). All individuals from the Eno River have similar PC1 scores. Three of the four sampled individuals from the Colonial period Fredricks site (Or231) have slightly higher PC1 scores than the two sampled Wall site individuals (Or11). These data should be interpreted cautiously given the low sample size and the unknown formation rate of calculus. The Fredricks site was only occupied for a short period of time between AD 1690 and 1710 by the Occaneechi, who moved to the Eno River from the Roanoke River following Bacon's Rebellion (Davis, 2002; Dickens et al., 1987; Lefler, 1967; H. T. Ward & Davis, 2001). It is possible that the calculus isotopic values preserve a dietary signal more representative of their diet while they lived within the Roanoke River drainage rather than their diet while they lived on the Eno River. The plant samples, by contrast, reflect their subsistence activities while living on the Eno River.

Longo (2018) suggested that sites in the northwest region of the study area – Stockton (Vir231) and Leatherwood Creek (Vir196) – included more bird remains than sites from other river drainages. And the Eno River Wall (Or11) and Fredricks (Or231) sites included more

reptile remains. The Stockton site individuals had the highest $\delta^{13}C$ of all individuals whose dietary isotopic ratios were measured, indicating that their protein profile likely came from a ^{13}C-enriched food web, perhaps from eating animals that ate maize, or from eating fish from a ^{13}C-enriched aquatic ecosystem.

Though a limited sample size, none of the sampled bird remains from archaeological contexts (four turkeys, one passenger pigeon) had high $\delta^{13}C$ values (Appendix 2). My interpretation is that their high organic $\delta^{13}C$ more likely derives from fish consumption. They do not have as high a $\delta^{15}N$ value as we would expect from people eating more fish given the local interpretive baseline (Figure 6.5), but it is possible that they were eating lower trophic level fish than the ones sampled for the interpretive baseline (two largemouth bass, two unidentified fish but of a size similar to largemouth bass) (Appendix 2). Holm (1994) reported that shad, which feeds at a lower trophic level than bass, was an important fish resource because they concentrate during spawning and are able to be caught in large quantities during that time (Lefler, 1967; Swanton, 1946). If the individuals from Stockton did consume more fish, then this would add to our understanding of Siouan animal foodways. Faunal remains from both the Stockton and Leatherwood Creek sites were hand collected, possibly leading to the underrepresentation of fish remains in the zooarchaeological record for those sites (VanDerwarker, 2001).

Reptiles have high $\delta^{15}N$ but low $\delta^{13}C$, and variation in reptile consumption would most likely be captured by PC2. Some of the Eno River individuals (three individuals from the Fredricks site) do have lower PC2 values (suggesting higher $\delta^{15}N$) than other sampled individuals, but fall within the PC2 range of several individuals from the Dan River. There is not enough data to support the hypothesis that Eno River individuals ate more reptiles than Dan

River individuals. The variation in $\delta^{15}N$ among sampled individuals is interesting, yet does not appear to follow a clear temporal or ecological pattern. It will be a focus of future study.

There is not currently enough resolution in the data to test whether people ate different proportions of nuts, since nuts are lumped with other C_3 plants. Nor is there enough resolution to differentiate deer from other terrestrial herbivores. And, I did not sample shellfish for the interpretive baseline, so it is also not possible to test whether shellfish consumption changed over time. In a future study, I will add shellfish to the baseline and use additional methods that can add nuance to the interpretation of dietary stable isotope data. For example, I plan to apply stable isotope mixing models with concentration dependence factors. Stable isotope mixing models are Bayesian models and have the ability to add priors to the model, such as which foods we expect to contribute more to the diet (informed by the archaeobotanical and zooarchaeological records) and the expected macronutrient content of the foods (Fernandes, Millard, Brabec, Nadeau, & Grootes, 2014; Phillips et al., 2014). It would also allow me to factor the uncertainty of using dental calculus into the model, since the dietary origins of dental calculus $\delta^{15}N$ and $\delta^{13}C$ have not been studied through animal feeding experiments like other tissues such as bone have.

6.5 Conclusion

Siouan groups from the Piedmont region of north-central North Carolina and southern Virginia appear to have consumed similar diets, best characterized as a mix of C_3 and C_4 plants, and aquatic and terrestrial resources. However, the slight dietary isotopic differences between the Siouan communities may reveal nuances in foodways practices, a possible result of diverse responses to the socioeconomic, geopolitical, and epidemiological changes attendant to colonialism. Variation among Piedmont Siouan individuals was best explained by $\delta^{13}C$, reflecting differences in maize consumption. Dan River communities, who had strong dietary

contributions from maize during the Late Woodland period, consumed less maize and more C_3 plants and terrestrial protein in the Colonial period. Colonial period Eno River communities potentially consumed more maize than the earlier occupants of the Eno River, but more data is needed to corroborate this.

CHAPTER 7 (PAPER 3): THE RELATIONSHIP BETWEEN DIET, IMMUNOSTIMULATION, AND NUTRITION AMONG PIEDMONT SIOUAN COMMUNITIES (AD 800 – 1710)

7.1 Introduction

In this paper, I investigate the causes and consequences of nutritional change among Piedmont Siouan communities who lived during AD 800–1710, the Late Woodland to Colonial periods. To that aim, I quantify skeletal manifestations of nutritional deficiencies and test theoretical associations between risk factors that potentially contribute to poor nutrition. Figure 7.1 shows the framework that guides this section and the relationships that will be tested. This framework involves two main pathways that contribute to nutritional epidemiology (dietary composition and immunostimulation) and one pathway (growth outcomes) that may result from nutritional change.

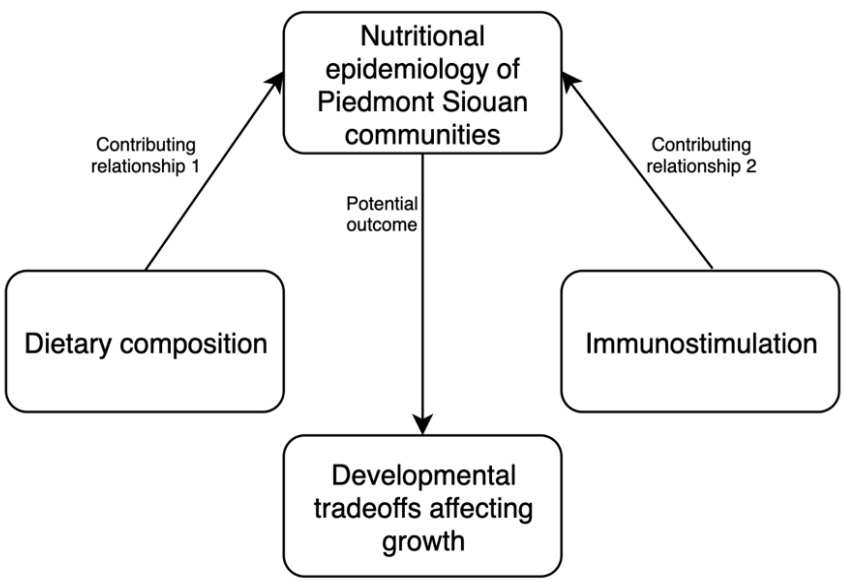
A person's diet provides nutrients to their body to fuel a multitude of metabolic and physiological reactions that sustain life, such as the immune system, which is energetically- and nutritionally-expensive for the body to maintain (McDade, 2003; Scrimshaw et al., 1968; Tomkins, 2003). Diet and immunostimulation are both important to study as part of the larger picture of nutritional health during colonialism. Dietary composition is an indicator of how communities creatively negotiated their daily lives and activities during colonial-attendant change, and phenotypes indicative of immunostimulation are useful to reconstruct changes in disease ecology associated with colonialism (Gosden, 2004; Hutchinson, 2006; Klaus & Tam, 2009; Larsen et al., 2001; Lightfoot et al., 2010; Silliman, 2005).

Nutrition also influences human growth and development (potential outcome, Figure 7.1), and I am particularly interested in how population-level shifts in nutritional epidemiology associated with colonialism may have impacted the health of subsequent generations. Intergenerational impacts stem from the long gestational period of humans, during which growth and reproductive potential, as well as the immune system, begin to develop (Long & Nanthakumar, 2004; McDade, 2003). The amount of energy a developing individual invests in each of these systems is influenced by cues received from the mother and her interaction with the environment (Barker, 1998; Burdge et al., 2011; Gluckman & Hanson, 2004; Kuzawa, 2005; McDade, 2003). If the mother signals an environment deficient in nutrients, for example, the infant may develop in ways that conserve energy by reducing investment in certain systems, such as immune system development or growth. The immune system continues to develop and mature through early childhood, and any nutrient stress experienced during early childhood (whether due to infection and/or diet) can therefore continue to affect immune system development and maturation (Barker, 2012; Gowland, 2015; McDade, 2003).

I test the relationships outlined in Figure 7.1 by examining temporal patterns in each of the variables – nutritional epidemiology, dietary composition, immunostimulation, and growth outcomes – as well as the relationship between each of these variables with nutrition.

Figure 7.1

Framework of the analysis for this chapter



7.2 Materials and methods

The sample for this chapter is composed of people who are observable for the variables of interest, which are outlined below. Observable generally means that the skeletal element of interest (for example, alveolar bone surrounding teeth) is preserved and can be assessed for lesion presence vs. absence. All of the tests presented in this paper either control for age as a covariate or divide the sample into age categories. This is done to account for the age-related differences in the likelihood and manifestation of nutritional and immunostimulatory lesions. Nutritional deficiency, for example, can affect someone at any point in their life, but may manifest differently in the actively growing skeletons of children compared to skeletally mature adults (Brickley & Ives, 2008; Lewis, 2017). Additionally, people have different nutritional needs depending on their life course stage (Agarwal, 2016; Herman et al., 2014).

Tests that control for age use the median estimated age of an individual. Tests that divide the sample into age bins use the following age categories: 0-5, 6-18, 19-34, and 35+, influenced

by Buikstra and Ubelaker (1994). These groups were established to both maximize within-group sample size as well as reflect the energy- and nutrient-demands of the different life history stages. Table 7.1 presents the descriptive statistics for the overall sample, per the main variables of interest examined in this study. I calculated the mean and median age of the sample using the midpoint of each person's estimated age range.

Age and sex estimation

Mixed metric and non-metric cranial and postcranial indicators were used to estimate the age and sex of adult individuals (Brooks & Suchey, 1990; Buikstra & Ubelaker, 1994; İşcan, Loth, & Wright, 1984; Klales, Ousley, & Vollner, 2012; Lovejoy, Meindl, Pryzbeck, & Mensforth, 1985; Meindl & Lovejoy, 1985; Walker, 2008). Age estimates are more specific for individuals who have not yet reached skeletal and dental maturation – e.g. their epiphyses are not fully fused and/or their third molars are not yet in occlusion (White & Folkens, 2005). Age ranges (spanning at least 10 years) were therefore used to estimate the age of skeletally-mature adults.

Dental eruption and epiphyseal fusion timelines were used to estimate the ages of developing infants, children, adolescents, and young adults (AlQahtani, Hector, & Liversidge, 2010; Buikstra & Ubelaker, 1994; Schaefer, Black, & Scheuer, 2009). All of the sex estimation methods used in this study are for skeletally-mature individuals, and I therefore did not estimate the sex of any individuals younger than 18 years old.

Table 7.1

Descriptive Statistics for each Variable Used in this Analysis

	Mean age	Median age	Age range	Sex ratio (women:men)	Sample size
Nutritional lesions	25.82	29	0-55	39:38	132
Isotopic ratios	34.61	37	19-53	7:12	21
PNB[†]	24.77	27	0-55	40:39	145
Kerr's score	35.35	37	9.5-55	23:29	64
Calculus score	32.58	35	6.5-55	27:30	87
Dental abscesses	27.22	30	1-55	32:36	109
Oral foramina[‡]	34.36	35	9.5-55	19:21	52
VNC dimensions[§]	35.58	35	19-55	13:12	25
Long bone ratios	35.63	40	19-50	8:16	26
LEH[¶]	24.24	25	0.5-55	31:37	144

[†]Periosteal new bone, [‡]Lesions of the mental and mandibular foramina, [§]Vertebral neural canal dimensions, [¶]Linear enamel hypoplasias

Skeletal lesions of nutritional deficiency

Nutritional lesions were recorded as "present" if a person has porous or vascular lesions on their cranial bones, and/or irregular cortical thickening or pitting of cranial bones, which are both indicative of remodeled lesions (Wilczak, 2011). All skeletal lesions were observed macroscopically using measurement guidelines established by Ortner (2003), Wilczak (2011), Brickley and Ives (2008), and Lewis (2017) as measurement guides. I recorded the extent of the lesion (percent of the skeletal element affected) for all lesions, as well as pore size, pore density, and activity (active vs. healing) for porous lesions (Wilczak, 2011).

The greater wings of the sphenoid bone, the pars orbitalis (eye orbits) of the frontal bone, and the parietal bones are used as the primary indicator bones of nutritional deficiency for this study, as all three skeletal elements are involved in multiple nutrition-related processes that create osseous lesions. These processes include the expansion of spongy bone for increased red blood cell production, hypomineralization of new bone, and hemorrhage and attendant

inflammation of cranial soft tissues (Brickley & Ives, 2008). Vitamins C, D, B_{12}, and iron may be implicated in these processes; see Chapter 4 for more information.

Dietary isotopic ratios

I tested the relationships between diet and nutritional lesions (contributing relationship 1 in Figure 7.1) using the dental calculus $\delta^{13}C$ and $\delta^{15}N$ of 21 people. Two criteria were used to select people to sample for dental calculus: (1) the presence of appreciable deposits of dental calculus for analysis (>8mg); and (2) river drainage and temporal period affiliation, with the aim of sampling people across the multiple time periods and different sociocultural groups included in this study. The organic and mineral biofractions of dental calculus were extracted and purified in the Paleobiogeochemistry Laboratory at the University of Illinois Urbana-Champaign (UIUC) according to methods outlined in Chapter 5 (Paper 1). The dental calculus $\delta^{13}C_{carbonate}$, $\delta^{13}C_{organic}$ and $\delta^{15}N$ were measured at the Stable Isotope Lab of the Illinois State Geological Survey, also at UIUC.

Organic biofraction $\delta^{13}C$ and $\delta^{15}N$ were measured using a Carlo-Erba NC 2500 Elemental Analyzer (EA) coupled to a Finnigan MAT 252 isotope ratio mass spectrometer (IRMS) or a Delta V Advantage IRMS using either a Conflo II or IV interface, respectively. The amino acids thiourea, serine, and methionine were used as internal working standards, and USGS 40 and USGS 41 were used for two-point machine calibration standards. Carbonate $\delta^{13}C$ was measured using a Kiel Carbonate Device III coupled to a MAT 252 IRMS. NBS 18 and NBS 19 were used for machine calibration and as within-run correction standards. Each EA and Kiel run included three sample replicates.

Skeletal proxies of immunostimulation

Periosteal new bone and lesions indicative of periodontal disease are both suggested to result from an immune system reaction, specifically inflammation (Hajishengallis, 2015; Klaus, 2014; Lieverse, 1999; Waldron, 2009; see also Chapters 4 and 8). I collected information on periosteal new bone following Ragsdale (1993) and Wilczak and Jones (2011), and recorded the following phenotypes that are possibly indicative of periodontal disease: Kerr's score of alveolar resorption; degree of calculus deposition; dental abscess presence; and pathological porosity and/or enlargement of the mental, mandibular, and incisive foramina (DeWitte & Bekvalac, 2011; Hillson, 2000; Kerr, 1988; Kingsmill, 1991; Lavigne & Molto, 1995; Tomczyk et al., 2017; Tuggle & Watson, 2019; Wasterlain et al., 2011). Each of these measures are explained below.

Kerr's score observes alveolar recession from the cemento-enamel junction as well as alveolar bone texture and combines those traits into a score of one to five for the interproximal alveolar bone of each tooth (Kerr, 1988; Tomczyk et al., 2017). This score is used to assess the degree of alveolar bone destruction that is possibly implicated in periodontal disease. I scored each observable interproximal region for an individual and calculated their average score per observable area.

Dental calculus deposition was scored according to Dobney and Brothwell's (1987) scoring system, which uses a score of zero to four to record the percentage of the tooth covered by calculus, as well as the thickness of the calculus deposit. I calculated average calculus scores for each individual as the average calculus score per all observable teeth. Large calculus deposits are often associated with periodontal disease (Wasterlain et al., 2011).

Abscesses were recorded as periapical abscesses (smooth-walled lesions at the base of the root that result from infection of the root canal) and periodontal abscesses (recession of the alveolar bone from the cemento-enamel junction usually attended by porous and/or jagged texture of the alveolar bone) (Griffin, 2014; Waldron, 2009). I treated abscesses as binary variables for this study; an individual was scored as either one (abscess present) or zero (abscess absent) for both categories of abscess. Lesions of the mental, mandibular, and incisive foramina were also recorded as binary variables; lesions were scored as present if the foramina were pathologically enlarged and/or if the bone immediately surrounding the foramina was porous or affected by periosteal new bone deposits.

Skeletal measurements of childhood growth velocity

Three variables were used to approximate childhood growth velocity: vertebral neural canal (VNC) dimensions, long bone lengths, and the presence of linear enamel hypoplasias (LEH). The VNC is the aperture that the spinal cord passes through, and this particular skeletal element fuses early in life –the lower vertebrae (the tenth through twelfth thoracic vertebrae and all five lumbar vertebrae) fuse to complete the VNC by the age of five (Clark, Panjabi, & Wetzel, 1985; R. Watts, 2011, 2013). Smaller VNC measurements may therefore indicate early-life growth faltering. I recorded VNC dimensions using a digital sliding caliper following Watts (2013).

Long bone lengths may also reflect growth faltering, especially the ratio of proximal-to-distal long bone lengths from the same limb (e.g., femur-to-tibia, humerus-to-ulna). Distal limbs are more sensitive to growth faltering, which Kemkes-Grottenthaler (2005:345) attributes to "greater plasticity and also a greater reaction to nutritional insults or health problems" of the distal compared to the proximal limb (Holliday & Ruff, 2011; Jantz & Jantz, 1999). I recorded

long bone lengths using an osteometric board following the measurement guidelines in Buikstra and Ubelaker (1994).

Linear enamel hypoplasias (LEH) result from a temporary disruption in enamel deposition due to infection, inflammation, malnutrition, trauma, or other physiological stressors (Armelagos, Goodman, Harper, & Blakey, 2009; Blakey & Armelagos, 1985; Goodman & Rose, 1990). I observed LEH macroscopically and treated LEH as a binary variable for this study – a person either had LEH (score of one) or they did not (score of zero).

7.3 Results and Interpretation

Proportion of people affected by lesions of nutritional deficiency

Figures 7.2–7.4 show the proportion of people affected by lesions of the orbits, parietal bones, and greater wings of the sphenoid bone. The sample is split into age categories (Figure 7.2, Table 7.2) as bone biology, metabolic demands, and biocultural exposures vary across the life course and can result in differential risk and manifestation of nutritional deficiencies (Brickley & Ives, 2008; Lewis, 2017). The sample is also split to show patterns in nutritional lesion prevalence among biological sex categories (Figure 7.3, Table 7.3). Given the limited number of people who were observable for nutrition-derived lesions, I compressed the temporal categories into Late Woodland (AD 800–1620) and Colonial (AD 1620–1710) to increase sample size within the demographic groups.

Figure 7.2

Prevalence of nutritional lesions by age and temporal group

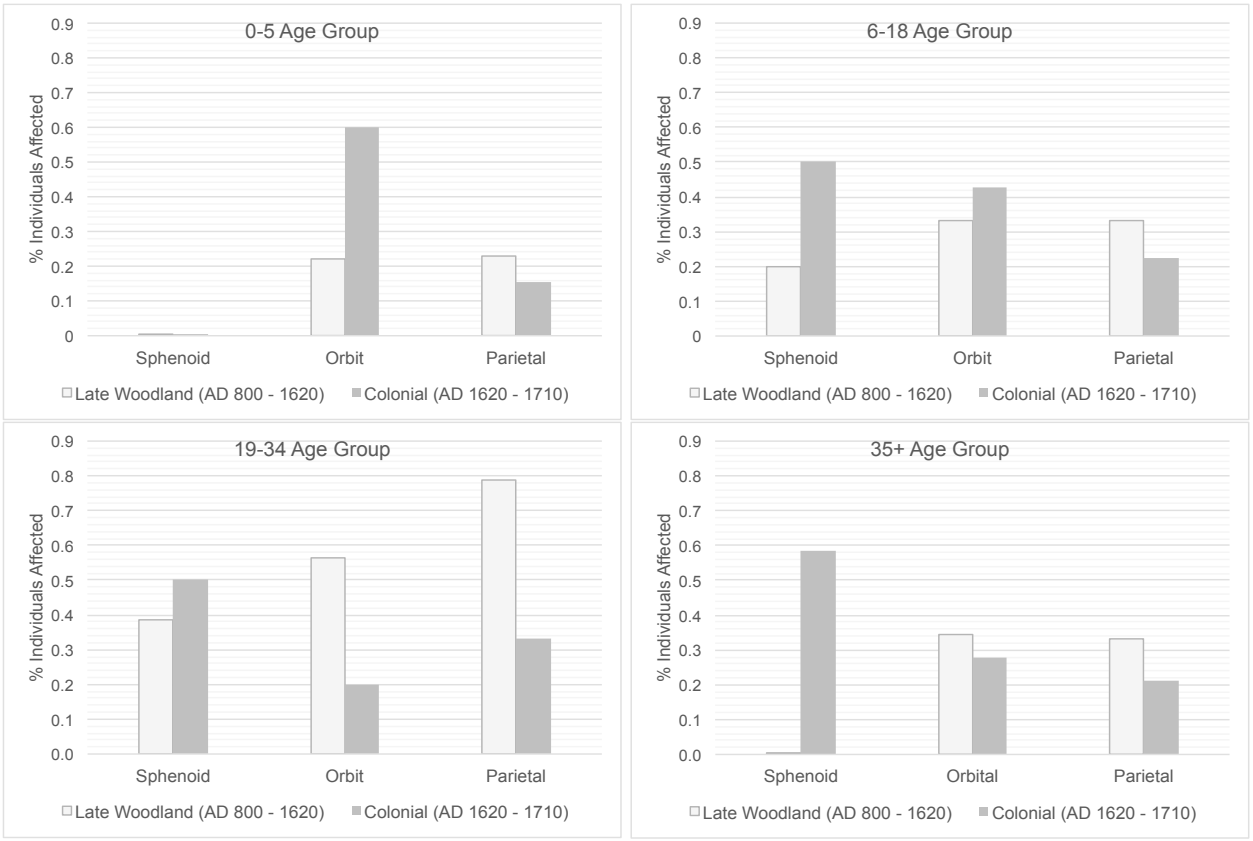


Table 7.2

Proportion of People with Nutritional Lesions, by Age, Bone, and Temporal Group

Age	Bone	Late Woodland (AD 800–1620)			Colonial (AD 1620–1710)		
		Present	Absent	%	Present	Absent	%
0-5	Sphenoid	0	8	0	0	3	0
	Orbit	2	7	6	6	4	60
	Parietal	3	10	23	2	11	15
6-18	Sphenoid	1	4	20	4	4	50
	Orbit	2	4	33	3	4	43
	Parietal	2	4	33	2	7	22
19-34	Sphenoid	5	8	38	3	3	50
	Orbit	9	7	56	1	4	20
	Parietal	15	4	79	3	6	33
35+	Sphenoid	0	8	0	7	5	58
	Orbit	10	19	34	5	13	28
	Parietal	10	20	33	4	15	21

Figure 7.3

Prevalence of nutritional lesions by bone, sex, and temporal period

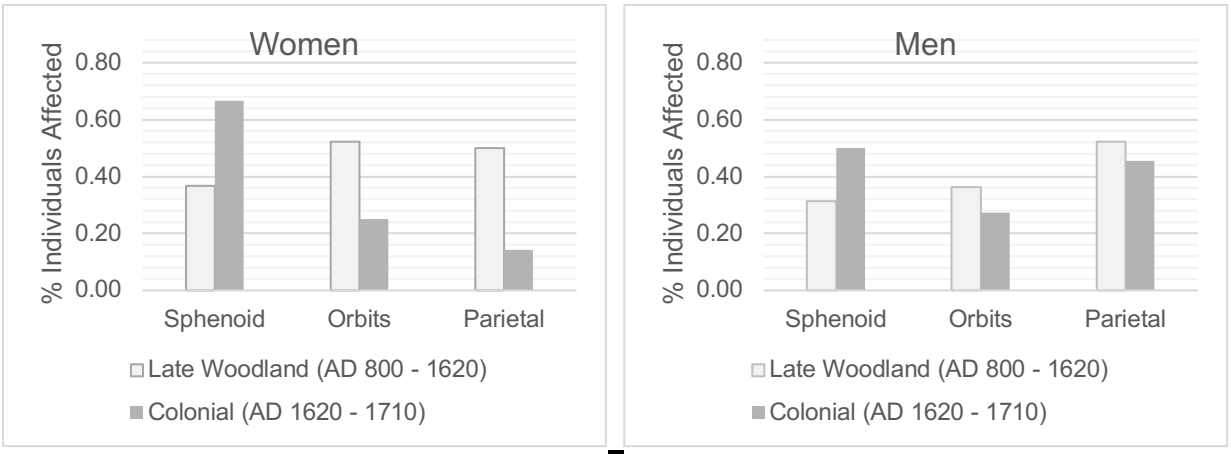


Figure 7.2 shows that more people from the Colonial period were affected by sphenoid lesions than Late Woodland people for all age categories except the 0-5 age group. Women were also more affected by sphenoid lesions than men in the Colonial temporal period (Figure 7.3, Table 7.3). Figure 7.4 and Table 7.4 show the prevalence of nutrition-derived lesions for the different river drainages, as diets, disease exposures, and experience with colonialism likely varied among the cultural groups occupying different river drainages (Eastman, 1999; Longo, 2018; Roark, 2020; H. T. Ward & Davis, 2001). Figure 7.4 suggests that Eno River occupants during the Colonial period (the Occaneechi at the Fredricks site) experienced a high prevalence of nutrition-related disease, particularly as it affected lesions on the sphenoid bone. Only four people from the Fredricks site had observable sphenoid bones, but all were affected by sphenoid lesions. I therefore included river drainage as a covariate in the multivariate statistical models to account for how the high prevalence of sphenoid lesions among Fredricks site occupants may skew the region-wide results.

