Isotopes of the Caribbean: An Investigation of Sample Pretreatment and Human Paleodiet at the Escape Site (AD 300-1000) on Saint Vincent, Lesser Antilles

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ABSTRACT

Isotopes of the Caribbean: An Investigation of Sample Pretreatment and Human Paleodiet at the Escape Site (AD 300-1000) on Saint Vincent, Lesser Antilles

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This research represents the first stable carbon and nitrogen isotope analysis of human bone collagen (n = 29) from the Escape Site (AD 300 - 1000), Saint Vincent. As a two-pronged investigation, this research had the following goals: (1) determining the ideal pretreatment for poorly preserved bones and (2) reconstructing the Escape Site sample population diet. By incorporating powdered specimens, shorter demineralizations and increased acid:sample exposure, higher collagen yields were produced, thereby expanding the sample size for isotopic analysis. Notably, the elemental data suggests that not all isolated collagen was biogenic and was perhaps contaminated by non-collagenous proteins. This highlighted the importance of using multiple criteria to rigorously evaluate collagen based on the full quality indicator profile. In the end, 5 individuals yielded useable isotope data which was consistent with a broad spectrum diet relying primarily on C_3 plants as well as terrestrial, reef, nearshore and freshwater fauna. Within the broad region, the Escape Site data was comparable to other islands from the Lesser Antilles and Cuba emphasizing the influence of regional biodiversity as well as the likelihood that the studied population contributed and benefitted from the extensive Saladoid trade networks which existed at the time.

Keywords: Stable isotope analysis, Carbon, Nitrogen, Collagen, Human diet, Caribbean, Saint Vincent, Lesser Antilles, Escape Site, Saladoid, Ceramic age

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Chapter 1 : Introduction

Humans as a collective are the product of their surroundings and environment, implicitly reflecting political, economic and larger cultural factors (e.g. tradition, hierarchies, taboos, socioeconomic status). Diet itself is a dynamic point of observation, embodying the interplay between biology and culture. The relative contributions of different foods represent an observable summary of cultural constraints and the specific choices taken beyond environmental restrictions on availability (Agarwal & Glencross, 2011; Larsen & Walker, 2010: pp. 381-382; Zuckerman & Armelagos, 2011). Within the Caribbean, early dietary assessments relied heavily on indirect evidence – manioc griddles, zooarchaeological remains, pathology as well dental wear patterns – pre-supposing shifts in diet as a consequence of population movements and resource exhaustion (Keegan & Hofman, 2017; Larsen & Walker, 2010: pp. 381-382). Given that zooarchaeological contexts overwhelmingly reflected marine resources which withstood the harsh preservation conditions of the Caribbean (e.g. mollusks, land crabs), it wasn't until direct methods were available that the scope of regional diet variability was better understood (Keegan & Hofman, 2017; LeFebvre, 2007; Newsom & Wing, 2004; Pestle & Colvard, 2012; Stokes, 1998; Wing & Wing, 2001). While indirect approaches infer diet, they do so based on relative concentrations of physical evidence which can mask more complex dietary trends. Benefitting from increased technological advancements and more nuanced bioarchaeological frameworks, research on prehistoric diet today is better able to mitigate some of the potential biases of indirect dietary investigations through specific technological quantification via gut content analysis, calculus assessment (e.g. SEM and

proteomics analysis) as well as stable isotope analysis (SIA) (Katzenberg, 2008; Mickleburgh, 2016; Larsen & Walker, 2010: pp. 381; Mickleburgh & Pagán-Jímenez, 2012).

Despite early foundational isotopic work focused within the region (Keegan & DeNiro, 1988), the decades since have produced more sporadic research mostly centred around the Greater Antilles (as reviewed in Pestle, 2013a). Focusing in particular on the island of Saint Vincent, this wider gap in available isotope literature becomes more evident. Previous work examining Saint Vincent has been largely archaeological in nature (e.g. site excavations) (Bullen & Bullen, 1972; Duval, 1996; Callaghan, 2007) with some samples being incorporated into larger regional studies focusing on dental wear and pathology (Mickleburgh, 2016; Mickleburgh & Pagán-Jiménez, 2012) as well as paleomobility strontium analysis (Laffoon, 2012). Given the limited availability of research, the 2009 excavation of the Escape Site and its associated burials provided an opportunity to apply carbon and nitrogen SIA to expand the understanding of diet on Saint Vincent as it relates to the wider context of the Caribbean (Moravetz, 2010; Morvetz & Callaghan, 2011). This thesis research represents the first application of carbon and nitrogen stable isotopes to investigate diet from a sample population from the early Saladoid period (AD 300 - 1000) interred at the Escape Site on Saint Vincent. Moreover, it represents a time period which hasn't been significantly studied within the southern Lesser Antilles beyond three samples which showed early ceramic or and possible Huecoid associations (Stokes, 1998) as well as recent radiocarbon studies (Fitzpatrick & Giovas, 2013; Hanna & Giovas, 2019).

1.1 Saint Vincent and the Escape Site

The Caribbean encompasses an array of environment types and ecological niches, where island geological classifications are defined by their environmental base – sedimentary or volcanic – with relative ages of islands driving the scope of terrestrial biodiversity. Amongst the southern islands of the Lesser Antilles lies Saint Vincent, a comparatively young geological island (CCA, 1991; Watts, 1987). As part of the volcanic arc of the Lesser Antilles, Saint Vincent microclimates and ecological niches are delineated by elevation occurring around the La Soufrière volcano in the northern quadrant of the island (Callaghan, 2007; CCA, 1991; Keegan & Hofman, 2017).

While early research noted the importance of the Escape Site (Duval, 1996), the site wasn't fully excavated until 2009 due to the impending construction of an international airport. Investigations at the Escape Site identified hundreds of features and thousands of pottery fragments which collectively pointed to an extended occupation by Saladoid populations from AD 300 to 1000 (Bright, 2011; Moravetz, 2010; Morvetz & Callaghan, 2011). While artifacts are predominately from the ceramic age, colonial artifacts were also found within some postholes emphasizing the long term use of the Escape Site area (Moravetz, 2010; Morvetz & Callaghan, 2011). Due to their extensive and early presence, the Saladoid culture group continues to be of interest for Caribbean archaeologists with ongoing debates concerning their specific migratory pathways and questions regarding the implications of ceramic age interaction spheres (Hofman & Hoogland, 2011; Keegan & Hofman, 2017; Rodríguez Ramos, 2010).

Previous Caribbean zooarchaeological assessments and SIA research have determined that subsistence is strongly tied to island size, island bedrock (e.g. volcanic or

sedimentary) as well as distance from larger source areas such as mainland South America or the larger islands within the Greater Antilles (Keegan & Hofman, 2017; Stokes, 1998). Such that populations who occupied geologically equivalent islands and had nearby access to similar resources were more likely to have comparable diets without significant influence from time period and socioeconomic factors. The results from this research follow these established trends whereby the Escape Site study population (n = 5) aligns to broad Lesser Antillean data (Laffoon et al., 2016; Norr, 2002; Stokes, 1998) as well as Cuba (Buhay et al., 2013; Chinique de Armas et al., 2015) and the Dominican Republic (Stokes, 1998) Given the determination of a Saladoid presence (Moravetz, 2010) as well as the identification of non-local individuals (Laffoon, 2012), the broad comparability of the Escape Site data emphasizes the movement of individuals both to and from Saint Vincent in addition to the participation in the extensive Saladoid trade network.

1.2 Research Objectives and Methods

Initially this investigation was focused on an in-depth assessment of the Saladoid sample population diet at the Escape Site using 29 human bone samples excavated during the 2009 excavations jointly undertaken by Bison Historical Services and the University of Calgary in co-ordination with local agencies and volunteers (Moravetz, 2010; Morvetz & Callaghan, 2011). As a result of the poor preservation of the archaeological human bones, the opportunity to include a methodological component was presented. Specifically, this research shifted to focus on the following four questions:

1. How does fraction size, demineralization and exposed sample surface area affect collagen yield and collagen quality in poorly preserved human bone?
2. What is the best pretreatment protocol for poorly preserved materials?
3. What foods were consumed by the individuals interred at the Escape Site?
4. How does the stable carbon and nitrogen isotope data from the Escape Site compare to regional and temporal Caribbean isotope data?

1.3 Significance

While borne out of limitation of useable data, the implications of a methodological assessment within the Caribbean cannot be ignored. In reviewing previously applied collagen isolation protocols, there is a notable inconsistency of approaches where the only regularly incorporated variable in bone collagen pretreatments was powdered bone specimens (Krigbaum, Fitzpatrick & Bankaitis, 2013; Laffoon et al., 2016; Norr, 2002; Pestle & Colvard, 2012). With methodological studies demonstrating the potential impacts on isotopic data, the need to establish best practices and unify future methods is essential to ensuring ongoing consistency and comparability of results (Collins & Galley, 1998; Tsutaya et al. 2017). The results from this research confirm the efficacy of using powdered (<1 mm) specimens, while also demonstrating how powdered samples when combined with shorter demineralization times, improves test results. When these variables are employed, researchers can expand the number of viable samples by increasing collagen yields in poorly preserved bones which are common throughout the region given the harsh environmental constraints on organic materials. Critically, the Escape Site elemental data brings into question if all the isolated collagen using this

method is biogenic, thereby emphasizing the importance of rigorously evaluating results prior to accepting stable isotope data.

While previous isotopic studies have been undertaken throughout the Caribbean, research has primarily been centred within the Greater Antilles and northern islands of the Lesser Antilles leaving a notable dearth of data concerning the early ceramic age within the southern Lesser Antilles (as reviewed in Pestle, 2013a; Stokes, 1998). Recent nearby radiocarbon and isotopic research has focused on the Grenadines, Saint Lucia and Grenada, however, Saint Vincent remains understudied (Fitzpatrick & Giovas, 2013; Krigbaum, Fitzpatrick & Bankaitis, 2013; Hanna & Giovas, 2019; Laffoon, 2012; Laffoon et al., 2016; Stokes, 1998). This absence of significant isotopic study on Saint Vincent can largely be attributed to a lack of available study materials due to the poor preservation conditions resulting from the highly acidic volcanic soils on the island (as reviewed in Pestle, 2013a; Moravetz, 2010). Moreover, much of the other work within the Lesser Antilles focuses on late ceramic, formative and post-colonial populations (c.f Krigbaum, Fitzpatrick & Bankaitis, 2013; Laffoon & de Vos, 2011; Laffoon et al., 2016; Norr, 2002; Stokes, 2005) despite evidence for an earlier presence in the area (Fitzpatrick, 2011; Hanna & Giovas, 2019). As such, this research is significant as it presents the first application of carbon and nitrogen stable isotopes to examine diet from a sample Saladoid population from Saint Vincent. Whereby this research serves to not only investigate a critical missing context within Caribbean literature but also examines the early prehistory of the Lesser Antilles. Given the question of specific migratory routes, the implications of diet at the Escape Site could further clarify the role of the southern Lesser Antilles to

early Caribbean populations. Through seafaring and trading, early populations had strong ties to both nearby islands and surrounding mainlands and establishing the scale of these interactions is needed to properly appreciate the precolonial people and cultures (Hofman & Hoogland, 2011; Keegan & Hofman, 2017; Rodríguez Ramos, 2010).

With the extensive resource exhaustion due to overfishing as well as the forceful transportation of enslaved African populations, the environment and population of the Caribbean has been irrevocably altered by European colonization (Fitzpatrick & Keegan, 2007; Giovas, 2016; Pestle, 2013b). The dynamic nature of the Caribbean cultural mosaic that existed prior to colonial interference, emphasizes the need to examine all available information to fully quantify and understand the long term impact of colonization (Allaire, 2003; 2013; Bullen & Bullen, 1972; Chanlatte Baik, 2003; Rouse, 1992). During the excavations at the Escape Site there was significant interest from the local population with many school trips visiting as well as volunteers actively engaging in the excavations. This high profile project generated increased awareness of Saint Vincentian cultural heritage, pre-colonial existence and archaeological practices (Callaghan, 2011; Moravetz & Callaghan, 2011). Given these implications, this thesis research provides additional information on the rich history of Saint Vincent and its earliest occupants, supplying another avenue for local engagement with their heritage.

1.4 Thesis Outline

This thesis is composed of seven chapters and three appendices. The general outline of chapters are as follows:

Chapter 2 provides background information pertaining to the geographic, environmental and cultural context of the Caribbean. This chapter starts with environmental information concerning the larger Caribbean region and Saint Vincent data before moving onto a broad discussion of the cultural prehistory of the Caribbean and the archaeological dynamics of the region. Implications for subsistence conclude the chapter by examining potentially imported foods as well as local flora and fauna of Saint Vincent.

Chapter 3 reviews SIA basic principles and bone biology. Specifically, this chapter explores carbon and nitrogen stable isotopes in both terrestrial and marine environments as well as the implications for interpreting ancient diet. Considerations for bone chemical composition and sources of contamination as it pertains to dietary assessment are also touched on.

Chapter 4 presents critical bioarchaeology theories as well as the methodologies employed for this research. Specific information regarding the Escape Site and the provided materials for analysis are outlined as well as the different protocols implemented.

Chapter 5 reviews the results from the methodological assessment regarding collagen isolation protocols as well as an overview of $\delta^{13}C$ and $\delta^{15}N$ data.

Chapter 6 examines more closely the implications of the results outlined from chapter 5 with regard to the research objectives as mentioned in section 1.2.

Chapter 7 provides concluding remarks and a summary of key findings, limitations of the current study as well as suggested avenues for future research.

Chapter 2 : Caribbean Archaeological and Geographic Background

To better understand the scope of dietary choices made by the Saladoid population on Saint Vincent, the geographic, climatic and biological conditions must be established. This chapter will serve to establish the environmental and cultural conditions which serve as a key foundation of this research; beginning with an overview of Caribbean background information and environmental contexts before discussing cultural evolutions. Once the larger environmental and cultural regional conditions have been provided, Saint Vincent local ecology and specific challenges therein for archaeological research will be discussed.

2.1 Environment

2.1.1 Caribbean Overview

The Caribbean basin covers an area from Florida to Venezuela and from the edges of the Antillean islands to the Yucatán peninsula, strictly encompassing the land masses touching the Caribbean Sea. As a region, the circum-Caribbean contains four major components: the Bahama archipelago, Greater Antilles, Lesser Antilles and the Leeward Antilles (Figure 2.1) (Watts, 1987: p. 3). The Lesser Antilles can be further divided into the Leeward (north of Martinique) and Windward Islands (south of Dominica). Saint Vincent, of central concern for this research, is located amongst the Windward Islands of the Lesser Antilles and is one of the geologically youngest major islands (CCA, 1991: p. 9). More specifically, Saint Vincent and the Grenadines (SVG) includes the main island of Saint Vincent as well as the 32 Grenadine islands to the south. Each of these subregions and specific islands have distinct patterns of geophysical and biogeographical

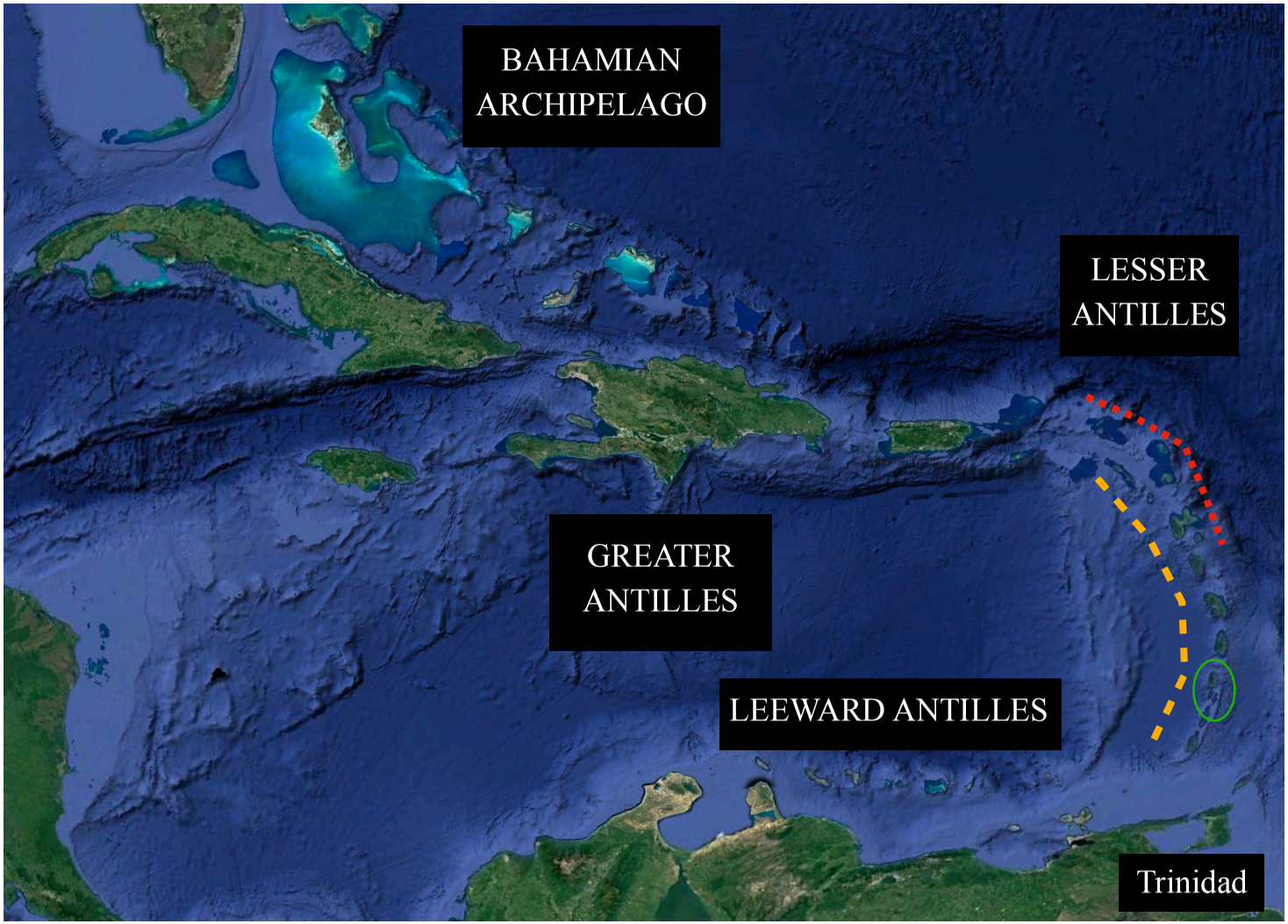

Figure 2.1 Map of the Caribbean. Saint Vincent is highlighted with the green circle. Sourced from Google Earth (Feb, 2020).

characteristics controlling the locally available flora and fauna (Burke, 1988; Newsom & Wing, 2004; Watts, 1987: p. 4-5).

The Greater Antilles are the northern islands of the Caribbean encompassing the larger and older islands of Cuba, Hispaniola, Jamaica and Puerto Rico. Starting as proto-antilles, following periodic submersion and re-emersion and gradual tectonic eastward movement, sediments were built up over time forming the Greater Antilles by the mid-Cenozoic (Newsom & Wing, 2004; Watts, 1987). Above the Greater Antilles, in the northern Caribbean region lies the Bahama archipelago which are a series of limestone islands with exceedingly prolific reef systems but a lack of terrestrial resources. Unique to the region, the Bahamian archipelago islands were never volcanic but display latitudinal variation in climate and biodiversity (Keegan & Hofman, 2017: p. 6; Newsom & Wing, 2004: p. 12). To the south, above the Venezuela mainland are Trinidad and Tobago to the east as well as the Leeward Antilles to the west (Aruba, Bonaire and Curacao) (Keegan & Hofman, 2017; Watts, 1987).

Beginning east of Puerto Rico, the Lesser Antilles span from the Virgin Islands and extend to the south of Grenada. These islands are the culmination of a series of complex geomorphological processes during the Eocene and Miocene epoch (Newsom & Wing, 2004: p. 12; Watts, 1987: p. 11-12). Notably the Lesser Antilles are geologically unstable, with a bow shaped zone of instability roughly aligning with the Atlantic edges of the insular Caribbean. This has been associated with the subduction of the South American plate and the uplift of the Caribbean plate which resulted in the double island arc seen today (Garmon, Allen & Groom, 2017: p. 10; Watts, 1987: p. 11). Between the

two arcs, the outer is approximately 40 million years old and consists of mostly extinct volcanoes which have, in their dormancy, transformed into vast marine reefs and are classified as sedimentary limestone islands with more depauperate terrestrial resources (Figure 2.1, red square dotted line). More specifically, this discontinuous island arc has a rock base level composed of ancient volcanic rock which has been covered by carbonates over the last 37 to 10 million years ago. In contrast, the younger inner arc was established during the last 20 million years and is still volcanically active with stark relief profiles and higher elevations (Figure 2.1, orange rectangle dotted line) (Garmon, Allen & Groom, 2017: p. 9-10; Newsom & Wing, 2004). Due to the much more productive volcanic soils, these islands (Guadeloupe, SVG, Saint Lucia) tend to have areas more conducive to agricultural practices as opposed to the limestone based islands (Antigua, Barbuda, US Virgin Islands) (Garmon, Allen & Groom, 2017; Stokes, 1998).

2.1.2 Saint Vincent Terrestrial Ecology

As a volcanic island, Saint Vincent has a max elevation of 1179 masl located at the peak of La Soufrière volcano in the northern quadrant of the island (Keegan & Hofman, 2017). Microclimates vary on islands with high reliefs depending on relative height and orientation influenced by rising sea air and moisture condensation. Looking at Saint Vincent, the mountainous island possesses many different environments including various forest types – cloud, rain and moist – which comprise a large majority of the higher central portion of the island with dry woodlands and cactus scrubs making up the coastal and immediate inland regions (Beard, 1949 as cited in CCA, 1991; Callaghan,

2007: p. 15; Watson et al. 1958 as cited in CCA, 1991). The native vegetation and its specific proliferation throughout the SVG region has undergone significant changes from the earliest occupants to colonial agricultural activities. Specifically, the rugged landscapes and relative small size meant Saint Vincent was not initially attractive for prospective large scale colonial plantations, however, by the late eighteenth century sugarcane was the dominant crop (CCA, 1991: p. 5). Today, the endemic flora reflects these continued changes with bananas, coconuts, cocoa, citrus and mangoes making up some of the staple crops (CCA, 1991: p. 6).

Throughout the Caribbean, temperatures remain fairly consistent, however, the specific climate varies between islands. While temperatures are tropical and generally stay within the range of 15ºC and 30ºC, precipitation is dictated by specific topographical profiles (Fielding & Olliviere, 2017: p. 227). Areas with the highest elevation on Saint Vincent, such as the peak of the La Soufrière volcano and associated mountainous regions on the north end of the island receive the highest amounts of rainfall while the low-lying coast and southern areas are far dryer (Fielding & Olliviere, 2017: p. 227). From June to November the island can get up to 190 - 215 mm per month during hurricane season which drops to 76 - 90 mm per month from December to May (Fielding & Olliviere, 2017: p. 226). Beyond topographical influences, there exists a dichotomy between the windward and leeward sides caused by the trade winds. While Saint Vincent still displays notable differences in terrestrial vegetation with the eastern coast being far dryer than the western, this is less severe than it could be due to the presence of Barbados. As the dominant wind blows from the windward or eastern direction, Barbados effectively

blocks a lot of the wind force despite its relatively flat profile (Fielding & Olliviere, 2017: p. 227; Newsom & Wing, 2004: p. 13). Furthermore, the windward side is composed of more rocky coasts and sharp cliffs while the leeward side in contrast shifts between rocky headlands and sandy beaches composed of volcanic materials transported down from la Soufrière (Fielding & Olliviere, 2017: p. 226).

2.1.3 Saint Vincent Marine Ecology

While the soils of Saint Vincent are fertile, the offshore marine ecosystems are not as prolific as throughout the nearby Grenadine islands (Fielding & Olliviere, 2017: p. 239; Nature Conservancy, 2016: p. 4). The Caribbean is a critical component within North Atlantic weather systems and is essential in driving the Gulf Stream. Northern and southern equatorial currents converge along the northern coast of South America resulting in strong currents moving clockwise from the Antilles to Central America. With these contrasting currents a zone of upwelling within the region of the Leeward Antilles is also present (Cooper, 2013: p. 53; Newsom & Wing, 2004: p. 15). With the consistent flow of equatorial waters and relative shallow depths through the region, consistent sea surface temperatures regulate larger climate trends (Cooper, 2013: p. 54). The specific prevalence and strength of the key marine ecosystems – coral reefs, seagrass meadows and mangroves – are driven by the strength of waves, currents and water depth within the region (Newsom & Wing, 2004).

Similar to the regional dichotomies of terrestrial environments based on trade winds, reefs surrounding Saint Vincent show an observable concentration on the southern

and western coasts where the wave energy is not as disruptive. The coral reefs throughout SVG are the most extensive within the eastern Caribbean encompassing an area of 168 km^2 (Nature Conservancy, 2016: pp. 2-4). On the eastern (windward) side there are wider shelves with minimal and short coral cover. In contrast, the western (leeward) side has more thorough and expansive coral reefs which occur deeper and along steeper underwater slopes (Nature Conservancy, 2016: p. 4). Both seagrass meadows and mangroves are much more restricted within the coastal regions of Saint Vincent being largely concentrated on the western and southern coasts and absent from the eastern region (Nature Conservancy, 2016: p. 4). Seagrass meadows are limited in their scope but are more prevalent encompassing 28 km^2 in contrast to the 0.7 km^2 of mangroves (Nature Conservancy, 2016: p. 4). Freshwater is found on Saint Vincent in the form of surface water – streams, rivers, springs – which are driven by the relative quantities of rainfall and elevation. Rivers on Saint Vincent are intermittent but are primarily short and straight transitioning from deep valleys in the mountain regions to small alluvial flats in the lowlands (CCA, 1991: p. 81). These rivers have been observed to flood quickly during rainy seasons with drastic water level changes (Bullen & Bullen, 1972).

2.1.4 Challenges of the Local Environment

Most of the local environmental challenges can be attributed to La Soufrière, which would have significantly impacted early populations (Allaire, 2003: p. 202). Archaeological evidence suggests that La Soufrière has a long history of volcanic events dating back to Saladoid occupations around AD 259 with further devastating activity post

1492 (Bullen & Bullen, 1972: p. 95; Callaghan, 2007: p. 13). Given the wider region of instability, there have been large scale eruptions causing mass evacuations as recent as April 2021 on Saint Vincent (Elliot, 2021). While volcanic soils are incredibly fertile, they are also subject to potentially extensive erosion. For pre-Columbian groups, however, this was likely not a significant concern as large scale land modifications are associated with colonial practices of vegetarian clearing (CCA, 1991: p. 16, pp. 22-23; Watts, 1987: p. 37).

Beyond the immediate implications of La Soufrière, additional challenges are presented in the form of hurricanes, earthquakes, tsunamis, landslides, rockslides and droughts (Allaire, 2003: p. 202; CCA, 1991: p. 23). While over the course of the last 6000 years the Caribbean has experienced a period of larger environmental instability with oscillating dry and wet periods, the climate has remained fairly stable which was essential for successful seafaring (Callaghan, 2003; 2013; Cooper, 2013: p. 53; Keegan & Hofman, 2017: p. 39). Historic trends of drought have been observed both pre-dating the and coinciding with larger migrational events within the region associated with both pre-ceramic and ceramic populations (Beets et al., 2006; Garmon, Allen & Groom, 2017: p. 14; Newsom & Wing, 2004: p. 13). Moreover, seasonally occupied coastal sites have likely been masked or destroyed due to ongoing environmental changes and rising sea levels (Beets et al., 2006; Cooper, 2013: pp. 53-54; Keegan, 1994: p. 264). While certainly major environmental events (e.g. hurricanes and flooding) presented direct risks to Caribbean populations it is variations in the lengths of the seasons that had the greatest impact (Cooper, 2013: p. 51; Newsom & Wing, 2004: p. 15; Watts, 1987: p. 5).

2.2 Prehistory of the Caribbean

Broadly speaking, archaeology in the Caribbean can be divided into two major time periods: prehistoric (6000 BC - AD 1492) and historic (AD 1492 - present) (Keegan & Hofman, 2017). While researchers have also relied on a five age system – lithic, archaic, ceramic, formative and historic – coinciding with technological changes (Keegan, 1994; Rouse, 1992), more recent work has emphasized the interplay between culture, place and time with emphasis placed on ceramic types to distinguish between cultures (Keegan & Hofman, 2017: p. 20). Moreover, distinctions between archaic and lithic ages have been increasingly discarded instead relying on a broad 'archaic' denomination (Fitzpatrick, Napolitano & Stone, 2021: 235; Keegan & Hofman, 2017: p. 21). While date ranges are provided (see Table 2.1), it is likely that the Caribbean region was settled via a series of discontinuous population movements from the surrounding mainland and dispersal dates are fluid (Fitzpatrick, Napolitano & Stone, 2021).

