



Universiteit  
Leiden  
The Netherlands

**Tooth be told. Exploring the additional use of bioapatite carbon stable isotopes in incremental dentine sampling of an enslaved African in the Colonial Dutch-Caribbean.**

Johnston, Roshanna

**Citation**

Johnston, R. (2023). *Tooth be told.: Exploring the additional use of bioapatite carbon stable isotopes in incremental dentine sampling of an enslaved African in the Colonial Dutch-Caribbean.*

Version: Not Applicable (or Unknown)

License: [License to inclusion and publication of a Bachelor or Master Thesis, 2023](#)

Downloaded from: <https://hdl.handle.net/1887/3640829>

**Note:** To cite this publication please use the final published version (if applicable).

# *TOOTH BE TOLD*

Exploring the additional use of bioapatite carbon stable isotopes in incremental dentine sampling of an enslaved African in the Colonial Dutch-Caribbean.

*Roshanna Johnston*

*Universiteit Leiden / Faculty of Archaeology*



**Tooth Be Told**

***Exploring the additional use of bioapatite carbon stable isotopes in incremental dentine  
sampling of an enslaved African in the Colonial Dutch-Caribbean.***

**By R. Johnston**

**Bachelor Thesis**

**1083VBTHEY\_2223\_HS**

**Supervisor: Dr. J. Laffoon**

**Leiden University, Faculty of Archaeology**

**Utrecht, 15/06/2023**

## Acknowledgements

I would like to thank my supervisor, Dr. Jason Laffoon, for his patience, time, and clear explanations. Thank you for always replying to my emails quickly, reading all my drafts and attempts as soon as I sent them and just generally being flexible and available. Armed with some very creative drawings, I always left our meetings feeling more confident, inspired, and excited about the subject.

I would also like to thank my mum, who folded my washing and cooked me dinner when I ran out of time or energy and assured me I could do it at every turn. Your unwavering love and support in everything I undertake inspires and motivates me to do my very best.

## Table of Contents

<b>Acknowledgements</b>	<b>3</b>
<b>List of tables and figures</b>	<b>5</b>
<b>Chapter 1: Introduction</b>	<b>6</b>
1.1 Research problem	7
1.2 Research Questions	7
1.3 Thesis outline	9
<b>Chapter 2: Background Isotopes</b>	<b>10</b>
2.1 Underlying principles	10
2.2 History of isotope research	12
2.2.1 Discovering isotopes and making mass spectrometers	12
2.2.2 Making its way to archaeology	14
2.3 Applications in archaeology	16
2.3.1 Carbon - Diet	16
2.3.2 Nitrogen - Diet	17
2.3.3 Other uses for carbon and nitrogen isotopes	18
2.3.4 Oxygen and Strontium - Mobility	19
2.4 Tissues	20
2.4.1 Tooth and bone	20
2.4.2 The organic and inorganic components	22
2.4.3 Incremental dentine sampling techniques	23
2.4.4 Diagenesis and discrimination variations	24
2.5 Bivariate and multivariate models	26
2.5.1 Bioapatite – collagen spacing.	26
2.5.2 Cluster analysis	27
<b>Chapter 3: Background case study</b>	<b>29</b>
3.1 Geographical context	29
3.1.1 Physical environment	29
3.1.2 Current political and social climate	31
3.2 Archaeological context	32
3.2.1 Slavery in archaeology	32
3.2.2 Spring Bay Flat	32
<b>Chapter 4: SB007</b>	<b>36</b>
4.1 Samples	36
4.2 Methods and measurements	37
4.2.1 Sampling strategies	37
4.2.2 Analytical parameters	37
4.2.3 Data selection	37
<b>Chapter 5: Results</b>	<b>39</b>
<b>Chapter 6: Discussion</b>	<b>41</b>
6.1 SB007	41
6.2 Advantages and disadvantages of analyses	47

6.3 Context in archaeological research	48
<b>Chapter 7: Conclusion</b>	<b>49</b>
7.1 Summary of findings	49
7.2 Implications for further research	50
7.2.1 Multiple isotopes from incremental dentine samples	50
7.2.2 Isotopes in archaeology	51
<b>Abstract</b>	<b>52</b>
<b>References</b>	<b>53</b>