Table 7.3

Proportion of People Affected by Cranial Porosity, by Bone, Sex, and Temporal Period

Sex	Bone	Late Woodland (800-1620)			Colonial (1620 – 1710)		
		Present	Absent	%	Present	Absent	%
Female	Sphenoid	7	12	37	6	3	67
	Orbit	12	11	52	3	9	25
	Parietal	12	12	50	2	12	14
Male	Sphenoid	5	11	31	4	4	50
	Orbit	8	14	36	3	8	27
	Parietal	12	11	52	5	6	45

Figure 7.4

Prevalence of nutritional lesions by bone, temporal period, and river drainage

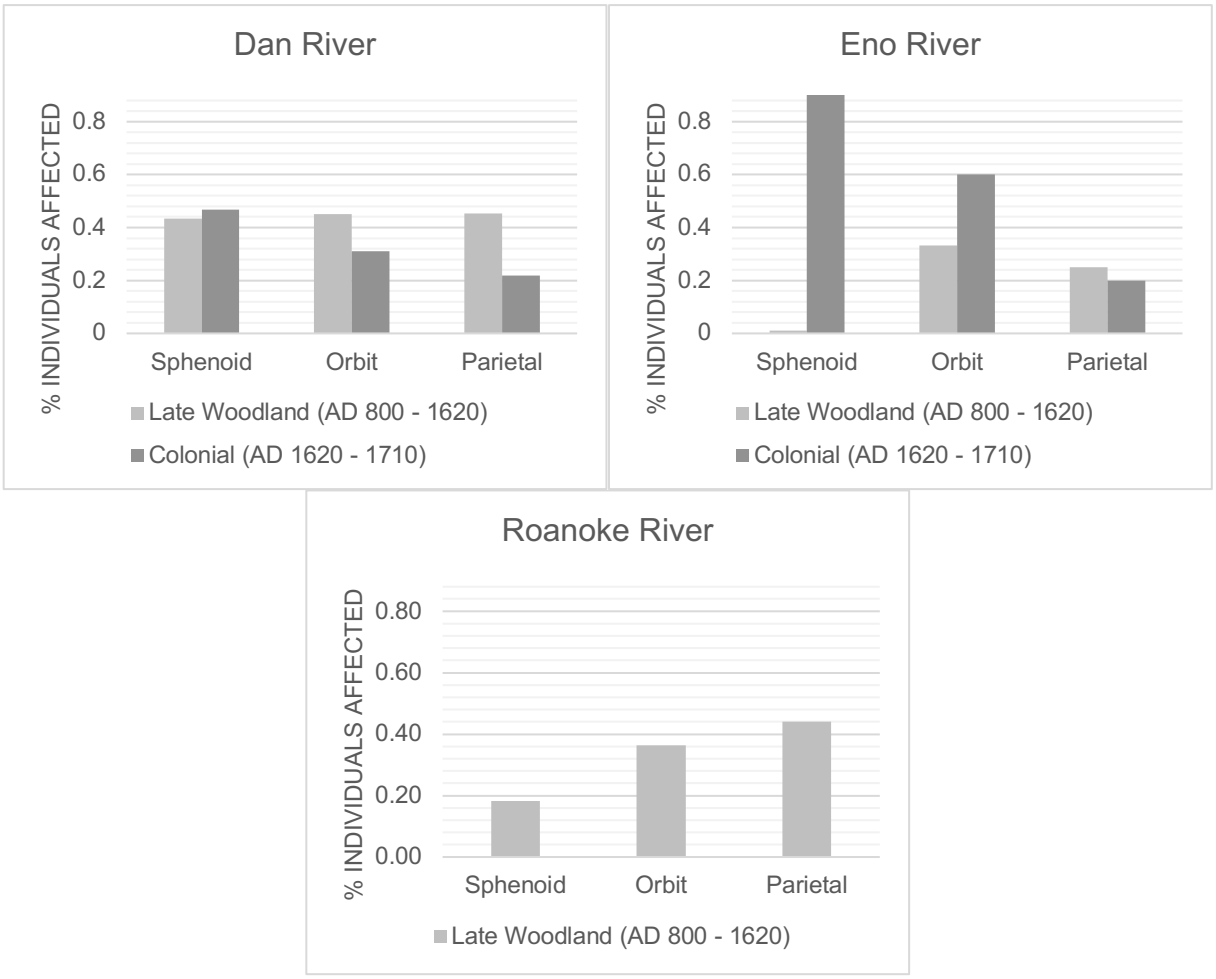


Table 7.4

Proportion of People affected by Cranial Porosity, by Bone, Sex, and Temporal Period, Split into the Relevant River Drainages

Age	Bone	Late Woodland (AD 800–1620)			Colonial (AD 1620–1710)		
		Present	Absent	%	Present	Absent	%
Dan River	Sphenoid	10	13	43	7	8	47
	Orbit	18	22	45	9	20	31
	Parietal	19	23	45	9	32	22
Eno River	Sphenoid	0	3	0	4	0	100
	Orbit	1	2	33	6	4	60
	Parietal	1	3	25	2	8	20
Roanoke River	Sphenoid	2	9	18	-	-	-
	Orbit	8	14	36	-	-	-
	Parietal	11	14	44	-	-	-

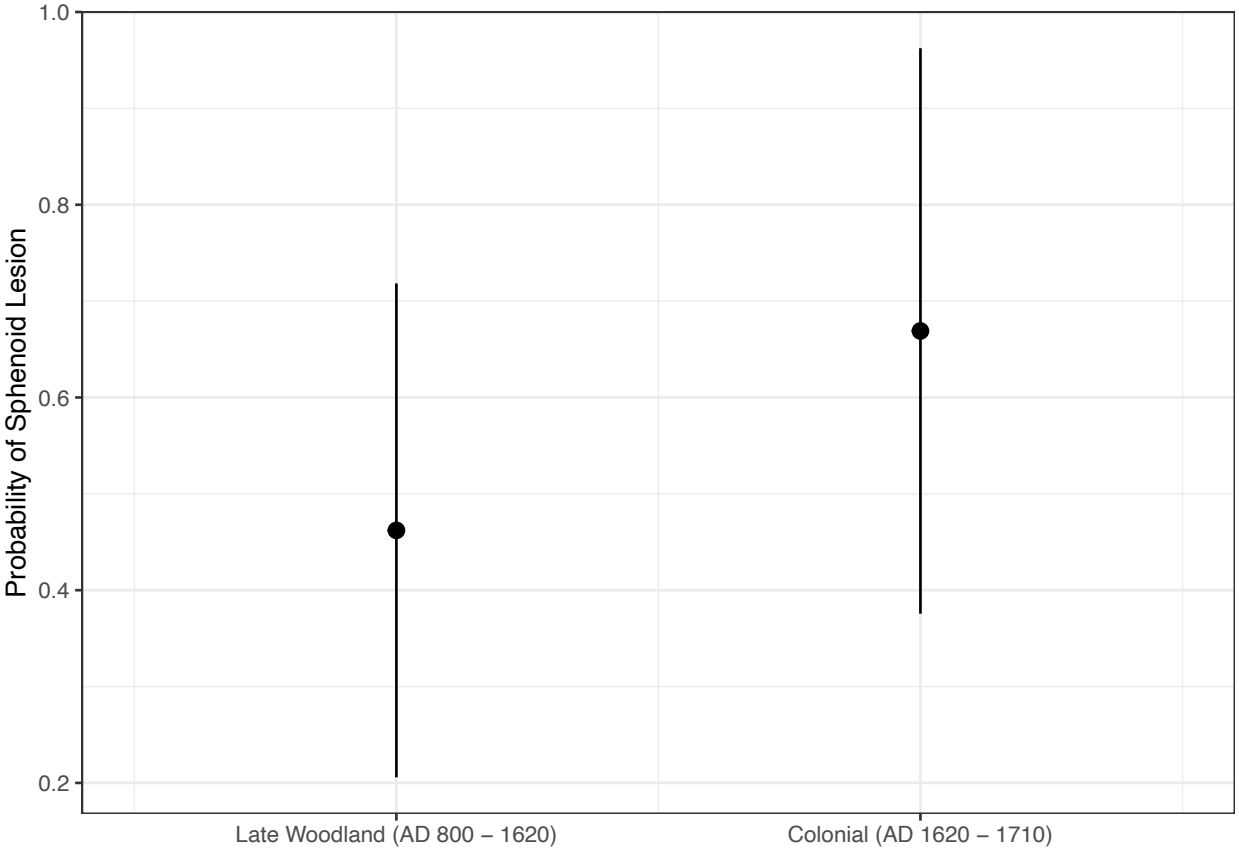
I also used a logistic regression model to test the significance of the patterns shown in Figures 7.2–7.4 to include multiple variables in one model. Since sphenoid lesion prevalence appears to be higher among Siouan people who lived during the Colonial period compared to those who lived during the Late Woodland period, I specifically tested the effects of temporal period on sphenoid lesion presence while controlling for biological age and sex as covariates.

The temporal categories are lumped in Figure 7.5 to better compare results to the above bar plots; by comparison, Figure 7.6 shows the temporal categories stratified to better capture biocultural changes in the Siouan lived experience over time. While stratifying the sample does split an already small sample size, the standard error ranges shown on the plot helps to visualize possible trends while recognizing the uncertainty associated with small sample sizes. The model that uses two temporal categories (Late Woodland and Colonial) indicates that people from the Colonial period were more likely to have sphenoid lesions than people from the Late Woodland period. This relationship, at $p < 0.1$ is suggestive but not significant. The model that uses stratified temporal categories indicates that the high prevalence of sphenoid lesions during the late Contact period (AD 1670–1710) may be driving this trend. People from the late Contact

period were more likely to have sphenoid lesions than people from the earlier periods. This relationship is significant at $p < 0.05$.

Figure 7.5

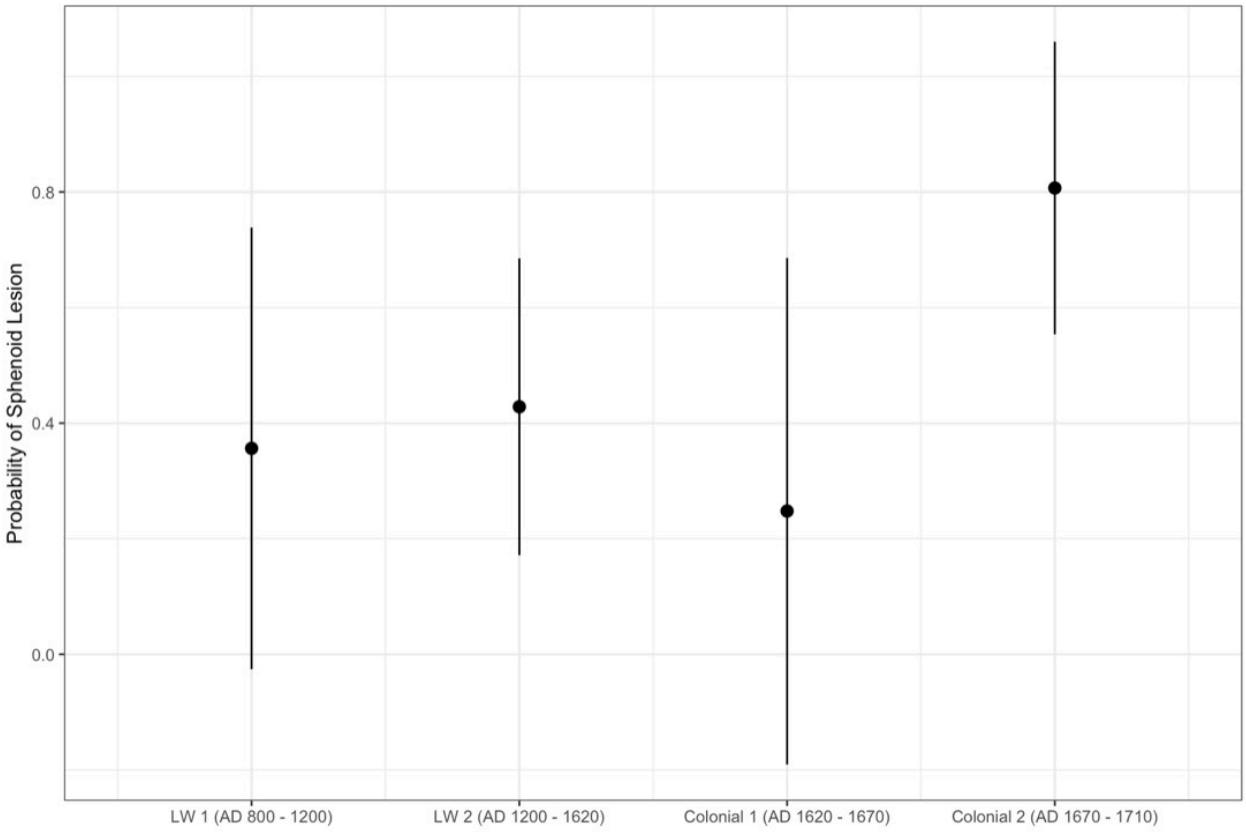
Predicted probability of sphenoid lesions by lumped temporal group, controlling for age and sex.



Late Woodland period people are less likely to have sphenoid lesions than Colonial period people (beta-coefficient: -1.05, standard error: 0.62, $p < 0.1$)

Figure 7.6

Predicted probability of sphenoid lesions by stratified temporal group, controlling for age and sex.



Late Contact period people are more likely to have sphenoid lesions than people from earlier periods (beta-coefficient: 2.54, standard error: 1.32, $p < 0.05$)

Figures 7.2–7.6 all suggest that sphenoid lesions were more prevalent in the Colonial period, particularly the late Colonial period (AD 1670–1710). The high prevalence of sphenoid lesions among the Occaneechi buried at the colonial period Fredricks site partially drove this trend, as the significance falls out when including river drainage as a covariate in the logistic regression model (beta-coefficient 1.22, standard error 1.01, $p = 0.22$). Sphenoid lesions remained less common among the Late Woodland than Colonial period groups when controlling for age and river drainage (beta coefficient -0.73, standard error 0.55, $p = 0.19$), but the model

indicated that river drainage was a confounding variable in the relationship between temporal period and sphenoid lesion prevalence.

However, it should be noted that splitting the research population into river drainage categories affects the direct comparability of the samples, as there are fewer people from the Eno River than the Dan River. Also, there are no represented Colonial period people from the Roanoke River, as Vir150 (the only Roanoke River site included in the sample) dates to the Late Woodland period. Overall, the salient trend that I cautiously interpret is that sphenoid lesion prevalence increased in the Colonial period compared to the Late Woodland period, especially for the latter half of the Colonial period and for the Occaneechi at the Eno River Fredricks site. I aim to further investigate this trend alongside other archaeological and ethnohistoric information from the Piedmont region,

Sphenoid lesions as possibly indicative of vitamin C deficiency

Ortner and Ericksen (1997) suggest that lesions on the greater wing of the sphenoid are pathognomonic of vitamin C deficiency; however, Waldron (2009) argues that more clinical studies are necessary to clarify the association between vitamin C deficiency and sphenoid lesions. Vitamin C deficiency weakens collagen and increases the risk of soft tissue hemorrhage, particularly of the chewing muscles (Crandall & Klaus, 2014; Fain, 2005). Hemorrhage and the attendant inflammatory response of the immune system disrupt bone metabolism and cause lesions of abnormal bone formation or bone loss (Berger et al., 2021; Takayanagi, 2005). Osseous lesions that result from hemorrhage of the chewing muscles have been identified on the maxilla (especially the posterior maxilla), the mandibular ramus, the orbits, the temporal bone, and the anterior and posterior surfaces of the zygomatic bone (Brickley & Ives, 2008; M. Brown

& Ortner, 2011; Crandall & Klaus, 2014; Geber & Murphy, 2012; Klaus, 2014b, 2017; Ortner, 2003; Ortner & Ericksen, 1997).

To critically examine the use of sphenoid lesions as an indicator of vitamin C deficiency, I tested whether sphenoid lesions are associated with lesions on other bones that are part of the chewing muscle complex. Figure 7.7 and Table 7.5 show the results of the logistic regressions between sphenoid lesion as the dependent variable and other cranial lesions as the independent variables. All models used age as a covariates. The relationships are displayed in Figure 7.7 as point estimates (correlation coefficients) with standard error ranges, and significant relationships have standard error ranges that do not cross the zero line (vertical line). The significant associations between sphenoid lesions and maxillary and zygomatic lesions ($p < 0.05$ and $p < 0.001$, respectively), and the near-significant associations between sphenoid lesions and temporal and parietal lesions ($p < 0.1$) show that sphenoid lesions co-occur with other lesions indicative of chewing muscle hemorrhage, thus strengthening the association between sphenoid lesions, chewing muscle hemorrhage, and the distal cause of hemorrhage – vitamin C deficiency.

Figure 7.2 shows that children aged 0-5 were the only age category where sphenoid lesions were *not* more prevalent in the Colonial period compared to the Late Woodland period. However, this may be due to the way that nutritional deficiencies impact the growing skeleton as opposed to the mature skeleton. Lambert (2000:180) noted that, "vitamin C deficiency may also cause porous lesions to form in the eye orbits, particularly in the fast-growing bones of infants and children." It is therefore possible that the higher prevalence of orbital lesions among children under five from the Late Woodland lends further support to the interpretation that vitamin C deficiency was higher in the Colonial than Late Woodland period.

Figure 7.7

Correlation coefficients and standard errors for logistic regression models between sphenoid lesions and other cranial lesions, controlling for age

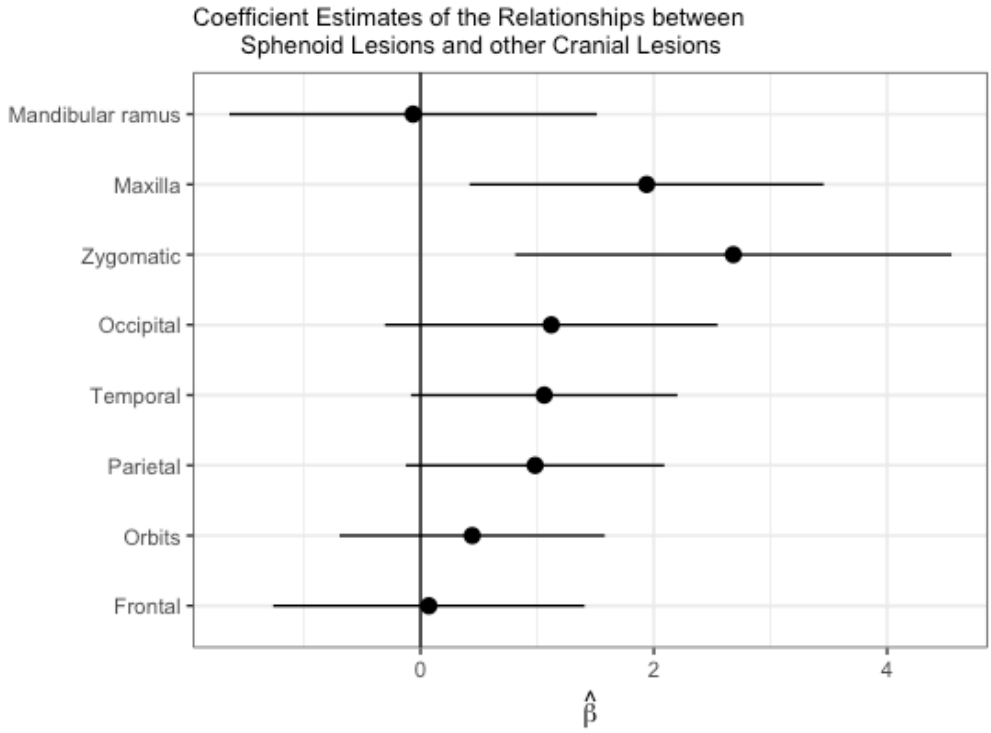


Table 7.5

Summary Statistics from the Logistic Regression Models Displayed in Figure 7.5

Independent variables	ß-coefficient	Std. error	p-value	Sample size
Mandibular ramus	-0.06	0.80	0.94	74
Maxilla	1.94	0.77	0.01*	73
Zygomatic	2.77	0.94	0.00**	65
Occipital	1.12	0.73	0.12	79
Temporal	1.06	0.58	0.07˙	81
Parietal	0.98	0.57	0.08˙	79
Orbits	0.44	0.58	0.44	73
Frontal	0.07	0.68	0.92	80

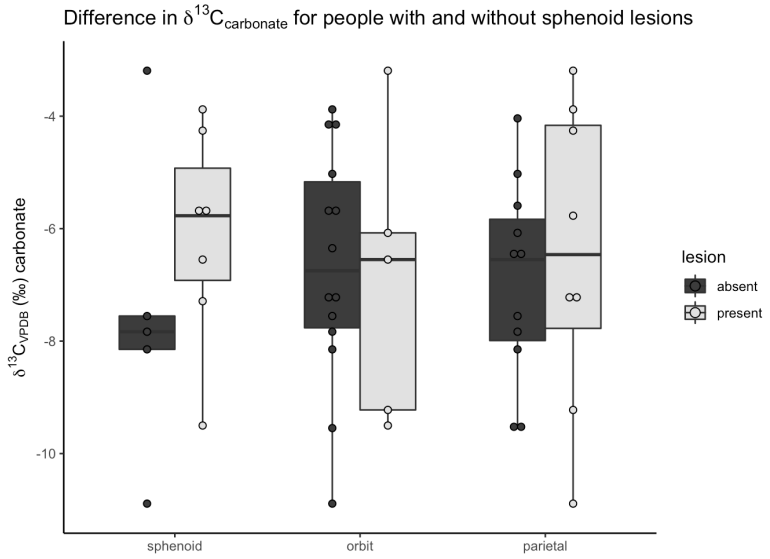
Age as a covariate; **p < 0.01, *p < 0.05, ˙p < 0.1

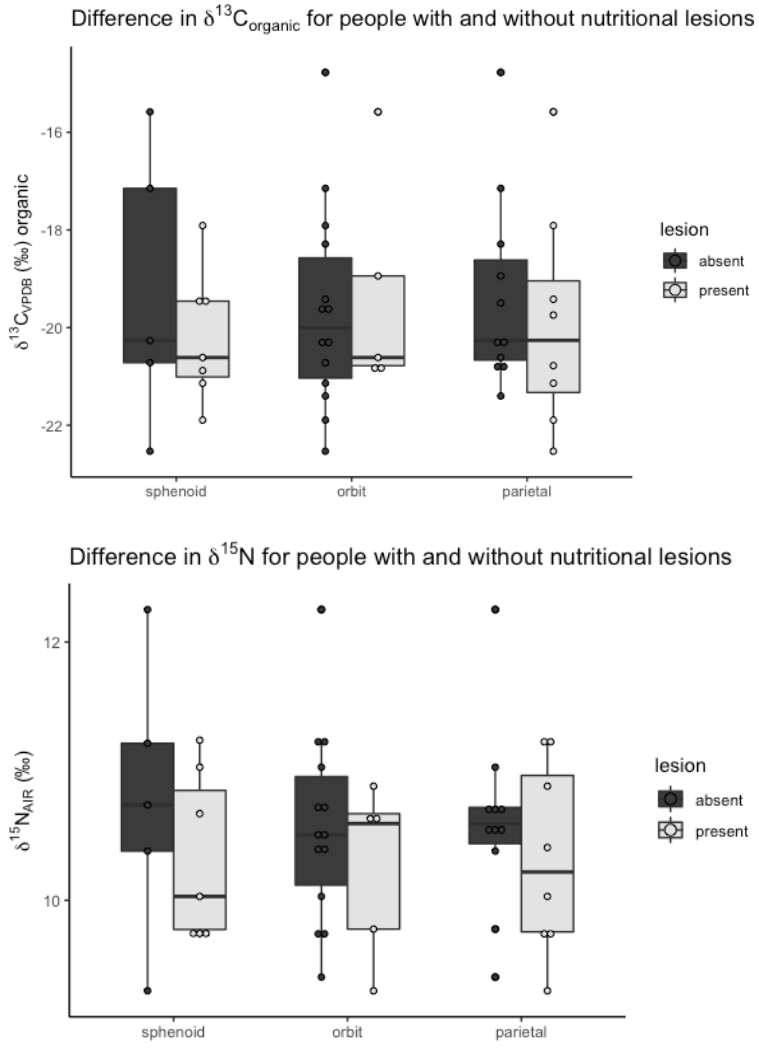
Contributing relationship 1: Associations between dietary isotopic ratios and nutritional lesions

Table 6.2 in Chapter 6 includes information on the dietary $\delta^{13}C$ and $\delta^{15}N$ of sampled Piedmont Siouan people. Carbonate $\delta^{13}C$ reflects the carbon profile of the whole diet, while organic $\delta^{13}C$ reflects the carbon profile of the protein portion of the diet (Ambrose & Norr, 1993; Jim et al., 2006). Information of the protein portion of the diet is further nuanced by $\delta^{15}N$ measurements, which can be used to interpret the trophic level and/or ecological source of the protein portion of past peoples' diets (Ambrose, 1991, 2000; DeNiro & Epstein, 1981). Figure 7.8 shows the relationship between nutritional lesions and dietary isotopic measurements ($\delta^{13}C_{carbonate,}$ $\delta^{13}C_{organic}$, and $\delta^{15}N$). It appears that people with sphenoid lesions have more ^{13}C-enriched $\delta^{13}C_{carbonate}$ than people without sphenoid lesions. In terms of food, this may translate to more consumption of C_4 plants, principally maize. Maize is the only C_4 plant commonly consumed by Piedmont Siouan groups (Gremillion, 1989; Roark, 2020; VanDerwarker et al., 2007).