Table 2.1 Summary overview of discussed culture group series and sub-series (Fitzpatrick, Napolitano & Stone, 2021; Keegan, 1994; 2000; Keegan & Hofman, 2017; Reid, 2009; Rouse, 1992)

Series "-oid"	Sub-Series "-an"	Age	Dates	Region
Casimiroid	Casmirian [x]	Archaic/ Lithic	4000 - 2000 BC	Cuba, Hispaniola
	Courian		2030 BC - AD 145	Haiti
			2660 BC - AD 240	Dominican Republic
	Redondan		2050 BC - AD 1300	(W) Cuba

Series “-oid”	Sub-Series “-an”	Age	Dates	Region
Ortoiroid	Undifferentiated ˣ		5000 - 200 BC	Hispaniola, Puerto Rico Trinidad, Windward Islands
Barrancoid	Undifferentiated	Ceramic	Post 1500 BC	Lower Orinoco River, Venezuela
Saladoid	Huecan ˣ	Ceramic	170 BC - AD 400	Puerto Rico
	Cedrosan ˣ		500 BC - AD 600	Lesser Antilles, Puerto Rico
Troumassoid	Troumassan	Ceramic	AD 500 - 1000	Lesser Antilles (not including US Virgin Islands)
	Suazan (Suazey, Suazoid)		AD 1000 - 1450	Windward Islands
Ostionoid	Ostionan	Ceramic	AD 600 - 1200	Cuba, Hispaniola, Jamaica, (W) Puerto Rico
	Palmetto *		AD 600 - 1200	Bahamas
	Meillacan		AD 600 - 1500	Hispaniola, Puerto Rico, US Virgin Islands
	Chican		AD 1200 - 1500	(S/ E) Hispaniola, (E tip) Cuba, US Virgin Islands, Puerto Rico, Bahamas, Northern Leeward Islands
	Elenan *		AD 600 - 1200	(E) Puerto Rico, US Virgin Islands
Taíno	Western	Formative	AD 1200 - 1500	Bahamas, (central) Cuba, Jamaica, Turks and Caicos
	Eastern		AD 1200 - 1500	Leeward Islands (north of Guadeloupe)

Series "-oid"	Sub-Series "-an"	Age	Dates	Region
	Classic		AD 1200 - 1500	(E tip) Cuba, Hispaniola Puerto Rico, US Virgin Islands
Guanahatabey	Undifferentiated	Formative	AD 1300 -	(W) Cuba
Island Carib	Undifferentiated[X]	Formative	AD 1450 -	Guadeloupe, Trinidad, Tobago, Windward Islands

X Denotes a cultural evolution driven primarily from a migratory event, does not preclude local influence
* Refers to groups that have been described as no longer relevant, however, this is not universally considered
'Undifferentiated' is listed when there are not relevant sub-series associated with the larger series

2.2.1 First Wave: the Earliest People of the Caribbean

The earliest populations of the Caribbean likely arrived in Cuba from Belize marking the start of the archaic pre-ceramic period (Keegan, 1994; Keegan & Hofman, 2017; Rouse, 1992). More recent discoveries of early pottery use, however, have challenged the pre-ceramic and aceramic description for the archaic age which were based primarily on the absence of pottery (Chanlatte Baik, 2013; Hofman, Bright & Rodríguez Ramos, 2010; Newsom & Wing, 2004: p. 24; Reid, 2009: p. 82; Rodríguez Ramos et al., 2008: p. 49; Rodríguez Ramos, Pagán-Jiménez & Hofman, 2013). Within the Greater Antilles, the earliest archaic groups fall under the umbrella of the Casimiroid series which has three distinguishable sub-series: Casimirian (4000 - 2000 BC), Courian (2660 BC - AD 240) and Redondan (2050 BC - AD 300) (Figure 2.2) (Keegan, 1994: pp. 268-269; Keegan & Hofman, 2017: p. 41; Rouse, 1992: pp. 51-61). Originating points from the Yucatán peninsula have been identified based on the observable similarity of their lithic tool kit (e.g. macroblade technology) and the close geographic proximity to

Cuba (Callaghan, 2003; Rodríguez Ramos, Pagán-Jiménez & Hofman, 2013: p. 129; Rouse, 1992). The subsequent emergence of the Courian and Redonadan within Hispaniola and Cuba respectively are associated with changes in the archaeological record circa 2000 BC resulting from continued regional influences and influxes of new populations (Figure 2.2, black arrows) (Callaghan, 2003; Keegan, 1994; Rodríguez Ramos, 2010; Rodríguez Ramos, Pagán-Jiménez & Hofman, 2013; Rouse, 1992).

Simultaneous to the development of the Casimiroid within the Greater Antilles, the Ortoiroid series were hypothesized to have emerged within the Trinidad and the Leeward Islands areas. More specifically, the Ortoiroid migration pathway starts with some of the earliest sites on Trinidad (~ 5500 BC) prior to travelling directly to Guadeloupe, bypassing the southern Lesser Antilles (Figure 2.2, red arrow) (Antczak et al., 2018: p. 121; Callaghan, 2013: p. 284; Hofman et al., 2019: p. 245; Keegan & Hofman, 2017: p. 48; Napolitano et al., 2019; Rouse, 1992). Despite the relative comparability of the tool kit, signifying a separate series from the Casimiroid, the associations continue to be debated. Indeed, Ortoiroid connections to Trinidad and beyond are more tenuous and thin, leading researchers to consider that perhaps they were simply an extension of the Casimiroid and not an additional series representing local technological evolution instead (Callaghan, 2013: p. 284; Fitzpatrick, Napolitano & Stone, 2021; Keegan & Hofman, 2017: p. 48). Critically, while radiocarbon dates from Trinidad indicate that it was the earliest settled island within the Caribbean basin circa 6470 - 5345 BC, due to its close proximity to South America this population movement is

often considered distinct from other migrations within the Lesser Antilles as it may not have required seafaring capabilities (Fitzpatrick, Napolitano & Stone, 2021: pp. 237-238).

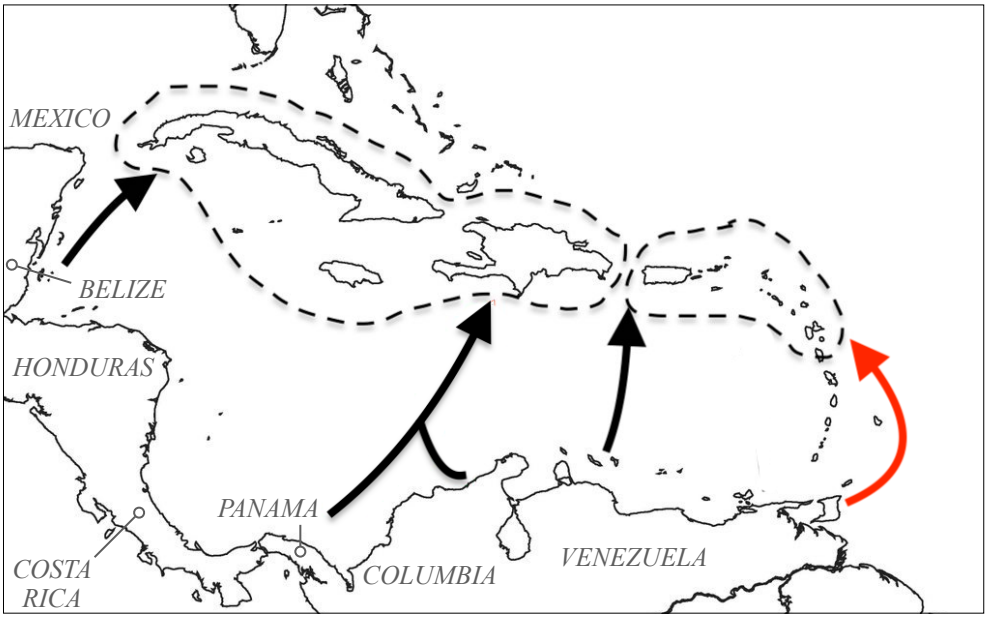

Figure 2.2 Preceramic migration trends 6000 - 200 BC. The black and red arrows respectively indicate the proposed Casimirian and Ortoiroid migratory routes (Image created by author using information from Keegan, 1994; Keegan & Hofman, 2017; Rouse, 1992)

2.2.2 Second Wave: Dynamics of the Ceramic Age

While early research attributed the ceramic age and its associated agricultural and technological advancements strictly to the Saladoid culture group, the dynamics of this period are far more complex (Chanlatte Baik, 2013; Keegan, 2000; Rouse, 1992). Initial generalizations have since been disputed and further clarified, instead emphasizing, the influence of archaic-ceramic interaction spheres and ties to South American through continued migration waves (Allaire, 2003; Rodríguez Ramos, 2010; Rouse, 1992; Siegel et al., 2018). As the name would suggest, the ceramic age is largely defined by diagnostic pottery designs associated with the Cedrosan Saladoid (500 BC - AD 600) (Figure 2.3,

green circle) and Huecoid (170 BC - AD 400) (Figure 2.3, blue circle) (Chanlatte Baik, 2003: p. 231; 2013; Keegan & Hofman, 2017: pp.67-68). Where the Cedrosan Saladoid are more strongly tied to the white-on-red patterning (WOR), the Huecan ceramic styles denote zone-incised-crosshatching (ZIC), with most Saladoid sites showing some level of WOR and ZIC commingling (Boomert, 1999; Chanlatte Baik, 2003: p. 232; 2013; Keegan, 2000; Reed & Petersen, 1999; Rouse, 1992).

Settlement sites during the Saladoid occupations of the ceramic age reflect a larger cultural transition from mobile bands to more permanent sedentary lifestyles (Bright, 2011; Keegan, 2000; Rouse, 1992). Previous research has considered a wide range of possible social structures for the Saladoid ranging from complex tribes, big man collectives to egalitarian societies (Boomert, 1999), however, recent archaeological research contradicts these ideas (Bérard, 2013; Bright, 2011: p. 2; Keegan & Hofman, 2017: pp. 76-77). Specifically, the general site organization and centralized burial practices observed suggest some level of ancestral veneration and ranked hierarchy which largely dispute egalitarian associations (Keegan, 2009; 2018: p. 9). Recent analysis has instead shifted to an exogamous matrilineal societal organization, where extensive trade networks were maintained through kinship and marriage. Similar matrilineal hypotheses have likewise been associated with earlier archaic groups and the Taíno (Ensor, 2013: p. 84). Within this frame, the delineation of gender roles and labour has men more associated with the water (e.g. fishing, trading) and women with the land (e.g. horticulture). Such that while remaining at established sites, women could still reliably maintain subsistence requirements in the absence of men during trading voyages (Ensor,

2013; Keegan, 2018: p. 11; Keegan & Hofman, 2017: pp. 77-78). Interior incisors wear patterns points to the additional role that women had in processing fibrous plant materials (Keegan & Hofman, 2017: p. 65). Critically, specifics regarding settlement hierarchies and societal organization are difficult to solidify in the absence of more contextualized archaeological sites (Bright, 2011: p. 6).

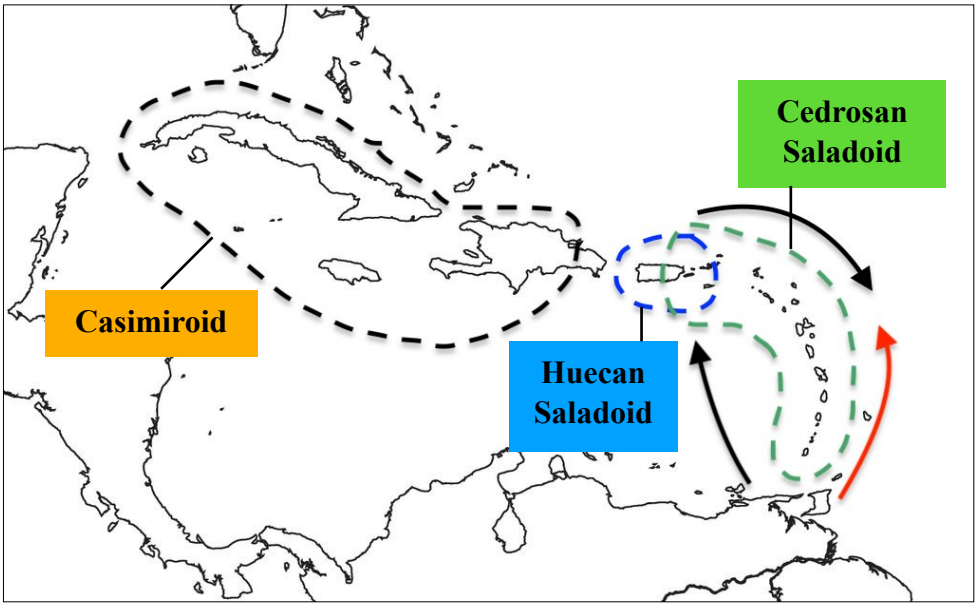

Figure 2.3 Early Ceramic (800 - 200 BC) migration trends. Colour version of this can be found online. (Image created by author using information from Keegan, 2000; Napolitano et al., 2019; Rouse, 1992).

With increasing reliance and clarification of radiocarbon dates, the narrative regarding the specific outset and tempo of the Saladoid expansion has shifted (Fitzpatrick, 2006; Giovas & Fitzpatrick, 2014; Hanna & Giovas, 2019; Napolitano et al., 2019). While initial research logically assumed a northern pathway through the Lesser Antilles via the the stepping stone (SS) pathway (Figure 2.3, red arrow) (Fitzpatrick, 2013: p. 201; Rouse, 1992), early dates from Puerto Rico and apparent commingling with Huecoid ceramics eventually gave rise to the the southern route hypothesis (SRH). This hypothesis

suggests a direct migration route to Puerto Rico from South America before gradually moving down into the Windward Islands (Figure 2.3, black arrows) (Callaghan, 2013; Fitzpatrick, 2013; Giovas & Fitzpatrick, 2014: p. 6; Hanna & Giovas, 2019; Napolitano et al., 2019). While the Windward Islands were likely bypassed during initial migration waves, Barbados as well as Grenada may have been colonized earlier during the first waves of archaic population movements (Napolitano et al., 2019: p. 9). Likely, Huecoid and Cedrosan distinctions are a result of both separate farmer-potter migrations as well as continued influence from Puerto Rican archaic groups (Chanlatte Baik, 2013: p. 173; Keegan & Hofman, 2017: p. 68; Rodríguez Ramos, 2010).

2.2.3 Third Wave: Post-Saladoid and Colonial Dynamics

With the end of the Saladoid series around AD 600 there were both local and larger sociocultural evolutions diverging the cultural landscapes of the Greater and Lesser Antilles (Keegan, 2000: p. 145). Coinciding with the late Saladoid period, influences from mainland Barrancoid (AD 350 - 500) appear first with subsequent development of the Troumassan Troumassoid (AD 500 - 1000) and Suazan Troumassoid (AD 1000 - 1450) ceramic styles (Allaire, 2003; Bright, 2011; Keegan, 2000: pp. 145-146; Rouse, 1992). It is during this time we also can see breakdowns in the longstanding trade networks which were a key component of the Saladoid cultural time period (Allaire, 2003; Bright, 2011). Following “a long pause”, potentially hindered by an archaic-ceramic age frontier, ceramic populations expanded into the larger islands of the Greater Antilles (1350 - 350 BP). Whether the transition from Suazey to Island Carib in the

Lesser Antilles was the result of displacement or conquest remains to be seen, however, a notable discontinuity in assemblages emphasizes that these were two separate populations and not another in-situ evolution (Allaire, 2003; Boomert, 1986). Perhaps one of the best examples of this is the Cayo Complex from Saint Vincent where there exists concurrent evidence of both local/Suazoid and late prehistoric mainland ceramic elements (Boomert, 1986; Duval, 1996). The Island Caribs were the last notable wave of late prehistoric migrations from South America into the Windward Islands prior to the arrival of Columbus and were essential to the colonial resistance efforts therein (Figure 2.5, blue circle) (Allaire, 2003; 2013; Bright, 2011; Rouse, 1992).

Simultaneous to the Post-Saladoid period of the Troumassoid, the Ostionoid regional ceramic styles (AD 600 - 1200) emerged throughout the Greater Antilles and Leeward Islands (Figure 2.4) (Chanlatte Baik, 2003; Keegan, 2000; Rouse, 1992). Consequently, the Taínos (AD 1200 - 1500) (Figure 2.5, red circles) evolved out of regional influences from both Ostionoid and Casimiroid interaction spheres by 1492 (Figure 2.4, Figure 2.5) (Keegan, 2000; Reid, 2009: p. 74; Rouse, 1992). While fully established by the arrival of Columbus within the Caribbean, the Taíno marked the shift from the ceramic to the formative age (Allaire, 2003; Chanlatte Baik, 2003; Keegan, 2000; Rouse, 1992). Due to the perspective of historical accounts from this time, a strong divisional narrative between the Taíno and Island Carib was established (Hofman, 2013). Broadly speaking, the Island Caribs were described as *invaders* who quickly established themselves within the region as aggressive and combative against both the Taíno and eventual European colonialists. Moreover, to colonial groups the Island Caribs

represented the direct counter-point to the *peaceful* Taíno (Allaire, 2003: pp. 216-218; Duval, 1996: p. 13). The Taíno and Island Carib were the key contact indigenous groups at the beginning of the historic age with Columbus' arrival (Allaire, 2003; Chanlatte Baik, 2003; Rouse, 1992).

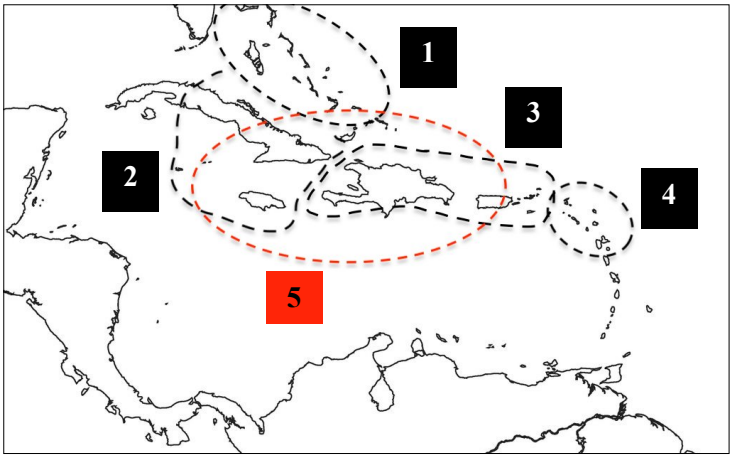

Figure 2.4 Ostionoid landscape. (600 - 1200 AD). (1) Palmetto (2) Meillacan (3) Chican (4) Elenan (5) Ostionan. Colour image available online. (Image created by author using information from Keegan & Hofman, 2017; Rouse, 1992).

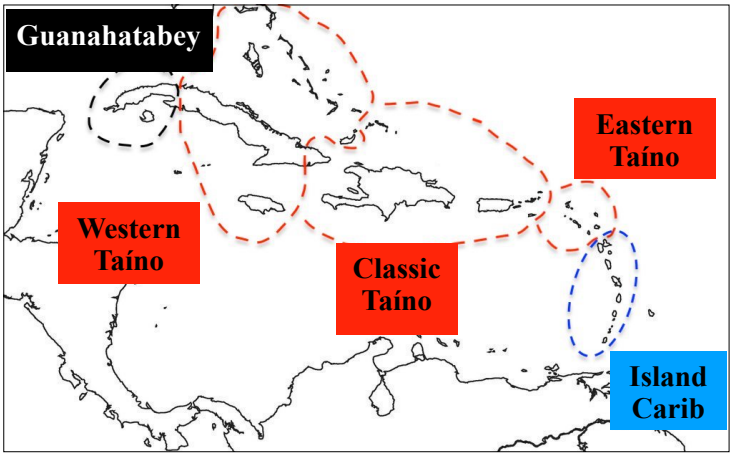

Figure 2.5 The organization of insular Caribbean cultural landscape at the time of Columbus' arrival. Colour image available online. (Image created by author using information from Keegan & Hofman, 2017; Rouse, 1992).

Subsequent colonial interventions within the Caribbean irrevocably impacted both the environment and the indigenous Taíno and Island Carib populations occupying the region (Bright, 2011: p. 37; Hofman et al., 2019). While in the Greater Antilles there are historical accounts of early Spanish settlers marrying into Taíno hierarchies (Sued-Badillo, 2003: p. 268), within the Lesser Antilles there was significant indigenous resistance which greatly hindered colonial attempts until at least the 1700s (Allaire, 2003:

p. 218; Bullen & Bullen, 1972; Duval, 1996: pp. 13-14; Hofman et al., 2014; 2019). In particular, both Saint Vincent and Dominica presented critical strongholds for Island Caribs, where escaped slaves were noted to have fled, creating a new cultural identity in the Black Carib (Allaire, 2013; Bullen & Bullen, 1972; Hofman et al., 2019). Critically, within the Caribbean it is believed that populations and cultures never expanded to city-states due to the unprecedented influence that Columbus and other colonial actors had on the entire region (Chanlatte Baik, 2003: p. 245).

2.3 Archaeological Dynamics of the Lesser Antilles

The pre-colonial Caribbean is defined by extensive trading, mobility networks and interaction spheres that spanned both the insular islands as well as the surrounding mainlands of Central and South America and eventually Europe (Hofman et al., 2014; Rodríguez Ramos, 2010). Where certainly in other regions water can act as a division, the open water of the Caribbean has been referred to as an aquatic motorway linking communities (Hofman, Bright & Rodríguez Ramos, 2010). Initial exploratory voyages in search of raw material during the archaic age cemented trade routes and contacts between regions, ensuring the success of subsequent migratory waves by providing a lifeline of sorts (Hofman, Bright & Rodríguez Ramos, 2010; Hofman & Hoogland, 2011: p. 30). Continued cultural influence from earlier groups as well as those from mainland interaction spheres had significant impacts on artifact trends and sociocultural changes (Antczak et al., 2018; Keegan, 2000; Rodríguez Ramos, 2010). Beyond long-standing dichotomies of the Greater and Lesser Antilles, the Leeward Islands are often associated

more so with the earlier cultural spheres of the Greater Antilles than those from the Windward Islands (Allaire, 2003; Chanlatte Baik, 2003; Hofman, 2013; Keegan, 1994; 2000; Rouse, 1992).

Given the fact that the vast majority of recovered artifacts from the Lesser Antilles are pottery, the ceramic age and the Saladoid in particular have garnered significant research focus (Curet, 1996; Keegan, 2000: p. 135; Keegan & Hofman, 2017: p. 74; Rouse, 1992). Beyond diagnostic ZIC and WOR ceramic patterns, zoomorphic adornos associated with ceramic vessels are also emblematic of Saladoid occupations (Keegan & Hofman, 2017: p. 80). Critically, Saladoid assemblages are fairly consistent resulting from their united and common cultural identity which was maintained through extensive trade networks strengthened by kinship ties (Curet, 1996; Keegan, 2000; 2018). Perhaps one of the most essential ceramic vessels related to the Saladoid and indicative of food processing techniques, beyond staple vessels such as jugs or bowls, is the griddle. Initial assessments concerning its use were tied strictly to manioc and cassava bread as it had been for Saladoid sub-series that had remained on the mainland (Newsom & Wing, 2004; Reid, 2009; Rouse, 1992). Recent starch analysis has consequently expanded its use to other root crops and plants, with maize having particular significance (Ciofalo, Sinelli, & Hofman, 2019; Keegan & Hofman, 2017: p. 92; Mickleburgh & Págán Jiménez, 2012; Págán Jiménez et al., 2015). Despite the larger trends of ceramic simplification post-Saladoid, during the late ceramic age griddles and other vessels were fitted with legs, pedestals or ringed bases (Keegan, 2000; Reid, 2009; Rouse, 1992).

While ceramic assemblages represent the most immediate evidence for associated Saladoid exchange networks, other components of subsistence economy and sociocultural organizations are reflected in relative changes to material culture (Keegan & Hofman, 2017; Rodríguez Ramos, 2010). Larger observations of habitation sites and villages, with changing organization and burial practices have served to emphasize evolving cultural priorities. In terms of burial practices for example, from the Saladoid to later series within the Greater and Leeward Islands there is a shift from communal ancestral veneration – centralized burials – to more personal observations of familial lineages – burials within habitations areas (Hoogland & Hofman, 2013; Keegan, 2018). Saladoid communities benefitted from larger regional stability, such that local populations could expand specialized land modification and raw material procurement strategies (Hofman & Hoogland, 2011: p. 30 Rodríguez Ramos, 2010). Other critical archaeological evidence depicts shifting religious practices through zemis, adornos, incense burners and three-pointed stones (Allaire, 2003; Keegan, 2000; Keegan & Hofman, 2017: pp. 74-76). Furthermore, raw materials, tools, ornaments and iconographic themes are consistent both within the Insular Caribbean and mainland South America reflective of the permanent trade routes in-between (Hofman & Hoogland, 2011: p. 30). Observable changes in material culture did not result in immediate and abrupt changes, but instead were the consequence of ongoing relations and ties within the Caribbean and mainland Central and South America (Hofman, Bright & Rodríguez Ramos, 2010; Rodríguez Ramos, 2010). Post-Saladoid, the regions of the Greater Antilles and Leeward Antilles continued to co-exist strengthening their larger communities. In contrast, within the Lesser Antilles the

permanent association with South America is evidenced through their comparable material culture, however, they did not abandon ties entirely with the northern circum-Caribbean (Hofman & Hoogland, 2011: p. 31).

2.4 Caribbean Subsistence

2.4.1 Imported Foods

Diet in the Caribbean has been inferred in a multitude of ways including archaeological evidence, biochemical analysis of remains, provenance of raw materials and shared themes in iconography implicitly tied to the trading networks throughout the Caribbean (Hofman, Bright & Rodríguez, 2010; Newsom & Wing, 2004). Current understandings of prehistoric regional plant use has been sourced from both European records as well as archaeobotanical evidence (Callaghan, 2011; Hofman et al., 2019; Rodríguez Ramos, 2010: p. 28; 2013: p. 157; Pagán Jiménez, 2013; Rodríguez Ramos, Pagán-Jiménez & Hofman, 2013: p. 130; Stokes, 1998: pp. 21-22). To some extent, Caribbean research still relies heavily on European narratives and records, however, new technological methods (e.g. stable isotope analysis, starch grain analysis) have allowed discussions regarding early diet to shift away from strict archaeological or written records (Pagán Jiménez, 2013). Based on an overall dearth of critical large scale domesticates and paucity of important cultivars, early archaic and ceramic age populations relied on the transportation of plant taxa and small mammals (Keegan & Hofman, 2017; Stokes, 1998).

Without the benefit of previous mainland connections through vicariance (e.g. Greater Antilles) or land bridges (e.g. Trinidad), the overall diversity of Saint Vincent and

other islands within the Lesser Antilles is limited (CCA, 1991; Keegan & Hofman, 2017; Newsom & Wing, 2004: pp. 10-12). As such, animals species on Saint Vincent include invertebrates, amphibians, reptiles, birds and mammals encompassing both those native to the islands (e.g. rice rat, hutia, various bat and bird species) as well as those likely introduced by migrating early populations (e.g. agouti, opossum, guinea pig, dog) (CCA, 1991; Giovas, 2017; 2019; Giovas, Lebvre & Fitzpatrick, 2012; Simmons and Associates, 2015). In contrast to the autonomy of birds and reptiles to transport themselves to previously uninhabited islands, the scope of nonnative mammalian movement throughout the Caribbean was largely driven by human intervention (Giovas, 2017: p. 2; Keegan & Hofman, 2017). While the zooarchaeological evidence of transported animals is minimal on Saint Vincent, nearby evidence from Saint Lucia, The Grenadines, Grenada and Carriacou indicates the presence of agouti, dog, guinea pig, pecarry, opossum, armadillo and deer (Giovas, 2017: p. 5; Giovas, Lebvre & Fitzpatrick, 2012). Of key importance here is that not all of these animals were live transports and instead were animal products in the form of raw material for artifact production (Giovas, 2017: p. 2; LeFebvre, 2007; Rainbow & Giovas, 2021). Within the Lesser Antilles, there is a lack of large-scale comprehensive studies regarding this and as such distinguishing between live and selective product transportation requires further species specific research (Giovas, 2017: p. 10)

Beyond understandings of imported animals and products, the Saladoid also brought with them a number of agricultural practices and technologies to more effectively use their environment. In terms of land modification, Saladoid groups utilized house

gardens, cultivated fields and gardens on higher slopes as well as incorporating slash and burn practices (LeFebvre & deFrance, 2018; Newsom & Wing, 2004; Reid, Thomas & Fitzpatrick, 2018; Pagán Jiménez, 2013; Rivera-Collazo & Sánchez-Morales, 2018). As well, food processing technologies are emphasized through the presence of griddles as previously mentioned. With regard to maize, recent work has more closely explained its dispersal throughout the Caribbean suggesting a much earlier (~350 BC) arrival within the insular Caribbean (Ciofalo, Sinelli & Hofman, 2019; Mickleburgh & Pagán Jiménez, 2012; Rodríguez Suárez & Pagán-Jiménez, 2008; Pagán-Jiménez et al., 2015). Plants that were used for potential medicinal use and ceremonial importance have also been noted throughout the Caribbean (e.g. trianthema, primrose, fish poison, tobacco) although to a much smaller scale within the Lesser Antilles (Pagán-Jiménez, 2013: pp. 397-398; Stokes, 1998: pp. 24-25).