Figure 7.8

Difference in $\delta^{13}C_{carbonate}$, $\delta^{13}C_{organic}$, and $\delta^{15}N$ of people with and without lesions of nutritional deficiency





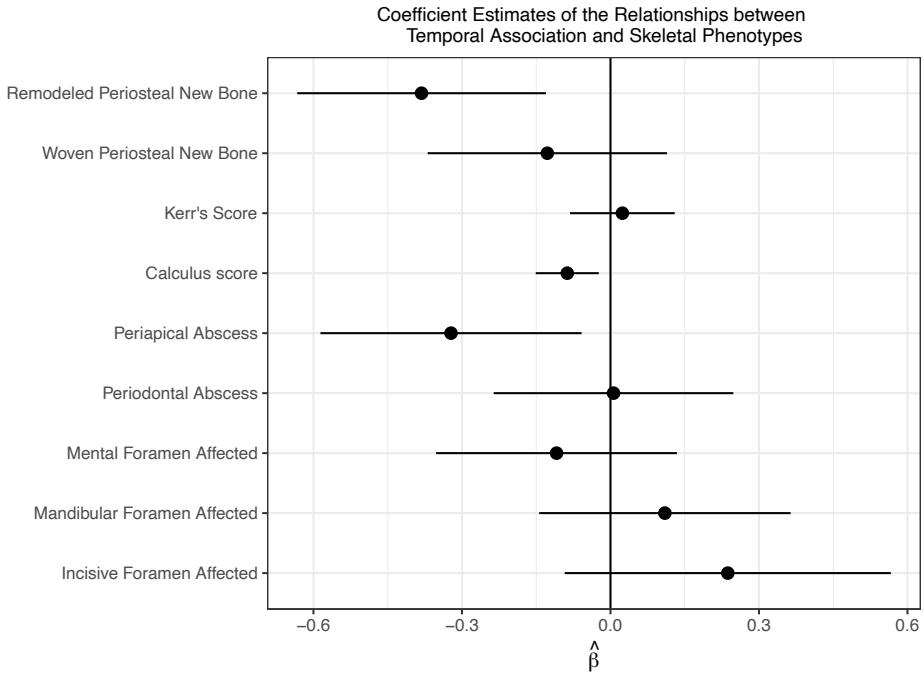
Contributing relationship 2: Associations between proxies of immunostimulation and nutritional lesions

Two models were used to test the relationship between immunostimulatory lesions and micronutrient deficiencies. The first model examined temporal trends in skeletal proxies of immunostimulation (Figure 7.9, Table 7.6), and the second tested the association between skeletal proxies of immunostimulation and sphenoid lesions (Figure 7.10, Table 7.7). Figure 7.9 shows the correlation coefficients and standard errors of the logistic and ordinary least squares (OLS) regression models used to test the association between proxies of immunostimulation (dependent variables) and temporal associated (independent variable). All models also used age

and river drainage as covariates, except the model with incisive foramen lesions as the dependent variable, due to a small sample size.

Figure 7.9

Correlation coefficients and standard errors for logistic and OLS regression models between proxies of immunostimulation and temporal association, controlling for age and river drainage



Correlation coefficients to the left of the zero line in Figure 7.9 suggest that the lesion is associated with earlier dates, while correlation coefficients to the right of the zero line suggest that the lesion is associated with later dates. None of the observed skeletal proxies of immunostimulation are significantly associated with a later date; rather, periosteal new bone lesions (especially remodeled lesions), dental calculus, and periapical abscesses are all significantly more prevalent during earlier compared to later temporal windows ($p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively).

Table 7.6

Summary Statistics from the Logistic and OLS Regression Models Displayed in Fig. 7.7

Dependent variables	ß-coefficient	Std. error	p-value	Sample size	Covariates
Remodeled PNB	-0.38	0.13	0.00**	145	Age, river drainage
Woven PNB	-0.13	0.12	0.30	145	Age, river drainage
Kerr's score	0.02	0.05	0.66	64	Age, river drainage
Calculus score	-0.09	0.03	0.01**	87	Age, river drainage
Periapical abscess	-0.32	0.13	0.02*	108	Age, river drainage
Periodontal abscess	0.01	0.12	0.96	109	Age, river drainage
Mental foramen lesion	-0.11	0.12	0.38	52	Age, river drainage
Mandibular foramen lesion	0.11	0.13	0.40	46	Age, river drainage
Incisive foramen lesion	0.24	0.17	0.16	32	Age

PNB = Periosteal new bone; **p < 0.01, *p < 0.05, ˙p < 0.1

Figure 7.10 shows the correlation coefficients of the logistic and OLS regression models that test the association between sphenoid lesions (dependent variable) and lesions indicative of immunostimulation (independent variables). The models also included age and river drainage as covariates (or just age, if the sample size was too small for multiple covariates). Table 6.7 details the standard error of the correlation coefficients, the p-values of the relationships, and the sample sizes used for each regression model. People with higher scores of dental calculus and/or mandibular foramen lesions are more likely to also have sphenoid lesions, though only the relationship between mandibular foramina and sphenoid lesions is marginally significant ($p < 0.01$). It should be noted that dental calculus scores are significantly correlated with porous lesions of the maxilla (1.28 ± 0.59, $p < 0.05$, controlling for age), and maxillary and sphenoid lesions are significantly associated (Figure 7.7).

Figure 7.10

Correlation coefficients and standard errors for logistic and OLS regression models between sphenoid lesions and proxies of immunostimulation, controlling for age and river drainage

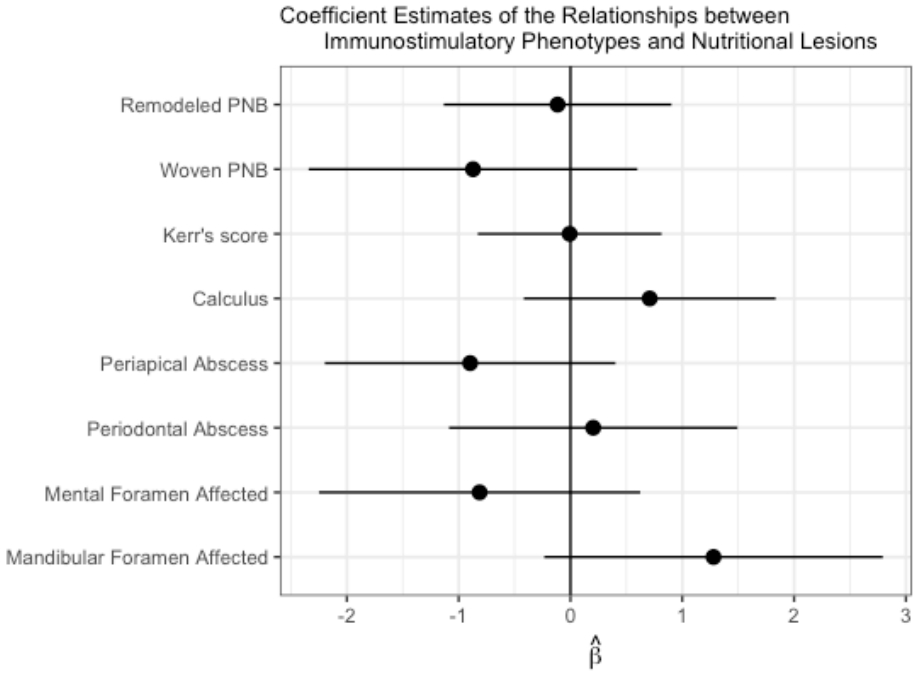


Table 7.7

Summary Statistics from the Logistic and OLS Regression Models Displayed in Fig. 7.10

Independent variables	ß-coefficient	Std. error	p-value	Sample size	Covariates
Remodeled PNB	-0.12	0.52	0.82	82	Age, river drainage
Woven PNB	-0.87	0.75	0.25	82	Age, river drainage
Kerr's score	-0.01	0.42	0.99	38	Age
Calculus score	0.71	0.57	0.22	53	Age, river drainage
Periapical abscess	-0.90	0.66	0.18	69	Age, river drainage
Periodontal abscess	0.20	0.66	0.76	69	Age, river drainage
Mental foramen lesion	-0.81	0.73	0.27	32	Age
Mandibular foramen lesion	1.28	0.77	0.10˙	31	Age
PNB = Periosteal new bone; ˙p < 0.1					

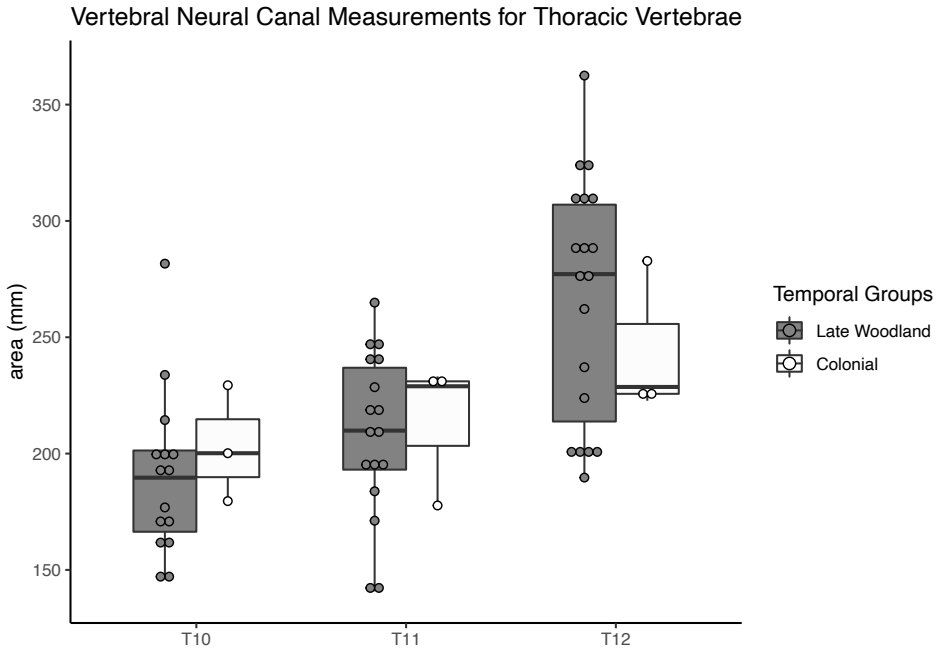
Potential outcome: Developmental trade-offs affecting skeletal growth

Figure 7.11 shows VNC dimensions by temporal period, and Figure 7.12 shows the predicted probability that a person from the Late Woodland, Protohistoric, or Colonial period would have LEH. The predicted probability was estimated from a logistic regression model with LEH as the dependent variable, temporal group as the independent variable, and age as a covariate.

Long bone ratios had very few observations for the Colonial period (due to differential preservation of skeletal remains, see Chapter 4 for more information) and I therefore did not split the variable of long bone ratios into temporal categories for analysis. Differential preservation also complicated the sample size for VNC dimensions. Current data indicates that there are no clear differences in either VNC dimensions or LEH prevalence over time.

Figure 7.11

VNC dimensions by vertebra and temporal group



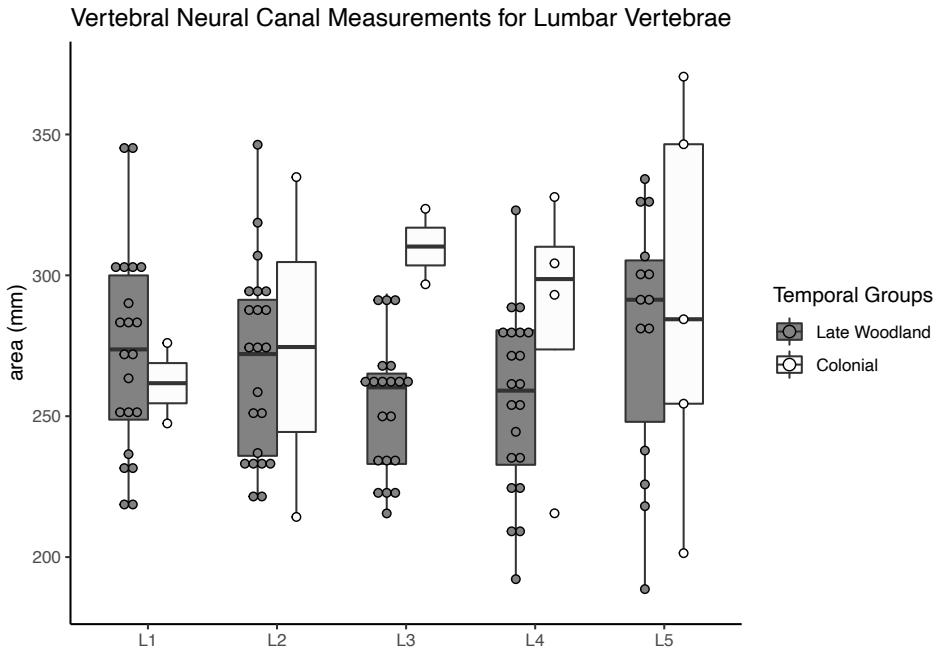
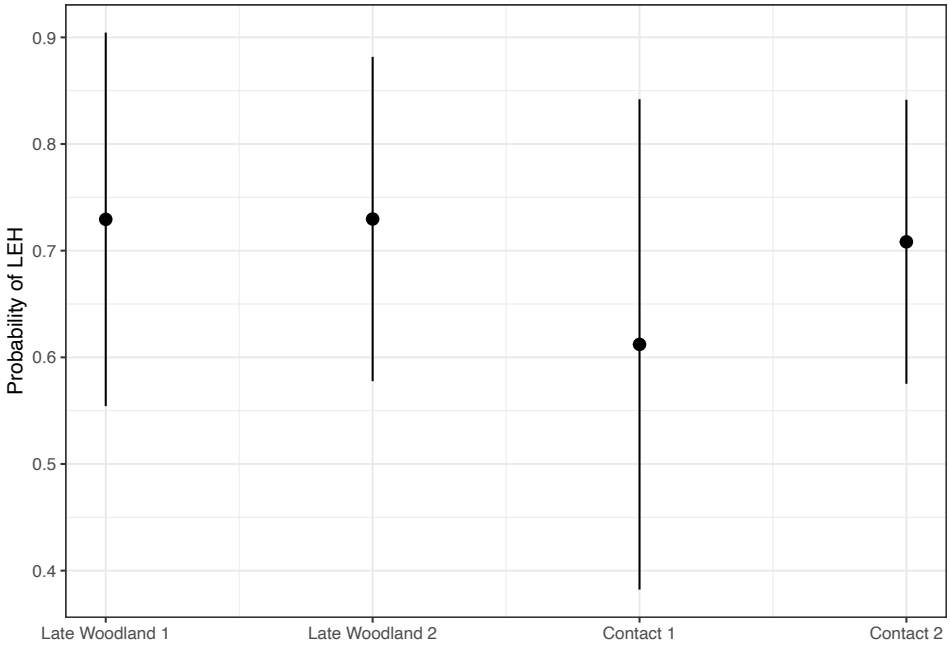


Figure 7.12

Predicted probability of LEH by temporal group, controlling for age



Beta-coefficient: 0.42, standard error: 0.59, p = 0.47

7.4 Discussion

Summary of contributing relationship 1: Diet and nutritional lesions

There is a possible relationship between carbonate $\delta^{13}C$ and sphenoid lesions – people with sphenoid lesions may have consumed more maize than people without sphenoid lesions. (Figure 7.8). A potential relationship between higher maize consumption and nutritional deficiency may result from the low nutritional composition of maize, but more likely from a combination of the low nutritional composition of maize and the biocultural consequences of the factors that encouraged an increased reliance on maize (M. N. Cohen & Armelagos, 1984; Crandall, 2014; Paine & Brenton, 2006). For example, Roark (2020) suggested that Dan River communities may have intensified their maize cultivation and deemphasized acorn foraging to more efficiently produce food for the larger population sizes of the Dan River groups compared to groups from nearby river drainages. Larger population sizes may support more communicable disease, which can affect the body's nutrient needs and absorption. Maize consumption, and diet in general, reasonably seems to directly and indirectly affect nutritional deficiency, but it is not the sole cause of nutritional deficiency. Instead, I argue that more influences than diet alone affect nutrition (see Figure 7.1).

Though only 12 people had both observable sphenoid bones as well as measured isotopic ratios, there are multiple indicators that suggest that diet is only part of the equation contributing to increased sphenoid lesions in the Colonial period. First, the person with the most ^{13}C-enriched carbonate $\delta^{13}C$ measurement does not have sphenoid lesions. And, archaeobotanical data as well as the isotopic data measured for this dissertation (see Chapter 6) support that people from the Dan River consumed more maize than people from the Eno River. Roark (2020) identified this as one of the most notable trends in the plant data from the Piedmont. Yet people from the Colonial

period in the Eno River have a high sphenoid lesion prevalence (Figure 7.4, Table 7.4). If diet alone contributed to sphenoid lesions, then I would expect that people from the Dan River who grew and consumed more maize would have a higher prevalence of sphenoid lesions. However, the sphenoid lesion data does not support that.

Summary of contributing relationship 2: Immunostimulation and nutritional lesions

I hypothesized that dietary composition is only one of the contributing factors to the nutritional epidemiology of Piedmont Siouan groups, and that the nutritional and energetic demands of immune system stimulation also contribute to nutritional epidemiology. Two models were used to test this hypothesis. The model used to generate Figure 7.10 indicated that people with dental calculus and mandibular foramen lesions (both immunostimulatory lesions) were more likely to have sphenoid lesions. These relationships were suggestive, not statistically significant.

However, neither dental calculus nor mandibular foramen lesions were more prevalent in the Colonial period compared to the Late Woodland period (Figure 7.9). It is interesting and seemingly contradictory that the phenotypes that co-occur with sphenoid lesions are not more prevalent in the Colonial period. I discuss potential explanations below.

Summary and reframing of growth outcomes

The sample size was not large enough to observe whether there were significant trends in growth velocity over time among Piedmont Siouan communities, and it is therefore impossible to test the hypothesis that population-level changes in nutrition affected developmental trade-offs in a way that led to growth stunting. It *is* possible, however, to test how a person's early-life exposures affected their likelihood of experiencing nutritional deficiencies. This is possible because skeletal development happens across the life course. Elements that develop and/or fuse

early in life (e.g. teeth and lower spine VNC) serve as a record of early-life experiences that is measurable even in the adult skeleton.

To evaluate the effects of early-life conditions on the development of lesions of nutritional deficiency, I tested whether LEH presence, VNC dimensions, or long bone ratios are associated with sphenoid lesions. Figure 7.13 shows VNC dimensions and Figure 7.14 shows long bone ratios, both by sphenoid lesion presence; however, there is not enough data to observe any clear trends. There was enough data to test the relationship between LEH and sphenoid lesions, and Figure 7.15 shows the predicted probability of a person having sphenoid lesions if they also have LEH. The predicted probability is estimated from a logistic regression model that uses sphenoid lesions as the dependent variable, LEH as the independent variable, and age and temporal period as covariates. LEH is significantly associated with sphenoid lesions. The relationship has a beta-coefficient of 1.89 (±0.84) and is significant at $p < 0.05$.

The association between LEH and sphenoid lesions suggests that it may be useful to revise the analytical framework outlined in Figure 7.1. Instead of measuring the outcomes of nutritional change for later generations, it may be productive to consider how early-life influences are either an additional contributing relationship to nutritional deficiencies, or how they may modulate the effect of contributing relationship 2 (the relationship between immunostimulation and nutrition).

Figure 7.13

VNC dimensions by vertebra and sphenoid lesion presence

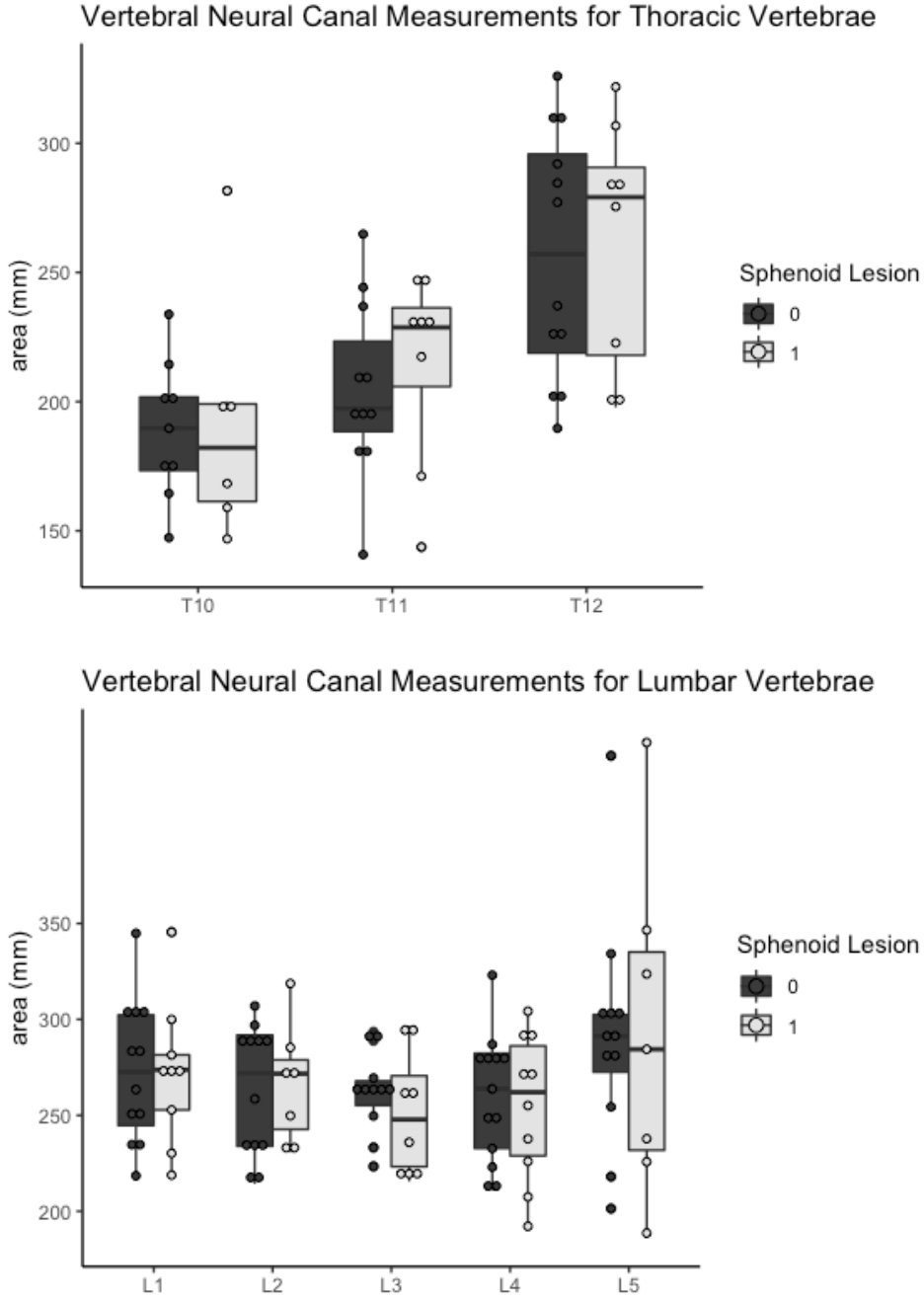


Figure 7.14

Humerus:Ulna ratios (left) and Femur:Tibia ratios (right), both by sphenoid lesions presence

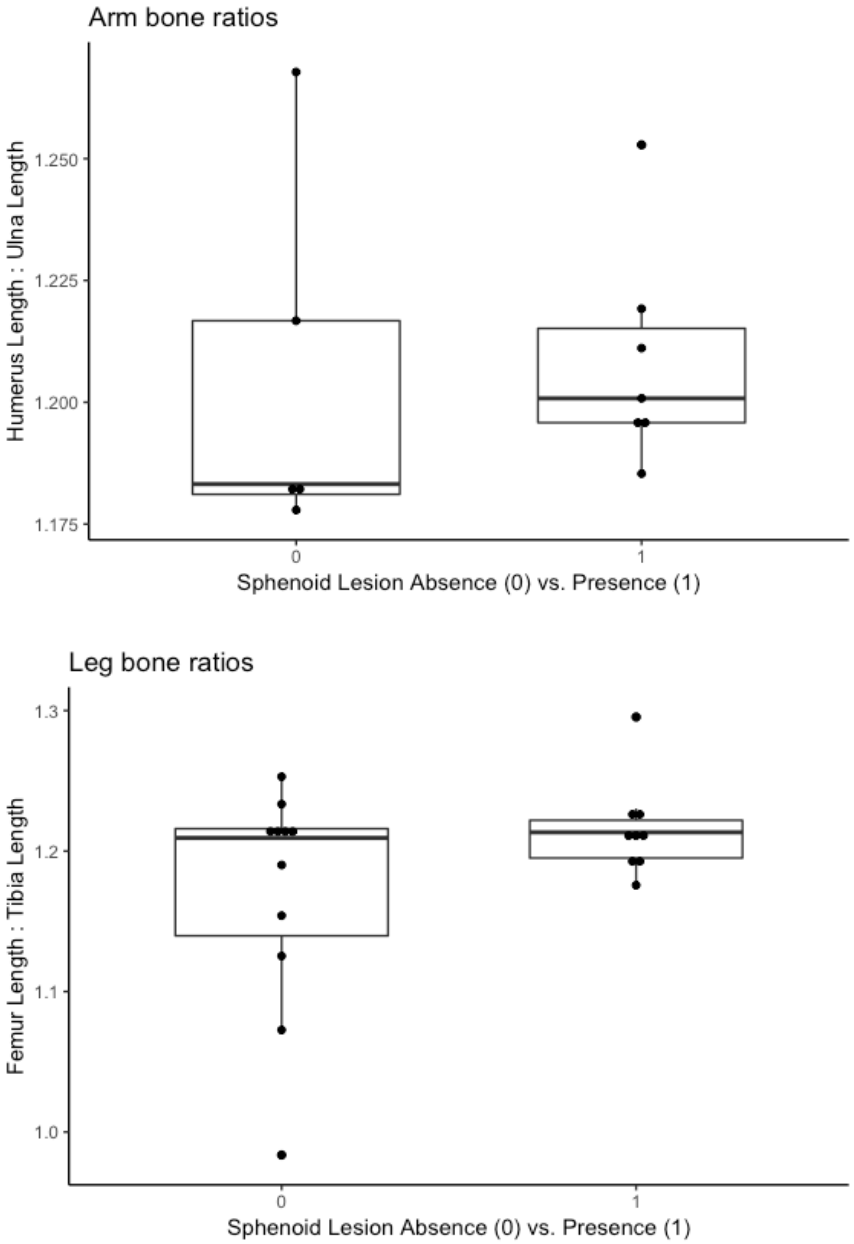
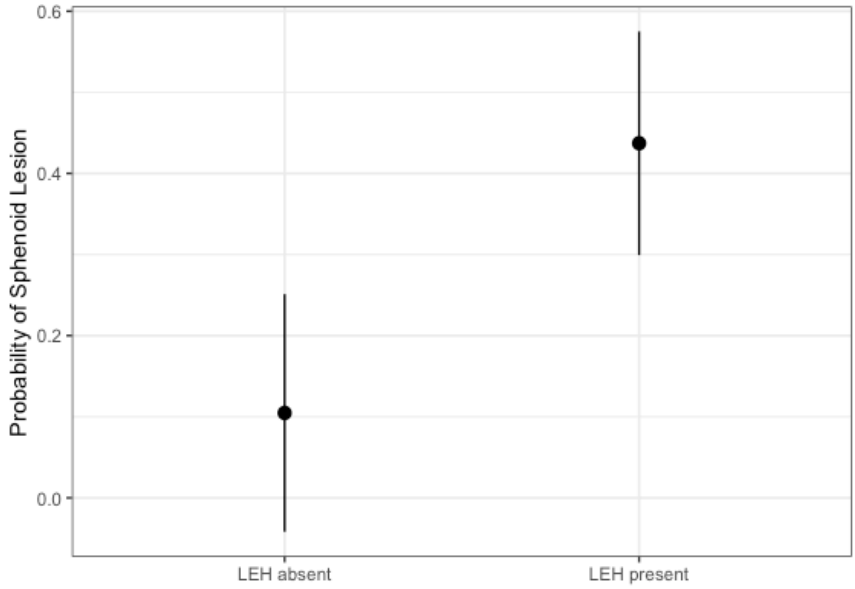


Figure 7.15

The predicted probability of sphenoid lesion presence by LEH absence/presence



Overall interpretations

In this section, I discuss two potential scenarios that would result in some of the seemingly-contradictory results, especially the discrepancy between the peri-Colonial increase in sphenoid lesions that occurred without a concurrent increase in dental calculus, mandibular foramen lesions, or LEH prevalence, all of which are associated with sphenoid lesions.

Scenario 1: Missing covariates

Many biocultural exposures are difficult to examine in the skeleton, and this inevitably leads to missing covariates in the reconstruction of disease in past populations. It is certainly possible that covariates not captured by the models included in this paper contributed to the increase in sphenoid lesions in later compared to earlier Piedmont Siouan communities. Potential covariates include infectious disease, pipe smoking, practices related to food preparation, and general malnutrition (possibly associated with food insecurity or famine).

Colonialism introduced new infectious diseases to the disease ecology of North America, including some that caused epidemics. Some researchers argue that a smallpox epidemic spread through southeast North America following Hernando de Soto's 16th-century trail through the Southeast (Dobyns, 1993; Smith, 1987), as de Soto's path included western North Carolina (though not the Piedmont region) (Hudson, 1990; Swanton, 1985; H. T. Ward & Davis, 1999). Others argue, however, that factors such as population size and density, sociopolitical organization, cultural practices, topography and ecology, and immune system resilience (immunostimulation and allostatic load) intersected to create differential population susceptibility to disease prior to direct interaction between indigenous and colonizing groups (Hutchinson, 2016; Larsen, 1994; Milner, Anderson, & Smith, 2001; H. T. Ward & Davis, 1999).

Kelton (2007) cites ethnohistoric evidence of a smallpox epidemic in 1696 that likely affected the Piedmont Siouan communities, and Lawson noted epidemic-related population loss in the Piedmont in his 1701 reports (Lefler, 1967). Mortuary patterns at Colonial period Piedmont Siouan archaeological sites support the likelihood of late 17th century infectious disease epidemics, as people began to position graves away from houses and bury the deceased without attendant mortuary rituals (H. T. Ward & Davis, 1993, 2001). Additionally, Roark (2020) found that medicinal taxa increased over time in the archaeobotanical record, starting around ~AD 1540–1620 and increasing through the Colonial period. Infectious diseases may cause LEH and/or nutritional deficiencies for those who survived the disease episode, depending on their age when they were affected by disease, and could therefore be a missing covariate in the immunostimulatory category (contributing relationship 2 in Figure 7.1).

Pipe smoking could also be an additional missing covariate in the model, and may possibly represent a missing contributing relationship. Smoking both increases periodontal disease susceptibility and severity, as well as decreases the body's vitamin C levels (Barbour et al., 1997; Nishida et al., 2000; Pamuk et al., 1994). Researchers of Piedmont Siouan material culture suggest that pipe smoking increased during the end of the Late Woodland period through Colonial period (H. T. Ward & Davis, 1993, 1999). However, if smoking significantly contributed to the increase in sphenoid lesions in the Colonial period, then periodontal disease indicators should increase in the Colonial period, which they do not (Figure 7.9). Additionally, sphenoid lesions were higher in all age groups except 0-5, and higher among peri-Colonial women than men, so a better understanding of the cultural practice of pipe smoking is necessary to critically evaluate the role of smoking in sphenoid lesion etiology.

Fresh fruits and leafy greens, as well as fish roe are all sources of vitamin C available to Piedmont Siouan communities (Gremillion, 1989; Holm, 1994, 2002; Lefler, 1967; Swanton, 1946; VanDerwarker, 2001; VanDerwarker et al., 2007). Vitamin C degrades upon exposure to light and heat, and food preparation practices like drying foods for storage therefore reduce the vitamin C content of foods. If Siouan groups relied more on stored versus fresh food in the Colonial period compared to earlier periods, then this may increase their likelihood of vitamin C deficiency. Storage can be evaluated via the archaeological record and change in food storage practices is a question for future research.

While general malnutrition associated with food insecurity or even famine would contribute to vitamin C deficiency, it would also cause other micronutrient deficiencies and likely manifest as lesions elsewhere on the cranium (e.g. eye orbits and parietal bones). Of the people who lived during the Colonial period and had sphenoid lesions, only 25% and 38.5%

have orbit and parietal lesions, respectively, and only 16% have lesions on all three cranial bones. Therefore, it is unlikely that general malnutrition led to the Colonial period increase in vitamin C deficiency. Infectious disease and food practices are therefore the most likely missing covariates that influenced the increase in sphenoid lesion prevalence.

Scenario 2: A dysregulated immune response

Scenario 1 outlines missing variables that potentially contribute to the peri-Colonial increase in sphenoid lesions, while Scenario 2 considers variables that may modify the relationship between the immune system and nutritional lesions. The magnitude, duration, and regulation of immune responses to physiological insults vary among people – some people will experience an aggressive immune response that causes damage to their tissues, while others experience an immune response that is insufficient to heal or ward off infections (M. N. Cohen & Armelagos, 1984; S. Cohen & Herbert, 1996; Holt & Jones, 2000; McDade, 2003, 2005). The theory of ecological immunology identifies multiple influences on the immune response, including early life effects on immune system development, as well as the adaptability of the immune system to conserve energy when the body experiences competing demands for its limited energetic resources. Ecological immunology could explain why the prevalence of immunostimulatory lesions did not change over time among Piedmont Siouan groups, but the prevalence of sphenoid lesions did.

The immune system develops and matures according to early life exposures, either in utero or during early childhood (Barker, 2012; Holt & Jones, 2000; McDade, 2003), and the body may make tradeoffs during immune system development to best prepare the developing child for survival. If the cues suggest a need for a well-developed specific immune response (e.g., the ability to make antibodies to fight off infectious disease), then the body may develop

the specific immune response at the expense of the nonspecific immune response, which may result in a poorly-regulated (and nutritionally-expensive) inflammatory response (Holt & Jones, 2000; McDade, 2005). Archaeological and ethnohistoric evidence suggest that Piedmont Siouan groups may have experienced increased infectious disease during the Colonial period. Such an epidemiological shift has the potential to affect the cues that children received from their mothers and their early life exposures, leading their immune response to develop in ways that were expensive for the body's resources, including vitamin C.

The immune system retains some degree of plasticity even after it has developed, and it is able to adapt to competing demands for the body's resources by other physiological systems. For example, an immune response is expensive for the body to mount and sustain, but so is a hormone-mediated stress response (McDade, 2005). Psychosocial stress negatively impacts the immune response (Glaser & Kiecolt-Glaser, 2005; Kemeny & Schedlowski, 2007; Padgett & Glaser, 2003; Peruzzo et al., 2007; Rosania et al., 2009), and one potential explanation for this negative effect of stress on immune function is the competition of different systems of the body for resources. Piedmont Siouan people who lived during the Colonial period may have experienced increased psychosocial stress, as these periods involved ecological, geopolitical, sociocultural, and epidemiological changes, and some of these factors (e.g., increased morbidity and mortality of community members, threats of raiding, disruption of social roles and kin structures) may have elicited a stress response.

A person who experiences psychosocial stress may have a poorly-regulated immune response to additional sources of immunostimulation such as periodontal disease and injury to soft tissues. A poorly-regulated immune response has the potential to be expensive for metabolic resources, including micronutrients. While it is not possible to reconstruct whether past Piedmont

Siouan individuals experienced psychosocial stress, it may be a missing variable in the larger picture of sphenoid lesion etiology. People across all temporal periods experienced immunostimulation; however, people in the Colonial period may have had a more severe or poorly-regulated immune response to immunostimulation, resulting in an increased likelihood for nutritional deficiency. In the future, I will employ microbiome analysis to evaluate the likelihood that Piedmont Siouan communities experienced an immunological shift, as functional analysis of the oral microbiome can reveal insights to person's pathogen-stimulated immune response (Warinner et al., 2014; Warinner et al., 2015).

7.5 Conclusion

A higher proportion of people from the Colonial period had sphenoid lesions compared to people from the earlier periods, and sphenoid lesions reflect vitamin C deficiency. Both diet and immune system stress likely contributed to vitamin C deficiency in Piedmont Siouan people who lived during the Colonial period. People with sphenoid lesions may have consumed more ^{13}C-enriched energy sources (probably maize) than people without sphenoid lesions, and people with sphenoid lesions were also more likely to have high scores of dental calculus and lesions of the mandibular foramina (both proxies of immunostimulation), as well as LEH (a proxy for childhood growth disruption). However, though sphenoid lesions were more prevalent in the Colonial period, other skeletal lesions associated with sphenoid lesions (dental calculus, pathological mandibular foramina, and LEH) did not significantly increase over time.

Two scenarios may explain this temporal discrepancy in sphenoid lesion prevalence compared to the prevalence of dental calculus, pathological mandibular foramina, and LEH. The first is missing covariates: other covariates not included in our models, including infectious disease and food preparation practices, may have contributed to the Colonial period increase in

vitamin C deficiency. The second is immunological shifts. Either developmental exposures and/or immune dysregulation may have led to population-level shifts in the immune response to biocultural stressors. A poorly-regulated (e.g., overactive) immune response has the potential to deplete vitamin C, as the immune response is expensive for vitamin C (see Chapter 8).

Overall, the conceptual framework used to guide this study (Figure 7.1) is oversimplified – results indicate that more contributing relationships should be considered, as well as more interconnections and interactions among the relationships. The models used to test the conceptual framework do show that nutritional lesions indicative of vitamin C deficiency increased in the Colonial period compared to earlier temporal periods. This nutritional shift has implications for how well peoples' musculoskeletal systems, immune systems, and other systems of the body are able to remain resilient to the increased biocultural stressors associated with European colonialism.

CHAPTER 8 (PAPER 4): BIOCULTURAL PATHWAYS LINKING PERIODONTAL DISEASE EXPRESSION TO STRESS, IMMUNE DYSREGULATION, AND NUTRITION

8.1 Introduction

Periodontal disease is measurable in both living and past populations and is therefore a phenotype that can reveal evolutionary trends in the relationships between humans and their biocultural contexts (Feldman, Douglass, Loftus, Kapur, & Chauncey, 1982; Hillson, 2000; Tomczyk et al., 2017). Research indicates that psychosocial stress is an important contributing factor for periodontal disease (Akcali et al., 2013; Rosania et al., 2009) and that the immune system impacts of periodontal disease contribute to tissue damage throughout the body (Scannapieco, 2013; Seymour et al., 2007). Studies of living populations have identified associations between periodontal disease and other inflammatory diseases, including obesity (Chaffee & Weston, 2010; Genco et al., 2005), cardiovascular disease (Beck, Garcia, Heiss, Vokonas, & Offenbacher, 1996; Scannapieco et al., 2003), rheumatoid arthritis (Kaur et al., 2013; Scher et al., 2012), and Alzheimer's disease (Kamer et al., 2009; Stein et al., 2012; A. Watts, Crimmins, & Gatz, 2008), as just several of the interactions between periodontal disease and other organ systems.

Meanwhile, studies of the osseous tissue of past populations have identified that periodontal disease and dental calculus are associated with periosteal new bone elsewhere in the body and increased mortality risk (DeWitte & Bekvalac, 2011; Yaussey & DeWitte, 2019). However, gaps remain in our understanding of the pathways that mediate psychosocial stress and periodontal disease, as well as periodontal disease and other skeletal and physiological

phenotypes (Akcali et al., 2013; Crespo et al., 2017; LeResche & Dworkin, 2002). The goal of this paper is to test theoretical pathways in the etiology and systemic impacts of periodontal disease. Additionally, this paper aims to clarify the impacts of periodontal disease on nutrition, an important phenotype in the human biology of both past and present populations.

Periodontal disease pathophysiology

Periodontal disease is a chronic infection perpetuated by pathogenic bacteria that disrupt the composition and functioning of dental plaque, a community of bacteria that lives on the surfaces of teeth and performs many functions to maintain health, such as protecting tooth surfaces from harmful exposures and contributing to circulating levels of nitric oxide necessary for vascular health (Wade, 2013; Warinner et al., 2014). The disruption of dental plaque by pathogenic bacteria stimulate human innate immune system receptors, which signal for the production of antimicrobial proteins, activate an inflammatory response by host cells, and prompt an adaptive immune system response (Hajishengallis & Lambris, 2012; Herrero et al., 2018; Lamont et al., 2018).

The immune response to pathogenic bacteria results in damage and loss of periodontal tissue through the increased production of tissue-damaging reactive oxygen species and the activation of enzymes that degrade structural proteins (Graves, 2008; Hajishengallis, 2015; Nanci & Bosshardt, 2006; Potempa, Banbula, & Travis, 2000). Bacteria involved in periodontal disease, such as *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia,* and *Treponema denticola*, benefit from and perpetuate the inflammation-induced breakdown of periodontal tissues. The bacteria use inflammatory byproducts such as degraded proteins as metabolites, and they excrete harmful byproducts that kill other bacteria and further damage host tissues (Hajishengallis, 2014; Hajishengallis & Lambris, 2012; Jenkinson, 2011; Lamont et al.,

2018). This creates a positive feedback cycle between periodontal disease-causing pathogens (periodontopathogens) and inflammation-associated host tissue breakdown.

Moreover, pathogenic bacteria have adapted to make environments even more favorable for their virulence. They manipulate and disrupt the function and regulation of innate immune receptors, prompting the host to mount an inflammatory response that is unchecked by normal suppressive mechanisms of the immune system (Darveau, 2014; Hajishengallis & Lambris, 2012). Not only does chronic inflammation in periodontal tissues lead to heightened osteoclastogenesis and thus loss of bone surrounding teeth (Graves, 2008; Nanci & Bosshardt, 2006), but it may also stimulate an acute-phase immune response that involves the synthesis of hepatic proteins [such as C-reactive protein (CRP)], which have systemic immunomodulatory effects (Hajishengallis, 2015).

The plasticity of periodontal disease etiology: Psychosocial stress and periodontal disease

The degree of immune response stimulated by periodontopathogens is not uniform. Some people will experience a higher immune response that exacerbates the cycle between tissue damage and bacterial proliferation (Akcali et al., 2013; Kemeny & Schedlowski, 2007; LeResche & Dworkin, 2002; Rosania et al., 2009). Stressors such as social inequalities may play a role in altering the immune response to pathogens, as psychological and physiological pathways respond to each other in complex ways (Goodman & Leatherman, 1998; Gravlee, 2009; LeResche & Dworkin, 2002; McDade, 2009; Wutich & Brewis, 2014), mediated by cultural buffering systems and social support (Ham, Temple, Klaus, & Hunt, 2021; LeResche & Dworkin, 2002; Reitsema & McIlvaine, 2014).

The stress response involves neuroendocrine and immune mediators that have the potential to disrupt healthy functioning of the body (LeResche & Dworkin, 2002). Stress is

thought to lower the immune activity of both the innate and acquired immune systems, increasing the risk of infection (Crespo et al., 2017; Irwin et al., 1990; Kemeny & Schedlowski, 2007; Rosania et al., 2009). This relationship between stress and immune system activity is mediated by the interactions of the hypothalamic-pituitary-adrenal-axis (HPA-axis), the central nervous system (CNS), the autonomic nervous system (ANS), and the immune system (Akcali et al., 2013). Stress exposure activates the HPA-axis, which results in a higher release of glucocorticoids such as cortisol (Akcali et al., 2013; Glaser & Kiecolt-Glaser, 2005; Kemeny & Schedlowski, 2007; Rosania et al., 2009). Cortisol and other glucocorticoids: (1) decrease the production of T helper type 1 (Th1) pro-inflammatory cytokines interleukin (IL)-12, IL-2, and tumor necrosis factor (TNF)-α; (2) increase the production of T helper type 2 (Th2) cytokines IL-10 and IL-4; and (3) alter lymphocyte activity, including altering neutrophil chemotaxis (Glaser & Kiecolt-Glaser, 2005; Kemeny & Schedlowski, 2007; Peruzzo et al., 2007). Neutrophil chemotaxis describes the directed movement of neutrophils from the bone marrow (where they are produced) to the tissues with the highest concentration of chemical signals for neutrophil recruitment (Nuzzi et al., 2007).

The ANS produces the catecholamines adrenaline and noradrenaline (Akcali et al., 2013). Catecholamines reduce the production of pro-inflammatory cytokines interferon(IFN)-γ, IL2, IL6, IL-12, and TNF-α (Akcali et al., 2013; Hänsel, Hong, Cámara, & Von Känel, 2010). They also reduce the production of lymphocytes and antibodies, and suppress cytolytic activity (Akcali et al., 2013; Ben-Eliyahu, Shakhar, Page, Stefanski, & Shakhar, 2000; Padgett & Glaser, 2003). While catecholamines reduce pro-inflammatory mediators, the ANS also stimulates the CNS, resulting in the production of substance P, which up-regulates pro-inflammatory cytokines and can lead to an exaggerated or dysregulated inflammatory reaction (Breivik, Thrane, Murison, &

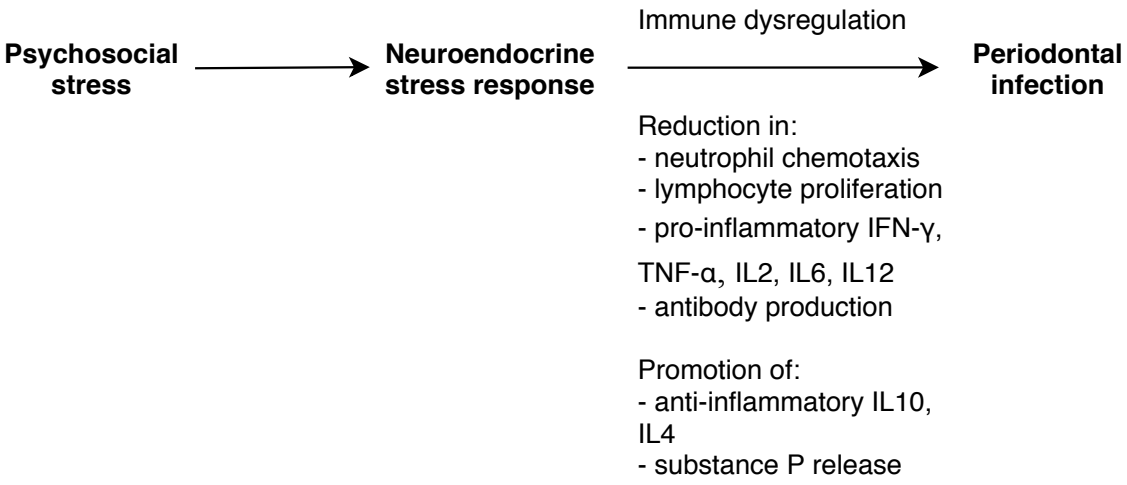
Gjermo, 1996; Kemeny & Schedlowski, 2007; Rosenkranz, 2007). The conflicting actions of catecholamines versus substance P illustrate the complexity of neuroendocrine-immune pathways that respond to stress, which are highly variable among people.

The negative effect of psychosocial stress on immune function can be interpreted using human ecological immunology, a framework which suggests that the immune system is one of several systems with competing demands for the body's finite resources (McDade, 2003, 2005). The body will optimize allocation of resources among those systems, and the systems themselves have mechanisms for energy regulation and resource conservation (e.g., trade-offs inherent to the polarization of the Th1 versus Th2 immune response) (Long & Nanthakumar, 2004). The body may reallocate energetic resources to mitigate psychosocial stress, possibly routing those resources from the immune system (McDade, 2005).

Figure 8.1 provides a conceptual model of the theoretical pathway linking psychosocial stress to its proximate impacts on the immune system and distal impacts on periodontal disease susceptibility and progression. Akcali et al. (2013:60) note, "mechanisms explaining the possible relationship between stress and increased susceptibility to periodontal disease remain poorly understood," and this paper therefore aims to test whether statistical models support theoretical mechanisms to better interpret periodontal disease epidemiology in biocultural context.

Figure 8.1

Theoretical pathway A: The relationship between psychosocial stress, immune mediators, and increased risk of periodontal infection



The systemic skeletal impacts of periodontal disease: Periodontal disease and Vitamin C

Periodontal disease feeds back into the immune system and possibly contributes to osseous impacts elsewhere in the body, including inflammatory arthritis and periosteal new bone (DeWitte & Bekvalac, 2011; Kaur et al., 2013; Scher et al., 2012). Periodontal disease may also affect nutritional status (Hujoel & Lingström, 2017; Ritchie & Kinane, 2003), which has the potential to affect skeletal tissues (Brickley & Ives, 2008; Genuis & Bouchard, 2012). Theoretical explanatory pathways linking periodontal disease to whole-body sequelae include traveling bacteria and hyperinflammation (high pro-inflammatory immune responses that exceed the balancing potential of anti-inflammatory responses) (Crespo et al., 2017; LeResche & Dworkin, 2002). Both traveling bacteria and hyperinflammation fall under the umbrella of oral focal infection theory, which posits that infections of oral tissues have immune system impacts that are not just confined to the mouth, but have impacts on whole-body physiology (Han & Wang, 2013; Offenbacher & Beck, 2014).

The perspective of periodontal disease in whole-body context is especially useful for life-course approaches to the skeletal biology of past populations, as periodontal disease potentially mediates some of the mosaic of skeletal lesions that occur over a person's life (Agarwal, 2016; Gowland, 2015; Stodder & Palkovich, 2012). In particular, periodontal disease may mediate skeletal phenotypes associated with micronutrient deficiencies. Chronic, systemic immune activation – such as that stimulated by periodontal disease – is metabolically expensive and requires micronutrients to sustain (Scrimshaw et al., 1968; Tomkins, 2003). Vitamin C is especially critical for immune reactions, and serum vitamin C may become depleted with chronic immune activation. Vitamin C is also essential for soft-tissue maintenance (Carr & Maggini, 2017; Crandall & Klaus, 2014) and deficiency in this micronutrient can lead to osseous impacts that are measurable through serum biomarkers as well as dry bone phenotypes.

Vitamin C plays a multifaceted role in periodontal disease; lower levels of vitamin C both contribute to as well as result from the disease. Low vitamin C levels disrupt collagen synthesis and thus compromise the integrity of soft tissues in the mouth, including the gingival and periodontal tissues. Periodontopathogens are more likely to infect compromised tissues than tissues with intact mechanical and immune defenses; therefore, low vitamin C levels increase the risk of oral infection by pathogens (Nishida et al., 2000). Low levels of vitamin C can also exacerbate the impact and progression of periodontal disease once oral tissues become infected. Vitamin C acts as an antioxidant that mitigates the effect of tissue-damaging reactive oxygen species produced by the immune reaction to pathogenic bacteria (Nishida et al., 2000; Ritchie & Kinane, 2003). It is also an important cofactor for immune processes, particularly for the function of neutrophils. Neutrophils are the most common leukocyte found in response to periodontal infection (Brock, Butterworth, Matthews, & Chapple, 2004; Hajishengallis, 2015),

and sufficient levels of vitamin C are required for leukocyte function and recruitment to infected sites (Nishida et al., 2000; Padayatty & Levine, 2016).