2.4.2 Local Fauna and Flora

The Saladoid diet on Saint Vincent was largely defined by the local and nearby island biodiversity, however, archaeobotanical and zooarchaeological remains within the Lesser Antilles are scarce (Krigbaum, Fitzpatrick & Bankaitis, 2013; Laffoon et al., 2016; Stokes, 1998). Pre-colonial populations relied heavily on both imported cultivars as well as terrestrial mammals in the absence of large scale domesticates (LeFebvre & deFrance, 2018). Evidence suggests that initially, populations were much more reliant on terrestrial species including rice rats (*Oryzomyini* sp.), agouti (*Dasyprocta* sp.), armadillo (*Dasypus novemcinctus*), iguana (*Iguana* spp.), opossum (*Didelphis marsupialis*) and potentially

guinea pigs (*Cavia porcellus*) with land crabs (Gecarcinus, Cardisoma) also representing an essential species (Keegan & Hofman, 2017; LeFebvre & deFrance, 2018; Newsom & Wing, 2004; Stokes, 1998). Beyond endemic bat species, a wide variety of bird species (dove, owl, pigeon) may also have been exploited (LeFebvre, 2007; Stokes, 1998). Within the zooarchaeological record, bird specimens are poorly represented at the Grand Bay site in Carriacou producing only a single pigeon bone making definitive conclusions regarding contributions to diet difficult (LeFebvre, 2007: p. 940). While the presence of manioc *(Manihot esculenta),* sweet potato *(Ipomoea batata)* and arrow root *(Maranta arundinacea)* were noted within colonial records, as previously mentioned these cultivars were likely introduced much earlier. Given the presence of griddles at the Escape Site, likely manioc and possibly maize could have contributed to Saladoid diet (Moravetz & Callaghan, 2011; Rodríguez Ramos, 2010; Rodríguez Ramos, Pagán-Jiménez & Hofman, 2013).

In contrast to the depauperate terrestrial species on Saint Vincent primarily limited to small mammals (rodents, amphibians, reptiles), the scope of biodiversity and available fauna within the various marine ecosystems is independent from direct human intervention and represents an incredible array of species (CCA, 1991: p. 61; Giovas, 2016). Within the various ecosystems encompassed within SVG there are over 800 species of marine invertebrates, reptiles (sea turtles, iguanas), shellfish (molluscs, freshwater shrimps, conch, crabs) and hundreds of fish species (CCA, 1991: p. 50; LeFebvre, 2007; Simmons and Associates, 2015 pp. 17-22). Certainly, while aquatic species – tuna, skipjack, jack, conch, lobster, crab, shrimp – tend to be associated with

specific habitats, each environment plays a critical role (e.g. nursery habitats, feeding grounds) and fish move interchangeably between them depending on their life stage (CCA, 1991: p. 50). Beyond established marine species, there are larger migratory mammals such as sharks (*Carcharhinus* sp.) and whales (*Cetacea* sp.) which occupy and pass through deeper pelagic waters (CCA, 1991: p. 98). Indeed, to some extent these species all appear within zooarchaeological contexts on Carriacou (LeFebvre, 2007). Additionally, monk seals (*Monachus tropicalis*), whales and porpoises (*Cetacea* sp.) were potentially consumed so long as conditions were favourable or opportunistic (e.g. beached). Based on its variety of uses for both food and raw materials, the queen conch (*Strombus gigas*) mollusk was also potentially a critical component to early diets (Keegan & Hofman, 2017: 74; Stokes, 1998). Looking at prehistoric archaeological assemblages from the Leeward Islands and Puerto Rico it is possible that for the Saladoid occupying the Escape Site, earlier occupants may show a stronger association to reef resources. With time, there may have a been an increasing emphasis on deeper pelagic fish moving away from shallow water species (Wing & Wing, 2001). Unfortunately, the intricacies of Saint Vincent aquatic fish species continue to be understudied (CCA, 1991: p. 50; Simmons and Associates, 2015: p. 21).

Chapter 3: Archaeological Applications of Stable Isotopes

The following discussion introduces the principles of stable isotopes while contextualizing them within the scope of archaeological applications before providing more specific information regarding carbon and nitrogen stable isotope analysis. By first discussing the overviews of both carbon and nitrogen, variations within the biosphere are better established and understood. This chapter will conclude with an examination of key tissues and biomolecules of interest – collagen and structural carbonate – as well as the sources of contamination and key markers for quality and distinguishing diagenetic influences.

3.1 Stable Isotope Analysis

3.1.1 Principles of Stable Isotopes

Isotopes are elements which possess the same number of protons in the nucleus but a variable of neutrons. As this definition does not necessarily account for mono-isotopic elements, Sharp (2017: p. 2-1) instead describes isotopes as, "a particular form of an element defined by a specific number of neutrons" (Sharp, 2017: p. 2-1). Importantly, the combination of protons and neutrons in this context yield either stable or radioactive isotopes, with both expressions having their own research use and applicability. For example, carbon has three associated isotopes – ^{12}C, ^{13}C and ^{14}C – and it is the unstable combination of protons and neutrons in ^{14}C that allows for radiocarbon dating of organic materials (Arnold & Libby, 1949). Conversely, ^{12}C and ^{13}C are stable with relative differences being the result of fractionation from being incorporated into biological systems (Fry, 2006; Sharp, 2017).

Fractionation is understood as the selective routing of heavier and lighter isotopes within systems which are preferentially incorporated by different substrates (Urey, 1947). Heavier and lighter isotopes are the product of relative quantities of protons and neutrons which result in same elements having minutely different overall masses. While slight, these differences in weight drive larger chemical and biological processes (Fry, 2006: p. 27; Sharp, 2017: p. 2-4). With the analogy of rolling a ball up a hill, the larger and denser the ball the more energy is required; the same can be said for chemical reactions and the uptake of isotopes into biological substrates. It is the intrinsic behavioural differences between 'heavy' and 'light' isotopes that drive their relative concentrations (Hoefs, 2009: p. 11; Sharp, 2017). Such that, lighter isotopes are energetically easier to uptake and are preferentially incorporated throughout the body in contrast to heavier isotopes (Sharp, 2017; Young, Galy & Nagahara, 2002).

There exists two types of fractionation – equilibrium and kinetic – with both reflecting transformations within open or closed systems, as quantified by an isotopic fractionation factor (α) which reflects the ratio of light to heavy isotopes in any two substances (Sharp, 2017: p. 2-8; Urey, 1947). Equilibrium fractionation is a chemical process in which there is no overall net change despite all compounds within that space fully reacting. Essentially it operates within a closed system resulting in a negligible fractionation factor ($\alpha = 0$) (Sharp, 2017: p. 1-13). Conversely, kinetic fractionation is far more prevalent and affiliated with incomplete reactions in open systems. Ultimately, in these situations no equilibrium is ever reached due to changing reaction rate constants and

external unidirectional processes with the associated fractionation factor, α, being of some positive value (Sharp, 2017: p. 1-14).

3.1.2 Isotope Ratio Mass Spectrometry

When examining stable isotope ratios, the observable weight differences in natural concentrations are so slight that they must be measured in relative rather than absolute terms (Fry, 2006: p. 27; Sharp, 2017: p. 2-4). The notation required when measuring isotopes is the delta (δ) symbol reflecting the minute differences as per mil or parts per thousand (‰). International standards are incorporated to better precisely contextualize the small changes between samples and standards (Sharp, 2017: p. 2-4). Such that, δ is calculated through the following equation:

$$\delta = \frac{R_x - R_{std}}{R_{std}} * 1000$$

Whereby 'R' denotes the ratio of heavy to light isotopes with 'x' reflecting the sample analyzed and 'std' being the international standard used for calibration. Notably, while the inclusion of the 1000 multiplication has been deemed erroneous, as it is still regularly mentioned by researchers and better reflects the diminutive variation between samples and standards, it has been included here (Szpak, Metcalfe & Macdonald, 2017). Should the sample be enriched in the heavier isotope relative to the standard (e.g. the 'R' is higher for the sample than the standard), δ will be positive. When the opposite is true, δ will be negative (Sharp, 2017: p. 2-3).

Standards employed are internationally recognized and are used in all labs not only to standardize measurements between labs but also to better facilitate inter-laboratory comparisons. For the purposes of this research, standards for both carbon (Craig, 1957) and nitrogen (Mariotti, 1983) as established by international governing bodies – International Atomic Energy Agency, the United States Geological Survey and the National Bureau of Standards – are relevant (IAEA, 1995). The PDB standard from limestone, aptly named for the Pee Dee formation in South Carolina, was used by early researchers to examine carbon stable isotope ratios (Craig, 1957), however, due to resource exhaustion this standard has shifted to VPDB. Through secondary standards LSVEC and NBS 19, VPDB links back to PDB (Brand et al., 2014; Coplen, 1995; Coplen et al., 2006). Most biological material is depleted of ^{13}C relative to the reference material resulting in most carbon isotope ratios being negative (O'Leary, 1988). Conversely, nitrogen analysis is not dependant on material resources instead relying on atmospheric nitrogen, denoted as AIR. As will be more fully examined in section 3.4, nitrogen fractionates within biological systems incrementally increasing such that ratios are generally expressed as positive values relative to the standard (Mariotti, 1983).

IRMS analysis provides raw isotopic data which must be calibrated and assessed for precision, accuracy and reproducibility prior to any further investigation. Based on the range of expected data points, calibration standards of known value at the high and low end are selected to allow for two-point calibration which is more precise than linear and one point calibration. In addition to the calibration standards, three internal check standards are included for analysis; these samples are chosen because their isotopic and

elemental composition falls within the expected range of the specimens being analyzed (Carter & Fry, 2013; Skrzypek, 2012; Szpak, Metcalfe & Macdonald, 2017). The calibration and check standards – representing at least 10 % of the samples being analyzed – are placed at the beginning, middle and end of the run to check for instrumental drift over the course of the analytical session. Internal check standards with known isotope ratios are analyzed as unknowns to determine the accuracy of the analytical session. Where analytical accuracy using internal check standards is concerned with systematic errors, precision is focused on random errors and the reproducibility of standards and is calculated from the standard deviation of the calibration standards, check standards and duplicates (Szpak, Metcalfe & Macdonald, 2017).

3.1.3 Isotopic Fractionation

It is the relationship between producers and consumers as well as the intricate fractionation of carbon and nitrogen within biological systems that is central for researchers concerned with determining paleodiet. Foodstuffs are not directly comparable to human isotope data and to properly ascertain their correct contributions, diet to consumer fractionation must be accounted for. Through experimental research, fractionation between consumers and diet has been modelled for plants at 5 ‰ and 0.5 ‰ for animals for carbon (Bocherons & Drucker, 2003; DeNiro & Epstein, 1978; 1981; Hedges & Reynard, 2007; Tieszen & Fagre 1993). Conversely, nitrogen fractionations are represented as a range of 3 to 5 ‰, although 4 ‰ is regularly applied (Bocherons &

Drucker, 2003; Keegan & DeNiro, 1988; Minagawa & Wada, 1984; Schoeninger & DeNiro, 1984).

While the quantitative relationship between carbon and nitrogen isotope ratios to the tissues of consumers and their diet has been established using controlled dietary studies, it is the observable and apparent enrichment factors between predator-prey pairs that requires further discussion. Specifically, trophic enrichment factors (TEF) denote the observable isotopic shift of $\delta^{13}C$ and $\delta^{15}N$ between consumer and dietary tissues. This is not a strict processual measurement but rather an 'apparent' fractionation requiring the notation of Δ as opposed to α (Bocherens & Drucker, 2003). Early controlled feeding studies demonstrated TEFs of approximately + 1 ‰ and + 3 ‰ for carbon and nitrogen respectively (DeNiro & Epstein, 1978; 1981). Subsequent studies have expanded these ranges from 0 to 2 ‰ for carbon and 3 to 5 ‰ for nitrogen (Bocherens & Drucker, 2003). Considering that later research focused on Type I collagen – hereafter referred to simply as 'collagen' – between trophic levels, the small $\delta^{13}C$ fractionation has been tied to the similarity of amino acid compositions transferred between trophic levels. Notably, the same can not be said concerning the larger variable TEF range for nitrogen (Ambrose & Norr, 1993). Given the scarcity of available human material, however, the scope of TEF related research had been overwhelmingly limited to faunal materials and assumed nitrogen TEF ranges (Hedges & Reynard 2007). While earlier research applied the previously established range of 3 to 5 ‰, more recent research proposes ~ 6 ‰ is more reflective of human-prey pairs (O'Connell et al. 2012). With this more elevated TEF, it is hypothesized that previous archaeological investigations may have overestimated the

animal protein consumption within paleodiet (Hedges & Reynard 2007; O'Connell et al. 2012).

3.2 Archaeological Applications

3.2.1 History of Stable Isotope Analysis in Archaeology

Stable isotope analysis as we apply it to archaeology was first identified at with radiocarbon research in the late 1960s. Researchers at the time were surprised to find unexpected discrepancies in dates between corn and wood specimens, which while not understood at the time, was caused by the different photosynthetic pathways used (Bender, 1968; Hall, 1967; van der Merwe, 1982; Vogel, 1993). Specifically, plants which used the C_4 pathway (e.g. corn) yielded younger radiocarbon dates than those using the C_3 photosynthetic pathway (e.g. wood) (Bender, 1968; Hall, 1967). Researchers in 1960s widely believed that the Calvin (C_3) cycle was the only method by which plants incorporated carbon, however, studies on sugar cane indicated the presence of second photosynthetic pathway. While initially thought to be unique to be sugar cane, eventually it was determined that most tropical plants and grasses also followed the same chemical process establishing the Hatch-Slack (C_4) photosynthetic pathway (Hatch & Slack 1966; Hatch et al. 1967). The observation that plants varied in their carbon isotope ratios as a result of their uptake of carbon, laid the groundwork for the earliest archaeological applications of SIA.

Once the contrast in $\delta^{13}C$ between C_3 and C_4 plants was established, it was proposed that within a C_3 region, the consumption of C_4 plants would be obvious (van der

Merwe, 1982; Vogel & van der Merwe, 1977). It was this notion that prompted the first major archaeological application of stable isotope analysis to investigate the spread of maize within North America (Vogel & van der Merwe, 1977). Around the same time, DeNiro and Epstein demonstrated that diet had a direct influence upon the isotopic ratios of consumer tissues through controlled feeding experiments (DeNiro & Epstein, 1978; 1981). While their work explored both carbon and nitrogen, it was the observation of trophic related increases for nitrogen that was critical. Where carbon could indicate plant protein sourcing, nitrogen could further clarify the consumption of animal protein which, with carbon alone, is indiscernible (Keegan & DeNiro, 1988; Minagawa & Wada, 1984; Schoeninger & DeNiro, 1984; Schoeninger, DeNiro & Tauber, 1983). For archaeological researchers, the early foundational work in the 1970s proved the applicability of collagen to decipher paleodiet, however, significant concerns regarding preservation pushed researchers to continue developing more methods of assessing ancient diet.

During the 1980s, to overcome preservation constraints researchers looked at analyzing new tissues as well as beginning to establish rigorous quality control measures (DeNiro, 1985; Krueger & Sullivan, 1984). Despite initial research proposing that collagen and structural carbonate could be interchangeably used (Sullivan & Krueger, 1981), further work instead clarified that structural carbonate and collagen reflect different components of diet. Where structural carbonate $\delta^{13}C$ represents whole dietary information (e.g. carbohydrates, lipids and proteins), collagen $\delta^{13}C$ and $\delta^{15}N$ denotes protein consumption (Ambrose & Norr, 1993; Hedges, 2003; Krueger & Sullivan, 1984; Masters, 1987; Tieszen et al., 1983; Tieszen & Fagre, 1993). Critically, while structural

carbonate has been referred to by many different descriptors (e.g. bioapatite, apatite carbonate) for this research going forward it will be referred to as ‘structural carbonate’. While today the theoretical understandings of collagen and structural carbonate are universally agreed upon, more minute technical and methodological aspects are still being debated (Garvie-Lok, Varney & Katzenberg, 2004; Koch, Tuross & Fogel, 1997; Snoeck & Pelligrini, 2015). For further discussion regarding these different components, refer to section 3.6 and 3.7.

3.2.2 Interpreting Isotope Data

While there are many methods available to ascertain paleodiet from isotope data, researchers must ensure that the right approach is implemented based on the unique circumstances of their data. Ranging from the qualitative to the statistical, dietary interpretations are dependant on the data and larger contextual constraints at hand. In recent years, Bayesian mixing models and various statistical software have become increasingly popular which incorporate stable isotope data within pre-established conditions of fractionation as well as potential source uncertainty (Laymen et al., 2012; Moore & Semmens, 2008). These methods systematically assess the scope of consumer and potential foodstuff combinations to see which most closely aligns with the study population isotope data. While there are many different statistical software – such as IsoSource, SOURCE, STEP, FRUITS and Baysesian mixing models – they are significantly hindered by the amount of knowledge required concerning behavioural and environmental contextual information to properly discern dietary inputs. Results from

these sources are probabilistic solutions and do not indicate specific dietary contributions from baseline foods and can sometimes be extremely complex to interpret (as reviewed in Layman et al., 2012: pp. 3-5). Moreover, excessive constraints and conditions placed on these determinations can often skew data unnecessarily (Cheung & Szpak, 2020; Fernandes et al., 2014).

Early research relied heavily on the assumption that there were distinct linear relationships between the $\delta^{13}C$ of collagen and whole diet isotopic information (Schwarcz, 1991). In this initial dietary interpretation model, diet was thought to be directly transmitted into the consumer's isotopic ratios, whereby carbon from diet was incorporated without alteration into the consumer tissues. With the clarification that structural carbonate was not interchangeable with collagen, Krueger and Sullivan (1984) noted that there was a consistent offset between the two components with the relative differences varying between diet types (e.g. omnivores vs carnivores). As such, the linear model was re-evaluated to account for the preferential routing of carbon isotope ratios between the different tissues depending on the composition of diets. Where the spacing between collagen and structural carbonate $\delta^{13}C$ pointed to the relative contributions of energy versus protein diet (Ambrose & Norr, 1993; Ambrose et al., 1997; Krueger & Sullivan, 1984; Tieszen & Fagre, 1993; Schwarcz, 1991). Building on this, in an effort to better explain energy and protein mixing, Kellner and Schoeninger (2007) proposed that alternatively $\delta^{13}C_{COL}$ and $\delta^{13}C_{SC}$ be compared once plotted on a bivariate regression model. Within this model, where the data points cluster along two parallel regression lines more accurately denotes C_3 vs C_4 energy (carbohydrate, lipid and excess protein) and

protein consumption (Kellner & Schoeninger 2007). Where the models proposed by Krueger and Sullivan (1984) as well as Kellner and Schoeninger (2007) strictly focus on carbon data, Froehle et al. (2012) expanded these models to incorporate nitrogen. Specifically, by incorporating carbon isotope ratios from collagen and structural carbonate in coordination with collagen nitrogen isotope ratios in a multivariate plot, researchers are better able to discern protein consumption (Froehle et al., 2012).

Considering the limited availability of contextual and local baseline information for the Escape Site, qualitative geometric methods for dietary interpretations will prove much more meaningful for this research. While basic statistics will be applied, qualitative approaches do not depend on these calculations. Through this approach, food webs are created by plotting averages and standard deviations for the $\delta^{13}C$ and $\delta^{15}N$ of plants and animals in their local environment which have been corrected by the appropriate TEF offsets. Human isotope data is then plotted on the food web and then used to determine likely dietary contributions (Layman et al., 2012). With regard to this research and Saint Vincent, the vast majority of baseline information available is non-local sourced from environments which is not congruent with the requirements of isotopic statistical softwares. Without high quality consumer and diet context data, any conclusions regarding the Escape Site diet using statistical software, such as Bayesian mixing models, will likely result in erroneous inferences (Layman et al., 2012). Moreover, as structural carbonate was not analyzed in this research, due to poor preservation, the associated interpretive tools for examining the relative contributions of energy versus protein diet

were not applied (Ambrose & Norr, 1993; Froehle et al., 2012; Kellner & Schoeninger 2007; Krueger & Sullivan, 1984).

Traditionally, animal and plant data has been plotted with human data being corrected to be comparable to likely foodstuffs, however, for this research human data was left unaltered and relevant food web data was corrected. TEF offset corrections to both animal (+ 0.5 ‰ and + 4 ‰) and plant (+ 5 ‰ and + 4 ‰) $\delta^{13}C$ and $\delta^{15}N$ data were applied (Ambrose & DeNiro, 1986; Bocherons & Drucker, 2003; Jim, Ambrose & Evershed, 2004; O'Connell et al., 2012). As isotope ratios are implicitly tied to unique environmental conditions – as is elaborated on throughout the rest of this chapter – using food web data sourced from local flora and fauna is critical. Given the extensive changes that a given landscape undergoes through time, archaeological zooarchaeological and archaeobotanical remains are the best sources of base diet. When unavailable, modern fauna can also be used once corrected for the Suess Effect (see section 3.3.2) (Dombrosky, 2019; Szpak et al., 2013). For the purposes of this research, isotopic data from archaeological and modern Caribbean samples from previous work which is non-local to Saint Vincent to will be used due to the absence of local data (Keegan & DeNiro, 1988; Norr, 2002; Pestle, 2013b; Schoeninger & DeNiro, 1984; Stokes, 1998).

3.3 Stable Carbon Isotopes

3.3.1 Overview of Variation in C_3 vs C_4 Plants

The primary source of variation in carbon isotope ratios is tied to photosynthetic pathways and how C_3, C_4 and CAM (crassulacean acid metabolism) plants individually

fix carbon. Plants that rely on the Calvin cycle and Rubisco for carbon fixation fall under the umbrella of C_3 plants while those that photosynthesize via the Hatch-Slack cycle are C_4. Conversely, the CAM photosynthetic pathway is unique as plants that use it can move between carbon cycles mirroring either C_3 or C_4 plants depending on their environment (Hatch & Slack, 1966; Nier and Gulbransen, 1939; O'Leary, 1988; Tieszen, 1991; Vogel, 1993). Generally C_4 and CAM plants are concentrated within hot and arid climates while C_3 plants tend to be present along coasts and in marshes. Although more uncommon, C_4 plants can also be found in temperate regions and C_3 trees and shrubs can be found in tropical locations (Chisholm, Nelson & Schwarcz, 1982; O'Leary, 1988; van der Merwe, 1982).

Differences in $\delta^{13}C$ between C_3 and C_4 plants are due to the enzymes associated with the uptake of CO_2 with consequent preferential uptake of ^{12}C and ^{13}C (van der Merwe, 1982; O'Leary, 1988). Most vegetables and trees as well as grains/grasses such as barley, rice, oats and wheat are classified as C_3 plants wherein the first photosynthetic product is a 3-carbon acid (Hobbie and Werner, 2004; O'Leary, 1988; Tieszen, 1991). During this process $^{12}CO_2$ is preferentially fixed resulting in $\delta^{13}C$ ranging between – 35 to – 25 ‰ (O'Leary, 1988; van der Merwe, 1982; Vogel, 1993). In contrast, many tropical grasses and cereals (e.g. maize and sugarcane) tend to be C_4 plants and are so named as their first product is a 4-carbon acid following initial carboxylation reactions (Hobbie & Werner, 2004; O'Leary, 1988; Tieszen, 1991; van der Merwe, 1982). Through the Hatch-Slack cycle, ^{12}C is discriminated against in favour of ^{13}C resulting in $\delta^{13}C$ ranges of – 15 to – 11 ‰ (Nier and Gulbransen, 1939; O'Leary, 1988; Tieszen, 1991; Vogel, 1993).

3.3.2 Historic Variation in Carbon Isotope Ratios

When examining modern day carbon isotope ratios it is important to consider the ongoing influence from increased levels of atmospheric fossil fuels. Following the Industrial Revolution, the burning of fossil fuels released increased amounts of ^{13}C depleted CO_2 which has had a large scale impact on atmospheric CO_2 $\delta^{13}C$ compositions. This phenomenon is referred to as the Suess Effect. As a consequence of increased ^{12}C being released into the global carbon cycle, modern materials and their $\delta^{13}C$ need to be adjusted for atmospheric influences; the corrections vary by time (see Dombrosky, 2019). Depending on seasonal shifts and habitat type adjustments of up to + 2 ‰ can be made (Dombrosky, 2019). As an additional consideration, given the present day impacts of climate change on the global community and more importantly within the Caribbean region, modern baseline faunal material faces further scrutiny (Taylor et al., 2012).

3.4 Stable Nitrogen Isotope Ratios

3.4.1 Trophic Position

Controlled dietary studies have demonstrated that there is an observable stepwise increase in nitrogen isotope ratios between consumers and diet. Transitioning between trophic levels – from primary to secondary consumers – $\delta^{15}N$ increases by 3 to 5 ‰ with each step up the food chain (DeNiro & Epstein, 1981; Minagawa & Wada, 1984; Schoeninger & DeNiro, 1984). Trophic related fractionation is caused by the transamination and deamination of the nitrogenous waste excreted by animals in the form of ammonia, urea and uric acid (McCutchan et al., 2003; Steele and Daniel, 1978; Tieszen

et al., 1983). During these processes ^{15}N is preferentially retained while the lighter isotope is excreted, resulting in a relative ^{15}N enrichment of amino acids as transamination tends to favour amine groups with relatively higher ^{14}N concentrations (Adams & Sterner, 2000; Gaebler, Vitti & Vukmirovich, 1966; Gannes, Martinez del Rio & Koch, 1998). While both terrestrial and marine food webs follow in line with the step-by-step trophic level increases, marine $\delta^{15}N$ tend to be higher due to the additional trophic level of secondary carnivores. The presence of ammonium and nitrate within the aquatic nitrogen cycle also contribute to the more elevated marine $\delta^{15}N$ (Chisholm, Nelson & Schwarcz, 1982; Schoeninger & DeNiro, 1984; Montoya, 2008).

Trophic level effects can also be observed in the relationship of breastfeeding and weaning. The tissues of infants whose primary source of nutrition is breast milk, are enriched in ^{15}N relative to their mother's (Fogel, Tuross & Owsley, 1989). Specifically, this relationship depicts a trophic level interaction whereby the child is observed to be occupying a higher trophic position than the mother by consuming the mother's tissues via breastmilk. With weaning, infant $\delta^{15}N$ return to adult ranges (Fogel, Tuross & Owsley, 1989; Reynard & Tuross, 2015; Schurr, 1998).

3.4.2 Plant Nitrogen Isotope Variation

There are many different factors that affect the nitrogen isotopic compositions of plants such as the specific form of nitrogen (e.g. NH_4^+, NO_3^-) incorporated, the uptake pathway, the location where the nitrogen is assimilated and indeed variations between foliar and root plant components (Szpak, 2014). Critical transformations of nitrogen

within the soil each have an associated fractionation factors due to the exchange of elements to form different compounds (Craine et al., 2009; Högberg, 1997; Szpak, 2014). Fertilizers, plant organic matter, climatic conditions as well as water availability will all have a significant impact on these soil processes by either hindering or enhancing the systems and their associated rate limiting steps (Bogaard et al., 2013; Craine et al., 2009; Delwiche & Steyn, 1970; Handley et al., 1999; Szpak, 2014; Zerkle and Mikhail, 2017).

Although nitrogen isotope ratios do not distinguish between plant photosynthetic pathways, they can be used to clarify leguminous and non-leguminous plants. Leguminous plants (e.g. beans and chickpeas) successfully form symbiotic relationships with nitrogen fixing bacteria enabling the direct uptake of nitrogen producing nitrogen isotope ratios around 0 ‰ (Hobbie & Högberg, 2012; Mariotti et al., 1982). In contrast, non-leguminous plants require fungi operating at the soil-plant interface to allow for nitrogen incorporation. These fungi are further influenced by their significant variation in regional presentation, specific carbon requirements, host plant, diversity, and enzymatic nitrogen capabilities (Craine et al., 2009; Hobbie & Högberg, 2012; Szpak, 2014). Within the context of baseline nitrogen isotope compositions, additional effects from the pathway of nitrogen assimilation between mycorrhizal-associated, non-mycorrhizal associated and nitrogen fixing plants can be observed (Hobbie & Högberg, 2012). Where leguminous and nitrogen fixing plants result in $\delta^{15}N$ around 0 ‰, mycorrhizae result in consistently lower $\delta^{15}N$. These can range from the lowest $\delta^{15}N$ of approximately – 5 ‰ within ericoid mycorrhizae up to – 1.1 ‰ which are associated with arbuscular mycorrhizae (Craine et al., 2009: p. 985).

3.5 Variation in Carbon and Nitrogen Isotopes in the Biosphere

3.5.1 Terrestrial Ecosystems

Considerations for humidity, temperature and moisture availability are critical in assessing water use efficiency, stomatal conductance and photosynthetic rates which directly impact isotopic discrimination (Farquhar & Richards, 1984; Farquhar, von Caemmerer & Berry, 1980; Morison & Gifford 1983). Stomata are essential in this process, controlling the flow of both CO_2 and water between plants and the atmosphere. Indeed, the subsequent carbon isotope ratios are directly tied to the opening and closing of stomata where C_3 are open primarily throughout the day and C_4 are open during the night largely in response to light but also to temperature, pressure and water deficients. Moreover, CAM plants which can exist as both C_3 and C_4 plants, will reflect either photosynthetic pathway respectively based on their stomatal conductance (O'Leary, 1988). Generally speaking, C_3 plants have a higher water-use efficiency than C_4 plants as they discriminate less against ^{13}C resulting in their lower $\delta^{13}C$ (Farquhar & Richards, 1984; Vogel, 1993). During times of physiological stress in hot/arid environments, C_3 plants will more strongly limit the time their stomata are open in order to avoid water loss through photorespiration culminating in higher C_4-like $\delta^{13}C$ ranges (Farquhar, von Caemmerer & Berry, 1980; O'Leary, 1988). Critically, local environmental conditions contribute to the regional specificity of isotope ratios for both plants and animals by influencing baseline data (Craine et al., 2009; Hobbie and Högberg, 2012)

Where the range of $\delta^{13}C$ is related primarily to environmental variables, the causes of $\delta^{15}N$ variability is farm more complex and related to both environmental and non-

environmental factors (Craine et al., 2009; Handley et al., 1999; Hobbie and Högberg, 2012; Shearer & Kohl, 1988; Szpak, 2014; Yoneyama et al., 1991). Within more arid regions nitrogen isotope ratios increase due to ^{15}N being preferentially expelled from the soil nitrogen pool due to ammonium volatilization and denitrification nitrogen transformation processes. Both of which, have substantial fractionation factors (Szpak, 2014). This trend importantly extends to the consequences of water availability and temperature. In colder and wetter environments, both foliar and overall $\delta^{15}N$ decrease due to these regions being less susceptible to soil nitrogen loss and excessive ^{15}N loss (Handley et al., 1999; Szpak, 2014).