The chronic nature of periodontal disease involves continued production of reactive oxygen species and recruitment of neutrophils, which places high, sustained demands on vitamin C levels. Activated neutrophils accumulate and use large quantities of vitamin C (Elste et al., 2017; Nualart et al., 2002; Washko et al., 1993), which may decrease serum levels of vitamin C during acute infection (Padayatty & Levine, 2016). The increase in reactive oxygen species involved in the inflammatory response to bacteria also puts a high demand on vitamin C for its antioxidant function (Elste et al., 2017).

Figure 8.2

Theoretical pathway B: The relationship between periodontal disease, immune mediators, and low serum levels of vitamin C

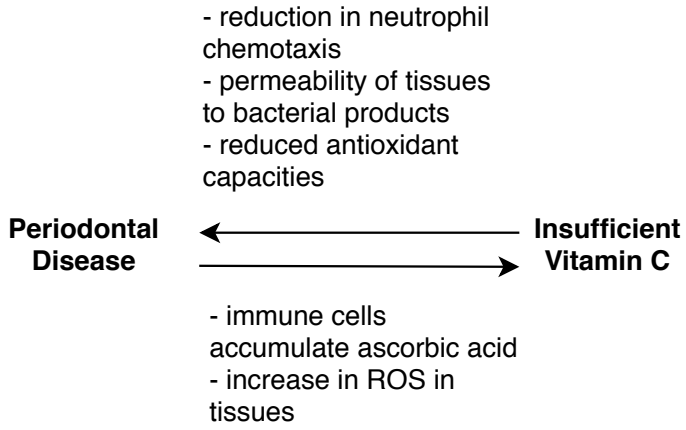
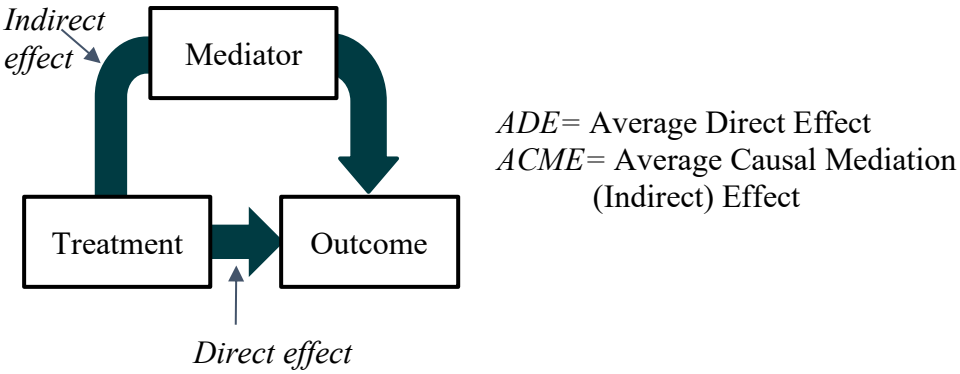


Figure 8.2 provides a conceptual model for the theoretical pathway linking periodontal disease, the immune response, and serum levels of vitamin C. Logistic and linear regression models are used to test pathway B as well as pathway A (Figure 8.1), and then examine the relationships in a causal mediation framework. Causal mediation analysis uses effect decomposition models to estimate how much of the effect of one variable on another is direct

versus mediated, and it is therefore a useful tool for testing pathways in the human body for which there are a set of theoretically-mediated relationships. Figure 8.3, adapted from Bejanin et al. (2017), provides a conceptual diagram for causal mediation models. This paper uses data from the National Health and Nutrition Examination Survey (NHANES) in regression and causal mediation models and focuses on variables that represent the main phenotype of interest – periodontal disease – as well as psychosocial stress, immune function, body composition, and nutrition.

Figure 8.3

Conceptual diagram of a causal mediation model



8.2 Materials: NHANES 2003-2004 data

NHANES is a program of the National Center for Health Statistics, part of the Centers for Disease Control and Prevention. NHANES uses combined interview, laboratory, and examination methods to collect data on the health and nutrition of people in the United States. Table 8.1 includes the descriptive statistics of the NHANES variables used for the statistical models in this study.

Periodontal disease assessments

I use the NHANES variable "periodontal needs" as a proxy for periodontal disease. Trained dentists and oral health recorders assessed "periodontal needs" using the combined indicators of periodontal pockets, gingival recession, loss of attachment between teeth and periodontal tissues, and gingival bleeding. Reference examiners observed and replicated assessments by dental examiners to improve standardization of recording (CDC & NCHS, 2006).

According to NHANES participant screening protocols, participants who were younger than 13 years old were excluded from the periodontal needs assessment, as were participants with heart problems, rheumatic fever, kidney disease requiring dialysis, hemophilia, and pacemakers or automatic defibrillators. Participants were also excluded if a dentist or doctor had previously recommended that they take antibiotics prior to a dental appointment (CDC & NCHS, 2006).

Table 8.1

Descriptive Statistics for Variables used in Regression Models

Variable	Mean	Range
Age‡	36.64	8–85
Gender‡†	1.51	1–2
Race‡†	-	1–5
Household income‡†	6.78	1–13
Smoking status‡†	0.45	0–1
Time since last dental visit‡†	2.70	1–7
Food insecurity†	1.52	1–4
Periodontal needs†	0.15	0–1
BMI‡	26.35	12.99–64.97
Neutrophils§	4.22	0.50–18.10
White blood cells§	7.20	2.3–99.99
Monocytes§	0.54	0.1–4.4
Lymphocytes§	2.18	0.4–89.7
CRP§	0.38	0.01–25.4
Vitamin C	56.49	0.6–274.2

n = 7656, † = categorical variable, ‡ = pretreatment covariate, §log-transformed for regression models

Psychosocial stress questionnaire data

The NHANES variable of "Household Food Security" was used as a proxy for psychosocial stress to test theoretical pathway A. Other potential proxies of psychosocial stress were available via the NHANES 2003-2004 variable set; however, these variables were assessed using only a subset of the whole pool of NHANES participants and would therefore bias the tests of periodontal disease pathophysiology and sequelae.

People who are food secure have predictable access to safe, nutritious, and sufficient quantities of food that meets their biological needs, cultural preferences, and social norms. Food security also involves predictable, culturally- and socially-acceptable avenues to acquire food (FAO, 2002; Hadley & Patil, 2008; Wutich & Brewis, 2014). Food insecurity, alternatively, occurs when access and avenues become unpredictable or uncertain. Research indicates that anxiety and depression co-occur with food insecurity (Hadley & Patil, 2008; McLaughlin et al., 2012; Weaver & Hadley, 2009), and that food insecurity elicits a neuroendocrine stress response (McClain et al., 2018; Pike & Williams, 2006).

Due to the constraints on variable options and the relationships outlined above between food insecurity and the neuroendocrine stress response, household food security is an appropriate proxy for psychosocial stress (and attendant neuroendocrine response) in the statistical models included in this paper. Additionally, a logistic regression model showed that the relationship between food insecurity and periodontal disease remained significant (0.20 ± 0.07, $p < 0.01$) even after including serum levels of vitamin C, vitamin D, vitamin B_{12}, and calcium as covariates, and I argue that a neuroendocrine stress response associated with food insecurity is responsible for the proportion of the relationship between food insecurity and periodontal disease that is not explained by the model covariates and nutrition variables.

NHANES calculated household food security from participants' responses to questions regarding multiple aspects of food insecurity. Questionnaire administrators read statements and asked participants whether they found the statement to often, sometimes, or never apply to their household over the last 12 months. Examples of these statements include: "[I am/we are] worried that [my/our] food would run out before [I/we] got money to buy more," and "[Child's name was / the children were] not eating enough because [I/we] just couldn't afford enough food." NHANES grouped participants into categories of household food security using the aggregate of their responses to the survey questions. These categories range from full food security (score of one) to very low food security (score of four) (CDC & NCHS, 2007). Participants 15 years and older were administered the household food security questionnaire. Participants were excluded if their household income was at least four times higher than the poverty guidelines of the U.S. Department of Health and Human Services (DHHS, 2007; CDC & NCHS, 2007)

Serum measurements of infection, inflammation, and vitamin C

Serum measurements of immune markers (counts of white blood cells, monocytes, lymphocytes, segmented neutrophils, and CRP) were available for all participants aged 1 year and older (CDC & NCHS, 2007a). Counts were measured in duplicate using calibrated equipment with reproducibility checks (CDC & NCHS, 2009).

Participants were excluded from blood sample collection if they had hemophilia; received chemotherapy during the four weeks prior to sample collection; had rashes, swelling, burns or scarred tissue, open sores and wounds, or bandages in the area of venipuncture on both arms; had casts, tubes, missing or paralyzed limbs, shunts or intravenous lines, damaged or occluded veins on both arms; or had allergies to the cleaning products used before venipuncture (CDC & NCHS, 2009).

Serum vitamin C (ascorbic acid) measurements were available for all participants aged 6 years and older, except for those excluded from blood sample collection, outlined above (CDC & NCHS, 2009). Vitamin C was measured using isocratic high performance liquid chromatography. Protocol steps including specific information on buffers, reagents, and temperatures can be found in the NHANES description of laboratory methodology (CDC & NCHS, 2006)

8.3 Methods and Results

Regression models

I applied logistic and linear regression models to test theoretical pathways A and B. Continuous variables with highly skewed distributions were log-transformed (indicated in Table 8.1), and all other continuous variables were rescaled to transform their means to zero and standard deviations to one. Covariates may explain some of the relationships between periodontal disease and other variables implicated in the theoretical pathways. The covariates included in the regression models are listed in Table 8.1.

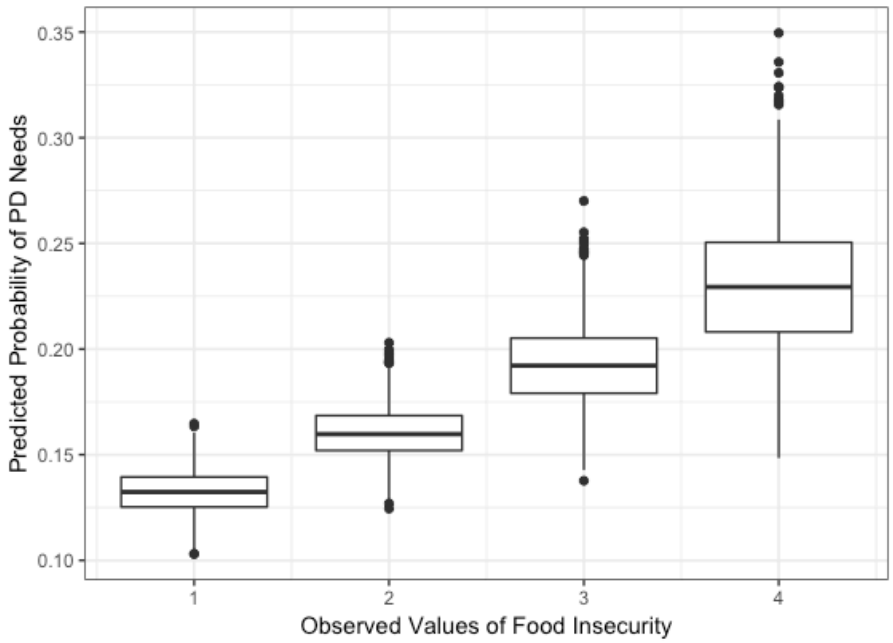
Regressions between food insecurity, immune biomarkers, and periodontal disease

Theoretical pathway A was tested using a logistic regression model with household food security as the exposure variable and periodontal disease as the outcome variable. The relationship between household food insecurity and periodontal disease was statistically significant at $p < 0.001$ and has a beta-coefficient of 0.22 ± 0.07 (odds ratio: 1.25). For every one-unit increase in food insecurity, there is a 0.22 increase in periodontal disease. Figure 8.4 shows the predicted probability that greater food insecurity contributes to periodontal disease; the model accounts for the covariates indicated in Table 8.1, with the addition of vitamin C as a covariate. Individuals who live in households with very low food security (category four on the

x-axis) have the highest predicted probability of periodontal needs, followed by individuals with low food security (category three), marginal food security (category two), and full food security (category one).

Figure 8.4

The predicted probability of periodontal needs for each category of food security

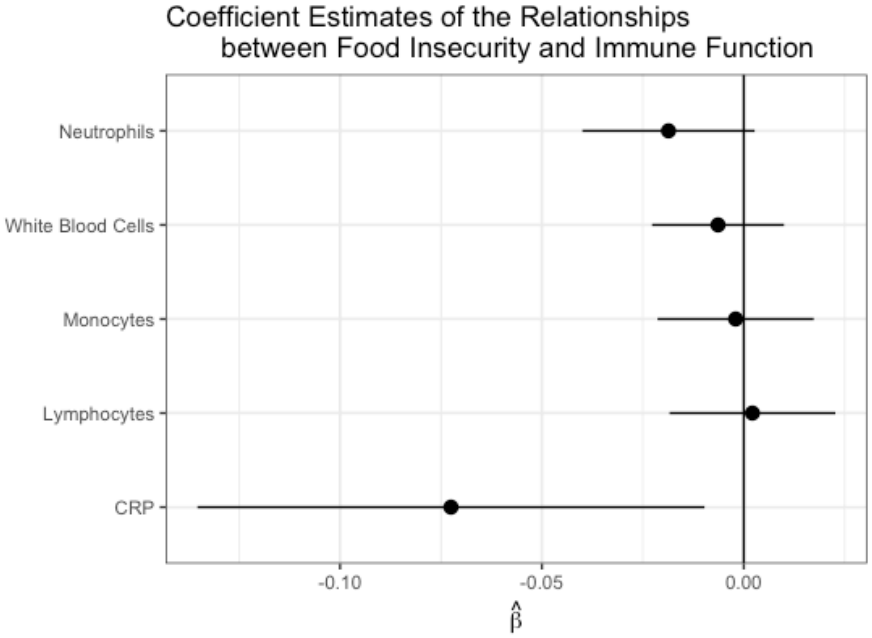


(1 = full food security, 2 = marginal food security, 3 = low food security, 4 = very low food security)

I used a regression model to test whether psychosocial stress does impact immune function and used household food security as the independent variable and proxies for immune activity as the dependent variables (Figure 8.5). The summary statistics of the regression models are included in Table 8.2.

Figure 8.5

Beta-coefficients and standard error ranges for the regression models using household food security as an independent variable and immune function proxies as the dependent variable.



Each row represents a different regression model, all using the same covariates (see Table 8.2 for information on covariates)

Table 8.2

Results of the Coefficient Models Between Food Security (Independent Variable) and Immune Biomarkers (Dependent Variables)

Dependent variable	ß-coefficient (standard error)	p-value
Neutrophils†	-0.019 (0.011)	0.09•
White blood cells†	-0.006 (0.008)	0.44
Monocytes†	-0.002 (0.010)	0.84
Lymphocytes†	0.002 (0.010)	0.84
CRP†	-0.073 (0.032)	0.02*

• $p < 0.1$, * $p < 0.05$, †log-transformed
Covariates: age (scaled), gender, race, time since last dental visit, smoking status, BMI (scaled), household income, serum vitamin C (scaled), periodontal needs

Regression between periodontal disease and vitamin C

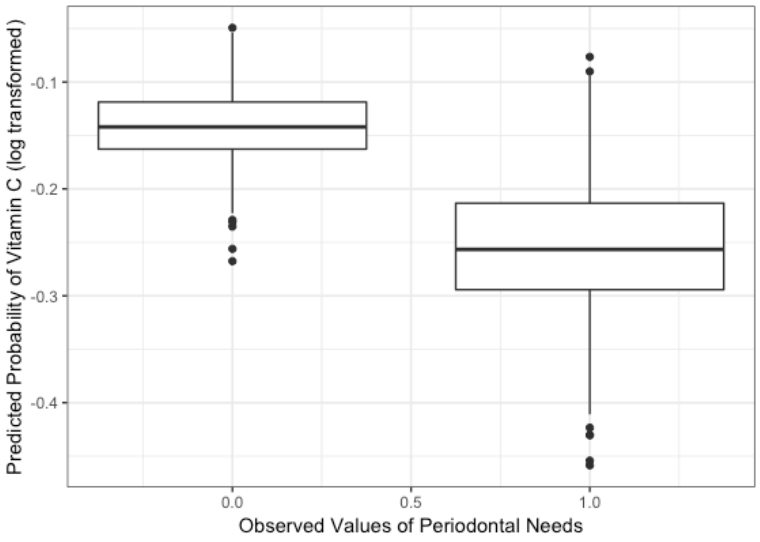
To test theoretical pathway B, I ran regression models with periodontal disease as the exposure variable and serum vitamin C measurements as the outcome variable. Periodontal

disease is negatively correlated with serum vitamin C measurements. A one-unit increase in periodontal disease is associated with a decrease of 0.14 (± 0.06) standard deviations for serum vitamin C. This effect is significant at $p < 0.05$. This model included the same covariates indicated in Table 8.1, with the addition of neutrophil counts (log-transformed). Figure 8.6 shows the predicted value of periodontal needs on serum vitamin C measurements. I numerically scaled the vitamin C variable, so the mean is zero and the standard deviation is one. Predicted serum vitamin C measurements are lower for periodontal needs (score of one on the x-axis) than no periodontal needs (category zero on the x-axis).

Immune cells, specifically neutrophils, theoretically mediate the relationship between periodontal disease and nutritional effects. Regression models with periodontal needs as the independent variable and immune biomarkers as the dependent variables are shown in Figure 8.7, and summary statistics of these models and information on covariates are included in Table 8.3.

Figure 8.6

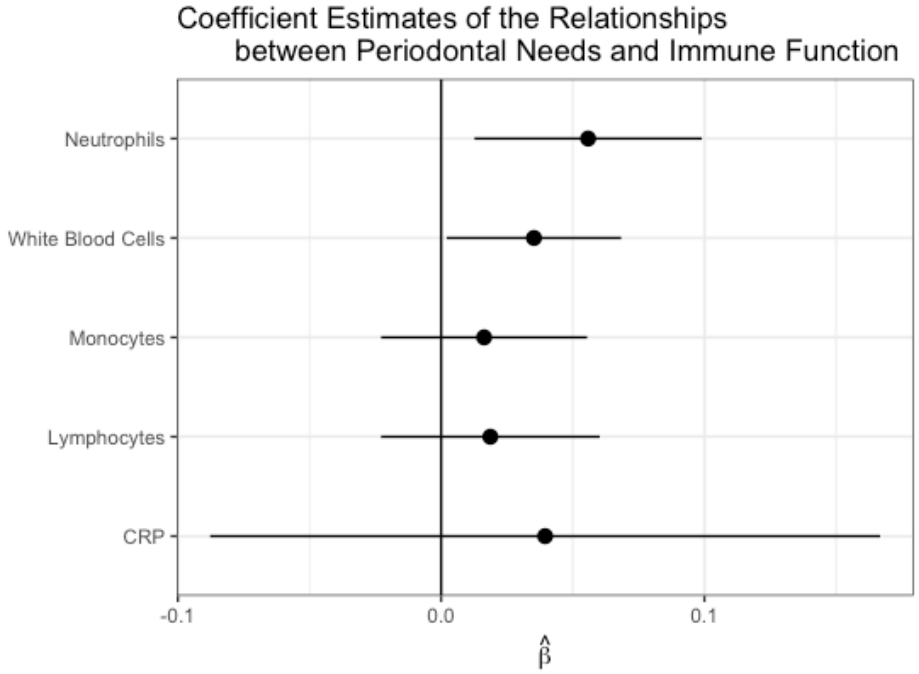
The predicted value of scaled serum vitamin C measurements by periodontal needs



Periodontal needs (category 1) vs. no periodontal needs (category 0)

Figure 8.7

Beta-coefficients and standard error ranges for the regression models using periodontal needs as an independent variable and immune biomarkers as the dependent variables.



Each row represents a different regression model, all using the same covariates (see Table 8.3 for information on covariates)

Table 8.3

Results of the Coefficient Models between Periodontal Needs (Independent Variable) and Immune Biomarkers (Dependent Variables)

Dependent variable	ß-coefficient (standard error)	p-value
Neutrophils†	0.056 (0.022)	0.01*
White blood cells†	0.035 (0.017)	0.04*
Monocytes†	0.016 (0.020)	0.41
Lymphocytes†	0.019 (0.021)	0.38
CRP†	0.039 (0.065)	0.54

* $p < 0.05$, †log-transformed

Covariates: age (scaled), gender, race, time since last dental visit, smoking status, BMI (scaled), household income, serum vitamin C (scaled), household food security

Causal mediation analysis

The package "mediation" for R statistical software (a causal mediation analysis package) (Tingley, Yamamoto, Hirose, Keele, & Imai, 2014) was used to test whether pathways A and B

are mediated according to the theoretical assumptions outlined in Figures 8.1 and 8.2. Causal mediation analysis was designed for randomized control trials. However, cross-sectional data, such as that available through NHANES, can be considered conditionally ("as-if") random as long as any pretreatment variables that would non-randomly distribute the dependent variable are controlled for (Greenland & Mansournia, 2015).

Neutrophils serve as an example: everyone has circulating levels of neutrophils, but some people have higher levels. We can consider people with higher levels of neutrophils to be the "treatment" (versus "control") group, as long as we include covariates that may be affecting neutrophils in our model. Causal mediation analysis is appropriate for conditionally random cross-sectional data. The pretreatment variables included in our causal mediation models are indicated in Table 8.4.

The "mediation" R package includes sensitivity analysis, which is used in conjunction with causal mediation models to estimate the strength of a hypothesis that a relationship is direct or mediated, and to evaluate the likelihood that the relationship is resilient to the effects of missing covariates. I trimmed the causal mediation dataset to individuals who had all of the variables of interest, which resulted in a sample size of 1853 individuals (Table 8.4). All continuous variables were rescaled or log-transformed. The trimmed dataset used for causal mediation analysis is much smaller than the untrimmed dataset, due to including a variable on cigarette smoking status. While reducing the sample size in this way may introduce bias, cigarette smoking is a critical variable to include, due to its effect on immune and nutrient levels (Barbour et al., 1997; Nishida et al., 2000; Pamuk et al., 1994).

Causal mediation analysis of pathway A

I applied causal mediation analysis to test the theoretical pathway linking psychosocial stress, mediated by the integrated activity of the HPA-axis, ANS, CNS, and immune system, to immune function and periodontal disease. This involved two models with different mediators: first with household food security as the exposure variable, log-transformed serum neutrophil concentrations as the mediator, and periodontal needs as the outcome; and the second with log-transformed serum CRP concentrations as the mediator. I selected these two mediators according to the relationship between food security and immune markers (Figure 8.5). Covariates for these models are indicated in Table 8.4, with the addition of vitamin C (scaled).

Table 8.4

Descriptive Statistics for Trimmed Dataset Used for Causal Mediation Models

Variable	Mean	Range
Age‡	52.34	20–85
Gender‡†	1.41	1–2
Race‡†	-	1–5
Household income‡†	6.62	1–13
Smoking status‡†	0.45	0–1
Time since last dental visit‡†	3.09	1–7
Food insecurity†	1.44	1–4
Periodontal needs†	0.22	0–1
Neutrophils§	4.64	0.5–18.0
CRP§	0.47	0.01–25.40
Vitamin C	50.49	0.6–274.20
BMI‡	28.28	16.01–64.97

† = categorical variable, ‡ = pretreatment covariate, n = 1853
§log-transformed in the causal mediation model

Causal models did not support that the relationship between food security and periodontal disease is operating through reduced neutrophil production and activity. The direct effect (between household food insecurity and periodontal disease) is significant at $p < 0.001$ with a beta-coefficient of 0.03 ± 0.02. The mediated effect of neutrophils is not significant (-9.69×10^{-4}

± -0.003, p = 0.15). The mediated effect of CRP is also not significant (-2.44 x 10^{-4} ± -0.002, p = 0.71).

Sensitivity analyses indicate that the model of the direct effect of food security on periodontal needs is sensitive to missing covariates that could alter the effect of the relationship. Missing covariates likely include neuroendocrine signals such as cortisol, as people have a varying neuroendocrine response to psychosocial stress, including food insecurity (Laraia, 2013; Maniam, Antoniadis, & Morris, 2014; McClain et al., 2018; Piperata, Schmeer, Rodrigues, & Torres, 2016). The mediated effect of food security on periodontal needs (operating through neutrophils or CRP) is more robust, and any missing covariates are unlikely to change the effect size of the relationship.

Causal mediation analysis of pathway B

I ran a causal mediation model to test the hypothesis that the immune response stimulated by periodontal disease is expensive for the body's vitamin C stores. The model included periodontal needs as the exposure variable, log-transformed serum neutrophil concentrations as the mediator, and numerically scaled serum vitamin C measurements as the outcome variable. Covariates are indicated in Table 8.4, with the addition of food security. The direct relationship between periodontal disease and vitamin C is negative, with an estimate of -0.12 ± -0.22. This relationship is significant at $p < 0.05$. The mediated relationship is also negative (estimate of -0.01 ± -0.02) and nearly significant at p = 0.07. The total effect is also significant at $p < 0.05$. The proportion of the relationship that is mediated is estimated to be 6.06% (p = 0.11). The results therefore suggest that the immune response likely plays a role in the effects of periodontal disease on serum vitamin C.

Sensitivity analysis indicates that the model accounted for most of the variation in the neutrophil-mediated relationship between periodontal needs and serum vitamin C levels, and additional covariates would not change the effect of this relationship. However, the direct relationship between periodontal disease and vitamin C is more sensitive to missing covariates, which possibly include reactive oxygen species or bacterial products.

8.4 Discussion

Susceptibility to periodontal disease is plastic, and psychosocial stress may play an important role in shaping the expression of this phenotype. Untreated periodontal disease also affects other skeletal and physiological phenotypes, particularly through its stimulation of a systemic immune response and its impacts on nutrition. Though periodontal disease is an infection of oral tissues, it impacts serum levels of multiple biomarkers. Periodontal disease can be assessed through salivary biomarkers for living populations (Javaid, Ahmed, Durand, & Tran, 2016; Kinney et al., 2011) and dry-bone observations for past populations (DeWitte & Bekvalac, 2011; Tomczyk et al., 2017; Tuggle & Watson, 2019). The phenotype of periodontal disease provides useful context for the interpretation of nutrition and immune biomarker levels, as well as skeletal lesions occurring elsewhere in the body.