What is also important to assess in regard to nitrogen transformations are the potential anthropogenic practices that can skew relative changes in $\delta^{15}N$. In regions where manuring is prominently used to help fertilize crops, increases in $\delta^{15}N$ can be significant (Bogaard et al., 2013; Szpak, 2014; Szpak et al., 2014). The degree to which manuring was utilized within the Caribbean basin is debatable given the lack of available large scale farming domesticates beyond the smaller animals introduced to region (e.g. guinea pigs, hutia). Consequently, it is unlikely that manuring would be reflected in the $\delta^{15}N$ of the Saladoid population at the Escape Site (LeFebvre and deFrance, 2018: p. 150). Importantly, there is some evidence that early Saladoid populations employed strategic crop burning practices so its influence is important to consider (Siegal et al., 2018: p. 62). Whether accidental or intentional, the burning of fields and land can result in consequential changes to nitrogen isotope ratios between burned and unburned resources which can be as much as 2 to 8 ‰, although the exact disparity can vary (Szpak, 2014).

Specifically this has been associated with low levels of nitrate assimilation. Nitrate strongly discriminates and is relatively ^{15}N depleted as a result of nitrification fractionation from increases in ammonium (Grogan, Bruns & Chapin, 2000; Cook, 2001).

Considering the high elevation profile of Saint Vincent and the possibility that early populations preferentially exploited land higher up on the volcanic slope, the impact of altitude and the canopy effect must be accounted for (Callaghan, 2007; Fielding & Ollivierre, 2017; Hofman & Hoogland, 2012; LeFebvre and deFrance, 2018). While still minimally understood, studies have demonstrated an associated shift in both $\delta^{13}C$ and $\delta^{15}N$ from coastal to highland areas. Research on this phenomenon within the Andes by Szpak et al. (2013) found that this elevation driven shift in isotope data was tied to water availability and its subsequent variation at different heights. Specifically, higher $\delta^{15}N$ was associated with dryer coastal regions while C_3 foliar $\delta^{13}C$ was positively correlated with mean annual precipitation (MAP) and altitude (Körner, Farquhar & Wong, 1991; Szpak et al., 2013). While the island of Saint Vincent may not have a drastic elevation change like the Andes, there is still a notable environmental shift between dryer coastal flats and tropical forests higher up on the volcano (CCA, 1991; Fielding & Ollivierre, 2017). Coinciding with the presence of tropical forests at higher elevations, the canopy effect must be considered. What this effect describes is that low growing plants closer to the ground have lower $\delta^{13}C$ when compared to those grown in open uncovered conditions (Farquhar, Ehleringer & Hubick, 1989). This is a result of recycling of ^{13}C within the forest ecosystems associated with decomposition of organic materials, as well as leaf level processes tied to shade. Consequently, this relative depletion of ^{13}C can be passed

onto higher consumers such as herbivores and carnivores feeding within the area (van der Merwe & Medina, 1991).

3.5.2 Marine Ecosystems

In a terrestrial context the $\delta^{13}C$ division between plants using the C_3 versus the C_4 photosynthetic pathway is fairly clear, however, this does not hold true in a marine context. For terrestrial resources, the primary source of carbon is via atmospheric carbon dioxide which exists at an approximate $\delta^{13}C$ of – 7 ‰. In direct comparison, marine environments do not have the clear photosynthetic distinctions between C_3 and C_4 plants that terrestrial conditions exhibit and the associated $\delta^{13}C$ ranges instead reflect the carbon sources within the different ecosystems (Chisholm, Nelson & Schwarcz, 1982; van der Merwe, 1982). While carbon sources are generally the same (e.g. carbonates, bicarbonates and dissolved carbon dioxide), freshwater and nearshore plants resemble the $\delta^{13}C$ range of terrestrial C_4 plants while pelagic waters fall in line with C_3 ranges (Sackett et al., 1965; Miller & Page, 2012; van der Merwe, 1982). Similar to terrestrial contexts, marine nitrogen isotope ratios are driven by the form of nitrogen assimilated. For example, cyanobacteria or blue-green algae, have $\delta^{15}N$ around 0 ‰ as dissolved N_2 within the ocean is the primary nitrogen source. Aquatic species also cannot take up dissolved nitrogen directly, instead relying on transformed compounds such as dissolved nitrate and ammonium which depend on local conditions to establish the scope of their final signature (Capone, Tayler & Tayler, 1977; Stewart, 1978). As such it is the

constraints of unique marine ecosystems which drives relative isotopic variation in both carbon and nitrogen.

With regard to Saint Vincent, the implications of coral reefs, seagrass meadows, as well as mangroves are of key interest (CCA, 1991; Keegan et al., 2008; Smikle, Christensen & Aiken, 2010). For Caribbean paleodietary research, reef systems present a critical factor for associated faunal baseline $\delta^{13}C$ and $\delta^{15}N$. Based on early baseline investigative work by Keegan and DeNiro (1988), the presence of blue green algae and sourcing of marine species from nearshore and shallow reef ecosystems was associated with lower $\delta^{15}N$ and higher $\delta^{13}C$ within human bone collagen (Keegan & DeNiro, 1988; Montoya, 2008). The majority of paleodietary reconstructions within the Caribbean highlight the direct influence of reef based systems in terms of relative variations of both $\delta^{13}C$ and $\delta^{15}N$, especially when in contrast to pelagic fish species (Keegan & DeNiro, 1988; Stokes, 1998; Stokes, 2005; Krigbaum et al., 2013). Applying isotopic analyses within near shore ecosystems has demonstrated that for species regularly occupying seagrass meadows and reef systems they have generally more positive $\delta^{13}C$ relative to offshore pelagic regions (Fry et al., 1982).

While certainly coral reefs as well as sea grass meadows likely provided the bulk of marine resources consumed by early populations given the highly productive shallow bank systems surrounding SVG, the potential impact of mangroves should also be accounted for (Keegan et al., 2008). While mangroves present isotopically complex ecosystems current research would not suggest that the intricate cycling of carbon and nitrogen has any influence on occupying fish species. Even when fish inhabit these

systems for daily sheltering or as a nursery habitat for juveniles, the majority will not consume resources from mangroves instead choosing to preferentially feed in nearby seagrass beds (Nagelkerken & van der Velde, 2004; Layman, 2007; Vaslet et al., 2012). For Saladoid groups moving throughout the Caribbean, mangroves are repeatedly highlighted by researchers as a region they exploited and often lived in close proximity to (Antczak et al., 2018; Boomert, 1999; Bright, 2011; Hofman et al., 2014). Indeed, given the emphasis placed on land crab exploitation, mangroves were an integral ecosystem for early populations and initial settlements (Boomert, 1999; Callaghan, 2007).

Moving beyond nearshore ecosystems, the dynamics of pelagic regions also warrant discussion. More specifically, how extensively species occupy and move throughout the water column of pelagic regions can impact $\delta^{13}C$ and $\delta^{15}N$. Moving from deep water into shallow reef regions allows for ^{15}N enriched materials from farther down in the water column to be reincorporated into new environments (Shipley et al., 2017). This gradient of change and movement has been used by researchers to highlight marine resource changes in paleo-dietary reconstructions, suggesting a shift to pelagic rather than shallow fish species being preferentially consumed (Keegan et al., 2008; Krigbaum et al., 2013; Giovas, 2016; Laffoon et al., 2016).

3.5.3 Physiological Effects

It is critical to not only consider the influence of external environmental conditions, but also the implications of physiological conditions on isotopic data which are largely driven by tissue turnover. Whereby tissues with a quick turnover, such as hair

or nails, are more susceptible to the minutiae of health status changes (D'Ortenzio et al., 2015; Fuller et al., 2005). Natural fixation processes and preferential retention of isotopes play key roles in metabolism which inherently reflect larger physiological changes (Adams & Sterner, 2000; Curto et al., 2019; Gaebler, Vitti & Vukmirovich, 1966). As such, analysis of carbon and nitrogen stable isotope ratios can point to internal nutrient resource allocation and changes in metabolic balances impacted by stress and physiological constraints (e.g. pathologies) (D'Ortenzio et al., 2015; Fuller et al., 2005; Reitsema, 2013). In situations where bone is irreversibly wasted resulting in consequent recycling of tissue protein, higher $\delta^{15}N$ are observed when samples close to pathological lesions are examined (Curto et al., 2019; Katzenberg & Lovell, 1999). Bones suffering from lesion producing illnesses can have carbon and nitrogen isotope ratios skewed as much as 0.6 and 2.5 ‰ respectively (Curto et al., 2019; Olsen et al., 2014). Of the investigated pathologies, nonspecific lesions associated with osteomyelitis (Olsen et al., 2014) and fracture callus' (Curto et al., 2019) produced the most significant changes to carbon and nitrogen isotope data and could indeed impact final paleodietary interpretations. As such, bones showing any signs of pathology should be steadfastly avoided to reduce potential influence on isotopic data (Katzenberg & Lovell, 1999; Olsen et al., 2014). No specimens from the Escape Site show any signs of pathology that would affect isotope ratios.

3.6 Bone Composition

3.6.1 Collagen

As a whole, bone is composed of both organic and inorganic components representing approximately 20% and 70% respectively with the remaining 10 % being bound water (Krueger & Sullivan, 1984: p. 209; Rogers, Weidmann & Parkinson 1952). The majority of the organic component of bone is Type I collagen, which is the most consistently used biomolecule for investigating paleodiet (Collins et al., 2002; Katzenberg, 2008; Rogers, Weidmann & Parkinson 1952). Collagen, is found within tendons, ligaments, tooth dentin and bone and is composed of repeating glycine, alanine, proline and hydroxyproline amino acids (Shoulders & Raines, 2009; Tuross, Fogel & Hare, 1988). As it contains both carbon and nitrogen, reflecting 35 % and 11-16 % by weight respectively, collagen remains of key interest for paleodietary research (van Klinken, 1999: p. 691).

The primary components of diets are carbohydrates, lipids and proteins with each reflecting specific metabolic pathways and the consequential routing of macronutrients. Such that the isotope ratio of collagen registers only the proteinaceous components of diet consumed (Ambrose & Norr, 1993; Krueger & Sullivan, 1984; Lee-Thorp, Sealy & van der Merwe, 1989), as a result of the synthesis and procurement of essential and non-essential amino acids (Ambrose et al., 1997; Schwarcz, 2000). Where non-essential amino acids can be synthesized via internal processes, essential amino acids must be obtained through diet and consequently undergo very little isotopic fractionation during uptake. In contrast, while non-essential amino acids can be produced internally via multiple

biosynthetic pathways and precursors, it is energetically more efficient to obtain them via diet. As such, it is the associated substrate and biological starting point that determines the level of fractionation from kinetic isotope processes (Schwarcz, 2000: p. 192-193). In diets with sufficient amounts of protein, the carbon will be preferentially incorporated into collagen for amino acid synthesis to effectively conserve energy. Conversely, when organisms are not reaching that threshold of minimum sufficient protein intake, collagen $\delta^{13}C$ values will be more comparable to whole diet composition (Ambrose & Norr, 1993; Ambrose et al., 1997; Krueger & Sullivan, 1984).

3.6.2 Structural Carbonate

Where collagen is the proteinaceous component of bone and reflects the associated protein dietary intake, structural carbonate is the mineral component providing support and structure to bone and teeth (Ezzo, 1994). This inorganic component is a calcium phosphate which when crystallized, and if pure, would be denoted by the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. Carbonate values are established through isotopic equilibrium between respired CO_2 and blood plasma bicarbonate sourced from the whole diet (carbohydrates, lipids as well as proteins) (Krueger & Sullivan, 1984; Wright & Schwarz, 1996). With researchers establishing the difference in collagen and structural carbonate, the observable relationship between $\delta^{13}C_{COL}$ and $\delta^{13}C_{SC}$ has been used to better understand paleodiet. To better clarify the protein and energy components of diet the 'spacing' between collagen and structural carbonate $\delta^{13}C$ ($\Delta\ ^{13}C_{SC\text{-}COLL}$) has been established as an essential tool to differentiate between mono-isotopic and multi-isotopic

diets by indicating whether the protein or whole diet $\delta^{13}C$ is more negative. For example, if the $\delta^{13}C$ of the protein component is more negative than the whole diet $\delta^{13}C$ (e.g. C_4 carbohydrates with C_3 protein) the $\Delta^{13}C_{SC\text{-}COLL}$ will be greater than 4.4 ‰. The opposite would occur in instances where the whole diet $\delta^{13}C$ is more negative than the protein component (e.g. diet with C_4 protein and C_3 carbohydrates) resulting in a $\Delta^{13}C_{SC\text{-}COLL}$ less than 4.4 ‰ (Ambrose et al., 1997: p. 351; Norr, 2002: p. 266).

3.6.3 Consequences of Bone Turnover

For the purposes of archaeological research, bone and teeth are the most widely used material in paleodietary research due to their long term preservation (Katzenberg, 2008). Bones are metabolically active biological tissues which are constantly forming in response to external stimuli either through modelling or remodelling. The general process follows that old bone is resorbed by osteoclasts and new bone is subsequently deposited in the lacuna by osteons (Collins et al., 2002; Ezzo, 1994). The formation of new bone is associated with age, health as well as biological sex (Fahy et al., 2017; Hedges et al., 2007). Due to its increased density and slow rate of bone remodelling, cortical bone is preferred to trabecular bone for stable isotope analysis (Robling et al., 2006).

Central to establishing diet is understanding the specific residency time of isotopic information within different tissues where tissues may either depict short term nutritional stresses or long term averages of diet (White, 1993; White & Schwarcz, 1994). Understanding what exactly is being studied is critical for accurate interpretations. Coinciding with early foundational stable isotope work, researchers were investigating

how long radioactive ^{14}C resided within different tissues as it pertains to turnover rates helping to inform dietary investigations. This work demonstrated that within bones, isotopic information reflected long term pooled average for up to 20 years (Hedges et al., 2007; Stenhouse & Baxter, 1977), although recent work has fine tuned this turnover rate to approximately 10 years for both structural carbonate and collagen within healthy adults (Hedges et al., 2007; Fahy et al., 2017). Isotopic data corresponds to an average of the last 10 years of diet and are not sensitive to short term changes in diet. For example, a shift from a C_3 based diet to one dominated by C_4 resources would not be immediately reflected within the stable isotope ratios of collagen and structural carbonate (Olsen et al., 2014; Fahy et al., 2017).

Critically, changes to turnover rates can occur depending on a wide range of factors such as health, age, genetic predispositions, mechanical stimuli as well as specific volume to surface ratios associated with different bones (Fahy et al., 2017; Hedges et al., 2007; Robling et al., 2001). The dynamics of secondary and fragmentary osteons are intricately tied to an individuals age, where with age new osteons remove traces of older ones (Fahy et al., 2017: p. 11). Importantly, it was observed that younger individuals and adolescents had a notably higher turnover rate than adults. Furthermore, research has shown that males remodel faster than females, with ideal Bone Mineral Density being achieved shortly following adolescence (Hedges et al., 2007). There has also been an observed decrease in $\delta^{15}N$ values with higher rates of remodelling, such that between ribs and femora the cross-examination between the elements is not necessarily proactive for fully formed investigations. As such consistency should be maintained for researchers

when possible to keep bone sampling to elements with similar turnover rates and surface to volume ratios (Fahy et al., 2017).

3.7 Tissues and Sources of Contamination

3.7.1 Chemical Characteristics of Collagen

Collagen is an intricate compound formed as a tightly woven triple helix structure made of parallel polypeptide strands (Shoulders & Raines, 2009; Vervloet & Brandenburg, 2017). The organization of these chains consists of amino acids ordered into a highly repetitive sequence, with the mandate that every third amino acid be glycine to better facilitate the tightly bound organization. As a result, collagen retains thermal stability and strength as well as being insoluble (Shoulders & Raines, 2009; Viguet-Carrin, Garnero & Delmas, 2006). Within the triple helix of collagen, there exists multiple types of bonds, which through the course of collagen isolation are systematically weakened and broken allowing for solubilization of collagen. This will be expanded upon further in section 4.4 (Shoulders & Raines, 2009; Szpak, 2019; Vervloet & Brandenburg, 2017).

3.7.2 Collagen: Sources of Contamination

There is an intrinsic difference between exogenous compounds that are incorporated into bones through contamination and post-mortem breakdown via diagenesis. These each respectively present regional problems based on key concerns for researchers; such that diagenesis is tied to poorly preserved samples and contamination is

more so associated with those that are well preserved. Critically, this does not preclude samples which are both poorly preserved and contaminated (van Klinken, 1999). Contamination refers to uptake of exogenous humic contaminants which are the acidic result of plants breaking down and the byproduct of microbial metabolism. Although the specific mechanism by which this occurs is still unknown (Szpak, Krippner & Richards, 2017), when humic contaminants leach into bone they are observed to confound isotopic results due to their relative enrichment of ^{13}C and depletion of ^{15}N (van Klinken & Hedges, 1995). Pretreatment with sodium hydroxide during the collagen isolation stage has been found to sufficiently remove these contaminants although this can also influence collagen yields (Ambrose, 1990; Szpak, Krippner and Richards, 2017). Additionally, humic contamination will result in bones that are observably darker brown in colour (van Klinken & Hedges, 1995; Szpak, Krippner & Richards, 2017). In contrast to contamination, diagenesis or post-mortem breakdown follows the critical chemical interactions by which bones lose initial soft tissues. Over the course of bones being buried, collagen molecules are chemically broken down due to the disintegration of α-chains and the peptide bonds between amino acids causing the overall structure of collagen to collapse (van Klinken, 1999). Specimens experience inherent alteration on many levels including molecular loss, elemental substitution, structural reorganization, increased porosity and overall disintegration of structural and molecular integrity (Nielson-Marsh & Hedges, 2000).

There are a variety of tests to ascertain whether the isotope ratios in bone reflect dietary information, rather than contamination or degradation. For assessing overall

quality of the collagen isolated, ranges of acceptable C/N ratios, %C, %N and collagen yields have been established (Ambrose, 1990; van Klinken, 1999; Neilsen-Marsh and Hedges, 2000; Dobberstein et al., 2009). Collagen yields are the first marker to evaluate preservation since it is obtained prior to IRMS analysis. This marker is a percentage based on how much collagen remained after being pretreatment and is calculated as follows:

$$\text{Collagen Yield (wt \%)} = \left(\frac{\text{(4 mL glass vial weight with collagen) - (glass vial weight)}}{\text{(starting sample mass)}} \right) \text{x } 100$$

Researchers accept that the minimum yield requirement is 1% before the subsequent isotopic data may become spurious and unreliable (Ambrose, 1990; van Klinken, 1999).

Where collagen yields indicate the scope of collagen breakdown (e.g. preservation), the remaining quality markers reflect potential contamination and are the result of the elemental analysis of the sample produced through the IRMS. Atomic C:N, wt% C and wt% N serve to examine the scope of change within the collagenous amino acid composition which have well understood carbon and nitrogen contributions (van Klinken, 1999). In modern, and consequently well preserved material, these contributions are approximately 15.3 to 43.2 % carbon and 5.5 to 17.3 % for nitrogen producing an atomic C:N ratio of 2.9 to 3.6. The atomic C:N ratio reflects these relative concentrations of carbon and nitrogen in the sample and are sensitive to the presence of exogenous compounds (Ambrose, 1990: pp. 437-438; van Klinken, 1999). Non-endogenous lipids or humic contamination would alter the specimen atomic C:N ratios, wt% C and wt% N highlighting those specimens as potentially problematic (Ambrose, 1990; van Klinken,

1999; Szpak, Krippner and Richards, 2017). While these quality control indicators provide essential guidelines for researchers to better determine useable specimens, more recent work suggests instead that the full QC profile needs to be assessed and less rigidly applied (Guiry & Szpak, 2021).

Chapter 4: Materials and Methods

This chapter will begin with an overview of the theoretical approach underpinning this research. Following that, there will be an overview of the archaeological contexts and materials used in this research. Relevant information from contemporaneous research will also be discussed to better frame the data gained from this analysis of the Escape Site. The final sections of this chapter will focus on methods such as sample pretreatment, variation based on fraction size/duration and the instrumental analysis.

4.1 Theoretical Approach

4.1.1 Bioarchaeology Theory and the Biocultural Approach

Human skeletal remains are distinct from other commonly examined elements of material culture as they are not only a physical manifestation of an individual's life but offer further evidence of cultural influences on both life and death (Agarwal & Glencross, 2011; Kakaliouras, 2017). As an essential bridge between anthropology and science, bioarchaeology links key concepts to provide a multidisciplinary approach to interpreting the past and lived experiences of early populations (Kakaliouras, 2017; Knüsel, 2009; Zuckerman & Armelagos, 2011). It is the critical combination of anthropological and sociocultural theory with archaeological data which inform bioarchaeological investigations (Buikstra, 2006; Kakaliouras, 2017). Bioarchaeology as such provides the tools to establish an individuals biological profile – sex, age and health status – but it is the use of the biocultural approach that enhances the collected data by placing these factors within population based patterns of variation. (Agarwal & Glencross, 2011; Kakaliouras, 2017; Zuckerman & Armelagos, 2011). These subsequent observations

inform larger cultural conclusions by contextualizing the individual data within a greater community. Where *community* refers to a dynamic concept of interpersonal relationships spanning from the farthest kin to the closest neighbours; it is the sum total of cultural influence (Becker & Juengst, 2017; Kakaliouras, 2017). Food and diet represent an important connection to larger cultural ideas as an implicit reflection of the wider collective. Despite the availability of a wide range of food, the active decisions behind what is consumed and by whom are more often the result of important socioeconomic factors like age, gender and social status (Agarwal & Glencross, 2011).

While important conclusions were drawn from early strict scientific observations of skeletal markers, their rigid examination of features did not scrutinize the influence of larger power dynamics on the individual experience (Goodman, 1998; Kakaliouras, 2017; Zuckerman & Armelagos, 2011). What has now become integral to a more fully formed understanding of past populations is the incorporation of the biocultural approach. The specific definition of the biocultural approach, and indeed what it means to have bioculturally oriented research, has been evolving over time in response to larger agendas and disciplinary theory paradigm shifts. Despite this, the generally accepted definition of this approach is in its explicit emphasis of the dynamic scope of humans within their cultural and physical constraints; highlighting the critical importance of context and continuous reflection (Becker & Juengst, 2017). It is the function of these 'unseen' sociological influences upon remains and the consequent community adaptations that are stressed rather than simply the physical. With the larger influences of the political landscape, this approach developed into a more engaged framework wherein the

consequences of social relations and power dynamics upon the human body are of central concern (Becker & Juengst, 2017; Kakaliouras, 2017; Zuckerman & Armelagos, 2011). What this approach emphasizes is an intricate focus on the interplay between culture and biology as well as the implications for the human condition across both time, space and environment (Kakaliouras, 2017; Zuckerman & Martin, 2016).

Understanding the scope of the human experience requires the tandem use of both bioarchaeology and the biocultural approach. While the biocultural approach is universally established, the definition of bioarchaeology has experienced significant methodological paradigm shifts over the last century (Knüsel, 2009; Zuckerman & Armelagos, 2011). It was E.A Hooton's *The Indians of Pecos Pueblo* (1930), which represented the first earnest attempt at implementing a more thorough investigation. Prior to that, skeletal examinations had revolved around strict dichotomous characteristics used to reinforce racial classifications and stereotypes in order to rationalize the "othering" of populations (Armelagos, 2003; Goodman, 1998). Beyond heavy reliance on typological assessments, Hooton placed paleopathological frequency within temporal and cultural contexts (Hooton, 1930 as reviewed in Armelagos, 2003). This represented an essential paradigm shift from the culture-historical diagnostic-clinical approach to processual archaeology. These new methods emphasized a perspective driven by process rather than description (Armelagos, 2003; Goodman & Leatherman, 1998; Zuckerman & Armelagos, 2011). While bioarchaeology was first coined and applied by Grahame Clark (1972) referring to zooarchaeological remains at the Star Carr site, bioarchaeology as it is understood today, was developed by Jane Buikstra (1977) (Buikstra, 1977 as reviewed in

Buikstra, 2006; Clark, 1972 as reviewed in Knüsel, 2009). Work through the 1980s continued to move away from strict typological assessments, however, new debates within the 1990s began to question assumed dichotomies of health and non-healthy individuals (Wood et al., 1992). Further applications of bioarchaeological theory have incorporated significant scientific technological capabilities such as ancient DNA analysis, non-invasive observatory methods as well as stable isotope analysis (Agarwal & Glencross, 2011). Of primary concern for this thesis is the combination of bioarchaeological observations with stable isotope analysis, an overview of which was previously detailed and can be found in Chapter 3.

4.1.2 Challenges of Studying the Dead

Certainly one of the most significant challenges in bioarchaeological research is the question of sample bias. In ideal research conditions, samples would reflect the entire interred population with each individual being included in the analysis, however, this is rarely the case. Researchers are constrained by what has been found, what is recovered, what is preserved and how representative these individuals are of the larger population (Argarwal & Glencross, 2011; Blom, 2017; Jackes, 2011; Kakaliouras, 2017). Indeed, to better investigate past groups, a distinction must be made between *population* and *sample* whereby *population* more accurately denotes the full group, while *sample* describes the specimens researchers are limited to for any number of reasons. Understanding and identifying this critical limitation is essential to better discussing final conclusions that have any relevance in reality (Blom, 2017; Jackes, 2011; Kakaliouras, 2017).

This question of implicit sample bias and overall population representation ties in strongly with the osteological paradox. It is the notions of "selective mortality" and "hidden heterogeneity" with regard to frailty and illness that form the basis of that conundrum (Argarwal & Glencross, 2011: p. 2; Wood et al., 1992: p. 343). Selective mortality refers to the fact that we only have the individuals who died at a given time and place whereby they do not necessarily reflect the experiences of the individuals who survived to enter the mortuary assemblage at an older age (Jackes, 2011; Kakaliouras, 2017; Siek, 2013; Wood et al., 1992). Not all individuals within a given population are equally at risk for death or disease and it is difficult to determine if an individual with no signs of disease was healthy or if they were already frail and died quickly before any lesions could form representing a hidden heterogeneity (Siek, 2013; Wood et al., 1992). Ultimately, placing the individuals within the sociocultural conditions of their environment via the biocultural approach and assessing all available human, animal, plant, chemical and archaeological provenance information is critical to the accurate interpretation of past populations (Larsen & Walker, 2010; Siek, 2013)

Considering the long term use of the Escape Site in contrast to the relatively strict association of the burials and artifacts to the early ceramic age, the paleodietary conclusions from this research are understood to represent only a small proportion of the population who both lived on Saint Vincent and occupied the Escape Site with a sample set of 29 specimens from 27 individuals. Despite the remains being too damaged and incomplete to develop full biological profiles, all samples used in this thesis are considered to be adults. What these limitations mean is that this investigation is restricted

not only by the small sample size that's been provided but also by the lack of contextual population level data (Callaghan, 2011; Moravetz & Callaghan, 2011). Implicitly understood is that this limited group does not adequately reflect the full scope of lived experiences on Saint Vincent. Moreover, given the question of infant or subadult diet trends without any available materials to study, these results cannot speak to any age related dietary conclusions.

4.2 Materials

4.2.1 Archaeological Context

The Escape Site (KuCe-5), is located within the south easterly quadrant of Saint Vincent on a stretch of relatively flat land on the coast (Figure 4.1 B) (Bright, 2011: p. 242). Overall, Saint Vincent has a rugged general topography with few level regions, making the low relief of the Escape Site and the immediate surrounding areas critical to early populations as evidenced by the concentration of nearby sites (Figure 4.1 A) (Callaghan, 2007; Fielding & Ollivierre, 2017). While the Escape Site is of primary interest for this research, continued excavations at the nearby Argyle Sites (JtCe-1 and JtCe-2) have recovered similar ceramic material culture suggesting that for early Saladoid populations this flat landscape was critical. Moreover, it has been suggested that Argyle and Escape could represent a larger habitation settlement as opposed to two unrelated archaeological sites (de Guzman & MacKay, 2011; de Guzman, Johannesson & MacKay, 2012).