Pathways connecting food insecurity to periodontal disease

Contrary to theoretical pathway A, the results do not support that serum neutrophil or CRP levels significantly mediate the relationship between household food security and periodontal disease, despite the negative relationship between food security and the serum measurements of neutrophils and CRP (Figure 8.5). Part of the null findings of the causal models may be rooted in a mismatch between the dataset and the research aims of this paper. While the data supports stress-based downregulation of pro-inflammatory immune reactions consistent

with energy conservation in human ecological immunology (McDade, 2005), the cross-sectional data do not separate people by the temporal duration of periodontal needs. The early stages of periodontal disease could be mediated by the suppressed immune functions associated with stress, while later stages of periodontal disease may shift energy allocation towards a pro-inflammatory immune reaction due to prolonged bacterial exposure. The literature on periodontal disease immunology supports a shift in immune response with periodontal disease: pro-inflammatory cytokines are present in periodontal tissues, where they work in concert with receptor activator of nuclear factor kappa-B ligand (RANKL) to mediate the alveolar bone loss that is pathognomonic of periodontal disease (Barros, Da Silva, Somerman, & Nociti Jr., 2003; Berger et al., 2021; Kawai et al., 2006).

Additionally, this paper used the variable household food security as a proxy for psychosocial stress in our models. The questionnaire used to categorize individuals into levels of household food security included statements that the participant was asked to agree or disagree with (see materials section) (CDC & NCHS, 2007), and these statements may have biased responses and missed important "cultural idioms of distress" (Pike and Williams, 2006:732). Another limitation is the cross-sectional nature of NHANES data; causal mediation analysis is optimally used with data from randomized control trials (Tingley et al., 2014), and pathways linking food insecurity to perceived stress and physiological outcomes are best evaluated using longitudinal data (Stevenson & Hadley, 2014). Overall, the models that examine the relationship between psychosocial stress and periodontal disease would be improved by additional physiological covariates not accessible via NHANES (such as the biomarkers of the neuroendocrine response to stress, including cortisol), longitudinal data, and the addition of variables useful for inferring cultural and social context, examples of which are outlined above.

Pathways linking periodontal disease and vitamin C

The immune involvement of periodontal disease has whole-body effects, one including reducing physiological vitamin C availability. Periodontal disease may affect serum vitamin C levels through multiple pathways, and the findings of this paper model identified neutrophils as a potential mediator between periodontal disease and vitamin C. The host immune response to bacterial products includes inflammation and antibody release, which stimulates neutrophil chemotaxis (Warinner et al., 2014). Neutrophils are produced in the bone marrow and have a very short lifespan once released into the blood (several hours to one day) (Nauseef & Borregaard, 2014; Warinner et al., 2014). Neutrophils are only released from the bone marrow into circulating blood (and directed to affected tissues) during chemotaxis.

The short life span of neutrophils makes it disadvantageous for them to be released from the bone marrow unless they are released in response to an immune insult. Therefore, high serum neutrophil levels reflect active infection and/or inflammation (Nauseef & Borregaard, 2014). For example, the inflammatory reactions of rheumatoid arthritis (RA) recruit neutrophils to the synovial fluid of affected joints. People with RA have higher serum neutrophil levels as a result of this stimulation and chemotaxis of neutrophils, especially during an arthritic flare (Foell et al., 2004). This paper shows that people with periodontal disease have higher serum neutrophil levels than people without periodontal disease. Therefore, serum neutrophil levels indicate an active immune response in periodontal tissues.

The results of the causal mediation models indicate that serum vitamin C may be depleted by periodontal disease through the promotion of neutrophil chemotaxis and activation. The effects of periodontal disease on serum vitamin C are consistent with oral focal infection theory, which postulates that the immune system involvement of oral infections has systemic impacts

(Han & Wang, 2013; Kumar, 2017; Offenbacher & Beck, 2014). Some studies apply oral focal infection theory to suggest that periodontal disease may contribute to a hyperinflammatory phenotype, in which pro-inflammatory immune mediators are not adequately regulated by anti-inflammatory mechanisms and thus have the capacity to aggravate inflammation-associated tissue damage throughout the body (Crespo et al., 2017; Garcia, Henshaw, & Krall, 2001; Offenbacher & Beck, 2014; Shaddox et al., 2010).

Interestingly, I did not find a significant association between periodontal disease and CRP, a biomarker of systemic inflammation (Du Clos, 2000) (Figure 8.7), though my results did indicate significantly heightened serum neutrophils in people with periodontal needs compared to those without. This may be consistent with Hajishengallis et al.'s (2016) identification of a hyperinflammatory *neutrophil* phenotype associated with periodontal disease.

A hyperinflammatory neutrophil phenotype is caused by the chronic stimulation of neutrophil production, activation, and chemotaxis, which is potentially seen with periodontal disease (Hajishengallis et al., 2016). A hyperinflammatory neutrophil phenotype may exaggerate tissue damage by neutrophils, contributing to cardiovascular disease (Hajishengallis et al., 2016; Yasunari, Watanabe, & Nakamura, 2006), as well as muscle damage following injury (Rigamonti, Zordan, Sciorati, Rovere-Querini, & Brunelli, 2014). Hyperreactive neutrophils and lower serum vitamin C levels are both probable consequences of periodontal disease that have direct effects on musculoskeletal tissues, as well as indirect effects through disruption of healthy immune system function (Carr & Maggini, 2017; Crandall & Klaus, 2014; Fain, 2005; Scrimshaw, 2003).

Putting the pathways together: Immune dysregulation and trade-offs in physiological resource allocation

Both theoretical pathways that we tested in this paper involved neutrophils as a potential mediator. In pathway A, higher food insecurity is associated with a greater likelihood of periodontal needs and lower serum neutrophil levels. In pathway B, periodontal disease is associated with lower serum vitamin C levels and higher serum neutrophil concentrations. The directionality of my findings – lower neutrophil concentration with food insecurity, but higher neutrophil concentration with periodontal disease – may seem discrepant. However, immune dysregulation does not involve mutually exclusive immune suppression or hyperreactivity. Rather, a person's immune response may be suppressed in certain instances and hyperreactive in others, per biocultural exposures and trade-offs in energy allocation (Boguniewicz & Leung, 2011; Edes & Crews, 2017; Liston, Enders, & Siggs, 2008).

Thus, a person with a dysregulated immune system could experience heightened susceptibility to infection, as well as heightened tissue damage upon infection. The effects of periodontal disease on neutrophils possibly indicate immune dysregulation, consistent with oral focal infection theory. Immune dysregulation caused by periodontal disease may be compounded by other influences such as psychosocial stress.

If it were true that stress-induced immune dysregulation compounds the immune dysregulation caused by chronic periodontal infection, people who experience food insecurity and periodontal disease would have higher serum neutrophils than people who have periodontal disease but are food secure. I tested this using a multiple regression model with an interaction term between periodontal needs and household food security and the same covariates as in the previously described regression models (Table 8.1, with the addition of vitamin C).

The interaction between food security and periodontal needs did not have a significant effect on log-transformed serum neutrophil levels (0.015 ± 0.021, $p = 0.48$). Therefore, these data do not support that people who are food insecure mount a higher immune response to periodontal disease as measured by serum neutrophil levels. The null results regarding an interaction between stress and the magnitude of neutrophil response may be an artifact of using cross-sectional data, as there are temporal elements to stress-induced immune dysregulation that cannot be gauged with cross-sectional data (e.g. the magnitude and duration of heightened glucocorticoid production during stress), or the results may suggest that other mechanisms connect food security and periodontal needs. In sum, the results of this paper indicate psychosocial stress associated with food insecurity possibly increases the risk of periodontal disease (though the mechanism remains to be identified). Periodontal disease, in turn, puts a strain on the body's finite micronutrient resources through continued immune activation.

8.5 Conclusion

The models I used to test theoretical pathway A (Figure 8.1) identify a relationship between psychosocial stress and periodontal disease, though they did not indicate that immune function (of the immune cell counts available through NHANES) mediates this relationship. The models used to test theoretical pathway B (Figure 8.2) identify a relationship between periodontal disease and serum vitamin C levels and indicate that this association may be partly mediated by serum measurements of neutrophils.

NHANES data are cross-sectional, and the investigation of causal and mediated relationships is best informed by longitudinal and/or randomized control trial studies. These findings on periodontal disease expression should be viewed as models that could be further tested and refined using expanded variable sets from additional research design formats.

Research design that includes variables on early life exposures would be particularly useful to critically test these models. Early-life exposures that alter resource-allocation to different developmental pathways may affect a person's later-life capacity for and regulatory mechanisms of a neuroendocrine immune stress response (McDade, 2005). Bioarchaeological research design has the potential to measure and compare early-life indicators to later-life outcomes within biocultural contexts and is therefore well-suited to test the models identified in this study (Gowland, 2015; Temple, 2019).

Research on the complex etiology and sequelae of periodontal disease is productive for understanding how humans embody their adaptations to biocultural contexts, and re-allocate physiological resources to mitigate stress (Abrams & Miller, 2011; Longman et al., 2018; McDade, 2003; McNamara & Buchanan, 2005). Testing the pathways that connect periodontal disease to biocultural exposures and to whole-body systems will improve the application and potential of the periodontal disease phenotype. Datasets such as NHANES and tools such as causal mediation analysis are productive avenues to test theoretical assumptions and refine models for experimental research design. More research into the causes and effects of periodontal disease, as well as standard data collection on its presence and severity, will allow direct comparison between past and present populations and will therefore elucidate evolutionary trends in the relationship between immune function, oral microbial communities, nutrition, and disease

CHAPTER 9: DISSERTATION SYNTHESIS

The research reported in this dissertation includes stable isotope analysis, paleopathology, and contemporary nutrition and health data in order to investigate how colonialism affected the nutrition of Siouan communities who lived in the Piedmont region of North Carolina and Virginia between AD 800 and 1710. This span of time included the early (AD 800–1200) and late (AD 1200–1620) Late Woodland periods, and the early (AD 1620–1670) and late (AD 1670–1710) Colonial periods.

During the Late Woodland period, Siouan groups gradually transitioned from seasonal settlements to the permanent settlements more characteristic of the latter half of the Late Woodland. They also likely focused on intensive maize agriculture, built palisades around their settlements, and may have experienced climate unpredictability (Holm, 1994; D. W. Stahle et al., 1988; H. T. Ward & Davis, 1999, 2001).

Siouan groups who lived during the early Colonial period engaged in indirect trade with European colonists (H. T. Ward & Davis, 1993, 1999). Groups who lived during the late Colonial period traded directly with Europeans and endured threats of raiding and violence due to European commercial enslavement and trade of captive Indigenous people. They merged with other groups and/or migrated elsewhere in response to socioeconomic opportunities, epidemic disease, and heightened violence (Davis, 2002; Eastman, 1999; Merrell, 2009; H. T. Ward & Davis, 1993).

I found that the prevalence of people affected by lesions on the greater wing of the sphenoid, likely indicative of chewing muscle hemorrhage associated with vitamin C deficiency, increased in the Colonial period compared to the Late Woodland, especially in the late Colonial period. Several factors may have intersected to influence the prevalence of vitamin C deficiency, including a possible shift in foodways, as well as immune system stress related to introduced infectious diseases. Each of the papers included in this dissertation contribute to the study of nutritional change among Siouan groups over time, and I outline the specific contributions of the individual papers below.

I used dental calculus, a secondary biomaterial that accumulates on teeth and is primarily composed of bacteria, as a substitute for bone for stable isotope analysis. Chapter 5 (Paper 1) presented results from the stable isotope analysis of dental calculus, which indicate that all three isotopic measurements used to reconstruct diet – $\delta^{13}C_{carbonate}$, $\delta^{13}C_{organic}$, and $\delta^{15}N$ – are significantly associated with bone $\delta^{13}C_{carbonate}$, $\delta^{13}C_{collagen}$, and $\delta^{15}N$. The significant association between calculus and bone highlights the potential of calculus as an ethical alternative to bone in dietary reconstruction via stable isotope analysis.

Chapter 6 (Paper 2) applied isotopic analysis of dental calculus to compare the dietary composition of Piedmont Siouan people who lived in different areas of the Piedmont and during different time periods. Results indicate that $\delta^{13}C_{carbonate}$ and $\delta^{13}C_{organic}$ most differentiated groups, and likely correspond to differences in degree of maize consumption. Of the people whose diets were measured through stable isotope analysis, those who lived in the Dan River drainage consumed more C_4 energy, likely maize, than people who lived in the Eno River drainage, which is consistent with Roark's (2020) archaeobotanical findings.

There is no evidence to support that maize increased over time in the Dan River drainage, as Upper Saratown (Sk1a) inhabitants from the late Colonial period had lower $\delta^{13}C_{carbonate}$ than people from Stockton (Vir231, AD 1300–1400) and the one tested individual from the Madison site (Rk6, early Colonial). For Eno River groups, it is possible that maize consumption was higher among late Colonial Fredricks site inhabitants than terminal Late Woodland Wall site inhabitants, but the sample size is small and additional corroborating evidence is therefore needed. Colonial period Upper Saratown individuals show more terrestrial protein than earlier Dan River groups (possibly lower trophic level protein such as herbivores), while Fredricks individuals were eating higher trophic level terrestrial protein (possibly reptiles) compared to contemporaneous Upper Saratown individuals.

Chapter 7 (Paper 3) examined the association between dietary composition, skeletal lesions that indicate an immune system response, and nutritional lesions. Sphenoid lesions, which I interpret as indicative of nutritional deficiency (likely including vitamin C deficiency), were significantly more prevalent in people from the late Colonial period. The results also show that, for the Piedmont skeletal sample, sphenoid lesions are associated with high $\delta^{13}C_{carbonate}$, which probably represents high maize consumption. However, high maize consumption does not explain all of the manifestations of sphenoid lesions. The high prevalence of sphenoid lesions in the late Colonial period likely had multiple influences.

I also tested lesions indicative of immunostimulation and found that sphenoid lesions are associated with periodontal disease as well as LEH, a manifestation of episodic growth disruption during early life. Like isotopic evidence of maize consumption, periodontal disease and linear enamel hypoplasia are not consistently higher in the late Colonial period than earlier temporal periods. I suggest that there are likely missing covariates that affect dietary nutritional

availability and/or peoples' nutritional needs or absorption. Perhaps there was a shift in foodways practices that affected the micronutrient content of foods, or there was an immunomodulatory factor that affected the magnitude of the immune response and thus its nutrient requirements.

Chapter 8 (Paper 4) used soft tissue and serum measurements from living people recorded in the National Health and Nutritional Examination Survey (2003-2004) to support the finding that periodontal disease is associated with nutrition, particularly vitamin C, and that this relationship is likely mediated by the immune system. People with periodontal disease have significantly lower vitamin C levels, and neutrophils – an immune cell that uses vitamin C to function – may mediate the relationship between periodontal disease and vitamin C (Brock et al., 2004; Washko et al., 1993).

The findings of this paper also indicate that food insecurity is significantly associated with periodontal disease, even after controlling for frequency of dentist care, nutrition, household income, and smoking status, and I suggest that the psychosocial stress response associated with food insecurity may dysregulate the immune response and make a person more susceptible to periodontal disease (Akcali et al., 2013; Glaser & Kiecolt-Glaser, 2005; Gowda et al., 2012; McClain et al., 2018; Wutich & Brewis, 2014). This raises interesting questions for further study regarding how compounding sources of stress may affect a person's immune response, susceptibility to infection, and severe health consequences of infection. It may also provide information about immunomodulation and nutrient needs that supports Paper 3.

9.1 Future research

It is important to note that my interpretations will likely evolve as I pursue additional research questions raised during the process of this study. Additional samples will improve the

analysis of dietary change presented in chapter 6, and my continuing research will therefore prioritize mass spectrometry methods and equipment that can accommodate smaller sample sizes of calculus, as the requisite analytical mass of dental calculus for stable isotope analysis (≥8mg) was a limiting factor. The use of stable isotope mixing models may also improve dietary interpretations, as the mixing models used to estimate dietary composition can account for the uncertainty of using dental calculus as an analytical material, since dental calculus is more heterogeneous in composition than bone collagen, which is very uniform in composition (Ambrose, 1990; Fernandes et al., 2014; Phillips et al., 2014; Warinner et al., 2015).

Stable isotope mixing models become more precise with informed priors, and information regarding not only what foods were available to past communities, but also how those foods were cooked can make mixing models more informative. Mixing models may therefore be a powerful tool to better integrate stable isotope information with other archaeological lines of information about past foodways.

Chapter 7 focuses mainly on population epidemiology to identify that sphenoid lesions, possibly indicative of vitamin C deficiency, were more prevalent in the Colonial period than earlier periods. This raises two future research questions: 1) Which members of Colonial period communities are most affected by sphenoid lesions?, and 2) How does the co-occurrence and intersection of physiological stressors affect the likelihood of nutritional deficiency? Colonialism did not uniformly affect all community members; rather, some members of communities were likely more at risk of health disparities than others (Ferris, Harrison, & Beaudoin, 2014; Klaus & Tam, 2009; Voss, 2008). It would be interesting to see how demographic patterns of health disparities changed over time from the Late Woodland period to the Colonial period.

Additionally, people with multiple intersecting sources of physiological stress may experience a different immune response and therefore more severe nutritional consequences than people with fewer sources of physiological stress (Crespo et al., 2017; McClain et al., 2018; McDade, 2005). In future research, I aim to expand my statistical models to better capture a person's immune burden. Rather than the one-to-one relationships I analyze in Chapter 7 (e.g. predicted probability that a person with periodontal disease, or with periosteal new bone, has sphenoid lesions), I could calculate an immunostimulatory index to measure peoples' different immune burdens and test the relationship between index score with vitamin C deficiency. Expanding the present study to include other populations would strengthen this aim, as sample sizes are currently too small to move beyond one-to-one relationships.

Finally, Chapter 8 raised several questions about the complex relationship between micronutrients and the immune system. For example: which micronutrients support which immune functions, and what happens when micronutrients are not in sufficient supply? Do people with evidence of early life physiological stress have altered immune responses compared to people without evidence of early life physiological stress? Does an altered immune response affect micronutrient metabolism? I will further research theoretical frameworks and purported mechanisms of nutritional immunology to better understand the complicated nexus of nutrition and the immune system.

9.2 Overall conclusions

Isotopic measurements of Late Woodland people from archaeological sites within the Dan River drainage system show that maize was an important part of Dan River Late Woodland groups' diets, which supports the interpretation that Dan River communities practiced larger scale maize agriculture than groups from other river drainages (Gremillion, 1989; H. T. Ward &

Davis, 1999). Late Woodland people from the Dan River drainage system also have a high prevalence of periosteal new bone, supporting Lambert's (2000) and Vogel's (2004) identification of nonvenereal endemic treponemal disease among the Late Woodland Piedmont Siouan groups[6]. The bacteria that cause treponemal disease are transmitted via the skin or mucous membranes, and the likelihood of contact with the disease is shaped by the local disease ecology (the intersection of climate and the susceptibility of the human population), which is influenced by immunological resilience as well as cultural practices (Bogdan & Weaver, 1992; Lambert, 2000; Powell, 2003). Both high maize consumption and immunostimulation due to chronic infections such as treponemal disease may have influenced the prevalence of orbital, parietal, and sphenoid lesions among the Late Woodland Dan River communities.

Wilson (1983) and Longo (2018) suggested that Dan River communities from the latter half of the Late Woodland period expanded their use of resources to include a more diverse subsistence base compared to earlier communities, and dietary isotopic data from the Wall site on the Eno River suggest that Wall site occupants consumed less maize than Dan River communities from the latter half of the Late Woodland period. It is therefore possible that communities from this time had more diverse diets and better nutrition than earlier Late Woodland communities. Additionally, the skeletal data do not indicate that people who lived during the second half of the Late Woodland period had a higher prevalence of immunostimulatory lesions than earlier groups (particularly for the Dan River communities);

[6] Lambert (2000:188) noted a sharp decline in the percent of people with tibiae lesions indicative of treponemal disease from the Late Woodland period to Colonial period (55 to 12%). She interpreted this possible decline in osteologically observable treponemal infections to "population resistance, evolution to less virulence, changes in the physical environment (climate change, particularly cooling), or social environment (clothing, changes in practices such as sweat baths)."

however, this does not necessarily mean that people from the early Colonial period were healthier.

While it appears that people from the terminal Late Woodland and early Colonial communities had a lower prevalence of nutrition-derived lesions than Late Woodland groups, interpretations about the early Colonial sample are complicated by small sample sizes. If the skeletal sample accurately represents the nutrition of the populations who lived at the early Colonial settlement sites, then the low prevalence of nutrition-related lesions could indicate either, a) people experienced less nutritional stress, or b) people died before they could manifest skeletal lesions of nutritional stress.

Lambert (2000:188) also noted that the proportion of people with periosteal new bone decreased across the Late Woodland period. She argued: "perhaps health improved, but the data on enamel hypoplasia do not support this hypothesis, and it may be that the changing patterns of morbidity and mortality had the effect of altering the visibility of this disease osteologically." The high proportion of deaths of children aged 0-5 compared to other age categories may support a shift in disease ecology. Overall, more research is needed to clarify the possible dietary, immune, and nutritional shifts that occurred from the across the Late Woodland into the early Colonial period.

Late Colonial communities (AD 1670–1710) show the highest proportion of sphenoid lesions, suggesting vitamin C deficiency, but have a lower prevalence of immunostimulatory lesions that earlier groups. Maize consumption was not consistently higher across all Colonial period groups, and Colonial period groups appear to have consumed relatively mixed C_3-C_4 diets, possibly indicative of dietary diversity. What, then, influenced the high prevalence of sphenoid lesions in the Colonial period? I suggest that infectious diseases as well as stressors

associated with increased geopolitical and socioeconomic risk converged to place additional demands on Colonial peoples' immune systems, which strained their physiological micronutrient resources.

Overall, the papers included in this dissertation provide several advancements in the study of diet and nutrition in past and present: 1) dental calculus is an ethical and productive alternative to bone in dietary reconstruction through stable isotope analysis, 2) psychosocial stress may be an important element to add to nutrition transition frameworks, and 3) consistent access to adequate nutrition and culturally appropriate food may prevent patterned health disparities in not only nutrition-related disease, but also susceptibility to infectious disease.