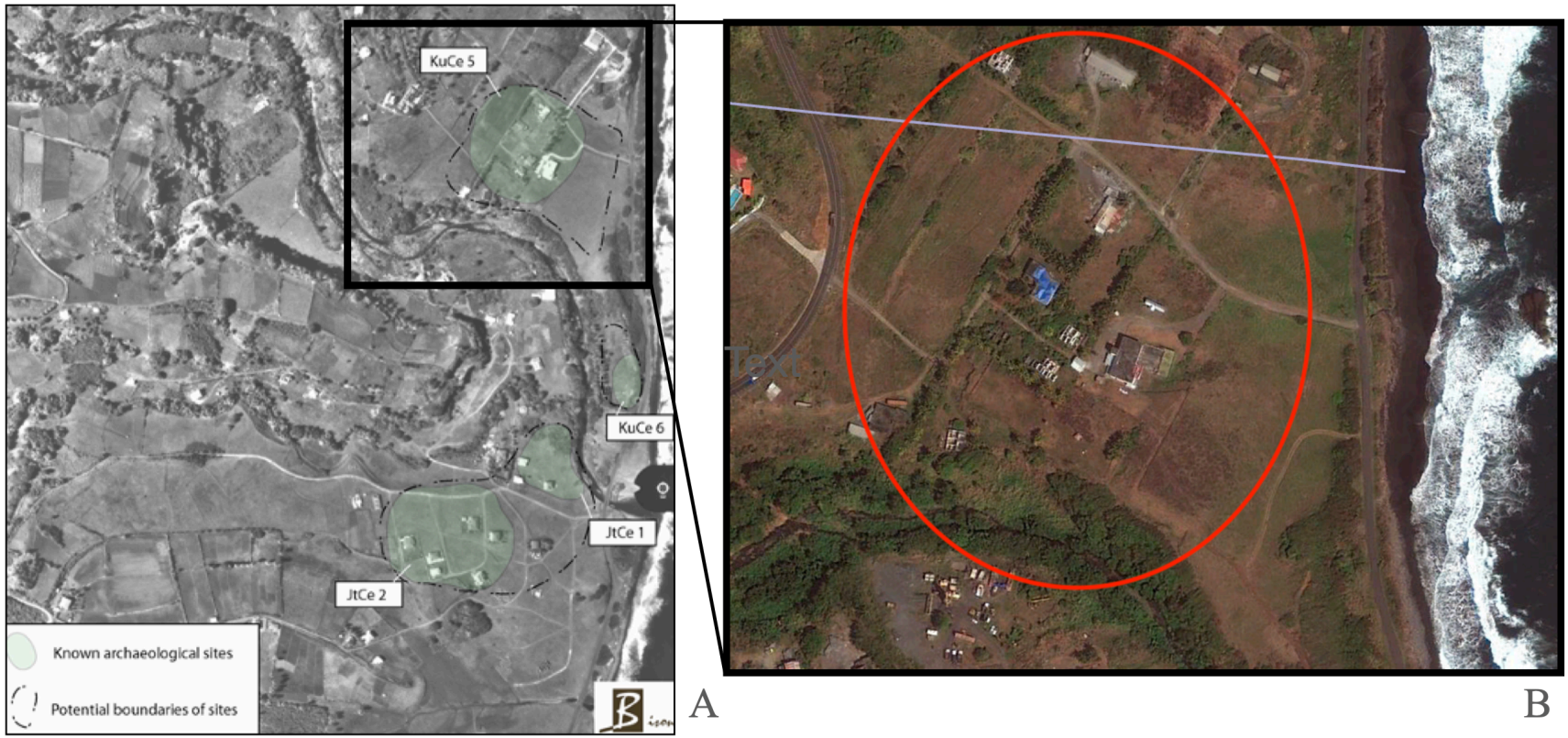

Figure 4.1 (A) depicts the location of nearby archaeological sites in green used with permission (Moravetz, 2010: p. 4) ; (B) terrestrial image of the Escape Site location sourced from Google Earth (2020).

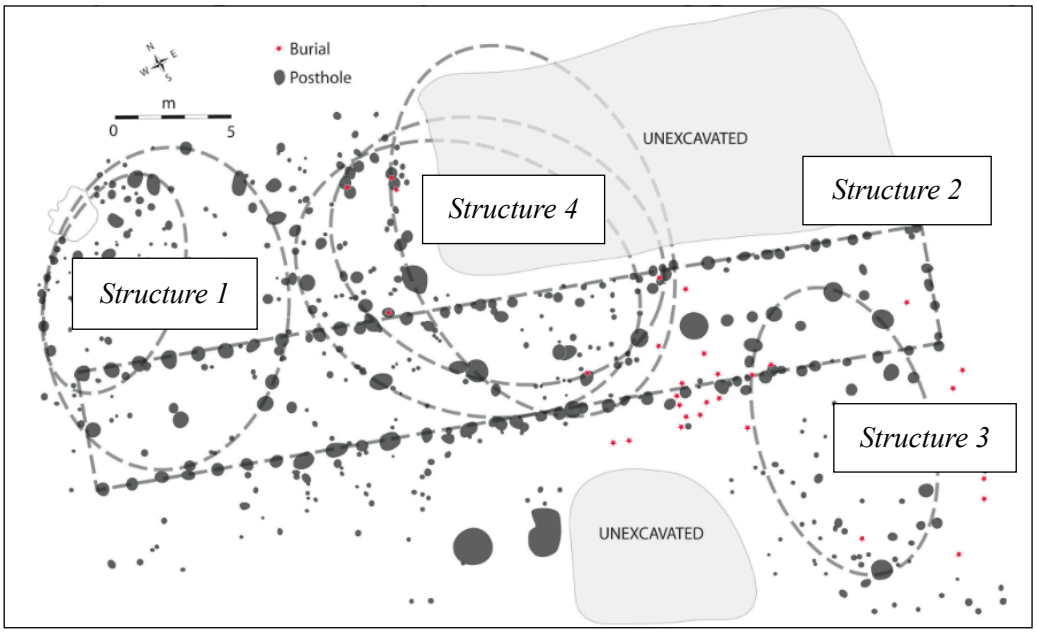

Figure 4.2 Escape Site overview with post holes and proposed structures (Moravetz & Callaghan, 2011: p. 650). Image used with permission. While structure 4 was not labelled in the original report it has been here for ease of discussion. For further information regarding burial locations, see Figure 6.3.

While the region had been studied over the course of the last four decades, it wasn't until 2009 that the site was fully excavated (Figure 4.1). These excavations uncovered more than 620 features, with the majority being post holes associated with a large rectangular structure located on the eastern side of the site with six other oval structures also tentatively identified (Figure 4.2) (Moravetz, 2010: pp. 8-12). Excavations unearthed thousands of pottery fragments overwhelmingly associated with early Saladoid culture groups, which were used to date the site. While generally a good indicator of occupational time periods, ceramic dating alone can potentially mask more complicated migratory events (Fitzpatrick, 2013; Moravetz & Callaghan, 2011). Despite this, it would appear that the Escape Site was used over the course of 2000 years by shifting populations as a habitation settlement (Moravetz, 2010; Moravetz & Callaghan, 2011).

Early work considered the Saladoid period to span 200 BC to AD 600 on Saint Vincent (Duval, 1996), however, further research has suggested instead that initial occupations and associated burials should be tied to the Early Ceramic Age between AD 300/400 and AD 700. This could extend to after AD 700 but no later than AD 1000 (Bérard, 2013; Bright, 2011; Duval, 1996; Moravetz & Callaghan, 2011).

During the excavations, the post holes were a critical discovery and were essential to classify this site as a habitation settlement. The largest feature identified based on the post holes is a large rectangular structure (Structure 2; Figure 4.2) measuring 36 m east to west and 5.5 m north to south that has both perimeter walls and interior central support posts. Within the Lesser Antilles this kind of structure is fairly unique and was the first of its kind found on Saint Vincent (de Guzman & MacKay, 2011; Moravetz, 2010; Moravetz & Callaghan, 2011). Beyond the longhouse, six other structure outlines were tentatively identified from the post holes roughly aligning into three circular structures (Structures 1, 3 and 4; Figure 4.2), with the close concentration of postholes uncovered potentially reflecting structure remodelling through time (Moravetz, 2010: p. 8). Artifacts are predominately Early Saladoid with some Late Saladoid and Suazey pottery also being found, although they were spatially separated. Suazey artifacts, representing a later occupation were concentrated in the western area of the site closer to Structure 1 which also had a potential refuse area which also contained Suazey pottery sherds and footed griddle fragments (Moravetz, 2010: p. 9). Conversely, while few artifacts were uncovered in the eastern section near Structure 3, they were all early Saladoid pottery fragments with ZIC and WOR patterning (Moravetz, 2010: p. 11).

Beyond pottery fragments, material culture was also identified in the form of personal adornments such as beads and pendants found in association with the burials (Moravetz, 2010; Moravetz & Callaghan, 2011). Initial excavations unearthed 24 burials which strongly align with Structure 3 (Figure 4.2, Figure 6.2), however, subsequent excavations by the National Trust and Leiden University uncovered more burials bringing the total found to 36. Focusing on the initial 24 burials, two were extended while the remaining 22 were in a flexed position without any consistent or standardized placements (Moravetz, 2010; Moravetz & Callaghan, 2011: p. 645). General body/element position was not considered influential due to the inconsistent nature and lack of overall patterning which is generally associated with Saladoid burial practices (Moravetz, 2010; Moravetz & Callaghan, 2011: p. 645). There was some evidence of secondary burials with commingled remains and haphazard concentrations of elements (e.g. crania alone, long bones with crania as well as long bones alone). Of the original 24 burials found, at least six were secondary burials (Moravetz, 2010: pp. 13-15). Given the close proximity of the burials to the tentatively identified structures as opposed strictly to a central plaza (Early Ceramic Age) or within dwellings (late Late Ceramic Age) the mortuary practices largely point to internments dating to the early component of the Late Ceramic Age (Hoogland & Hofman, 2013: p. 463). As with most organic materials from Caribbean archaeological contexts, the Escape Site skeletal materials were very friable and poorly preserved which precluded any osteometric analysis at the time of excavation (Moravetz & Callaghan, 2011; Pestle, 2013a).

Amongst the burials found during excavations, some were notable due to the artifact uncovered with only five graves yielding pottery and personal adornments. One of the more interesting burials B7 was an extended burial containing non-local personal adornments (two drilled beads, a quartz pendant and two zoomorphic pendants) suggesting the individual was interred wearing a necklace as well as four axes and a small collection of lithic debitage made from both local and non-local chert at their feet. Moreover, one the axes potentially appears to have been modified to look like a three pointed zemi (religious anthropomorphic or zoomorphic object) (Moravetz, 2010: p. 15). Another burial (B17) contained an intact St. Lucia incised bowl indicating a Saladoid individual (Moravetz, 2010: p. 15). Most relevant to this research is the burial which contained two highly friable griddle fragments placed on top of a skull (Moravetz, 2010: p. 15). Griddles are very commonly found in archaeological excavations throughout the Lesser Antilles and are typically associated with the processing of bitter manioc, a root crop common in South America and introduced to the West Indies (Ciofalo, Sinelli, & Hofman, 2019; Newsom & Wing, 2004; Rouse, 1992). In early surveys of the Caribbean, griddle fragments were found in areas associated with high fertility with nearby habitation sites (Duval, 1996: p. 160). The discovery of griddles in this context further supports the Saladoid designation of the site (Duval, 1996: p. 4).

For this research 30 bones samples were provided, which was ultimately reduced to 29 after initial assessment of the specimens. The 29 samples which were analyzed, are considered to represent 27 unique individuals, based on the provided burials IDs. Due to the poor preservation conditions of the Escape Site specimens, traditional methods of

aging and sexing were not possible but all are considered to be adults (Moravetz & Callaghan, 2011). Although it should be noted that Laffoon (2012) considered B20 a subadult during strontium analysis of materials from the Escape Site. All specimens were taken from mid-shaft cortical regions, as these were described as having the best preservation. More specifically, 15 femur, 5 tibia, 4 humerus, 1 fibula and 1 radius fragments as well as 4 unknown elements approximately 2 ½ - 3 ½ inches in length were provided for analysis. A more detailed, breakdown of materials included in this research can be found in Appendix A, Table A1. The majority of these specimens (n = 25) were uncovered during initial excavations at the Escape Site, with the remaining samples (n = 5) being from subsequent excavations. These 30 bone samples were selected and provided by the SVG National Trust in coordination with Kathy Martin who was involved with both excavations and continues to work on Saint Vincent preserving local heritage. Further clearance was provided under the guidance of Louise Mitchell-Joseph and Dr Roger Duncan. Permission for destructive analysis of these samples was likewise provided by the same institution, the SVG National Trust, and further approved through Trent University ethics board (see Appendix B for documentation).

4.2.2 Implications of Previous Caribbean Research

Coinciding with increases in technological capabilities and shifts away from strict zooarchaeological and archaeobotanical refuse assessments, diet throughout the Caribbean was found to be much more regionally defined; refuting earlier considerations that diet was largely defined by moving populations (Keegan & Hofman, 2017; Stokes,

1998). Large scale Caribbean investigations are limited primarily to Stoke's 1998 dissertation, which found that dietary trends were driven by unique regional conditions as opposed to being the result of cultural influences (anthropogenic changes on environment or demographic characteristics) or time (Norr, 2002; Stokes, 2005; Stokes, 1998). In particular, paleodiet seems to be driven by island size, general topography, biodiversity, distance from mainlands and source islands, island age and geological formation (Frielich, 1967; Stokes, 1998). Given the nature of island subsistence patterns and proximity to marine based resources, it is unsurprising that there is a general marine based economy within the Caribbean, however, isotope ratios suggest that there is regional variation in the degree to which a population relies on marine resources (Keegan & DeNiro, 1988; Stokes, 1998).

Generally speaking, larger islands amongst the Greater Antilles – source islands – had diets which relied heavily on terrestrial or a mix of terrestrial and marine proteins (Pestle, 2009; Stokes, 1998; 2005). Having formed much earlier than the Lesser Antilles and benefitted from previous mainland connections, the Greater Antilles have significantly more biodiverse regions where terrestrial fauna had time to establish themselves within the ecosystems of the individual islands (Garmon, Allen & Groom, 2017; Keegan & Hofman, 2017; Newsom & Wing, 2004). In contrast, populations on younger and smaller islands within the Lesser Antilles, with notably fewer terrestrial resources, relied more fully on marine resources to substantiate their diet (Krigbaum, Fitzpatrick & Bankaitis, 2013; Laffoon & de Vos, 2011; Laffoon et al., 2016; Stokes, 1998). Furthermore, smaller islands in close proximity to source islands will more so

reflect the resources available on nearby prolific and diverse islands due to the extent of nearby trade. As evidenced by the diet from the Tutu Site on St Thomas, which closely aligned to those from the nearby source island of Puerto Rico (Norr, 2002; Stokes, 1998). For smaller islands within the Lesser Antilles, additional differences in available food resources are observed based on the geological foundations and the degree of topographical relief (Stokes, 1998). Between the two island arcs of the Lesser Antilles, those with lower reliefs and limestone sedimentary bases are typically much less biodiverse, however, they tend to have much more prolific reef systems. In contrast, the volcanic islands with higher reliefs tend to have much better soils and consequently more diverse terrestrial fauna but more depauperate marine environments (Newsom & Wing, 2004; Stokes, 1998). This is not to say that populations weren't making more intricate cultural decisions regarding diet or trading foods but, that they simply cannot consume foods that were not there or that could not be successfully integrated or imported (Stokes, 1998: p. 8).

What these larger environmental contexts suggest is that to better establish a likely Saladoid diet at the Escape Site more environmentally comparable and nearby islands would be far more insightful than strict contemporaneous assessments. As environment can be a confounding variable to time within the Caribbean, examining both regionally similar as well as contemporaneous research is essential. For this reason, isotopic research from Carriacou (Krigbaum, Fitzpatrick & Bankaitis, 2013) and St. Lucia (Laffoon et al., 2016) as well as Guadeloupe to an extent (Laffoon & de Vos, 2011) would likely be more directly relevant for establishing diet (Stokes, 1998). This previous research has

demonstrated a mixed terrestrial-marine diet with an emphasis on C_3 plants and near shore reef fish – which produce high $\delta^{13}C$ and low $\delta^{15}N$ (Keegan & DeNiro, 1988; Krigbaum, Fitzpatrick & Bankaitis, 2013). Similar to other research from the Greater Antilles, these studies have shown a relative consistency of carbon and nitrogen stable isotope ratios regardless of age or biological sex (Krigbaum, Fitzpatrick & Bankaitis, 2013; Laffoon & deVos, 2010; Laffoon et al., 2016). Collectively, considering the scope of regional dietary trends as well as environmental constraints it seems likely that Saint Vincent will likewise reflect a heavy reliance on marine resources with some terrestrial protein contributions.

4.2.3 Overview of Previous Methodological Assessments

What is surprising given the broad applications of stable isotope analysis, is that there is generally a large scale lack of consensus regarding collagen extraction procedures. Methodical differences can have critical implications for precision and accuracy significantly hindering inter-lab comparisons (Carter & Fry, 2013; Pestle et al., 2014; Szpak, Metcalfe & Macdonald, 2017). While most procedures incorporate the same chemical steps – lipid extraction, demineralization, humic extraction and solubilization – each step has the potential to influence and reduce quality and quantity of collagen (Pestle, 2010; Tsutaya et al., 2017; van der Haas et al., 2018). Most labs follow a modified Longin (1971) collagen isolation methodology, however, this has not been rigorously and sufficiently tested with modifications being based on assumed optimal conditions. For example, the inclusion of ultrafilters are staples at some key laboratories

(e.g. Brock et al., 2010), but have been shown to hinder high quantity collagen yields (Sealy et al., 2014; Guiry et al., 2016; Szpak, Krippner & Richards, 2017). Given the exceedingly poor preservation observed throughout the Caribbean and certainly seen with the samples from the Escape Site, obtaining sufficient collagen yields is critical to determining any conclusions regarding diet (Pestle, 2013a).

Based on the findings of previous methodological research, this thesis incorporated an experiment designed to investigate how duration and particle size during demineralization influenced collagen yield. Demineralization is the process whereby the mineral component of bone is removed, isolating the the organic material, or 'pseudomorph'. During demineralization there is a physical change to bone which can be observed with increasing translucency and loss of rigidity due to the dissolution of the inorganic structural materials (Ambrose, 1990; Ambrose and Norr, 1993; Katzenberg, 2008). While previous research has been designed to better understand the implications of slight processual changes, these studies often lack sufficient sample size and assessment of key variables. This prompted research by Tessa Grogan and myself with support from Tess Wilson and Dr. Szpak to more closely examine the consequences of demineralization duration and specimen size. A major conclusion of this research is that it is possible to get good collagen yields with a decrease in fraction size and demineralization time (Grogan, et al., 2021).

4.3 Methods and Sample Preparation

4.3.1 Escape Site Materials

All 30 bone samples provided by the SVG National Trust were first documented and photographed prior to any destructive preparation and analysis in accordance with Trent Environmental Archaeology Lab (TEAL) protocols. Each bone sample was assigned a unique ID from the TEAL database and pertinent information for each specimen including material, provenience, TEAL ID as well as any additional available contextual data was recorded. This information was then written on an index card and photographed next to the associated sample before being uploaded to the photo database. Once all the provided bones were documented and catalogued, bones were cleaned of surface level contaminants (e.g. excess dirt or mold) using a NSK dental drill. Through the initial cleaning and documenting process it was observed that of the 30 bones within the collection, 15 of them had visible white mold spots on the surface. Emphasis was placed on avoiding this when possible through the removal of the dirt as well as removing chunks from the specimen away from any concentrations of mold. Although, in certain specimens the extent of the mold was spread across the entire surface and could not be avoided. In removing the exterior discolouration, no further mold spots were found within the bone or anywhere that did not have a direct soil presence. Notably though, specimen B15/ TEAL ID 12280 was excluded from further examination as it was too extensively damaged and co-mingled with dirt to adequately isolate the bone for further cleaning and pretreatment reducing the total sample size to 29.

In total, three collagen isolation protocols were implemented using the Escape Site materials to determine best practices for obtaining sufficient collagen yields with poorly preserved bones. All protocols were based on TEAL lab procedure (memorandum #17-02) with modifications to demineralization time, fraction size as well as sample to acid exposure as follows (Figure 4.3):

(1) > 2 mm chunks treated following TEAL lab protocol #17-02

(2) < 1 mm powder treated following TEAL lab protocol #17-02 with demineralization reduced to 30 minutes

(3) < 1mm powder treated following TEAL lab protocol #17-02 with demineralization reduced to 30 minutes and increasing HCl:sample exposure by dispersing total sample weight over four culture tubes

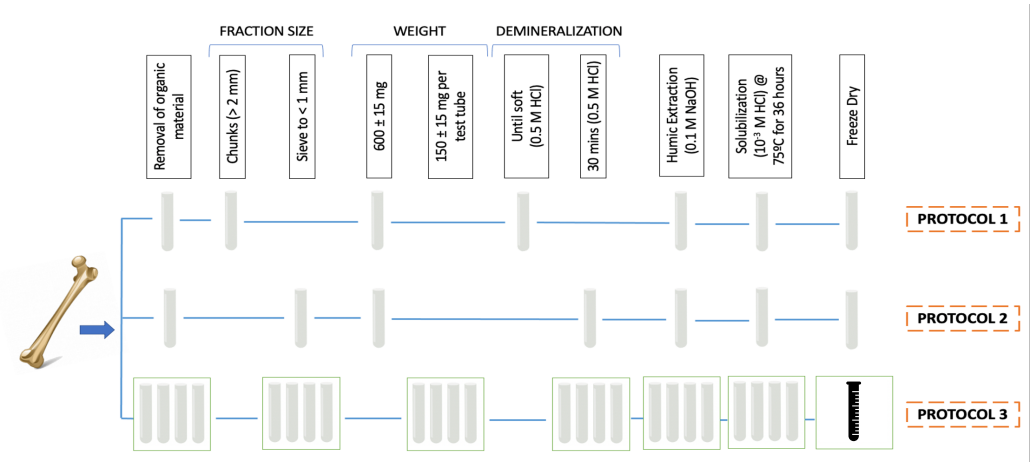

Figure 4.3 Overview of protocols implemented, demonstrating the changing variables (fraction size, acid:sample surface area, demineralization duration).

Each sample was assigned a unique TEAL ID for each run that was completed such that each bone would have four IDs. Considering the state of preservation of the specimens, the maximum recommended weight was chosen for all procedures to ensure

that as much collagen as possible could be isolated. All sample weights were 600 ± 15 mg regardless of protocol to avoid any possible influence on isotopic results (Carter & Fry, 2013). For protocols (2) and (3), bones were reduced in size using a Plattner's mortar and pestle and sifted through a geologic sieve to ensure consistent sizing. All surfaces and tools were cleaned between each bone using acetone and Kim wipes.

4.3.2 Collagen HCl Demineralization : Protocol 1

Protocol 1 followed the TEAL memorandum #17-02 (Figure 4.3) (Szpak, 2019). Bone fragments > 2 mm weighing 600 mg ± 15 mg were placed in a 10 mL glass culture tube and 9 mL of 0.5 M HCl was added using a 10 mL pipette to begin the demineralization process. Culture tubes were covered in aluminum foil, perforated and placed on the orbital shaker. After 24 hours, the HCl was decanted and specimens were prodded using metal tweezers, to examine if the sample was demineralized (e.g. soft). Tweezers were cleaned with acetone and a Kim wipe between samples to avoid cross contamination. If the bone samples remained hard, the HCl was replenished and left for another 24 hours and then rechecked for demineralization; these steps were repeated as many times as necessary to reach demineralization. This process was repeated 3 - 6 times. Once demineralized, samples were centrifuged at 3000 rpm for 15 minutes prior to decanting HCl to avoid potential sample loss. Samples were rinsed to neutrality with Type I water, centrifuged, decanted and repeated until the solution was pH = 6 and considered neutral; this generally took between 4 - 7 rinses. The pH was evaluated after every rinse using pH strips. Following neutralization, 8 mL of 0.1 NaOH was added to each culture

tube in order to remove humic acids. The tubes were covered in aluminum foil, perforated and placed on the orbital shaker for 30 minutes. The NaOH solution was decanted and changed every 30 minutes until solution remained clear; repeating this step between 1 - 5 times. Samples were rinsed to neutrality with Type I water, centrifuged and decanted and repeated until the solution pH was neutral ($pH \leq 8$), on average this took 2 - 5 repetitions. To ensure that the sample was acidic and no longer basic, a solution of 8 mL of Type I water with 2 mL of 0.5 M HCl was added to every culture tube and once centrifuged and decanted was rinsed twice more with 10 mL of Type I water.

To solubilize the collagen, 3.5 mL of 0.01 (10^{-3}) M HCl was added to the culture tube after the sample had been centrifuged and the Type I was decanted. The tube was covered with a loose plastic lid and placed on a heating block for 36 hours at 76˚C to transition the collagen into a soluble state. Following solubilization, the culture tubes were centrifuged at 3000 rpm for 15 minutes to settle any floating particulate matter. The solution was was then decanted, using a 9" Pasteur pipette to previously weighed and labelled 4 mL vials and covered with parafilm. Each vial was inserted into a cryobox and placed into the freezer for 24 hours. Once frozen, the parafilm was perforated, vials were returned to deep freeze for one hour and then vials were placed in the freeze dryer and left for 48 hours. Once dried, the vials were reweighed in order to determine collagen yields. The final step was to weigh out 0.5 - 0.6 mg of the collagen into tip caps using the microbalance which were then folded in on themselves and rolled into a tight ball to ensure that no sample would be lost and that there was no atmosphere within the foil. Tin

cups were then placed into a holder with the associated sample IDs and sent for isotopic analysis to the Trent Water Quality Centre.

4.3.3 Collagen HCl Demineralization : Protocol 2 and Protocol 3

Protocol 2 likewise followed TEAL memorandum #17-02 (Szpak, 2019) but with modifications to demineralization duration (Protocol 2; Figure 4.3) (Grogan et al., 2021). Approximately 600 mg ± 15 mg of bone < 1 mm was placed in 10 mL glass culture tubes and 9 mL of 0.5 M HCl was added using a 1-10mL pipette to begin the demineralization process. Culture tubes were covered in aluminum foil, perforated and placed on the orbital shaker for approximately 30 minutes and were considered done after this regardless of physical changes. All subsequent steps followed the protocol outlined in 4.3.1.

Protocol 3 also generally adhered to the TEAL memorandum #17-02 (Szpak, 2019) with modifications to demineralization duration, HCl:sample exposure as well as freeze drying (Protocol 3; Figure 4.3) based on results preliminary results from Protocol 2 and findings from Grogan et al. (2021). For each sample, four culture tubes were labelled with the associated TEAL ID and additionally identified with a letter between A-D. Four aliquots of 150 ± 15 mg were weighed out and associated with A-D, each specific weight was recorded such that the total weight was 600 ± 15 mg sized < 1 mm. This distribution of sample over four culture tubes was to ensure proper disruption of the nerst layer and exposure to the HCl during the shortened HCl demineralization. Demineralization, humic extraction and freeze drying procedure followed Protocol 2 with all associated cultures

being identically treated. All associated culture tubes (A-D) for each sample were combined in a single 15 mL plastic falcon tube using a 9” inch Pasteur pipette.

The initial collagen yields from Protocol 3 suggested that there was an issue with water loss from the plastic falcon tubes that were used. For this reason, the collagen from Protocol 3 was resuspended and transferred to 4mL glass vials as were used in both Protocol 1 and 2. To re-suspend the collagen, Type I water was heated on a hot plate until it reached 75 ºC - 85 ºC, allowing for 3.5 mL per sample. Using the 1-10 mL pipette, 3.5 mL of hot water was slowly added to the falcon tube making sure to melt all collagen on all sides of the the vessel. Once all the visible collagen appeared to be resuspended or within the Type I solution, a 9” Pasteur pipette was used transfer the liquid to the associated labeled and weighed 4 mL vial, making sure to use a new Pasteur pipette for each sample. To ensure that collagen was sufficiently heated and solubilized within the Type I water solution, the 4 mL vials were placed on the hot plate to allow any remaining collagen to re-suspend into the solution. The heating plate setting was kept to 1 and 2 in order to avoid excessive solution bubbling. Vials were left on the heating plate for roughly 10 minutes and then removed and left to cool off for another 10 minutes before parafilm was placed over each vial. Remaining steps followed the freeze drying protocol as outlined in section 4.3.1.

4.3.4 Statistical Analysis

To better quantify the significance of the Escape Site data non-parametric Kruskal-Wallis tests were done to examine the scope of variation within regional and

temporal data with relevant chi-squared values (x^2), degrees of freedom (df) and p-values provided. Further post hoc comparisons using a Dunn's Test with a Bonferroni correction were also done. Bonferroni corrections through RStudio are applied in order to reduce the possibility of Type I errors (false positive) whereby p-values from the pairwise comparisons are multiplied by the number of tests run. Results from the post hoc Dunn's Test are reported with the appropriate z and p values. For the purposes of this research and to ensure consistency in data analyzed, all relevant $\delta^{13}C$ and $\delta^{15}N$ data were analyzed in one test. Such that, when analyzing regional trends one test would include the Escape Site data as well as all available Caribbean comparative $\delta^{13}C$ data and the subsequent test would look at $\delta^{15}N$. The specific scope of these tests are outlined in Chapter 6.3.1.

4.4 EA-IRMS Analytical Uncertainty

Carbon and nitrogen stable isotope ratios determined at the Trent Water Quality Centre (WQC) with a NuHorizon continuous flow IRMS coupled with an Agilent 7890A GC. Results were calibrated to international standards – VPDB and AIR – through a two point calibration curve using USGS40 ($\delta^{13}C$ – 26.39 ± 0.04 ‰, $\delta^{15}N$ – 4.52 ± 0.06 ‰) (Qi et al., 2003) and USGS66 ($\delta^{13}C$ – 0.67 ± 0.04 ‰, $\delta^{15}N$ + 40.83 ± 0.06 ‰) (Schimmelmann et al., 2016). To establish accuracy and precision calculations, internal standards caribou bone collagen (SRM-1:$\delta^{13}C$ – 19.40 ± 0.08 ‰, $\delta^{15}N$ + 1.82 ± 0.11 ‰), walrus bone collagen (SRM-2: $\delta^{13}C$ – 14.82 ± 0.08 ‰, $\delta^{15}N$ + 15.59 ± 0.13 ‰), polar bear bone collagen (SRM-14: $\delta^{13}C$ – 13.68 ± 0.08 ‰, $\delta^{15}N$ + 21.61 ± 0.15 ‰), Histidine (SRM-21: $\delta^{13}C$ − 9.82 ± 0.05 ‰, $\delta^{15}N$ − 7.47 ± 0.07 ‰) and Glycine (SRM-23) were

placed throughout the microplate. Sample data from B11 and B14 duplicates were averaged by run for ease of discussion. These standards selected due to their similar material composition as well as their thoroughly established isotopic compositions.

Precision (u(Rw)) or random error was determined to be ± 0.14 ‰ for $\delta^{13}C$ and ± 0.39 for $\delta^{15}N$ on the basis of repeated measurements of calibration standards, check standards, and sample replicates. Accuracy or systematic error (u(bias)) was found to be ± 0.02 for $\delta^{13}C$ and ± 0.008 for $\delta^{15}N$ on the basis of the difference between the observed and known δ values of the check standards and the long-term standard deviations of these check standards. The total analytical uncertainty was estimated to be ± 0.06 ‰ for $\delta^{13}C$ and ± 0.39 for $\delta^{15}N$. These calculations were done in accordance with the equations as outlined in Appendix F from Szpak et al. (2017).

Chapter 5 : Results

This chapter presents the results of both the comparison of collagen isolation protocols as well as the Escape Site stable carbon and nitrogen isotope data. The first section of this chapter reviews the variability in collagen yields and other quality indicators for all specimens included in Protocols 1, 2 and 3 as well as a brief comparison by element. Following this, the second section will focus on the isotope data for the individual bone samples as representative of the individuals interred at the Escape Site.

5.1 Collagen Yield and Pretreatment Protocols

For each protocol a total of 29 samples were pretreated. The collagen yields for Protocol 1 ranged from 0.18 - 4.37 % with a mean of 0.93 ± 0.95 %. The yields for Protocol 2 ranged from 0.27 - 3.84 % with a mean of 0.67 ± 0.62 %. The yields for Protocol 3 ranged from 0.82 - 2.73 % with a mean of 1.88 ± 0.48 %. From this, eight samples from Protocol 1, one sample from Protocol 2 and 27 samples from Protocol 3 had collagen yields of at least 1 % and were then analyzed for the stable isotope ratios of carbon and nitrogen. Outliers from Protocol 3 – 12926 (B7), 12929 (B10) – with yields that fell just below the acceptable threshold were also included in IRMS analysis with yields of 0.89 and 0.96 % respectively (Table 5.1). All the data from Protocols 1, 2 and 3 can be found in Appendix A, Table A2.

Due to concerns about the quality of isolated collagen based on the poor preservation conditions on Saint Vincent as well as the extensive degradation that the samples have undergone, QC indicators were strictly applied to the Escape Site data. While in most cases collagen can be classified based on a broader scope of elemental

data, for the purposes of this research only samples which unanimously passed wt% C, wt% N and C:N atomic ratio assessment were accepted. In order to be incorporated in further analysis, sample elemental data had to have wt% C values higher than 13 and wt% N higher than 4.5 resulting in atomic C:N ratios between 2.9-3.6. Anything outside of these ranges for wt% C and wt% N were rejected and excluded from further discussion. For samples which were repeated between protocols, data which had relatively higher wt% C and wt% N were selectively incorporated as opposed to averaging repeated results.

Table 5.1 Escape Site stable isotope and elemental data. Descriptive statistics and a table legend can be found at the bottom of this table.

Site ID	TEAL ID	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{AIR}$ (‰)	Collagen Yield (%)	wt% C (%)	wt% N (%)	Atomic C:N	Protocol
B1	12861	– 18.37	10.41	1.88	**2.59**	0.89	3.39	3
B1A	12862	–14.99	13.77	1.15	14.56	5.47	3.10	1
B2	12863	– 21.09	10.23	1.22	**1.58**	**0.41**	**4.54**	3
B4	12864	– 18.49	8.54	1.56	**1.60**	**0.47**	**4.01**	3
B5	12865	– 18.54	10.21	2.01	**3.34**	**1.13**	3.45	3
B6	12866	– 21.83	5.82	1.66	**0.78**	**0.14**	**6.67**	3
B7	12867	– 16.61	11.94	**0.96**	**5.43**	**1.61**	**3.92**	3
B8	12868	– 25.41	– 6.32	2.26	**0.99**	**0.11**	**10.84**	3
B9	12869	– 23.42	3.07	2.03	**1.74**	**0.24**	**8.60**	3
B10	12870	-17.06	12.39	0.82	16.96	6.18	3.20	3
B11*	12871	-16.26	12.23	3.98	35.38	13.12	3.15	1
B12	12872	– 15.13	11.76	1.77	**6.65**	**2.44**	3.17	1, 3
B13	12873	– 21.48	0.89	1.80	**0.72**	**0.14**	**5.99**	3
B14**	12874	-15.47	11.94	2.12	34.24	12.44	3.21	3

Site ID	TEAL ID	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{AIR}$ (‰)	Collagen Yield (%)	wt% C (%)	wt% N (%)	Atomic C:N	Protocol
B16	12876	– 16.28	13.01	1.64	**8.84**	**3.18**	3.24	3
B17	12877	– 17.41	12.79	1.04	**6.06**	**1.95**	3.62	1
B18	12878	– 18.07	9.35	2.36	**2.25**	**0.77**	3.40	3
B19*	12879	-15.78	12.49	1.74	32.39	11.86	3.19	3
B20×	12880	– 16.85	13.03	1.07	**8.21**	**2.98**	3.22	1
B21A	12881	– 19.58	5.97	1.91	**0.92**	**0.28**	**3.77**	3
B21B	12882	– 23.74	– 1.05	2.31	**0.62**	**0.14**	**5.24**	3
B22	12883	– 20.63	6.45	2.38	**1.20**	**0.28**	**5.05**	3
B23	12884	– 18.56	10.15	2.73	**3.05**	**1.03**	3.46	3
B24	12885	– 18.33	9.60	2.17	**2.83**	**0.96**	3.43	3
B25	12886	– 20.91	7.06	1.03	**1.80**	**0.34**	**6.24**	3
B26	12887	– 20.9	4.20	2.31	**2.09**	**0.50**	**4.82**	3
B27	12888	– 28.61	– 10.05	2.07	**0.61**	**0.09**	**7.57**	3
B28	12889	– 20.48	3.65	2.12	**0.96**	**0.31**	3.66	3
B32	12890	– 24.04	– 7.23	2.31	**0.89**	**0.12**	**8.79**	3
Minimum		-17.06	11.94	0.82	14.56	5.47	3.10	
Maximum		-14.99	13.77	3.98	35.38	13.12	3.21	
Average		-15.91	12.56	1.96	26.71	9.81	3.17	
Standard Deviation		0.79	0.71	1.23	10.08	3.68	0.04	

* : optimal data preferentially selected between two protocols
** : optimal data preferentially selected between all three protocols
Bolded values fall outside acceptable ranges
Grey highlighted boxes denote bone isotopic data that was rejected and not incorporated in further analysis or in the summary statistics provided at the bottom of the table

5.2 Collagen Preservation by Protocol

For Protocol 1, eight samples (not including duplicates) were produced that met the minimum 1 % collagen yield. From these, four produced acceptable data with an average wt% C of 25.19 ± 9.48 (range: 14.56 - 35.39), average wt% N of 9.36 ± 3.51(range: 5.47 - 13.12), and average C:N atomic ratio of 3.14 ± 0.03 (range: 3.10 - 3.17). The remaining four samples were rejected based on their average wt% C of 8.41 ± 1.91 (range: 6.06 - 10.72), average wt% N of 3.03 ± 0.85 (range: 1.95 - 4.03) and average C:N atomic ratio of 3.28 ± 0.24 (range: 3.10 - 3.62). Protocol 2 only produced one sample that met the minimum 1 % collagen threshold yield and also indicated acceptable preservation with a wt% C of 31.55 ± 0.81, wt% N of 11.85 ± 0.41 and C:N atomic ratio of 3.11 ± 0.03. For Protocol 3, 29 specimens, which includes two samples with a yield under the 1% minimum threshold and excludes duplicates, were analyzed. Out of these samples, four had acceptable QC values with an average wt% C of 28.01 ± 7.75 (range: 16.96 - 34.24), average wt% N of 10.20 ± 2.83 (range: 6.18 - 12.44) and average C:N atomic ratio of 3.20 ± 0.01 (range: 3.19 - 3.21). These four samples were used in subsequent analysis. The remaining 25 samples were rejected based on unacceptable wt% C (average: 2.45 ± 2.01; range: 0.61 - 8.84) wt% N (average: 0.74 ± 0.76; range: 0.09 - 3.18) and atomic C:N ratio (average: 5.04 ± 2.07; range: 3.17 - 10.84). None of these samples were used in subsequent analyses (see Table 5.3). See Figure 5.1 for a summary of yield and QC indicators by protocol. For the full details of duplicates and their respective QC indicators, refer to Appendix A, Table A2.

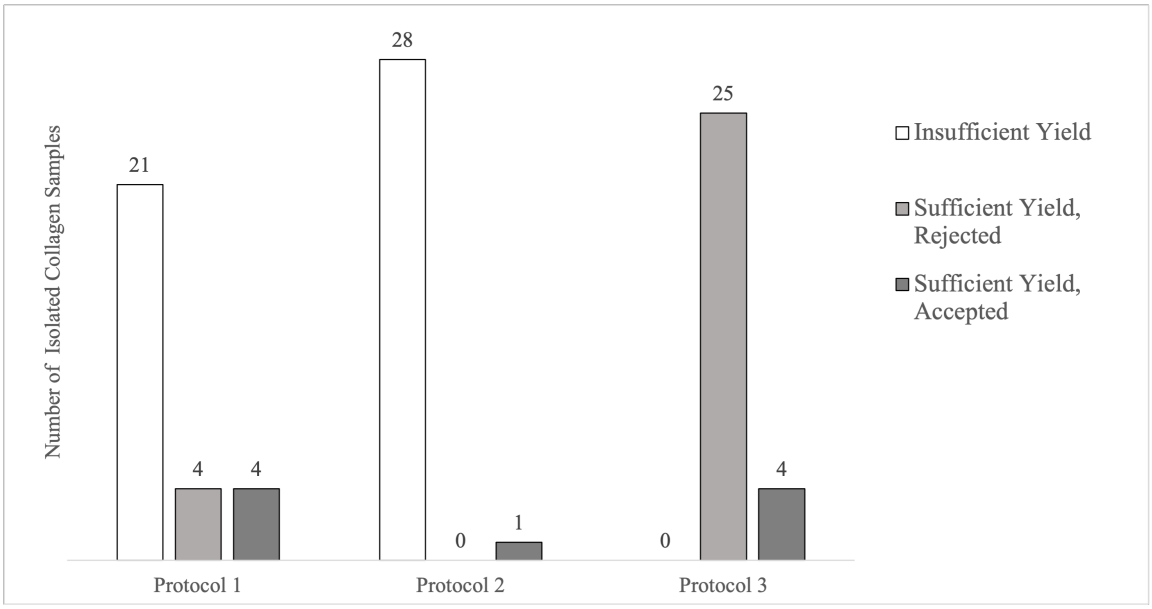

Figure 5.1 Collagen yield and preservation by protocol.

Despite the overlap of specimens between protocols, only 12874 (B14) had adequately preserved collagen across all three different protocols. Within this specimen, wt% C increased by + 3.72 from Protocol 1 to 3 (Figure 5.2, A). Wt% N also increased by + 1.07 between Protocol 1 to 3 (Figure 5.2, B). In contrast, collagen yields decreased by – 2.25 % from Protocol 1 to 3 (Figure 5.2, C). Atomic C:N ratios showed minimal changes, with a slight decrease of – 0.02 between Protocol 1 to 2 and increasing + 0.11 between Protocol 2 and 3 (Figure 5.2, D). While these trends represent the only sample replicated between all the three protocols, the other two samples duplicated between Protocols 1 and 3 (B11 and B19) also indicate inter-protocol changes. Collagen yields decreased from Protocol 1 to 3 for B11 and B14 but increased for B19. For both B14 and B19, wt% C and wt% N values increased from Protocol 1 to 3 and decreased for B11. Atomic C:N ratios all decreased from Protocol 1 to 3 for B11, B14 and B19 (Figure 5.3).

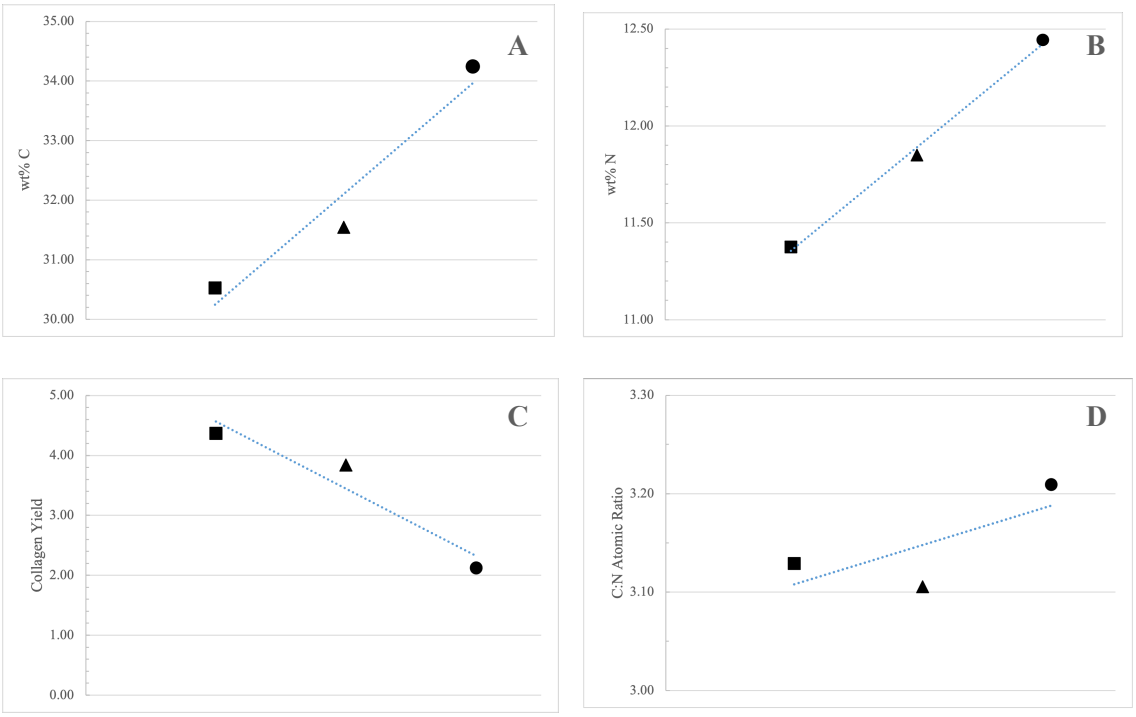

Figure 5.2 B14 Protocol comparison with trend lines. Protocol 1 (■), Protocol 2 (▲), Protocol 3 (•). (A) Protocols vs wt% C, (B) Protocols vs wt% N, (C) Protocols vs collagen yields, (D) Protocols vs atomic C:N ratio.

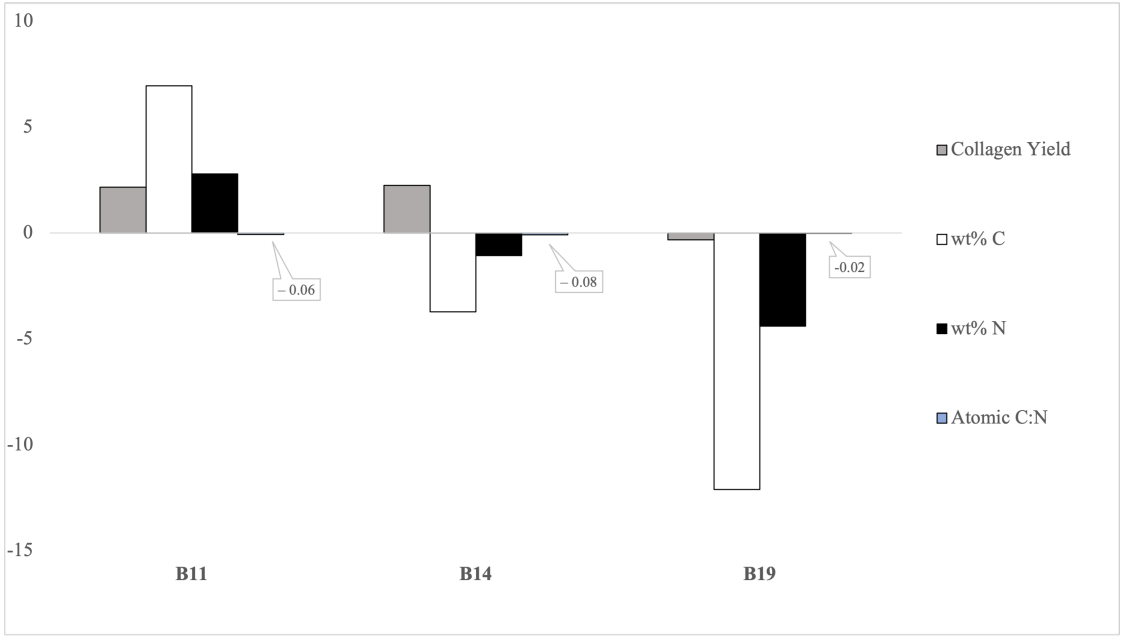

Figure 5.3 Relative difference of QC indicators between Protocol 1 and 3. Columns above the x-axis denote an increase in values from Protocol 1 to Protocol 3 while those below the x-axis reflect decreases. The differences in atomic C:N ratios are labelled as their values are so small they are not clearly visible.

5.3 Isotope Data by Individual

After extensive QC consideration, five specimens were considered acceptable for further analysis and included in this research. For these individuals the average $\delta^{13}C$ was – 15.05 ± 0.79 ‰ (range: – 17.06 to – 14.99 ‰) and $\delta^{15}N$ + 12.56 ± 0.71 ‰ (range: + 11.94 to 13.77 ‰) (Table 5.2 , Figure 5.4).

Table 5.2 Overview of Escape Site Isotopic Data

TEAL ID	Site ID	Element	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{AIR}$ (‰)	wt% C (%)	wt% N (%)	Atomic C:N	Protocol
12862	B1A	Unknown	– 14.99	13.77	14.56	5.47	3.10	1
12870	B10	Femur	– 17.06	12.39	16.96	6.18	3.20	3
12871	B11	Femur	– 16.26	12.22	35.38	13.12	3.15	1
12874	B14	Femur	– 15.47	11.94	34.24	12.44	3.21	3
12879	B19	Femur	– 15.78	12.49	32.39	11.86	3.19	3

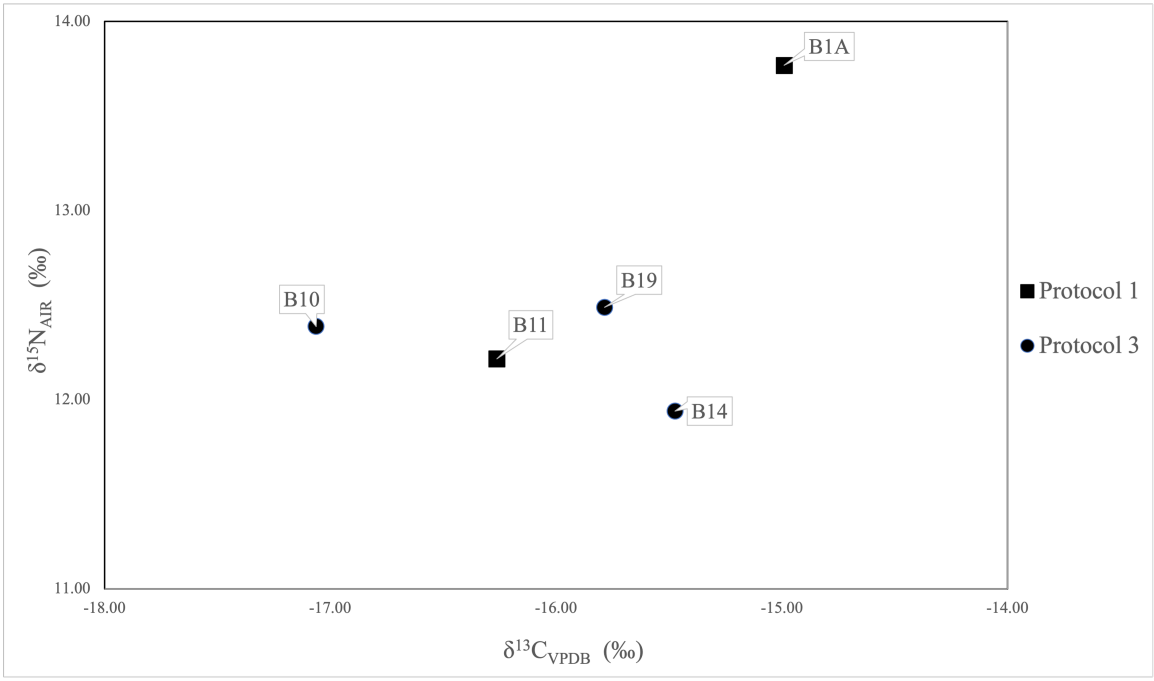

Figure 5.4 Escape Site $\delta^{13}C$ and $\delta^{15}N$ individual data, plotted by protocol.

Chapter 6 : Discussion

To assess the larger implications of the methodological and isotopic analysis of the Escape Site sample population, this chapters serves to answer the following questions:

1. How does fraction size, demineralization and exposed sample surface area affect collagen yield and collagen quality in poorly preserved human bone?
2. What is the best pretreatment protocol for poorly preserved materials?
3. What foods were consumed by the individuals interred at the Escape Site?
4. How does the stable carbon and nitrogen isotope data from the Escape Site compare to regional and temporal isotope data?

6.1 Methodological Implications for Poorly Preserved Bones

6.1.1 Collagen Yield and Quality

While this thesis research is limited by the incredibly small sample size, it nonetheless points to the viability of incorporating a shorter demineralization paired with a smaller fraction size (< 1 mm) (Protocol 3, see chapter 4.3.3). Moreover, powdered bone was most productively demineralized when the sample was spread out over multiple vials to maximize the surface area exposed to HCl; ensuring sufficient disruption of the nerst layer (Collins & Galley, 1998). Generally speaking, with modifications more collagen samples were produced, however, the inconsistency of QC indicators warrant further consideration.

While eight samples produced sufficient collagen from Protocol 1 using bone pieces > 2 mm and undergoing demineralization until physical signs of change were observed, only four samples above a 1% yield had acceptable QC markers once analyzed. Preliminary results from a larger methodological study – which provided the impetus for incorporating a shorter demineralization and powdered sample – demonstrated that when increased surface area (by means of reducing fraction size) is paired with decreased HCl demineralization length, 'peak' results can be achieved faster. Where 'peak' results were considered the 'true' specimen elemental data (Grogan et al., 2021). The necessity of ensuring adequate acid:sample exposure, however, was proven through the limited results of Protocol 2. Out of the three protocols, Protocol 2 was least successful overall with only one sample yielding sufficient collagen for analysis. In contrast, Protocol 3 was the most productive with regard to collagen yields emphasizing that incorporating small particle sizes and shorter demineralizations requires sufficient acid:sample exposure. The increased variation in wt% C, wt% N and C:N atomic ratios, however, caused the majority of Protocol 3 samples to be rejected from further analysis.

Given the well established chemical composition of collagen, elemental data is essential to examine the scope of sample degradation and contamination. Considering that the Escape Site materials have undergone extensive degeneration, the primary goal of QC assessment was determining if the 'collagen' isolated was in fact collagen and not merely what non-collagenous proteins (NCPs) or other materials remained. Such that, for this research a particular emphasis on strict wt% C and wt% N assessment was used to more reliably determine biogenic collagen samples (Guiry & Szpak, 2021; Schwarcz, 2000;

Shoulders & Raines, 2009). Looking at the three samples which were duplicated across Protocol 1 and 3 (Figure 5.3), the trends generally showed a relative increase in wt% C and wt%N from Protocol 1 to the modified Protocol 3 for both B14 and B19. With the magnitude of the relative differences being consistently most pronounced for wt% C. Previous research (Collins & Galley, 1998; Tsutaya et al. 2017) has demonstrated that changes in fraction sizes do not produce significant differences in isotope data and as such changing from chunks to powder cannot account for the differences in elemental data.

Considering the increase in yields and a decrease in overall quality within the Protocol 3 data, it is possible that with a shorter demineralization humic contaminants and NCPs were not fully and sufficiently removed or perhaps that selective amino acid deamination occurred (Grogan et al. 2021; Guiry & Szpak, 2021; Schwarcz, 2000; van Klinken & Hedges, 1995). The increased variation in QC indicators observed within samples from Protocol 3 results was certainly exacerbated by the acidic conditions on Saint Vincent and their impact on preservation (Grogan et al., 2021; Tsutaya et al. 2017); such that, the isolated material may contain non-collagenous contaminations.

6.1.2 Pretreatment Recommendations

Carbon and nitrogen isotope data from five samples were ultimately accepted, which included specimens from Protocol 1, Protocol 2 and Protocol 3. Critically, inter-procedural $\delta^{13}C$ and $\delta^{15}N$ variation for duplicated samples, with acceptable elemental data, did not exceed 1 ‰ (Grogan et al., 2021); above this threshold differences would

indicate change associated with excessive contamination (Guiry & Szpak, 2021; van Klinken & Hedges, 1995) or external influence (pathology, degradation) (Curto, 2019; D'Ortenzio et al., 2015; Guiry & Szpak, 2021; Olsen et al., 2014). Considering the scope of the methodological implications discussed herein, with the added benefit of previous methodological studies, it is reasonable to suggest that shorter demineralizations combined with powdered bone samples can effectively increase useable samples. Within the context of this preliminary investigation into optimal pretreatment protocols for poorly preservation bones, the method as outlined for Protocol 3 is recommended to increase viable collagen samples.

Within the Caribbean, concerns about QC indicators are not frequently highlighted, and when mentioned in any detail, collagen yields are most often cited (as detailed in Krigbaum, Fitzpatrick & Bankaitis, 2013; Laffoon et al., 2016; Mickleburgh & Laffoon, 2018; Norr, 2002; Pestle, 2009; Stokes, 1998; 2005). Given the harsh preservation conditions throughout the Caribbean, and certainly on Saint Vincent – being close to the coast and the acidic volcanic soils – it is reasonable to suggest that the materials from the Escape Site have undergone extensive diagenetic alteration and contamination. The limited number of specimens suitable for isotopic analysis, could be further explained by the age of the Escape Site (300 - 1000 AD) materials; as bones older than 300 - 400 AD are unlikely to produce reliable isotope data (Moravetz, 2010; Pestle & Colvard, 2012). One of the biggest aims for this investigation was to increase biogenic collagen samples, and by following Protocol 3, this was accomplished.

It is essential that researchers understand the potential risks associated with these procedural changes. As outlined previously, there is a possibility that the isolated collagen might not be entirely biogenic and the increased yields could instead be the result of retained non-collagenous proteins and insufficient removal of humic contaminants. It is essential that researchers rigorously evaluate all elemental data – atomic C:N ratios, wt% C, wt% N – for each specimen prior to further study. When considering the finite available material, which can often be highly friable, these concerns do not necessarily outweigh the value in increasing overall viable collagen samples. For this research, implementing a shorter demineralization with powdered specimen was critical in expanding the conclusions garnered by the cumulative isotope data. Without the constraints of poor preservation experienced with the Escape Site materials, it seems likely that other locations within the Caribbean could benefit from incorporating both shorter demineralizations with smaller fraction sizes.

6.2 Escape Site Diet

6.2.1 Escape Site Foodweb

For this research, comparative faunal and human isotope data was pulled from existing literature. Looking specifically at available food web data for the Caribbean, previous research has relied on the same resources which include zooarchaeological and modern samples from the Caribbean (Keegan & DeNiro, 1988; Norr, 2002; Pestle, 2013b; Schoeninger & DeNiro, 1984; Stokes, 1998) as well as some non-Caribbean data (Schoeninger & DeNiro, 1984). While previous research has traditionally relied on the

same food web depictions (see Norr, 2002: p. 265; Krigbaum, Fitzpatrick & Bankaitis, 2013: p. 215), based on updated information regarding TEF offsets and how to adjust plant and faunal data to be comparable, efforts were made to create a new food web image. All existing faunal isotope data within Caribbean literature was incorporated (Keegan & DeNiro, 1988; Norr, 2002; Pestle, 2013b; Schoeninger & DeNiro, 1984; Stokes, 1998) with any modern data which was not explicitly corrected for the Suess Effect being properly adjusted (Dombrosky, 2019). Concerning the non-local data, only pelagic species (e.g. assorted whales) and terrestrial species (e.g. birds and deer) which could have hypothetically been available on Saint Vincent or were comparable to species which would have been in the area were included (Schoeninger & DeNiro, 1984). The summary of comparative food web data can be found in Appendix C, Table C3 and C4.

In order to assess components of the Escape Site diet, human data was left unadjusted while both animal and plant data were corrected using TEF offsets of (+ 0.5 ‰ for $\delta^{13}C$ and + 4 ‰ for $\delta^{15}N$) and (+ 5 ‰ for $\delta^{13}C$ and + 4 ‰ for $\delta^{15}N$) respectively (Ambrose & DeNiro, 1986; Bocherons & Drucker, 2003; Jim, Ambrose & Evershed, 2004; O'Connell et al., 2012). Furthermore, comparisons were kept collagen to collagen as opposed to correcting for collagen to tissue fractionation, as the collagen-tissue offset is poorly understood (Figure 6.1).