APPENDIX 1: INDIVIDUAL DATA FOR CALCULUS ISOTOPIC MEASUREMENTS

For the first two tables in this section, brackets (e.g. [Diff $\delta^{13}C$]) indicate that the absolute difference between replicates was calculated; †Replicates were from the same organic biofraction purification reaction, but were analyzed on different mass spec runs (though with the same correction standards)

Replicates for Organic Calculus Samples											
Group ID	Lab ID	$\delta^{13}C_{VPDB}$ ‰	[Diff $\delta^{13}C$]	$\delta^{15}N_{AIR}$ ‰	[Diff $\delta^{15}N$]	Wt% C	[Diff Wt%C]	Wt% N	[Diff Wt%N]	C:N	[Diff C:N]
Coastal NC	SD17	-17.021	0.37	16.103	0.24	41.51	1.40	8.60	0.43	5.63	0.10
Coastal NC	SD17rep	-16.652		16.348		40.11		8.17		5.73	
IL Flood Plain	SD186	-24.521	0.21	11.449	0.30	46.56	1.08	8.79	0.41	6.18	0.15
IL Flood Plain	SD186rep	-24.311		11.145		45.47		8.38		6.33	
IL Flood Plain	SD188	-15.968	0.24	11.239	0.19	47.40	1.58	8.82	0.34	6.27	0.03
IL Flood Plain	SD188rep	-16.210		11.429		48.98		9.16		6.24	
Modern	SD111	-23.659	0.16	11.712	0.34	53.95	0.35	9.98	0.07	6.31	0.01
Modern	SD111rep	-23.502		11.372		54.29		10.06		6.30	
Modern	SD112	-23.309	0.07	13.020	0.11	51.71	2.42	10.49	0.54	5.75	0.03
Modern	SD112rep	-23.375		12.910		54.12		11.03		5.72	
NC-VA Piedmont	SD79	-15.581	3.11	9.299	2.17	43.51	3.21	9.19	0.06	5.34	0.63
NC-VA Piedmont	SD79rep†	-18.688		11.465		46.72		9.13		5.97	
NC-VA Piedmont	SD80	-20.881	0.35	9.777	0.59	49.05	0.38	9.32	0.09	5.94	0.19
NC-VA Piedmont	SD80rep†	-21.236		10.364		49.43		9.41		6.13	
NC-VA Piedmont	SD206	-17.148	0.87	12.252	1.83	42.84	1.09	9.19	0.06	5.44	0.18
NC-VA Piedmont	SD206rep	-18.018		10.418		43.93		9.13		5.62	

Replicates for Organic Calculus Samples Continued											
Group ID	Lab ID	$\delta^{13}C_{VPDB}$ ‰	[Diff $\delta^{13}C$]	$\delta^{15}N_{AIR}$ ‰	[Diff $\delta^{15}N$]	Wt% C	[Diff Wt%C]	Wt% N	[Diff Wt%N]	C:N	[Diff C:N]
NC-VA Piedmont	SD209	-20.337	0.96	10.497	0.40	35.50	8.01	5.85	2.16	7.08	1.61
NC-VA Piedmont	SD209rep	-21.301		10.893		27.49		3.69		8.69	
NC-VA Piedmont	SD196	-18.290	0.56	10.517	0.30	42.09	1.36	8.83	0.08	5.56	0.24
NC-VA Piedmont	SD196rep	-18.848		10.220		43.44		8.75		5.80	
NC-VA Piedmont	SD197	-17.911	2.61	9.771	1.05	14.01	14.29	2.69	5.60	6.08	2.10
NC-VA Piedmont	SD197rep	-15.298		8.717		28.29		8.29		3.98	
NC-VA Piedmont	SD74	-20.266	0.34	10.381	0.20	45.17	1.34	9.22	0.80	5.53	0.29
NC-VA Piedmont	SD74rep	-19.928		10.178		46.51		10.02		5.24	
Average			0.82		0.64		3.04		0.89		0.46
sd			1.00		0.68		4.09		1.60		0.68
Median			0.36		0.32		1.38		0.38		0.19
IQR			0.23 - 0.89		0.23 - 0.70		1.09 - 2.62		0.08 - 0.60		0.08 - 0.38
Min			0.07		0.11		0.35		0.06		0.01
Max			3.11		2.17		14.29		5.60		2.10

Mineral Biofraction Sample Replicates					
Group ID	Lab ID	$\delta^{13}C_{VPDB}$ ‰	[Diff $\delta^{13}C$]	Wt% C	[Diff Wt%C]
Coastal NC	SD62	-6.183	0.12	0.88	0.03
Coastal NC	SD62rep	-6.063		0.91	
IL Flood Plain	SD179	-0.086	0.34	4.48	0.75
IL Flood Plain	SD179rep	-0.426		3.73	
IL Flood Plain	SD191	-8.764	0.06	0.69	0.09
IL Flood Plain	SD191rep	-8.827		0.78	
IL Flood Plain	SD177	-1.987	0.00	0.55	0.01
IL Flood Plain	SD177rep	-1.990		0.56	
Historic NC	SD96	-6.665	0.14	0.64	0.15
Historic NC	SD96rep†	-6.523		0.49	
Modern	SD116	-11.719	0.11	0.47	0.03
Modern	SD116rep	-11.831		0.44	
NC-VA Piedmont	SD92	-7.554	0.31	0.50	0.11
NC-VA Piedmont	SD92rep†	-7.862		0.39	
NC-VA Piedmont	SD220	-4.039	0.15	0.34	0.00
NC-VA Piedmont	SD220rep	-3.890		0.34	
NC-VA Piedmont	SD235	-5.026	0.07	0.48	0.35
NC-VA Piedmont	SD235rep	-4.951		0.84	
NC-VA Piedmont	SD212	-6.348	0.09	0.60	0.12
NC-VA Piedmont	SD212rep	-6.440		0.48	
Bolivia Lake Shore	SD162	-9.673	0.08	0.26	0.01
Bolivia Lake Shore	SD162rep	-9.593		0.25	
Average (sd)			0.13 (0.10)		0.15 (0.22)
Median (min, max)			0.11 (0.0, 0.34)		0.09 (0.0, 0.75)
IQR			0.08 - 0.15		0.02 - 0.14

Organic Biofraction Samples													
Group ID	Calculus isotopic measurements									Bone isotopic measurements		Calculus-bone spacing	
	Lab ID	$\delta^{13}C_{VPDB}$ ‰	$\delta^{15}N_{AIR}$ ‰	Wt% C	VM44	Wt% N	VM28	C:N	%Recov	$\delta^{13}C_{VPDB}$ ‰§	$\delta^{15}N_{AIR}$ ‰§	$\delta^{13}C$	$\delta^{15}N$
Coastal NC	SD9	-16.48	16.48	44.23	2.03	9.21	0.60	5.61	4.30	-11.3	14.9	-5.15	1.58
Coastal NC	SD11	-16.66	17.32	45.27	2.07	9.55	0.62	5.53	3.33	-11.5	14.7	-5.16	2.62
Coastal NC	SD15	-16.39	16.36	37.91	1.93	8.23	0.60	5.37	6.85	-11.0	14.9	-5.39	1.46
Coastal NC	SD16	-15.30	15.71	45.56	2.34	10.10	0.75	5.26	5.19	-11.2	14.9	-4.21	0.91
Coastal NC	SD7	-16.07	16.40	51.87	4.36	10.91	1.38	5.54	5.58	-11.5	14.2	-4.57	2.20
Coastal NC	SD8	-16.69	15.52	50.72	3.31	10.84	1.06	5.46	5.85	-12.5	14.5	-4.19	1.02
Coastal NC	SD14	-17.27	16.64	46.87	2.22	9.69	0.68	5.64	5.94	-	-	-	-
Coastal NC	SD17†	-16.84	16.23	40.81	2.01	8.39	0.59	5.68	3.93	-	-	-	-
Coastal NC	SD18	-17.06	14.90	42.65	1.80	9.02	0.54	5.52	4.76	-	-	-	-
Coastal NC	SD19	-17.61	16.21	41.43	2.26	8.96	0.70	5.39	3.94	-19.6	12.4	1.99	3.81
Coastal NC	SD12	-18.12	14.21	48.31	2.45	8.91	0.65	6.33	5.00	-	-	-	-
IL Flood Plain	SD170	-14.31	10.61	28.29	2.28	5.05	0.66	6.53	6.25	-9.79	9.65	-4.52	0.95
IL Flood Plain	SD167	-20.52	11.28	40.62	2.36	6.98	0.66	6.79	2.38	-10.94	9.05	-9.58	2.23
IL Flood Plain	SD171	-15.15	8.50	41.99	3.14	8.91	1.09	5.50	6.00	-11.45	8.21	-3.70	0.29
IL Flood Plain	SD184	-14.54	10.72	47.62	3.44	9.19	1.22	6.05	3.66	-10.75	8.21	-3.78	2.51
IL Flood Plain	SD185	-23.76	10.25	47.18	2.73	9.76	1.03	5.64	4.74	-20.16	7.80	-3.60	2.45
IL Flood Plain	SD186†	-24.43	11.14	46.02	2.66	8.59	0.91	6.26	3.94	-20.55	7.43	-3.97	4.02
IL Flood Plain	SD187	-25.14	12.84	47.47	2.99	7.60	0.88	7.28	6.82	-21.15	9.22	-3.99	3.62
IL Flood Plain	SD188†	-16.03	11.49	48.19	3.15	8.99	1.08	6.26	3.98	-12.03	10.21	-3.94	1.03
IL Flood Plain	SD168	-17.98	11.40	37.98	2.47	5.89	0.62	7.52	8.00	-9.85	9.45	-8.13	1.95
Historic NC	SD58	-19.77	13.67	47.90	1.70	8.18	0.41	6.83	3.85	-15.14	10.70	-4.63	2.98

Organic Biofraction Samples Continued													
Group ID	Calculus isotopic measurements									Bone isotopic measurements		Calculus-bone spacing	
	Lab ID	$\delta^{13}C_{VPDB}$ ‰	$\delta^{15}N_{AIR}$ ‰	Wt% C	VM44	Wt% N	VM28	C:N	%Recov	$\delta^{13}C_{VPDB}$ ‰[§]	$\delta^{15}N_{AIR}$ ‰[§]	$\delta^{13}C$	$\delta^{15}N$
Historic NC	SD159	-22.79	10.84	21.37	2.01	10.60	0.85	4.44	4.92	-14.17	11.06	-8.63	-0.23
Historic NC	SD59	-18.82	11.69	46.20	1.99	7.75	0.48	6.96	2.79	-13.14	11.16	-5.67	0.53
Modern	SD106	-24.28	14.01	53.85	3.55	10.16	1.29	6.18	10.41	-19.52	11.99	-4.76	2.02
Modern	SD107	-25.78	11.81	51.62	3.71	10.97	1.49	5.49	8.37	-20.20	10.67	-5.58	1.14
Modern	SD108	-24.03	10.61	51.45	3.53	11.38	1.45	5.28	7.41	-20.55	10.37	-3.48	0.24
Modern	SD109	-24.12	10.40	52.35	3.47	10.91	1.38	5.60	6.80	-19.08	10.07	-5.04	0.33
Modern	SD110	-22.38	14.34	52.90	3.28	11.33	1.35	5.45	6.80	-19.54	11.73	-2.84	2.61
Modern	SD111[†]	-23.58	11.54	54.12	3.72	10.02	1.32	6.31	6.12	-	-	-	-
Modern	SD112[+]	-23.34	12.96	52.91	3.55	10.76	1.39	5.74	6.57	-20.30	10.49	-3.01	2.53
Modern	SD165	-22.89	13.01	50.64	3.84	10.14	1.26	5.82	7.08	-19.05	8.83	-3.84	4.18
Modern	SD113	-21.95	14.12	53.06	3.58	11.52	1.49	5.37	6.22	-17.72	12.32	-4.23	1.80
NC-VA Piedmont	SD74[‡]	-20.14	9.83	46.51	2.88	10.20	1.33	5.23	6.73	-	-	-	-
NC-VA Piedmont	SD75	-15.30	10.35	46.30	3.25	10.28	1.64	5.08	4.55	-	-	-	-
NC-VA Piedmont	SD76	-18.94	10.59	46.82	3.15	8.56	1.30	6.17	6.86	-	-	-	-
NC-VA Piedmont	SD77	-20.72	10.74	51.24	3.83	8.27	1.41	7.00	6.52	-	-	-	-
NC-VA Piedmont	SD78	-20.78	10.88	45.88	3.00	8.12	1.19	6.38	7.79	-	-	-	-
NC-VA Piedmont	SD79[†]	-17.13	10.43	45.11	3.53	9.16	1.48	5.66	4.58	-	-	-	-
NC-VA Piedmont	SD80[†]	-21.06	10.12	49.24	3.57	9.36	1.42	6.04	5.91	-	-	-	-
NC-VA Piedmont	SD206[†]	-17.58	11.34	43.39	2.95	9.16	1.20	5.53	5.51	-	-	-	-
NC-VA Piedmont	SD207	-18.49	9.93	42.20	3.10	8.22	1.16	5.99	9.18	-	-	-	-
NC-VA Piedmont	SD208	-21.40	10.70	38.20	3.24	7.90	1.30	5.64	6.53	-	-	-	-
NC-VA Piedmont	SD209[†]	-20.82	10.70	31.49	2.17	4.77	0.64	7.89	4.60	-	-	-	-
NC-VA Piedmont	SD222	-20.61	10.67	12.21	0.92	1.84	0.28	7.74	20.37	-	-	-	-

Organic Biofraction Samples Continued													
Group ID	Calculus isotopic measurements									Bone isotopic measurements		Calculus-bone spacing	
	Lab ID	$\delta^{13}C_{VPDB}$ ‰	$\delta^{15}N_{AIR}$ ‰	Wt% C	VM44	Wt% N	VM28	C:N	%Recov	$\delta^{13}C_{VPDB}$ ‰[§]	$\delta^{15}N_{AIR}$ ‰[§]	$\delta^{13}C$	$\delta^{15}N$
NC-VA Piedmont	SD228	-14.77	9.40	37.96	3.45	8.23	1.45	5.38	8.43	-	-	-	-
NC-VA Piedmont	SD224	-21.48	10.77	19.20	1.40	2.41	0.35	9.30	15.80	-	-	-	-
NC-VA Piedmont	SD195	-19.50	11.03	25.53	1.46	4.91	0.66	6.07	5.47	-	-	-	-
NC-VA Piedmont	SD196[†]	-18.57	10.37	42.77	2.48	8.79	1.21	5.68	5.47	-	-	-	-
NC-VA Piedmont	SD197[†]	-16.61	9.24	21.15	1.31	5.49	0.82	5.03	9.09	-	-	-	-
NC-VA Piedmont	SD198	-19.42	11.24	25.82	1.40	4.80	0.61	6.27	7.22	-	-	-	-
NC-VA Piedmont	SD199	-19.74	10.41	42.40	2.61	8.96	1.32	5.52	6.41	-	-	-	-
NC-VA Piedmont	SD205	-22.53	11.22	34.07	1.76	7.87	0.96	5.05	6.52	-	-	-	-

[†] = calculus sample is average of 2 replicates; [‡]= calculus sample is average of 3 replicates; [§]published values had a variable number of places after the decimal

Mineral Biofraction Samples									
Group ID	Calculus isotopic measurements						Bone isotopic measurements		Calculus-bone spacing
	Lab ID	$\delta^{13}C_{VPDB}$ ‰	Wt% C	VM44	%Recov	$\Delta^{13}C_{ca\text{-}org}$	$\delta^{13}C_{VPDB}$ ‰‡	$\Delta^{13}C_{ca\text{-}coll}$	$\delta^{13}C$
Coastal NC	SD3	-4.38	0.76	2.76	76.09	11.69	-6.8	4.7	2.4
Coastal NC	SD64	-5.52	0.84	1.21	77.27	11.17	-7.1	5.4	1.6
Coastal NC	SD4	-5.65	0.54	2.26	73.53	-	-7.6	3.3	2.0
Coastal NC	SD60	-4.15	0.67	1.46	74.19	-	-5.8	4.5	1.7
Coastal NC	SD61	-6.66	0.55	1.47	70.37	-	-7.0	4.2	0.34
Coastal NC	SD62†	-6.12	0.90	1.28	74.19	-	-5.8	5.0	-0.32
Coastal NC	SD63	-4.38	0.54	1.23	79.41	-	-7.0	4.4	2.6
Coastal NC	SD65	-6.25	0.63	1.45	75.41	-	-6.7	5.1	0.45
Coastal NC	SD67	-7.17	0.56	1.41	72.97	-	-7.3	6.7	0.13
IL Flood Plain	SD179†	-0.26	4.11	5.60	68.00	14.05	-1.96	7.82	1.71
IL Flood Plain	SD176	-7.49	0.67	2.58	62.16	13.02	-6.01	4.93	-1.48
IL Flood Plain	SD180	-4.62	0.66	2.16	54.84	10.54	-4.90	6.56	0.28
IL Flood Plain	SD190	-1.55	0.64	2.60	80.00	12.98	-5.41	5.34	3.86
IL Flood Plain	SD191†	-8.80	0.74	2.88	79.17	14.96	-10.95	9.21	2.16
IL Flood Plain	SD192	-8.02	0.49	2.16	81.58	16.41	-11.42	9.13	3.41
IL Flood Plain	SD193	-9.19	0.47	1.88	76.47	15.94	-9.01	12.14	-0.18
IL Flood Plain	SD194	-3.80	0.48	1.91	82.69	12.23	-6.83	5.19	3.04
IL Flood Plain	SD177†	-1.99	0.56	2.05	70.97	15.99	-3.63	6.22	1.64
Historic NC	SD96†	-6.59	0.57	1.97	70.37	12.22	-9.21	3.93	2.62
Modern	SD114	-12.51	0.59	1.98	63.79	11.78	-15.16	4.37	2.65
Modern	SD115	-12.79	0.25	1.13	64.29	12.98	-15.49	4.71	2.70
Modern	SD116†	-11.78	0.46	1.53	63.46	12.26	-15.48	5.07	3.71
Modern	SD117	-10.68	0.40	1.52	68.52	13.45	-14.77	4.31	4.10

Mineral Biofraction Samples Continued									
Group ID	Calculus isotopic measurements						Bone isotopic measurements		Calculus-bone spacing
	Lab ID	$\delta^{13}C_{VPDB}$ ‰	Wt% C	VM44	%Recov	$\Delta^{13}C_{ca\text{-}org}$	$\delta^{13}C_{VPDB}$ ‰‡	$\Delta^{13}C_{ca\text{-}coll}$	$\delta^{13}C$
Modern	SD118	-9.92	0.19	0.93	62.07	12.47	-15.03	4.51	5.11
Modern	SD119	-11.24	0.49	1.65	73.47	12.34	-	-	-
Modern	SD120	-12.07	0.52	1.65	69.77	11.28	-15.36	4.92	3.29
Modern	SD174	-7.93	0.44	1.67	59.26	14.96	-14.23	4.82	6.30
Modern	SD121	-11.280	0.52	1.73	68.33	10.68	-13.01	4.71	1.73
NC-VA Piedmont	SD89	-7.83	0.93	2.59	63.16	12.31	-	-	-
NC-VA Piedmont	SD90	-3.71	0.77	2.21	75.44	11.59	-	-	-
NC-VA Piedmont	SD91	-6.08	0.83	2.13	74.6	12.87	-	-	-
NC-VA Piedmont	SD92†	-7.71	0.45	1.58	71.43	13.01	-	-	-
NC-VA Piedmont	SD93	-9.22	0.56	1.74	72.73	11.56	-	-	-
NC-VA Piedmont	SD94	-3.19	0.61	1.89	75.00	13.93	-	-	-
NC-VA Piedmont	SD95	-9.50	0.57	1.87	68.75	11.56	-	-	-
NC-VA Piedmont	SD2	-6.97	0.42	1.88	78.79	15.79	-	-	-
NC-VA Piedmont	SD1	-8.07	0.49	1.93	80.00	-	-	-	-
NC-VA Piedmont	SD217	-8.15	0.50	1.95	73.53	9.44	-	-	-
NC-VA Piedmont	SD218	-8.23	0.52	1.88	67.74	10.26	-	-	-
NC-VA Piedmont	SD219	-9.55	0.47	1.87	76.14	11.85	-	-	-
NC-VA Piedmont	SD220†	-3.96	0.34	1.46	59.38	16.85	-	-	-
NC-VA Piedmont	SD235†	-4.99	0.66	2.17	64.71	9.78	-	-	-
NC-VA Piedmont	SD211	-5.59	0.72	2.77	64.52	13.91	-	-	-
NC-VA Piedmont	SD212†	-6.39	0.54	2.05	59.46	12.18	-	-	-
NC-VA Piedmont	SD213	-3.88	0.19	1.12	70.37	12.73	-	-	-
NC-VA Piedmont	SD214	-4.26	0.40	1.70	64.00	15.16	-	-	-

Mineral Biofraction Samples Continued									
Group ID	Calculus isotopic measurements						Bone isotopic measurements		Calculus-bone spacing
	Lab ID	$\delta^{13}C_{VPDB}$ ‰	Wt% C	VM44	%Recov	$\Delta^{13}C_{ca\text{-}org}$	$\delta^{13}C_{VPDB}$ ‰‡	$\Delta^{13}C_{ca\text{-}coll}$	$\delta^{13}C$
NC-VA Piedmont	SD215	-7.15	0.69	2.286	62.07	12.59	-	-	-
NC-VA Piedmont	SD216	-10.89	0.53	2.054	36.59	11.64	-	-	-
Bolivia Lake Shore	SD161	-9.61	0.31	1.471	80.00	-	-13.9	4.8	4.3
Bolivia Lake Shore	SD162†	-9.63	0.26	1.304	79.07	-	-13.8	4.8	4.2
Bolivia Lake Shore	SD164	-9.77	0.56	2.280	73.08	-	-14.8	3.7	5.0
Bolivia Lake Shore	SD163	-10.64	0.44	1.864	71.43	-	-	-	-

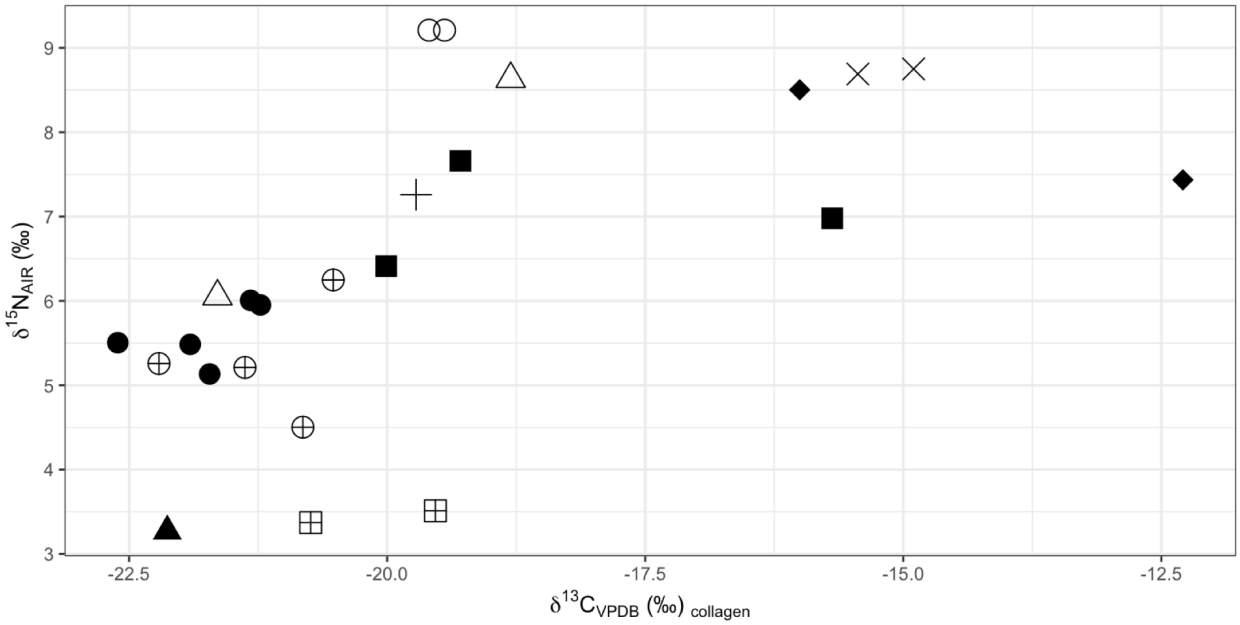
† = calculus sample is average of 2 replicates; ‡published values had a variable number of places after the decimal

APPENDIX 2: PIEDMONT PLANT AND ANIMAL DATA

Interpretive Baseline Samples					
Lab ID	Common name	Scientific name	$\delta^{13}C$	$\delta^{15}N$	Material
DH703	Beaver	*Castor canadensis*	-25.40	3.00	flesh
DH744	Beaver	*Castor canadensis*	-25.37	5.70	bone
DH603	Beaver	*Castor canadensis*	-24.44	1.29	flesh
DH681	Fish	unID	-16.00	8.50	bone
DH682	Fish	unID	-12.29	7.44	bone
DH748	Largemouth bass	*Micropterus salmoides*	-15.44	8.69	bone
DH749	Largemouth bass	*Micropterus salmoides*	-14.90	8.75	bone
DH629	Burdock	*Arctium sp.*	-29.21	1.96	plant
DH653	Chenopod leaves	*Chenopodium album*	-29.81	6.87	plant
DH657	Dandelion greens	*Taraxacum sp.*	-29.89	5.96	plant
DH689	Dandelion greens	*Taraxacum sp.*	-31.84	1.21	plant
DH624	Fiddlehead	*Polystichum acrostichoides*	-29.25	-0.91	plant
DH635	Fiddlehead	*Polystichum acrostichoides*	-29.36	-2.60	plant
DH628	Violet	*Viola sororia*	-29.43	2.52	plant
DH652	Pokeweed	*Phytolacca decandra*	-31.73	6.15	plant
DH636	Sorrel	*Rumex acetosa*	-29.52	0.19	plant
DH633	Wild mustard	*Sinapis arvensis*	-28.54	3.12	plant
DH640	Deer	*Odocoileus virginianus*	-21.32	6.01	bone
DH646	Deer	*Odocoileus virginianus*	-21.72	5.13	bone
DH647	Deer	*Odocoileus virginianus*	-21.91	5.48	bone
DH648	Deer	*Odocoileus virginianus*	-21.23	5.95	bone
DH680	Deer	*Odocoileus virginianus*	-22.61	5.50	bone
DH698	Deer	*Odocoileus virginianus*	-24.62	2.51	flesh
DH637	Dog	*Canis lupus*	-18.80	8.63	bone
DH645	Dog	*Canis lupus*	-21.64	6.05	bone
DH644	Duck	*Anatidae sp.*	-19.72	7.26	bone
DH700	Gray fox	*Urocyon cinereoargenteus*	-21.23	7.05	flesh
DH743	Red fox	*Vulpes vulpes*	-20.25	6.65	flesh
DH683	Largemouth bass	*Micropterus salmoides*	-29.24	14.36	flesh
DH684	Largemouth bass	*Micropterus salmoides*	-28.95	15.07	flesh
DH612	Bull chub	*Nocomis raneyi*	-25.69	9.43	flesh
DH622	Bluehead chub	*Nocomis leptocephalus*	-23.62	12.40	flesh
DH665	Bull chub	*Nocomis raneyi*	-24.29	9.75	flesh
DH666	Bull chub	*Nocomis raneyi*	-23.23	11.37	flesh
DH608	Bluegill	*Lepomis macrochirus*	-24.74	8.42	flesh
DH609	Sunfish	*Lepomis auritus*	-26.91	10.81	flesh
DH684	Largemouth bass	*Micropterus salmoides*	-29.09	15.44	flesh
DH685	Largemouth bass	*Micropterus salmoides*	-29.68	13.55	flesh
DH616	Black walnut	*Juglans nigra*	-29.61	1.07	plant

Interpretive Baseline Samples Continued					
Lab ID	Common name	Scientific name	$\delta^{13}C$	$\delta^{15}N$	Material
DH605	Hickory nuts	*Carya sp.*	-28.96	0.78	plant
DH604	Oak acorns	*Quercus rubra*	-27.06	1.45	plant
DH617	Oak acorns	*Quercus rubra*	-31.34	0.24	plant
DH615	Black walnut	*Juglans nigra*	-32.31	-0.18	plant
DH650	Hickory nuts	*Carya sp.*	-30.42	1.87	plant
DH701	Opossum	*Didelphis sp.*	-25.57	4.30	flesh
DH679	Passenger pidgeon	*Ectopistes migratorius*	-22.13	3.27	bone
DH642	Raccoon	*Procyon lotor*	-20.01	6.41	bone
DH670	Raccoon	*Procyon lotor*	-15.69	6.98	bone
DH747	Raccoon	*Procyon lotor*	-19.29	7.66	bone
DH695	Snake	unID (non-poisonous)	-19.19	8.13	bone
DH746	Snake	unID (poisonous)	-19.58	8.53	bone
DH673	Squirrel	*Sciurus carolinensis*	-19.53	3.51	bone
DH674	Squirrel	*Sciurus carolinensis*	-20.74	3.37	bone
DH639	Turkey	*Meleagris gallopavo*	-20.52	6.25	bone
DH643	Turkey	*Meleagris gallopavo*	-21.38	5.21	bone
DH671	Turkey	*Meleagris gallopavo*	-20.82	4.50	bone
DH694	Turkey	*Meleagris gallopavo*	-22.21	5.26	bone
DH704	Turkey	*Meleagris gallopavo*	-17.22	6.50	flesh
DH641	Box turtle	*Terrapene sp.*	-19.44	9.21	bone
DH693	Box turtle	*Terrapene sp.*	-19.59	9.21	bone
DH607	Striped mud turtle	*Kinosternon baurii*	-29.49	7.84	flesh
DH678	Turtle	unID	-19.54	9.64	bone
DH696	Turtle	unID	-19.60	9.82	bone
DH697	Turtle	unID	-21.73	6.01	bone
DH763	Maize	*Zea mays*	-11.13	1.12	plant
DH764	Maize	*Zea mays*	-11.31	0.13	plant
DH764	Maize	*Zea mays*	-11.15	0.25	plant