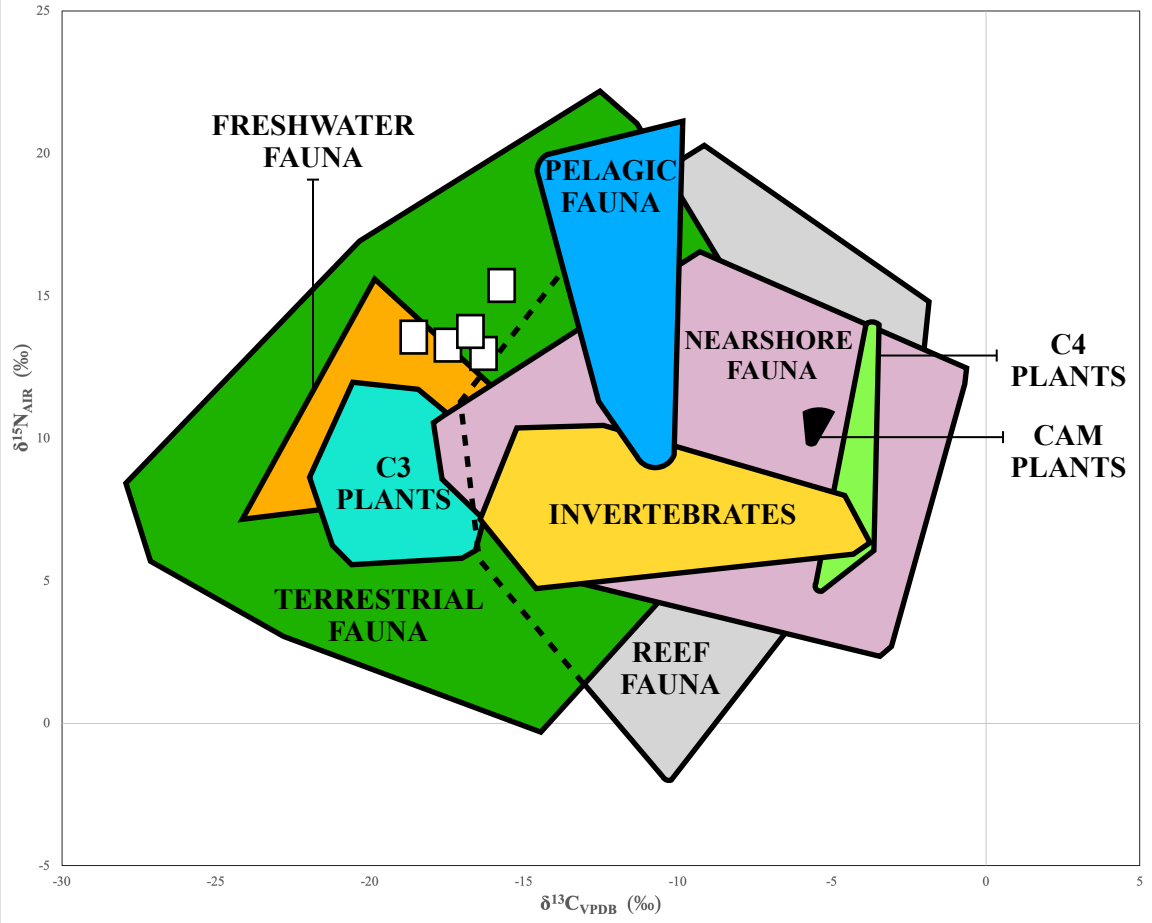

Figure 6.1 Updated Caribbean food web, TEF offset corrections to both animal (+ 0.5 ‰ for $\delta^{13}C$ and + 4 ‰ for $\delta^{15}N$) and plant (+ 5 ‰ for $\delta^{13}C$ and + 4 ‰ for $\delta^{15}N$) have been applied, with human Escape Site data plotted without any TEF adjustments (■) (Ambrose & DeNiro, 1986; Bocherons & Drucker, 2003; Jim, Ambrose & Evershed, 2004; O'Connell et al., 2012). Based on data collected from: Keegan & DeNiro, 1988; Norr, 2002; Pestle, 2013b; Schoeninger & DeNiro, 1984; Stokes, 1998.

6.2.2 Whats on the Menu? Dietary Insights at the Escape Site

When compared to the updated Caribbean food web (Figure 6.1), the Escape Site stable isotope data is consistent with a diet which incorporated proteins from a variety of sources. Considering that the food web within the Caribbean is not clearly delineated and many ecological niches significantly overlap (as reviewed in Pestle, 2013a), determining specific resource use with collagen isotope data alone is difficult, however, the Escape

Site isotope data points to a broad spectrum diet which heavily relied on C_3 plants (Figure 6.1).

Given the presence of ceramic griddles – both Saladoid and Suazey – food processing occurred at the Escape Site, however, the griddles could have been used to process a variety of foods such as manioc, yam, sweet potato or maize (Ciofalo, Sinelli & Hofman, 2019). With the available food web data it seems likely that the Saladoid population relied heavily on C_3 plants (e.g. manioc, yam, sweet potato) (Figure 6.1) although, occasional C_4 plant consumption cannot be disregarded entirely. Despite initial research suggesting that maize was a restricted resource, further work has found more widespread evidence of maize consumption as early as 350 BC - AD 150 (Chinique de Armas et al., 2015; Mickleburgh & Pagán-Jiménez, 2012; Pagán-Jiménez et al., 2015). Critically, sites which were contemporaneous and show similarities to the Escape Site isotope data, including the Tutu Site (St. Thomas), yielded evidence for maize consumption (Mickleburgh & Pagán-Jiménez, 2012). Considering the extensive Saladoid trade network, long-standing ties to the South American mainland as well as nearby evidence, it seems surprising that maize was not more prominent within the Escape Site diet. Some possible explanations for this discrepancy could include that that maize was a ceremonial or restricted resource, that it was hard to transport or perhaps that it was an occasional foodstuff that never came to dominate diet as it was unreliable compared to other established stable crops (Keegan & DeNiro, 1988; Newsom & Wing, 2004; Stokes, 1998).

For the Escape Site, the primary environmental niches that appear to have been exploited were freshwater, reef, nearshore and terrestrial. With the collective data falling on the boundary line of reef species. While certainly marine resources beyond freshwater, nearshore and reef fauna may have been consumed (e.g. pelagic or estuarine), they were incorporated more infrequently and, similar to C_4 plant resources (e.g. maize), never dominated the overall diet. Without Saint Vincent zooarchaeological data, supporting information is unavailable, however, nearby islands (e.g. Carriacou, St Lucia), suggest pelagic resource exploitation (LeFebvre, 2007; Wing & Wing, 2001). The observed $\delta^{15}N$ is not sufficiently high as to suggest that marine resources became the dominant component of the diet, and as well, the $\delta^{15}N$ are not low enough to indicate strictly herbivorous diets (Laffoon et al., 2016: p. 175). Considering that Saint Vincent is a small island, with nearby prolific marine environments the emphasis on terrestrial resources is surprising. Given the quality of the soil on Saint Vincent, due to its volcanic base, it is more plausible that inhabitants of the Escape Site were able to make use of land more immediately bolstered by the extensive Saladoid trade networks ensuring sufficient foodstuffs were obtained.

Ultimately, it would seem that the diet of the Escape Site population reflected a broad spectrum diet relying on many difference sources. Certainly C_3 plants were critical, with their well documented presence throughout the Caribbean, however, contributions from C_4 plants cannot be rejected (Mickleburgh & Pagán-Jiménez, 2012). Moreover, freshwater (e.g. crab, shrimps) and terrestrial fauna (e.g. tortoise, rock iguana, doves) would appear to be regularly incorporated with contributions from reef fish and nearshore

species. The Escape Site isotope data is consistent with a heavy reliance on these resources, although it does not preclude the possibility that other environmental niches may have substantiated the diet. As structural carbonate was not included in this analysis, the larger contributions of whole diet versus protein are not available to better tease out relative contributions of different resources.

6.2.3 Escape Site Burial Implications

As a whole, the Escape Site proved to be a dynamic habitation site with excavations uncovering hundreds of postholes resulting in both oval structures and a longhouse as outlined below and previously discussed (Figure 4.2; Figure 6.2) While it had been suggested that the longhouse was a colonial building used for drying tobacco, its location and available colonial records do not support this indicating this building likely has an earlier ceramic age association (Moravetz, 2010).Without the benefit of strict chronology or ^{14}C dates it is hard to say if perhaps the oval structures (Structures 1, 3, 4) came first or if the longhouse (Structure 2) did and moreover what that means for the Escape Site burials (Moravetz, 2010; Moravetz & Callaghan, 2011: p.649). Broadly speaking, when highlighted on the site map, the Escape Site sample population falls into alignment with Structures 2, 3 and 4, where stronger correlations are observed with the oval structures (Moravetz, 2010: p. 10) (Figure 6.2). Critically, the burial placement could implicate both Early Ceramic Age and early Late Ceramic Age internments.

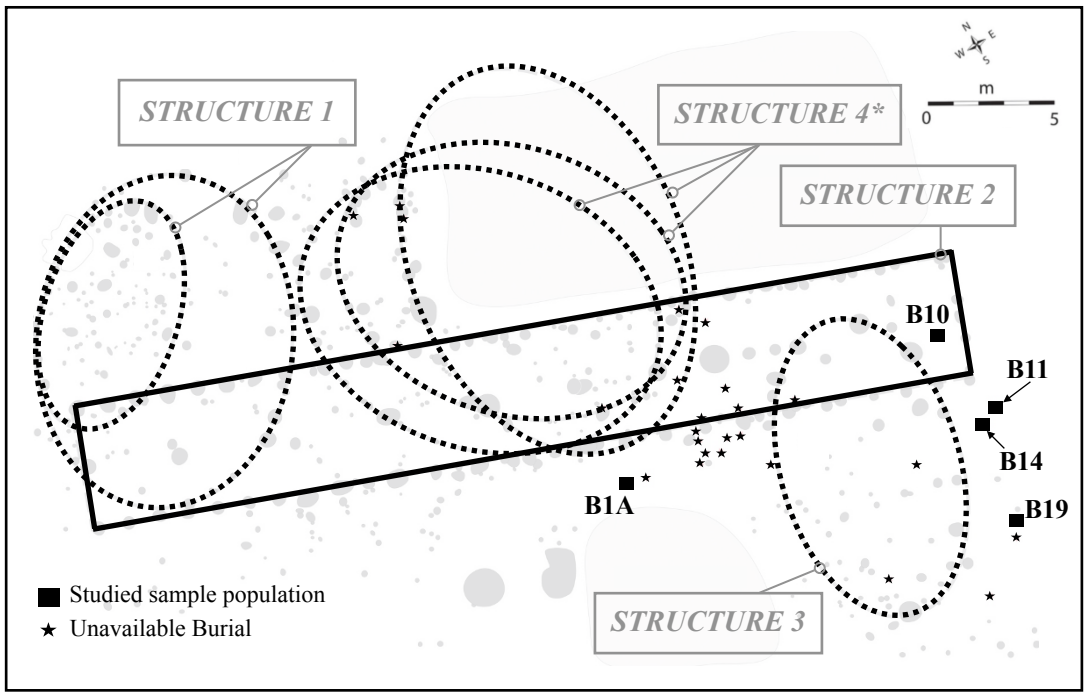

Figure 6.2 Site map including location of burials, postholes and proposed structures. Burials which were successfully analyzed have their associated burial ID. Modified image from Moravetz, 2010, p. 10.
*Structure 4 has been labelled here for ease of discussion, however, it was not labelled within the archaeological report.

Generally speaking, Saladoid mortuary practices show a gradual shift in burial location from central plaza locations and middens to habitation areas near home dwellings/structures to eventually under floors within homes (Hoogland & Hofman, 2013). With the burials largely aligning to the proposed oval buildings (Structure 3 and 4) at the Escape Site, these likely occurred contemporaneously during the Saladoid occupation. Considering the implications of ancestral veneration within Saladoid communities it is not surprising to see the Escape Site burials outside of dwellings which would allow for communal tribute (Keegan, 2009; 2018: p. 9). Although, the close proximity of B10, B11, B14 and B19 eastern edge of Structure in contrast to the relatively more central location of B1A, could indicate a temporal differentiation. Such that, B1A

could represent an internment during the Early Ceramic Age while B10, B11, B14 and B19 could be tied to the earlier phase of the Late Ceramic Age. (Keegan, 2009; 2018: p. 9). Moreover, returning to Figure 5.2 (Escape Site data scatter plot) and Figure 6.1 (Caribbean foodweb), we can see that B1A is not as tightly clustered as the remaining four samples. It could be suggested that these differences are tied to shifts in diet through time, however, without a larger study population these patterns cannot fully be established or solidified. Moreover, the differences between B1A and the remaining samples are not hugely significant with max offsets of 2.07 ‰ between B1A and B10 for $\delta^{13}C$ and 1.88 ‰ between B1A and B14 for $\delta^{15}N$ which cannot account for notable changes in diet. Further investigation would be required to examine this theory.

6.3 Isotopes of the Caribbean: Larger Implications of the Escape Site

6.3.1 Regional Comparisons

As previous research has emphasized the importance of regional levels of biodiversity and its subsequent impact on diet, the scope of dietary similarities for the Escape Site population were important to establish in order to assess the role of Saint Vincent within the larger region. Given the regional influence on diet, at the outset of this work it was hypothesized that the closest islands to Saint Vincent – St Lucia, Grenada, Carriacou – would have the most comparable diets. When analyzed within the context of the larger regional diet data, ultimately these trends held true as shown in Figure 6.3. Clear associations to some nearby islands including Saint Lucia and Grenada can be seen,

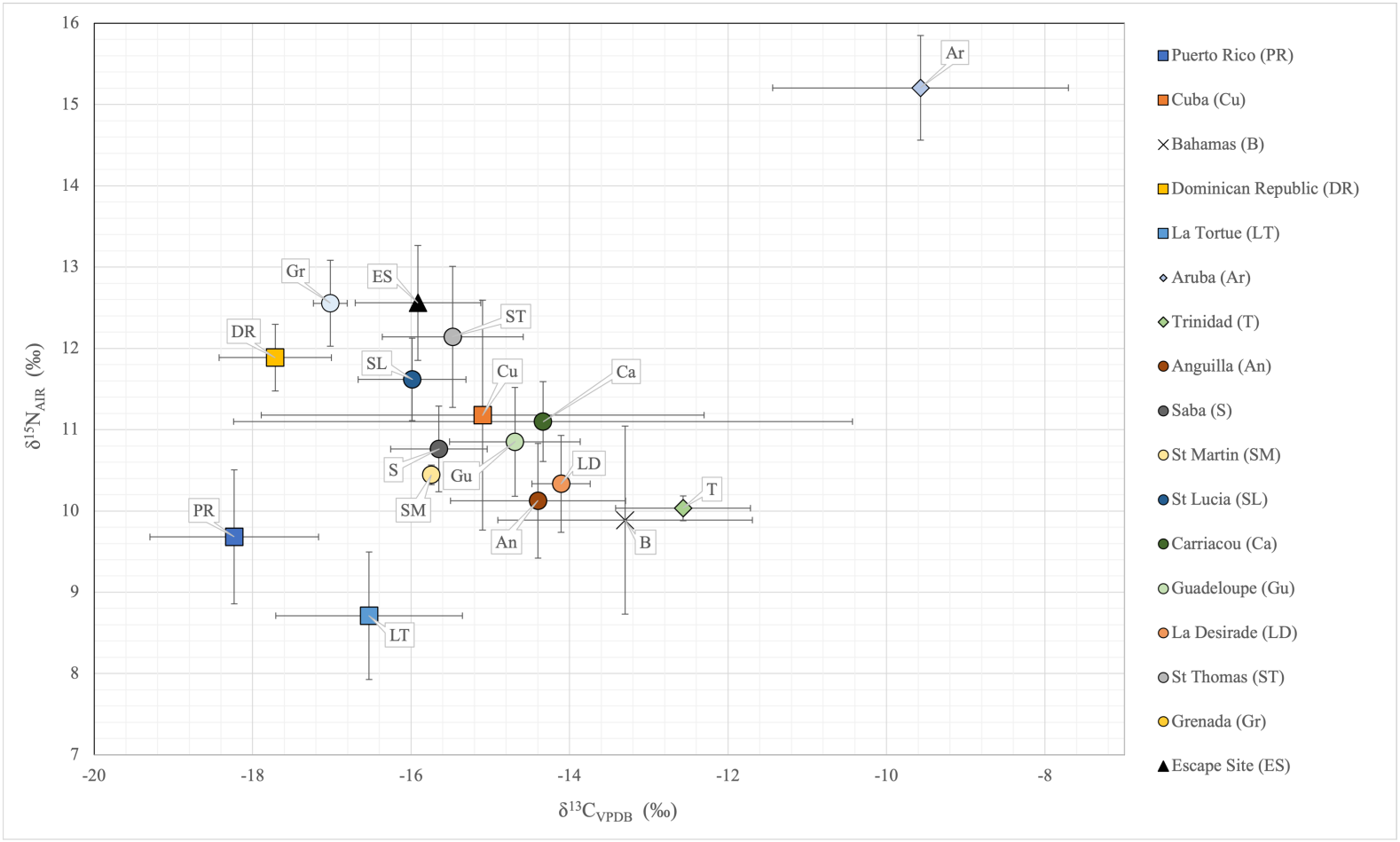

Figure 6.3 Bivariate scatter plot of regional averaged $\delta^{13}C$ and $\delta^{15}N$, not taking into account time period. The various regions are denoted as follows: Escape Site (Δ), Leeward Antilles (◇), Greater Antilles (□), Bahamas (X) and Lesser Antilles (O). Colour version available online. Refer to Appendix C, Table C1 for full overview of regional data (Buhay et al, 2013; Chinique de Armas et al, 2015; Healy, Keenlyside & Dorst, 2013; Keegan & DeNiro, 1988; Krigbaum, Fitzpatrick & Bankaitis, 2013; Laffoon & de Vos, 2011; Laffoon et al., 2016; Mickleburgh & Laffoon, 2018; Norr, 2002; Pestle, 2009; Pestle & Colvard, 2012; Stokes, 1998; 2005).

with additional similarities noted to farther islands such as Cuba and Saint Thomas. Refer to Appendix C, Table C1 for full overview of regional data.

To better examine regional trends, three Kruskal-Wallis tests were done comparing the Escape Site sample population against the following areas:

1. Broad regions (Aruba, Bahamas, Trinidad, Greater and Lesser Antilles)
2. Greater Antilles islands (Cuba, Puerto Rico, Dominican Republic, La Tortue)
3. Lesser Antilles islands (Anguilla, Carriacou, Grenada, Guadeloupe, La Desirade, Saba, St Lucia, St Martin, St Thomas)

Each test was done by compiling all relevant $\delta^{13}C$ and $\delta^{15}N$ data including the Escape Site results to ensure consistency in the analysis and tests run. Post hoc comparisons using a Dunn's test with Bonferroni corrected p-values were used to identify where exactly significant differences exist (Table 6.1). By completing these tests in this order we can establish which regions and consequently which islands can be considered comparable to the Escape Site.

When the Escape Site group data was compared to the broad regions, there were significant differences for both $\delta^{13}C$ ($x^2 = 419.47$, df = 333, p = 0.0009) and $\delta^{15}N$ ($x^2 = 345.39$, df = 284, p = 0.007). Further analysis using a post hoc Dunn's test, failed to identify any statistically significant differences between the Escape Site data and all of the regional $\delta^{13}C$ data. Statistical differences were found between the Escape Site $\delta^{15}N$ and data from the Bahamas and Greater Antilles (Test 1 Table 6.1). While similarities can be observed for the Lesser and Greater Antilles, there are clear differences between Aruba, the Bahamas and Trinidad, all of which have fairly distinct diets (Healy, Keenlyside,

Dorst, 2013; Keegan & DeNiro, 1988; Mickleburgh & Laffoon, 2018). For this reason, I suggest this statistical result is due to the extremely small sample sizes from Aruba (n = 4) and Trinidad (n = 3) as well as the Escape Site study population (n = 5). Concerning the Greater Antilles, when the Escape Site data was compared to Cuba, Puerto Rico, Dominican Republic and La Tortue data there were significant differences for both $\delta^{13}C$ ($x^2 = 275.41$, df = 212, p = 0.002) and $\delta^{15}N$ ($x^2 = 264.15$, df = 205, p = 0.003). Post hoc Dunn's test comparisons found that within the Greater Antilles, the Escape Site data was significantly different to Puerto Rico $\delta^{13}C$ and $\delta^{15}N$ data as well as La Tortue $\delta^{15}N$ (Test 2 Table 6.1).

When the Escape Site data was compared to other isotopic information from the Lesser Antilles, there were no significant differences found for both $\delta^{13}C$ ($x^2 = 146.12$, df = 125, p = 0.09) and $\delta^{15}N$ ($x^2 = 126.86$, df = 110, p = 0.13). Ultimately this would indicate that within the Lesser Antilles, broad regional $\delta^{13}C$ and $\delta^{15}N$ similarities are evident. Considering the extensive long range Saladoid trade network, the relative comparability of various islands to the Escape Site data serves to emphasize that populations from Saint Vincent likely benefitted from and contributed to trade within the region resulting in an overall similarity in diet (Hofman et al., 2014; Keegan, 2018; Keegan & Hofman, 2017; Rodríguez Ramos, 2010). Largely, the data from these islands point to an intermediate diet incorporating a broad spectrum terrestrial and marine based diet which was similarly consumed at the Escape Site (Laffoon & de Vos, 2011; Norr, 2002; Stokes, 1998). Moreover, with at least five non-local individuals being identified at the Escape Site (B4,

B6, B8, B20, B24), none of which produced useable collagen data, further signifies the larger regional ties that Saint Vincent had (Laffoon, 2012).

Table 6.1 Summary of post hoc comparisons using a Dunn's test with Bonferonni corrected p-values (p = 0.025) for the Escape Site data to regional isotope data calculated using RStudio.

Test	Island	Escape Site $\delta^{13}C$		Escape Site $\delta^{15}N$	
		Z	p-value	Z	p-value
1	**Bahamas**	1.90	0.43	– 3.79	0.001
	Greater Antilles	1.94	0.39	4.21	0.0002
	Lesser Antilles	– 0.86	1.00	1.88	0.45
	Aruba	2.06	0.29	0.29	1.00
	Trinidad	– 1.65	0.75	2.59	0.07
2	**Cuba**	– 0.46	1.00	– 1.64	0.51
	Puerto Rico	3.24	0.006	3.99	0.0003
	Dominican Republic	– 1.83	0.33	– 0.34	1.00
	La Tortue	0.62	1.00	5.25	0.00

Grey highlighted p-values indicate statistically significant differences.

6.3.2 Diet Through Time

Caribbean prehistory reflects a dynamic mosaic of culture groups ranging from the Casimiroid to the Saladoid to Taíno and Island Caribs. While debates regarding specific migratory pathways and definitive origins continue, unique series and regional sub-series were largely affected by both population pulses and regional interactions. Changing cultural traditions and tools were not immediate and abrupt, instead reflecting ongoing local and regional influences (Hofman, Bright & Rodríguez Ramos, 2010; Keegan, 1994; 2000; Rodríguez Ramos, 2010; Rouse, 1992). Time brought new people and technology throughout the Caribbean basin, providing the tools to enhance cultivation

strategies, domestication work as well as material culture (both functional and symbolic). Indeed, the fortitude of the pre-Colonial populations – and the Saladoid in particular – lay in their expansive trade networks and relations strengthened by familial ties as well as continued interaction spheres with mainland Central and Southern America (Hofman et al., 2014; Keegan, 2018; Keegan & Hofman, 2017; Rodríguez Ramos, 2010). Given the paucity of large domesticates and important cultivars, transportation of plant taxa and small mammals was essential to archaic and ceramic age populations (Keegan & Hofman, 2017). Within a historical ecology approach, new island settlers relied on more easily obtained nearshore marine resources (e.g. reef species), shifting to terrestrial agriculture once established. Such that on islands, like Saint Vincent, earlier migrations would consume higher relative quantities of marine resources than later populations (Keegan et al., 2008; Fitzpatrick & Keegan, 2007).

When comparing the available cumulative isotope data for the Caribbean, there appears to be a decrease in both $\delta^{13}C$ and $\delta^{15}N$ over time data (Figure 6.4, Figure 6.5). Generally suggesting a decline in nearby marine species and a shift to more terrestrial and freshwater resources aligning with previous zooarchaeological research. For example, sites which had both early and late occupations reflected relative changes in fish species sourced from different ecological niches (Wing & Wing, 2001). Decreases in size and mean trophic level have also been tied to overfishing, where early populations focused on nearby reef species and once established, shifted to new ecological niches (e.g. pelagic) for resource collection (Fitzpatrick & Keegan, 2007; Wing & Wing, 2001). To that end, the $\delta^{13}C$ and $\delta^{15}N$ time data would reinforce these ideas whereby the decreasing trends

suggest a shifting focus to species occupying lower trophic levels and reflecting C_3 like resources through time (Figure 6.4, Figure 6.5). When the Caribbean time period data was analyzed with Kruskal-Wallis non-parametric tests, statistical differences for $\delta^{13}C$ (x^2 = 105.7, df = 4, $p < 2.20 \times 10^{-16}$) and $\delta^{15}N$ ($x^2 = 49.82$, df = 4, $p = 3.93 \times 10^{-10}$) were found. Despite this, when further analyzed using post hoc comparisons using a Dunn's test, the Escape Site was only found to be statistically different to Saladoid and Post-Saladoid $\delta^{15}N$ data (Table 6.2). Considering the approximate association of the Escape Site burials to the Early Ceramic Age and the early Late Ceramic Age based on mortuary practices, it is surprising to see this difference.

Table 6.2 Summary of post hoc comparisons using a Dunn's test with Bonferonni corrected p-values (p = 0.025) for the Escape Site data to time period data calculated using RStudio.

Time Period	Escape Site $\delta^{13}C$		Escape Site $\delta^{15}N$	
	Z	**p-value**	**Z**	**p-value**
Pre-Ceramic	– 0.78	1.00	2.02	0.22
Saladoid (250 BC - AD 600)	1.01	1.00	3.71	0.001
Post-Saladoid (AD 600 - 1000)	1.72	0.43	3.80	0.0007
Formative (Post AD 1000)	– 0.71	1.00	2.41	0.08

Grey highlighted p-values indicate statistically significant differences.

While the $\delta^{13}C$ data remains fairly stable through time with the Escape Site data $\delta^{13}C$ falling with these ranges (Figure 6.4) and emphasized by the statistical results (Table 6.2), the Escape Site $\delta^{15}N$ skews slightly higher than most other time period data (Figure 6.5). This could suggest a higher consumption of higher trophic foodstuffs, however, given the previously discussed Caribbean food web and the primary dietary components

at the Escape Site this is not a parsimonious explanation. Certainly, given the small sample size of useable data from the Escape Site it is hard to say if these values are outliers and in fact the larger sample population did align with specific temporal trends. As well, it is possible that the Escape Site individuals were preferentially incorporating plants from dryer coastal areas which can results in relatively higher $\delta^{15}N$ data (Körner, Farquhar & Wong, 1991; Szpak et al., 2013), however, without local contextual data that cannot be definitely associated to these results.

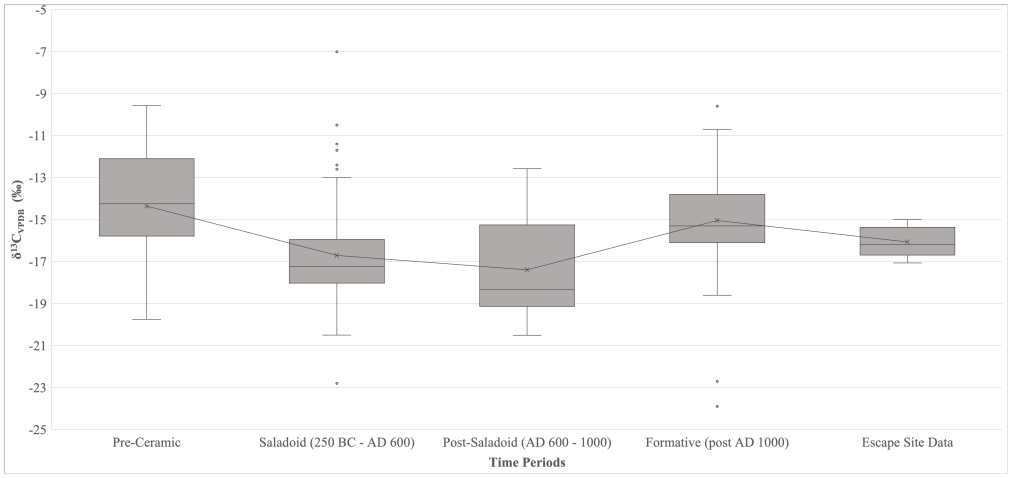

Figure 6.4 Boxplot diagram depicting relative changes in $\delta^{13}C$ across time periods in comparison to the Escape Site isotope data. For full scope of temporal data refer to Appendix C, Table C2.

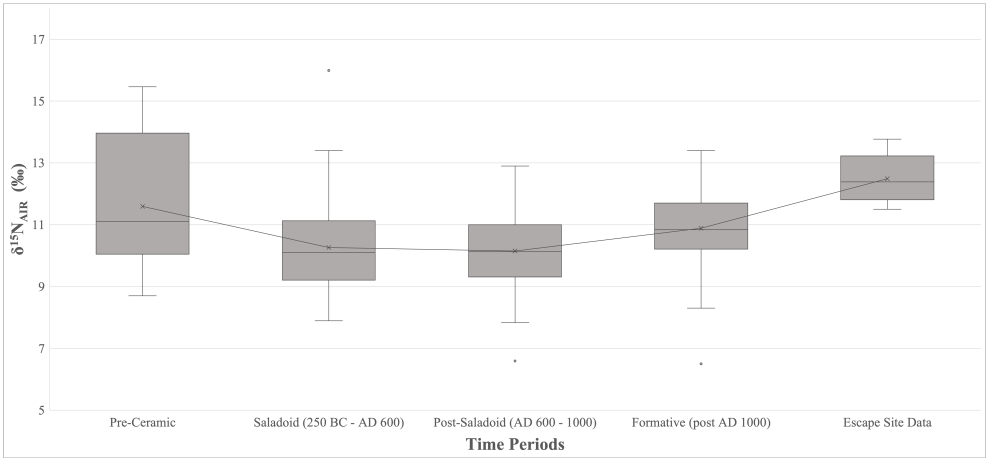

Figure 6.5 Boxplot diagram depicting relative changes in $\delta^{15}N$ across time periods in comparison to the Escape Site isotope data. For full scope of temporal data refer to Appendix C, Table C2.

6.4 Summary

The key methodological conclusions of this research suggest that implementing a shorter demineralization time in combination with a smaller fraction size can increase the overall amount of samples available for isotopic analysis when the sample is effectively exposed to HCl. While established protocols produced smaller overall viable samples, most were useable. Conversely, a modified protocol, greatly increased the collagen which passed the minimum threshold requirement, however, most samples were not useable based on their elemental data. Despite this, methodological changes were essential to producing a more fully formed picture of the dietary variation at the Escape Site and warrant further examination within poorly preserved samples.

Overall the Escape Site sample population consumed a broad spectrum diet with a heavy emphasis on terrestrial, freshwater, nearshore and reef fauna substantiated by C_3 plants. Contributions from pelagic species as well as C_4 plants may also have been consumed, however, with the intermediary $\delta^{13}C$ and $\delta^{15}N$ compositions, these resources never came to dominate the diet. Aligning within previous Caribbean research, the Escape Site data shows broad regional similarities to other isotope data from throughout the Lesser Antilles as well as Cuba and the Dominican Republic; serving to emphasize that Saint Vincent prehistoric populations likely contributed to and benefitted from the extensive Saladoid trade network which was prominent throughout the region.

Chapter 7: Conclusion

7.1 Summary of Findings

Given the primary research questions – as outlined in section 1.2 – of this thesis, there are both methodological and anthropological implications to review. While previous methodological studies have demonstrated that a material's isotope ratios can be altered with the inclusion of different variables (e.g. HCl concentration, sample size, preservation conditions), pretreatment protocols are largely inconsistent relying on anecdotal and perceived optimal conditions (Carrasco et al., 2018; Pestle, 2010; Sealy et al., 2014; Tomaszewicz et al., 2015; van der Haas et al., 2018). This prompted the DATA project (Grogan et al., 2021), which provided the conditions to further assess best practices for poorly preserved material within the Caribbean. Despite an increase in QC variation, the data points to the effectiveness of including shorter demineralizations and powdered specimens, especially when dealing with limited and degraded materials. These variables effectively doubled the useable collagen samples, expanding the dietary information available for the Escape Site. Critically, this is only advantageous when sufficient sample exposure is ensured. Considering the prevalence of poor preservation conditions, especially within the Caribbean (Pestle, 2013a), establishing pretreatment methods will help to ensure consistent results and increase data from limited sample sets. While this research has contributed to the debate, further work is still needed.

While the methodological component of this work was borne out of the limited number of useable samples initially produced, the central objective of this research focused on the anthropological examination of diet. Considering both the sporadic nature of Caribbean isotope data and the absence of any such research from Saint Vincent, this

investigation sought to contribute to the larger examination of regional diets and fill in a critical gap in the literature. Broadly speaking, the diet of the sampled individuals from the Escape Site largely consisted of C_3 plants as well as terrestrial, reef, nearshore and freshwater fauna. It was generally expected that the diet on Saint Vincent would reflect local resources, mirroring diets found on other nearby islands (St Lucia, Grenada and Carriacou). When analyzed within the context of available regional data, these trends held true with St Lucia, Grenada, St Thomas and Cuba showing the strongest similarities. Although certainly, given the environmental constraints of Saint Vincent, being smaller in size and located to nearby prolific reefs, it was surprising that marine resources did not significantly dominate the diet (Fielding & Olliviere, 2017; Moravetz, 2010).

7.2 Limitations and Future Avenues of Research

While interesting conclusions can be drawn from this work, it is not without limitations, both analytical and circumstantial in nature. From the outset, the sample size greatly impacted the strength of these conclusions and how broadly they could be applied to the entirety of the Escape Site population. With 29 specimens yielding sufficient collagen for isotopic analysis and only five samples being adequately preserved and included in this research, this study population does not fully reflect the extent of subsistence on Saint Vincent especially when you consider its long term occupation. Moreover, the relative contributions of energy and protein components are unclear as no structural carbonate data was available due to poor preservation, financial constraints and delays due to COVID-19. The sole reliance on bone collagen isotope data means the

Escape Site dietary reconstruction concerns only the proteinaceous component of diet, likely underestimating the full extent of dietary component (e.g. carbohydrates, lipids) (Krueger & Sullivan, 1984).

Another limitation to this research is the absence of definitive population demographics specifically regarding age and biological sex. Considering that generally age and biological sex do not impact dietary trends (as outlined in Krigbaum, Fitzpatrick & Bankaitis, 2013; Laffoon & de Vos, 2011; Laffoon et al., 2016; Mickleburgh & Laffoon, 2018; Norr, 2002; Stokes, 1998), they are unlikely to have influenced the Escape Site data, however, the influence of this cannot be tested within the constraints of this research. Another concern was that the comparable food web data included in this analysis was nonlocal to Saint Vincent and sourced from previous research (Keegan & DeNiro, 1988; Norr, 2002; Pestle, 2013b; Schoeninger & DeNiro, 1984; Stokes, 1998). Considering that isotope ratios reflect local conditions, it would have been optimal to construct the food web from local flora and fauna. Unfortunately, no zooarchaeological data was provided for this analysis and, to my knowledge, no zooarchaeological material from Saint Vincent has yet been analyzed to produce relevant $\delta^{13}C$ and $\delta^{15}N$ data with the closest local samples coming from Grenada (Keegan & DeNiro, 1988; Stokes, 1998). The data for the food web in this research represents the best food web data for comparison at this time and has been drawn from a similar geographical area and ecological niches to the Escape Site.

Immediate recommendations for future avenues of research primarily concern the anthropological component of this investigation. Certainly, producing the associated

$\delta^{13}C_{SC}$ compositions is considered one of the more critical next steps to better tease out the relative contributions of terrestrial and marine resources. Further scientific work including ^{14}C dating or DNA would also be beneficial to support potential conclusions regarding the origin of the respective Escape Site population and indeed their specific relationship (e.g. any familial ties). Establishing the temporal context of the site and the sequence of structures would help better contextualize the changing cultural identity of the inhabitants as well as their respective age (e.g. being earlier or later migrations).

With regard to the methodological component of this research, the question of what a potential minimum sample weight requirement may be was not investigated. Certainly when using the maximum suggested weight (600 mg) according to TEAL lab memorandum 17-02, there is a critical tipping point where the efficiency of using a decreased demineralization time no longer exceeds traditional collagen isolation methods. Although, this specific point remains to be seen, having 600 mg spread approximately even over four culture tubes allowed for this constraint to be overcome. Future work could attempt to identify this point to suggest the smallest reasonable weight that could be used while still performing at optimal capacities, to ensure minimal sample destruction and use.

Humans, as a collective, represent a dynamic point of observation implicitly reflecting their environmental and socioeconomic contexts where diet symbolizes a measurable quantification of these conditions. Within the Caribbean, dietary research has demonstrated that changing identities and connections during prehistory were far more dynamic than many would have realized. While it's known for beautiful climates and being a popular travel destination, the Caribbean has a long and resilient history in the face of colonial interventions showing it has far more to offer than beaches and vacation destinations.

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Appendix A - Data Set Details

Table A1 Contextual information and notes regarding provided samples and TEAL IDs.

Burial ID	Element	Side	TEAL ID	Protocol (1) ID	Protocol (2) ID	Protocol (3) ID
B1	Femur	L	12266	12891	12891	12920
B1A			12267	12892	12892	12921
B2	Femur	L	12268	12893	12893	12922
B4	Femur	R	12269	12894	12894	12923
B5	Tibia		12270	12895	12895	12924
B6	Femur	L	12271	12896	12896	12925
B7	Femur	L	12272	12897	12897	12926
B8	Femur	R	12273	12898	12898	12927
B9			12274	12899	12899	12928
B10	Femur	L	12275	12900	12900	12929
B11	Femur	L	12276	12901	12901	12930
B12	Tibia	R	12277	12902	12902	12931
B13	Tibia	R	12278	12903	12903	12932
B14	Femur	R	12279	12904	12904	12933
B15			12280	–	–	–
B16	Humerus	L	12281	12905	12905	12934
B17	Femur		12282	12906	12906	12935
B18	Humerus	R	12283	12907	12907	12936
B19	Femur	L	12284	12908	12908	12937
B20	Tibia	L	12285	12909	12909	12938
B21A			12286	12910	12910	12939
B21B	Humerus		12287	12911	12911	12940
B22	Humerus		12288	12912	12912	12941
B23	Radius	R	12289	12913	12913	12942
B24	Femur	L	12290	12914	12914	12943
B25	Femur		12291	12915	12915	12944
B26	Tibia?		12292	12916	12916	12945
B27	Fibula		12293	12917	12917	12946
B28	Femur	L	12294	12918	12918	12947

B32	Femur		12295	12919	12919	12948

Blank cells indicate that data was unavailable or unknown.

Table A2 Escape Site isotopic and chemical compositions, with sample averages provided in grey boxes

Site ID	TEAL ID	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{AIR}$ (‰)	Collagen Yield[1]	wt% C (%)[2]	wt% N (%)[3]	Atomic C:N[4]
B1	12861						
Protocol 1	11519			***0.43***			
Protocol 2	12891			***0.29***			
Protocol 3	12920	*– 18.37*	*10.41*	*1.88*	***2.59***	***0.89***	*3.39*
B1A *	12862	– 14.99	13.77	1.15	14.56	5.47	3.10
Protocol 1	11520	– 14.99	13.77	1.15	14.56	5.47	3.10
Protocol 2	12892			***0.62***			
Protocol 3	12921	*– 15.9*	*12.82*	*2.20*	***6.23***	***2.28***	*3.19*
B2	12863						
Protocol 1	11521			***0.25***			
Protocol 2	12893			***0.47***			
Protocol 3	12922	*– 21.09*	*10.23*	*1.22*	***1.58***	***0.41***	***4.54***
B4	12864						
Protocol 1	11522			***0.53***			
Protocol 2	12894			***0.54***			
Protocol 3	12923	*– 18.49*	*8.54*	*1.56*	***1.60***	***0.47***	***4.01***
B5	12865						
Protocol 1	11523			***0.96***			
Protocol 2	12895			***0.74***			
Protocol 3	12924	*– 18.54*	*10.21*	*2.01*	***3.34***	***1.13***	*3.45*
B6	12866						

Protocol 1	11524			***0.41***			
Protocol 2	12896			***0.61***			
Protocol 3	12925	*– 21.83*	*5.82*	*1.66*	***0.78***	***0.14***	***6.67***
B7	12867						
Protocol 1	11525			***0.62***			
Protocol 2	12897			***0.57***			
Protocol 3	12926	*– 16.61*	*11.94*	***0.96***	***5.43***	***1.61***	***3.92***
B8	12868						
Protocol 1	11526			***0.74***			
Protocol 2	12898			***0.41***			
Protocol 3	12927	*– 25.41*	*– 6.32*	*2.26*	***0.99***	***0.11***	***10.84***
B9	12869						
Protocol 1	11527			***0.18***			
Protocol 2	12899			***0.48***			
Protocol 3	12928	*– 23.42*	*3.07*	*2.03*	***1.74***	***0.24***	***8.60***
B10	12870	– 17.06	12.39	**0.82**	16.96	6.18	3.20
Protocol 1	11528	*– 16.89*	*11.89*	*1.12*	***10.72***	***4.03***	*3.10*
Protocol 2	12900			***0.59***			
Protocol 3	12929	– 17.06	12.39	**0.82**	16.96	6.18	3.20
B11*	12871	– 16.26	12.22	3.98	35.38	13.12	3.15
Protocol 1	11529	– 16.26	12.22	3.98	35.38	13.12	3.15
Protocol 2	12901			***0.56***			
Protocol 3	12930	– 16.12	12.00	1.81	28.42	10.33	3.21
B12	12872						
Protocol 1	11530	*– 15.02*	*12.23*	*1.06*	***8.65***	***3.18***	*3.17*
Protocol 2	12902			***0.53***			
Protocol 3	12931	*– 15.24*	*11.30*	*2.49*	***4.65***	***1.71***	*3.17*

B13	12873						
Protocol 1	11531			***0.24***			
Protocol 2	12903			***0.89***			
Protocol 3	12932	*– 21.48*	*0.89*	*1.80*	***0.72***	***0.14***	***5.99***
B14*	12874	– 15.47	11.94	2.12	34.24	12.44	3.21
Protocol 1	11532	– 16.32	11.50	4.37	30.53	11.38	3.13
Protocol 2	12904	– 16.13	11.59	3.84	31.55	11.85	3.11
Protocol 3	12933	– 15.47	11.94	2.12	34.24	12.44	3.21
B16	12876						
Protocol 1	11533			***0.68***			
Protocol 2	12905			***0.57***			
Protocol 3	12934	*– 16.28*	*13.01*	*1.64*	***8.84***	***3.18***	*3.24*
B17	12877						
Protocol 1	11534	*– 17.41*	*12.79*	*1.04*	***6.06***	***1.95***	*3.62*
Protocol 2	12906			***0.58***			
Protocol 3	12935	*– 19.41*	*8.33*	*1.71*	***2.12***	***0.62***	***3.99***
B18	12878						
Protocol 1	11535			***0.88***			
Protocol 2	12907			***0.81***			
Protocol 3	12936	– 18.07	9.35	2.36	**2.25**	0.77	3.40
B19*	12879	– 15.78	12.49	1.74	32.39	11.86	3.19
Protocol 1	11536	– 15.71	12.87	1.42	20.28	7.47	3.17
Protocol 2	12908			***0.59***			
Protocol 3	12937	– 15.78	12.49	1.74	32.39	11.86	3.19
B20	12880						
Protocol 1	11537	*– 16.85*	*13.03*	*1.00*	***8.21***	***2.98***	*3.22*
Protocol 2	12909			***0.69***			

Protocol 3	12938	*– 17.04*	*9.66*	*1.14*	***3.46***	***0.73***	***5.51***
B21A	12881						
Protocol 1	11538			***0.62***			
Protocol 2	12910			***0.56***			
Protocol 3	12939	*– 19.58*	*5.97*	*1.91*	***0.92***	***0.28***	***3.77***
B21B	12882						
Protocol 1	11539			***0.57***			
Protocol 2	12911			***0.46***			
Protocol 3	12940	*– 23.74*	*– 1.05*	*2.31*	***0.62***	***0.14***	***5.24***
B22	12883						
Protocol 1	11542			***0.54***			
Protocol 2	12912			***0.27***			
Protocol 3	12941	*– 20.63*	*6.45*	*2.38*	***1.20***	***0.28***	***5.05***
B23	12884						
Protocol 1	11540			***0.60***			
Protocol 2	12913			***0.54***			
Protocol 3	12942	*– 18.56*	*10.15*	*2.73*	***3.05***	***1.03***	*3.46*
B24	12885	– 18.33	9.60	2.17	2.83	0.96	3.43
Protocol 1	11541			***0.66***			
Protocol 2	12914			***0.49***			
Protocol 3	12943	– 18.33	9.60	2.17	**2.83**	0.96	3.43
B25	12886						
Protocol 1	11543			***0.60***			
Protocol 2	12915			***0.61***			
Protocol 3	12944	*– 20.91*	*7.06*	*1.03*	***1.80***	***0.34***	***6.24***
B26	12887						
Protocol 1	11544			***0.67***			

Protocol 2	12916			***0.56***			
Protocol 3	12945	*– 20.9*	*4.20*	*2.31*	***2.09***	*0.50*	***4.82***
B27	12888						
Protocol 1	11545			***0.54***			
Protocol 2	12917			***0.53***			
Protocol 3	12946	*– 28.61*	*– 10.05*	*2.07*	***0.61***	***0.09***	***7.57***
B28	12889						
Protocol 1	11546			***0.80***			
Protocol 2	12918			***0.53***			
Protocol 3	12947	*– 20.48*	*3.65*	*2.12*	***0.96***	***0.31***	*3.66*
B32	12890						
Protocol 1	11547			***0.36***			
Protocol 2	12919			***0.57***			
Protocol 3	12948	*– 24.04*	*– 7.23*	*2.31*	***0.89***	***0.12***	***8.79***
Minimum [5]		– 17.06	11.94	0.82	14.56	5.47	3.10
Maximum [5]		– 14.99	13.77	3.98	35.28	13.12	3.21
Average [5]		– 15.91	12.56	1.96	26.71	9.81	3.17
Standard Deviation [5]		0.79	0.71	1.23	10.08	3.68	0.04

***** indicates that data from multiple protocols was useable, however, results which higher wt% C and wt% N QC values were preferentially used
Bolded values fall outside acceptable ranges.
Italicized values indicate samples whose isotope ratios were rejected due to contamination/degradation. This data was excluded from all subsequent analyses and summary statistics at the bottom of the chart.
Empty cells indicate no isotope data exists.
Grey boxes denote the useable isotopic data from viable samples, when left blank the associated data was rejected.
[1] Collagen Yield range of acceptable values: 1 % ≤ ; outliers 12926 (B7), 12929 (B10) with yields 0.89 % and 0.96 % accepted
[2] wt% C range of acceptable values: 13 ≤
[3] wt% N range of acceptable values: 4.5 ≤
[4] C:N Atomic Ratio range of acceptable values: 2.9 - 3.6
[5] Only values within the grey boxes were included in associated summary statistics, n = 5

Appendix B - Escape Site Documentation and Ethics Forms

Figure B2 Permission for destructive analysis from Saint Vincent the Grenadines National Trust for the Escape Site samples.

TRENT UNIVERSITY

November 25, 2020

File #: 26397
Title: Exploring human diet and local ecology in the Early to Late Saladoid period (250 BC to AD 700) of the Lesser Antilles: Insights from the Escape Site, St. Vincent.

Dear Dr. Williams,

The Research Ethics Board (REB) has given approval to your proposal entitled "Exploring human diet and local ecology in the Early to Late Saladoid period (250 BC to AD 700) of the Lesser Antilles: Insights from the Escape Site, St. Vincent.".

When a project is approved by the REB, it is an Institutional approval. It is not to be used in place of any other ethics process.

To maintain its compliance with this approval, the REB must receive via ROMEO:

An Annual Update for each calendar year research is active;

A Study Renewal should the research extend beyond its approved end date of August 30, 2021;

A Study Closure Form at the end of active research.

This project has the following reporting milestones set:

Renewal Due-2021/08/30

To complete these milestones, click the Events tab in your ROMEO protocol to locate and submit the relevant form.

If an amendments to the protocol is required, you must submit an Amendment Form, available in the Events tab in your ROMEO protocol, for approval by the REB prior to implementation.

Any questions regarding the submission of reports or Event forms in ROMEO can be directed to Jamie Muckle, Certifications and Compliance Officer, at jmuckle@trentu.ca

On behalf of the Trent Research Ethics Board, I wish you success with your research.

Best Wishes,

Dr. Michele Janet McIntosh
REB Chair
Phone: (705) 748-1011 ext. 7507
Email: michelejmcintosh@trentu.ca

c.c.: Jamie Muckle
Certifications and Regulatory Compliance Officer

ST. VINCENT AND THE GRENADINES NATIONAL TRUST
P.O. Box 1538
Heritage Hall, Carnegie Building, Heritage Square, Kingstown, St. Vincent
Tel: (784) 451-2921
Email: svgntrust@vincysurf.com

Assistant Professor,
Department of Anthropology,
Trent University,
1600, West Bank Drive,
Peterborough,
ON K9J 7B8
Canada

23rd February 2010

Dear Dr. Williams,

Permission is hereby granted for the destruction of the bone samples from Escape in St Vincent and the Grenadines, which you have in your possession, during the process of diet analysis.

We will be happy for one of your post graduate students to perform this work and look forward to receiving the results in due course.

Yours truly,

Kathy Martin,
Chair of Archaeology Committee

Appendix C - Comparative Regional and Food-web Data

Table C1 Relevant mean $\delta^{13}C$ and $\delta^{15}N$ data from other islands within the Caribbean. Overall counts and sources are also listed. With the scope of data available from Puerto Rico, specific data data and counts have been provided and are italicized.

Region	Island	n = ?	$\delta^{13}C_{VPDB}$ (‰) [1]	$\delta^{15}N_{AIR}$ (‰) [1]	Source
Greater Antilles	**Cuba**	49	– 15.09 ± 2.79	+ 11.18 ± 1.41	Buhay et al., 2013; Chinique de Armas et al., 2015
	La Tortue	15	– 16.53 ± 1.18	+ 8.71 ± 0.79	Stokes, 1998
	Dominican Republic (all)	12	– 17.71 ± 0.71	+ 11.89 ± 0.41	Stokes, 1998
	Juan Dolio, DR	4	– 17.12 ± 0.69	+ 11.86 ± 0.52	Stokes, 1998
	El Soco I	7	– 18.04 ± 0.56	+ 11.91 ± 0.41	Stokes, 1998
	El Soco II	1	– 17.83	11.86	Stokes, 1998
	Puerto Rico (all)	221	– 18. 23 ± 1.06	+ 9.68 ± 0.82	Keegan & DeNiro, 1988; Stokes, 1998; 2005; Pestle & Colvard, 2012
	Maisabel, PR	18	– 18.07 ± 0.97	+ 9.59 ± 0.83	Stokes, 1998; 2005
	Paso del Indio, PR	96	– 19.09 ± 0.52	+ 9.71 ± 0.86	Stokes, 2005; Pestle & Colvard, 2012
	Punta Candelero, PR	50	– 17.46 ± 1.01	+ 9.85 ± 0.88	Pestle & Colvard, 2012
	Tibes, PR	46	– 17.59 ± 0.60	+ 9.49 ± 0.74	Stokes, 2005; Pestle & Colvard, 2012
	Los Indios, PR	10	– 17.04 ± 0.79	+ 9.61 ± 0.36	Pestle & Colvard, 2012
	Unlisted, PR	1	– 19.10	9.40	Keegan & DeNiro, 1988
Lesser Antilles	**Saint Lucia**	29	– 17.03 ± 1.24	+ 11.46 ± 1.31	Stokes, 1998; Laffoon et al., 2016
	Guadeloupe	80	– 14.69 ± 0.82	+ 10.85 ± 0.67	Stokes, 1998; Laffoon & de Vos, 2011
	La Desirade	3	– 14.11 ± 0.37	+ 10.33 ± 0.59	Stokes, 1998
	Carriacou	14	– 14.34 ± 3.91	+ 11.10 ± 0.49	Krigbaum, Fitzpatrick & Bankaitis, 2013

Region	Island	n = ?	$\delta^{13}C_{VPDB}$ (‰) [1]	$\delta^{15}N_{AIR}$ (‰) [1]	Source
	St Thomas	24	– 15.48 ± 0.89	+ 12.14 ± 0.87	Norr, 2002
	Grenada	2	– 17.02 ± 0.21	+ 12.56 ± 0.53	Stokes, 1998
	St Martin	2	– 15.75 ± 0.01	+ 10.45 ± 0.12	Stokes, 1998
	Saba	6	– 15.65 ± 0.61	+ 10.76 ± 0.53	Stokes, 1998
	Anguilla	6	– 14.39 ± 1.10	+ 10.13 ± 0.71	Stokes, 1998
Bahamian Archipelago	**Assorted Islands**	25	– 13.30 ± 1.61	+ 9.89 ± 1.16	Keegan & DeNiro, 1988; Stokes, 1998
Trinidad		3	– 12.57 ± 0.85	+ 10.03 ± 0.15	Healy, Keenlyside & Dorst, 2013
Aruba		5	– 11.12 ± 3.82	+ 14.38 ± 1.92	Mickleburgh & Laffoon, 2018

[1] Mean values presented

Table C2 Relevant mean $\delta^{13}C$ and $\delta^{15}N$ sorted according to time period within the Caribbean. Overall counts and sources are also listed.

Time Period	Islands	n = ?	$\delta^{13}C_{VPDB}$ (‰) [1]	$\delta^{15}N_{AIR}$ (‰) [1]	Source
Pre-Ceramic	Aruba, Cuba, St Martin	16	– 14.36 ± 2.81	+ 11.59 ± 2.26	Buhay et al., 2013; Chinique de Armas et al., 2013; Mickleburgh & Laffoon, 2018
Saladoid (250 BC - AD 600)	Anguilla, Aruba, Cuba, Grenada, La Tortue, Puerto Rico (Punta Candelero, Tibes), Saint Lucia (Grand Anse), St Martin, St Thomas (Tutu)	146	– 16.71 ± 2.15	+ 10.26 ± 1.34	Buhay et al., 2013; Chinique de Armas et al., 2013; Keegan & DeNiro, 1988; Mickleburgh & Laffoon, 2018; Norr, 2002; Pestle & Colvard, 2012; Stokes, 1998
Post-Saladoid (AD 600 - 1000)	Dominican Republic (El Soco I) Guadeloupe, Puerto Rico (Paso del Indio, Maisabel) St Thomas (Tutu)	200	– 17.39 ± 2.06	+ 10.15 ± 1.15	Laffoon & de Vos, 2011; Norr, 2002; Pestle & Colvard, 2012; Stokes, 2005; 1998

Time Period	Islands	n = ?	$\delta^{13}C_{VPDB}$ (‰) [1]	$\delta^{15}N_{AIR}$ (‰) [1]	Source
Formative (Post AD 1000)	Bahamas, Carriacou, Dominican Republic (El Soco II, Juan Dolio) Guadeloupe, Puerto Rico (Los Indios), Saint Lucia (Lavoutte), St Thomas (Tutu), Saba, Trinidad	127	– 15.04 ± 1.98	+ 10.89 ± 1.07	Healy, Keenlyside & Dorst, 2013; Keegan & DeNiro, 1988; Krigbaum, Fitzpatrick & Bankaitis, 2013; Laffoon et al., 2016; Norr, 2002; Pestle & Colvard, 2012; Stokes, 1998

[1] Mean values presented

Table C3 Mean fauna food web $\delta^{13}C$ and $\delta^{15}N$ isotope data by habitat. Modern samples $\delta^{13}C$ were corrected for the Suess Effect and TEF offsets of *0.5 ‰* for $\delta^{13}C$ and *4 ‰* for $\delta^{15}N$ are included in the data. Summary of data used to create food web image (Figure 6.1)

Habitat	n = ?	$\delta^{13}C_{DIET}$ (‰)	$\delta^{15}N_{DIET}$ (‰)	Source
Invertebrates	33	– 11.53 ± 3.20	+ 8.08 ± 1.34	Keegan & DeNiro, 1988; Pestle, 2013b; Stokes, 1998
Freshwater	25	– 19.41 ± 1.71	+ 11.86 ± 1.63	Pestle, 2013b; Stokes, 1998
Pelagic	19	– 11.94 ± 1.51	+ 16.72 ± 3.52	Keegan & DeNiro, 1988; Pestle, 2013b; Schoeninger & DeNiro, 1984; Stokes, 1998
Reef	146	– 9.47 ± 3.15	+ 10.75 ± 2.67	Keegan & DeNiro, 1988; Norr, 2002; Stokes, 1998
Terrestrial	74	– 18.38 ± 4.86	+ 9.65 ± 3.90	Keegan & DeNiro, 1988; Norr, 2002; Pestle, 2013b; Schoeninger & DeNiro, 1984; Stokes, 1998
Nearshore	37	– 10.02 ± 4.34	+ 9.84 ± 3.16	Keegan & DeNiro, 1988; Norr, 2002; Pestle, 2013b; Schoeninger & DeNiro, 1988; Stokes, 1998

Table C4 Mean flora $\delta^{13}C$ and $\delta^{15}N$ isotope data by type. Modern samples $\delta^{13}C$ were corrected for the Suess Effect and TEF offsets of *5 ‰* for $\delta^{13}C$ and *4 ‰* for $\delta^{15}N$ are included in the data.

Plant Type	n = ?	$\delta^{13}C_{Edible}$ (‰)	$\delta^{13}C_{DIET}$ (‰)	$\delta^{15}N_{Edible}$ (‰)	$\delta^{15}N_{DIET}$ (‰)	Source
CAM	3	– 10.49 ± 0.26	– 5.49 ± 0.26	+ 6.59 ± 0.46	+ 10.59 ± 0.46	Stokes, 1998
Seagrass	7	– 10.29 ± 4.19	– 5.29 ± 4.18	+ 4.21 ± 2.87	+ 8.21 ± 2.87	Keegan & DeNiro, 1988
C_3	25	– 24.57 ± 1.55	– 19.57 ± 1.55	+ 4.19 ± 1.69	+ 8.19 ± 1.69	Keegan & DeNiro, 1988; Stokes, 1988
C_4	4	– 9.29 ± 0.77	– 4.29 ± 0.77	+ 3.74 ± 4.21	+ 7.74 ± 4.21	Keegan & DeNiro, 1988; Stokes, 1998