Collagen $\delta^{15}N$ vs. $\delta^{13}C$ of archaeological faunal bone samples

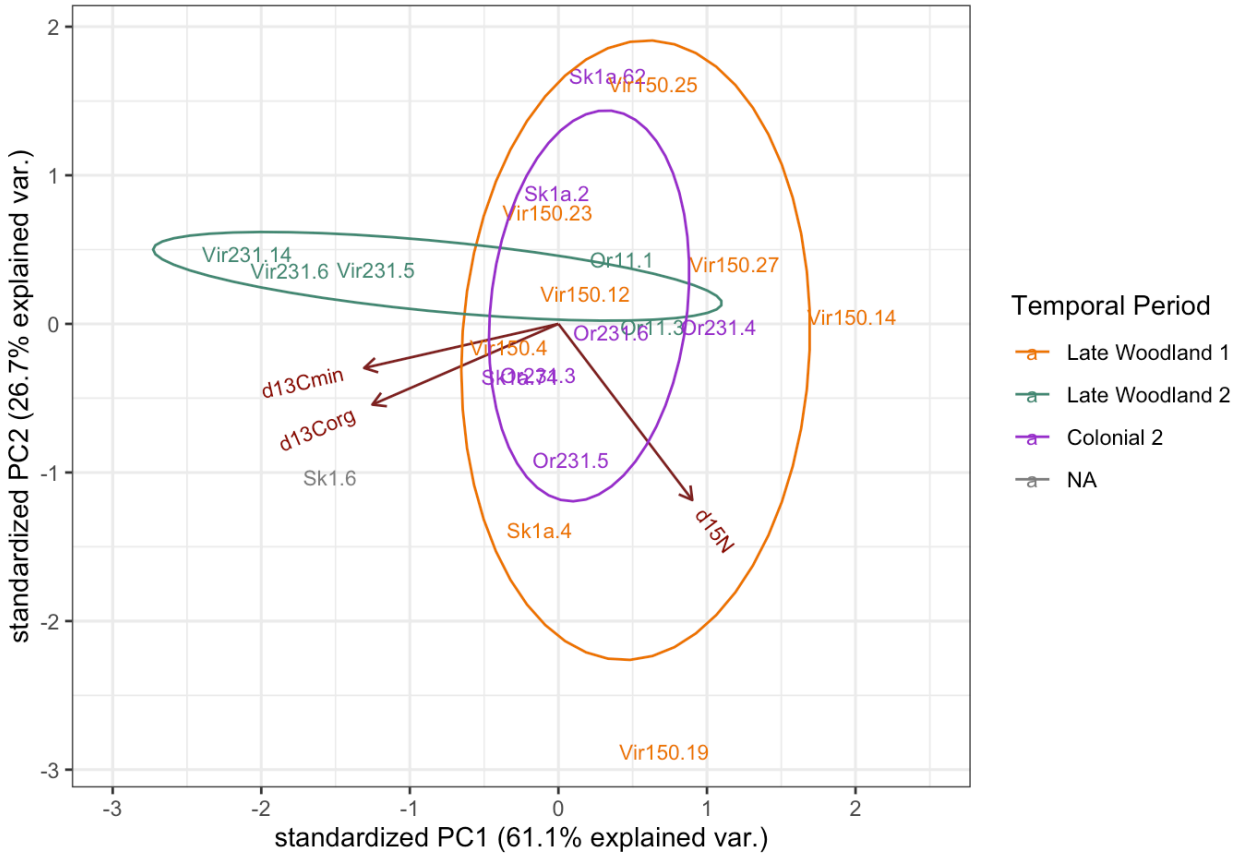


Species
- Box turtle *(Terrapene sp.)*
- Deer *(Odocoileus virginianus)*
- Dog *(Canis lupus)*
- Duck *(Anatidae sp.)*
- Fish *(Micropterus salmoides)*
- Fish *(UnID)*
- Passenger pidgeon *(Ectopistes migratorius)*
- Raccoon *(Procyon lotor)*
- Squirrel *(Sciurus carolinensis)*
- Turkey *(Meleagris gallopavo)*

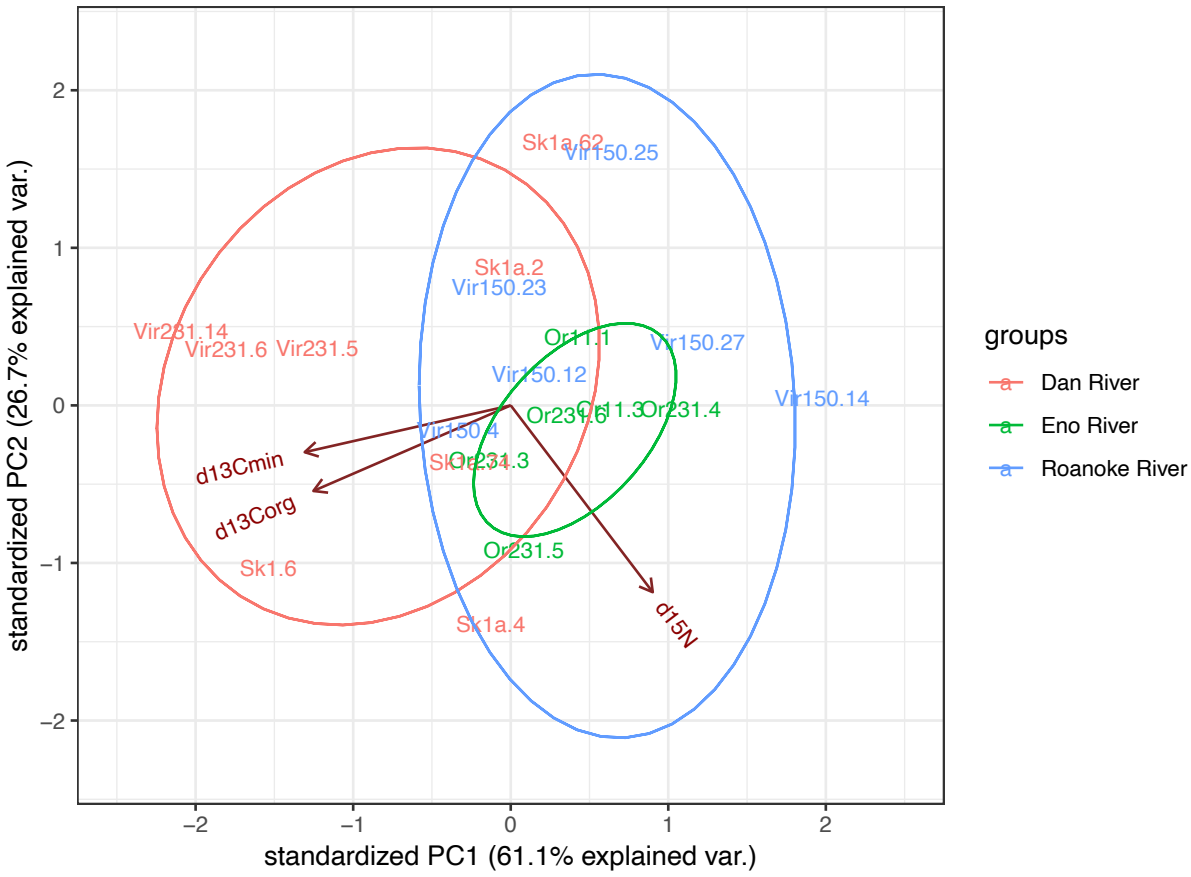
APPENDIX 3: RESULTS FROM PRINCIPAL COMPONENTS ANALYSIS WITH VIR150 INCLUDED

Results of principal components analysis with people from Vir150 included in the sample			
	PC1	PC2	PC3
Standard deviation	1.325	0.90	0.60
Proportion of variance	0.61	0.27	0.12
Cumulative proportion	0.61	0.88	1.000
$\delta^{15}N$	0.45	-0.89	-0.13
$\delta^{13}C$ carbonate	-0.65	-0.22	-0.73
$\delta^{13}C$ organic	-0.62	-0.41	0.67

Principal components plot, with people from Vir150 included in sample. Ellipses represent temporal periods



Principal components plot, people from Vir150 included in sample. Ellipses represent river drainage affiliation



APPENDIX 4: DEMOGRAPHIC AND DISEASE DATA BY INDIVIDUAL

Chapter 7 Sample by Individual, Including Demographic Information and Lesion/Measurement Presence (Marked as X)

Temporal Pd.	Site	Burial	River	Date	Sex	Age	PNB	Periodontal	NutrLes	VNC	LEH	LBratio	IsoRatio
LW 3	Or11	1	Eno	1500-1600	male	45-60	X	X	X	X	X		X
LW 3	Or11	2	Eno	1500-1600		2.5-3.5	X		X		X		
LW 3	Or11	2	Eno	1500-1600		11-14	X		X				
LW 3	Or11	3	Eno	1500-1600	male	35-45	X	X	X	X	X	X	X
LW 3	Or11	4	Eno	1500-1600	male	18-21	X	X	X	X	X		
Colonial 2	Or231	1	Eno	1690-1710		3.5-5.5	X		X		X		
Colonial 2	Or231	2	Eno	1690-1710		8-10	X	X	X		X		
Colonial 2	Or231	3	Eno	1690-1710	male	40-49	X	X	X	X	X		X
Colonial 2	Or231	4	Eno	1690-1710	male	20-28	X	X	X	X	X	X	X
Colonial 2	Or231	5	Eno	1690-1710	male	35-50	X	X	X	X	X	X	X
Colonial 2	Or231	6	Eno	1690-1710	female	30-44	X	X	X		X		X
Colonial 2	Or231	7	Eno	1690-1710		0-0.25	X						
Colonial 2	Or231	8	Eno	1690-1710		4-5	X		X		X		
Colonial 2	Or231	9	Eno	1690-1710	female	30-39	X	X	X	X	X		
Colonial 2	Or231	10	Eno	1690-1710		5-6	X				X		
Colonial 2	Or231	11	Eno	1690-1710		15-16	X	X	X		X		
Colonial 2	Or231	13	Eno	1690-1710	female	35-50	X	X	X		X		
LW 2	Rk12	1	Dan	1250-1450?	female	37-47	X	X	X	X	X	X	
Colonial 1	Rk6	3	Dan	1620-1670		2.5-3.5	X				X		
Colonial 1	Rk6	9	Dan	1620-1670		3-4	X		X		X		
Colonial 1	Rk6	46	Dan	1620-1670		20-30	X	X			X		

PNB = periosteal new bone, NutrLes = lesion of nutritional deficiency, VNC = vertebral neural canal measurement, LEH = linear enamel hypoplasia, LBratio = long bone ratio measurement, IsoRatio = isotopic measurements

Chapter 7 Sample by Individual, Including Demographic Information and Lesion/Measurement Presence (Marked as X) Continued													
Temporal Pd.	Site	Burial	River	Date	Sex	Age	PNB	Periodontal	NutrLes	VNC	LEH	LBratio	IsoRatio
indet	Rk6	79	Dan	indet	male	35-45	X	X	X		X		
Colonial 2	Rk6	83	Dan	1670-1710		2.5-4	X		X		X		
Colonial 1	Rk6	90	Dan	1620-1670		25-35	X	X	X		X		
Colonial 1	Rk6	107	Dan	1620-1670		25-35	X	X			X		
indet	Sk1-2342	1	Dan	indet		3-4	X		X		X		
LW 3	Sk1-2342	2	Dan	1450-1620		1-2	X		X		X		
LW 2	Sk1-2342	3	Dan	1250-1450	female	18-22	X	X	X	X	X	X	
LW 2	Sk1-2342	4	Dan	1250-1450		9-10	X	X	X		X		
indet	Sk1-2342	5	Dan	indet		0.75-1	X		X		X		
LW 2	Sk1-2342	6	Dan	1250-1450		4-6	X		X		X		
Colonial 1	Sk1-2365	2	Dan	1620-1670?		3-5	X		X		X		
Colonial 1	Sk1-2365	2	Dan	1620-1670?		35-50	X	X	X		X		
indet	Sk1-2365	3	Dan	indet	male	40-55	X	X	X		X	X	
indet	Sk1-2365	4	Dan	indet	female	30-35	X	X	X				
indet	Sk1-2365	6	Dan	indet	male	35-55	X	X	X		X		
Colonial 2	Sk1a	1	Dan	1670-1710	female	18-20	X	X	X	X	X		
Colonial 2	Sk1a	2	Dan	1670-1710	male	20-25	X	X	X	X	X	X	X
Colonial 1	Sk1a	3	Dan	1650-1670	male	40-55	X	X	X		X		

PNB = periosteal new bone, NutrLes = lesion of nutritional deficiency, VNC = vertebral neural canal measurement, LEH = linear enamel hypoplasia, LBratio = long bone ratio measurement, IsoRatio = isotopic measurements

Chapter 7 Sample by Individual, Including Demographic Information and Lesion/Measurement Presence (Marked as X) Continued													
Temporal Pd.	Site	Burial	River	Date	Sex	Age	PNB	Periodontal	NutrLes	VNC	LEH	LBratio	IsoRatio
LW 1	Sk1a	4	Dan	800-1200	male	25-30	X	X	X	X	X	X	X
Colonial 2	Sk1a	5	Dan	1670-1710		2-4	X		X		X		
Colonial 1	Sk1a	8	Dan	1650-1670		3-4	X		X		X		
Colonial 1	Sk1a	9	Dan	1650-1670		9.5-11	X		X		X		
Colonial 2	Sk1a	14	Dan	1670-1710	female	40-50	X	X	X		X		
Colonial 1	Sk1a	15	Dan	1650-1670		2-4	X		X		X		
LW 1-2	Sk1a	16	Dan	1100-1450		15-18	X	X	X		X		
Colonial 2	Sk1a	17	Dan	1670-1710	female	20-40	X	X			X		
Colonial 1	Sk1a	18	Dan	1650-1670	female	45-59	X	X	X		X		
Colonial 1	Sk1a	19	Dan	1650-1670	female	30-55	X		X				
LW 1-2	Sk1a	20	Dan	1100-1450		20-30	X	X	X		X		
Colonial 2	Sk1a	22	Dan	1670-1710		15.5-18	X	X	X		X		
Colonial 1	Sk1a	24	Dan	1650-1670	male	20-40	X	X	X		X		
LW 1-2	Sk1a	26	Dan	1100-1450		0-0.08	X		X				
Colonial 2	Sk1a	51	Dan	1670-1710	female	50-60	X	X	X	X	X		
Colonial 2	Sk1a	52	Dan	1670-1710		11-12	X	X	X		X		
Colonial 2	Sk1a	53	Dan	1670-1710		3-5	X		X		X		
Colonial 2	Sk1a	54	Dan	1670-1710		1-2	X		X		X		
Colonial 2	Sk1a	55	Dan	1670-1710		2-3	X				X		
Colonial 2	Sk1a	56	Dan	1670-1710	female	20-34	X		X	X			
Colonial 2	Sk1a	57	Dan	1670-1710		0.5-0.625	X				X		
Colonial 2	Sk1a	58	Dan	1670-1710	female	25-45	X	X	X		X		
Colonial 2	Sk1a	62	Dan	1670-1710	male	20-40	X	X	X		X		X
Colonial 1	Sk1a	65	Dan	1650-1670	female	30-40	X	X	X		X		

PNB = periosteal new bone, NutrLes = lesion of nutritional deficiency, VNC = vertebral neural canal measurement, LEH = linear enamel hypoplasia, LBratio = long bone ratio measurement, IsoRatio = isotopic measurements

Chapter 7 Sample by Individual, Including Demographic Information and Lesion/Measurement Presence (Marked as X) Continued

Temporal Pd.	Site	Burial	River	Date	Sex	Age	PNB	Periodontal	NutrLes	VNC	LEH	LBratio	IsoRatio
Colonial 2	Sk1a	68	Dan	1670-1710	male	25-55	X	X	X		X		
Colonial 2	Sk1a	69	Dan	1670-1710		5.5-7.5	X		X		X		
Colonial 2	Sk1a	73	Dan	1670-1710	male	45-55	X	X	X		X	X	
Colonial 2	Sk1a	74	Dan	1670-1710	male	35-45	X	X	X		X		X
Colonial 2	Sk1a	75	Dan	1670-1710	male	40-55	X		X		X		
Colonial 1	Sk1a	81	Dan	1650-1670		25-55	X	X			X		
Colonial 1	Sk1a	87	Dan	1650-1670	female	30-55	X		X		X		
Colonial 1	Sk1a	91	Dan	1650-1670	female	25-35	X		X	X			
Colonial 1	Sk1a	99	Dan	1650-1670		3-5	X				X		
LW 1-2	Sk1a	105	Dan	1100-1450		18-26	X		X			X	
Colonial 2	Sk1a	108	Dan	1670-1710	male	30-47	X		X		X		
Colonial 1	Sk1a	109	Dan	1650-1670		20-30	X	X	X		X		
LW 1-2	Sk1a	110	Dan	1100-1450	male	30-44	X	X	X				
Colonial 2	Sk6	1	Dan	1690-1710		15-18	X		X		X		
Colonial 2	Sk6	2	Dan	1690-1710		2-4	X		X		X		
Colonial 2	Sk6	3	Dan	1690-1710		2-3	X		X		X		
Colonial 2	Sk6	4	Dan	1690-1710		5.5-7.5	X		X		X		
Colonial 2	Sk6	6	Dan	1690-1710		0.5-1.5	X				X		
Colonial 2	Sk6	6	Dan	1690-1710		0.5-2.5	X		X		X		
Colonial 2	Sk6	7	Dan	1690-1710		30-55	X		X				
Colonial 2	Sk6	8	Dan	1690-1710	female	35-45	X		X				
Colonial 2	Sk6	9	Dan	1690-1710		9-10	X	X	X		X		
LW 1-2	Vir150	1	Roanoke	1000-1450	male	28-38	X	X	X	X	X	X	
LW 1-2	Vir150	3	Roanoke	1000-1450	male	35-45	X	X	X	X	X		
LW 1-2	Vir150	4	Roanoke	1000-1450	female	40-55	X		X				X

PNB = periosteal new bone, NutrLes = lesion of nutritional deficiency, VNC = vertebral neural canal measurement, LEH = linear enamel hypoplasia, LBratio = long bone ratio measurement, IsoRatio = isotopic measurements

Chapter 7 Sample by Individual, Including Demographic Information and Lesion/Measurement Presence (Marked as X) Continued													
Temporal Pd.	Site	Burial	River	Date	Sex	Age	PNB	Periodontal	NutrLes	VNC	LEH	LBratio	IsoRatio
LW 1-2	Vir150	5	Roanoke	1000-1450		3-4	X		X		X		
LW 1-2	Vir150	6	Roanoke	1000-1450		2-3	X		X		X		
LW 1-2	Vir150	8	Roanoke	1000-1450	male	30-40	X	X	X	X	X		
LW 1-2	Vir150	9	Roanoke	1000-1450	male	35-55	X	X	X	X	X	X	
LW 1-2	Vir150	10	Roanoke	1000-1450	female	35-45	X		X				
LW 1-2	Vir150	11	Roanoke	1000-1450	male	19-26	X	X	X	X	X	X	
LW 1-2	Vir150	12	Roanoke	1000-1450	female	20-30	X	X	X		X		X
LW 1-2	Vir150	13	Roanoke	1000-1450	male	30-39	X	X	X		X		
LW 1-2	Vir150	14	Roanoke	1000-1450	female	40-49	X	X	X	X	X		X
LW 1-2	Vir150	15	Roanoke	1000-1450		10.5-12	X	X	X		X		
LW 1-2	Vir150	16	Roanoke	1000-1450	female	30-45	X		X		X		
LW 1-2	Vir150	17	Roanoke	1000-1450	female	30-45	X	X	X		X		
LW 1-2	Vir150	18	Roanoke	1000-1450		0-0.25	X		X				
LW 1-2	Vir150	19	Roanoke	1000-1450	male	35-45	X	X	X	X	X	X	X
LW 1-2	Vir150	20	Roanoke	1000-1450		4-5	X		X		X		
LW 1-2	Vir150	21	Roanoke	1000-1450		0.79-1.17	X		X		X		
LW 1-2	Vir150	22	Roanoke	1000-1450	male	25-35	X		X	X	X	X	
LW 1-2	Vir150	23	Roanoke	1000-1450	male	17-21	X	X		X	X	X	X
Colonial 1	Vir150	23	Roanoke	1620-1670?			X						
LW 1-2	Vir150	24	Roanoke	1000-1450		1-2	X		X		X		
LW 1-2	Vir150	25	Roanoke	1000-1450	male	30-39	X	X	X	X	X	X	X
LW 1-2	Vir150	26	Roanoke	1000-1450		7-11	X		X		X		
LW 1-2	Vir150	27	Roanoke	1000-1450			X	X	X		X		X
LW 1-2	Vir150	29	Roanoke	1000-1450		20-28	X	X	X		X		
LW 2	Vir196	2	Dan	1200-1450	male	35-45	X	X	X		X		

PNB = periosteal new bone, NutrLes = lesion of nutritional deficiency, VNC = vertebral neural canal measurement, LEH = linear enamel hypoplasia, LBratio = long bone ratio measurement, IsoRatio = isotopic measurements

Chapter 7 Sample by Individual, Including Demographic Information and Lesion/Measurement Presence (Marked as X) Continued													
Temporal Pd.	Site	Burial	River	Date	Sex	Age	PNB	Periodontal	NutrLes	VNC	LEH	LBratio	IsoRatio
LW 2	Vir196	3	Dan	1200-1450	female	22-28	X	X	X	X	X		
LW 2	Vir196	4	Dan	1200-1450		8.5-9.5	X		X		X		
LW 2	Vir196	5	Dan	1200-1450		2-3	X		X				
LW 2	Vir196	6	Dan	1200-1450	female	25-29	X	X	X	X	X	X	
LW 2	Vir196	7	Dan	1200-1450	female	40-50	X	X	X		X		
LW 2	Vir196	8	Dan	1200-1450		5.5-7.5	X		X		X		
LW 2	Vir196	9	Dan	1200-1450	female	20-29	X	X	X		X		
LW 2	Vir199	10	Dan	1300-1400	male	45-60	X	X	X		X	X	
LW 2	Vir199	16	Dan	1300-1400	female	45-55	X	X	X	X	X		
LW 2	Vir199	18	Dan	1300-1400	male	24-34	X	X	X		X		
LW 2	Vir199	20	Dan	1300-1400	male	18-23	X	X	X		X		
LW 2	Vir199	Display	Dan	1300-1400	female	35-44	X	X	X	X	X	X	
LW 2	Vir231	3	Dan	1300-1400?	female	38-48	X	X	X	X	X	X	
LW 2	Vir231	4	Dan	1300-1400?		35-45	X		X			X	
LW 2	Vir231	5	Dan	1300-1400?	female	18-24	X	X	X		X		X
LW 2	Vir231	6	Dan	1300-1400?	female	23-29	X	X	X		X		X
LW 2	Vir231	7	Dan	1300-1400?		0.75-1.5	X		X		X		
LW 2	Vir231	8	Dan	1300-1400?	male	40-49	X	X	X	X	X		
LW 2	Vir231	9	Dan	1300-1400?	female	45-60	X	X	X		X		
LW 2	Vir231	10	Dan	1300-1400?	female	35-50	X	X	X		X		
LW 2	Vir231	11	Dan	1300-1400?		0-0.33	X		X				
LW 2	Vir231	12	Dan	1300-1400?	female	40-55	X	X	X	X		X	
LW 2	Vir231	13	Dan	1300-1400?	male	30-45	X	X	X	X	X		
LW 2	Vir231	14	Dan	1300-1400?	female	35-44	X	X	X	X	X	X	X
LW 2	Vir231	15	Dan	1300-1400?	male	20-40	X	X	X	X	X		

PNB = periosteal new bone, NutrLes = lesion of nutritional deficiency, VNC = vertebral neural canal measurement, LEH = linear enamel hypoplasia, LBratio = long bone ratio measurement, IsoRatio = isotopic measurements

Chapter 7 Sample by Individual, Including Demographic Information and Lesion/Measurement Presence (Marked as X) Continued													
Temporal Pd.	Site	Burial	River	Date	Sex	Age	PNB	Periodontal	NutrLes	VNC	LEH	LBratio	IsoRatio
LW 2	Vir231	17	Dan	1300-1400?	female	38-48	X	X	X	X	X		
LW 2	Vir231	18	Dan	1300-1400?	female	40-47	X	X	X	X	X		
LW 2	Vir231	20	Dan	1300-1400?	male	30-40	X	X	X	X	X		
LW 2	Vir231	21	Dan	1300-1400?	male	25-40	X		X	X		X	
LW 2	Vir231	22	Dan	1300-1400?		3-5	X		X		X		
LW 2	Vir231	Infant	Dan	1300-1400?		0.125-0.375	X		X				
LW 2	Vir231	X	Dan	1300-1400?	female	38-48	X	X	X	X		X	

PNB = periosteal new bone, NutrLes = lesion of nutritional deficiency, VNC = vertebral neural canal measurement, LEH = linear enamel hypoplasia, LBratio = long bone ratio measurement, IsoRatio = isotopic measurements

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