## List of tables and figures

Table 2.1 The abundance of natural isotopes relevant to archaeology and their atomic mass. Adapted from Ehleringer and Rundel (1989).	12
Figure 3.1 An example of Saba's climate and vegetation (Romeijn, 1989, p. 47).	30
Figure 3.2 Enslaved African Domestic Area at Spring Bay Flat (Laffoon et al., 2018, p. 353).	34
Figure 4.1 The dental elements found in the lockbox at Spring Bay Flat. (Laffoon et al., 2018, p. 354)	36
Table 5.1 Carbon and nitrogen stable isotope values and function scores (according to the method proposed by Froehle et al., 2012)	40
Figure 6.1 $\delta^{13}\text{C}_{\text{bioapatite}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ values plotted across dentine increments for SB007 from M1, M2 and M3.	41
Figure 6.2 $\Delta^{13}\text{C}$ values for SB007 across dentine increments from M1, M2 and M3.	42
Figure 6.3 $\delta^{15}\text{N}$ values for SB007 across dentine increments from M1, M2 and M3.	43
Figure 6.4 $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bioapatite}}$ values from SB007 M1, M2 and M3 across all dentine increments plotted on against a bivariate model as proposed by Kellner and Schoeninger (2007). The bottom shaded linearity represents a 95 % confidence interval of a $\text{C}_3$ protein dominated diet and the top shaded area a 95 % confidence interval of $\text{C}_4$ /marine protein dominated diet.	45
Figure 6.5 SB007 dentine increments of M1, M2 and M3 plotted using the discriminate function analysis model by Froehle et al. (2012) (For clusters see table 6.1). Data for Enslaved Africans, Saba and precolonial Lesser Antilles obtained from personal communication with J. Laffoon on April 14, 2023.	46
Table 6.1 Discriminate function clusters as proposed by Froehle et al. (2012).	46

## Chapter 1: Introduction

Multi-isotope analysis has undoubtedly revolutionised archaeological research concerning subsistence and migration patterns. Granting scientists the ability to collect and observe quantitative and high-resolution data, the discovery of this method has had an important impact on archaeology akin to other technological and analytical advances spilling over from neighbouring disciplines such as radiocarbon dating, CT scanning and 3D-modelling, and aDNA research. This has opened many exciting new avenues of questioning and reasoning (Makarewicz & Sealy, 2015, p. 147).

When John Vogel and Nikolaas van der Merwe published their article in 1977 studying carbon isotope values in bone collagen to research maize consumption, they knocked over the first domino in a long line of what would become multitudes of stable isotope research on bone collagen. The premise of stable isotope research rests on the fact that the ratio of light to heavy isotopes of a given element in what we eat is reflected in our tissue in a fixed manner. For example, with carbon and nitrogen stable isotope ratios, tissues become more enriched in the heavier isotope per trophic level. Michael DeNiro and Samuel Epstein (1976), two other pioneers in stable isotopes research in diet, famously said: “You are what you eat, plus a few per mil” (p. 834).

While bulk collagen sampling gained popularity in studying paleodiet, other tissues remained in the background until more than a decade later a breakthrough interest in enamel bioapatite occurred. The discovery that the isotopic signature in collagen is skewed towards the ratios in the protein aspect of the diet, whereas bioapatite tends to reflect a more holistic nutrient signature, led to extensive studies using bioapatite and collagen samples in tandem to realize more resolution in our understanding of dietary practices in the past (Lee-Thorp et al., 1989, p. 587).

Another recent development utilizes the nature of dentine formation in incremental regular layers and undergoing no geochemical biogenic alteration after formation, which has provided archaeologists with the potential for more detailed intra-individual research in childhood (Beaumont, Gledhill, et al., 2013, pp. 279-278). A pilot study conducted in 2021 by



Stantis et al. compared the isotope ratios of the organic and inorganic phases *within* these dentine layers, which has inspired this thesis. The use of stable multi-isotope and multi-tissue analyses in numerous case studies continues to improve our understanding of subsistence behaviour and strategies at the same time as gaining currency as a method.

## 1.1 Research problem

Despite the near ubiquity of multi-isotope research in the present day, as of yet studies combining data analysis from both the mineral and collagen components of the same dentine samples (such as the one mentioned above) have been scarce, and virtually lacking within archaeology (Makarewicz & Sealy 2015, p. 147; Clementz et al., 2009, p. 605). As we strive to fill in the lacunas in our understanding regarding the applications of multi-isotope research in archaeology, the importance of such comparative studies gains significance. The value of multi-isotope research in aiding archaeologists to form a more detailed view of an individual's diet and migration is evident and well-established, but the importance of studies such as this one extends beyond this and aims to also contribute to our understanding of the *extent* to which multi-isotope data can inform us of the past. Therefore, this thesis will not only aim to gain more insight into the diet of an individual case study, but also of the benefits of such further perusal in general.

## 1.2 Research questions

The main research objective is two-fold:

1. To provide an analysis of new data of carbon isotope values in the dentine bioapatite in tandem with previously published multi-isotope data from the dentine collagen in the individual SB007.
2. To explore the advantages and disadvantages of the analyses of collagen and bioapatite in dentine increments as a research approach and the potential benefits of comparative studies such as this one.

To provide scaffolding for the undertaking of this thesis, the above research questions will be split up into several sub-questions. For the first research question the focus lies on the case study of an enslaved African, dubbed SB007, forcibly moved to Saba during the Dutch colonial period. The multi-isotope values of the collagen component of the dentine increments belonging to this individual have already been extensively analysed by Laffoon and colleagues (2018). Thus, the first research question will endeavour to observe potential correlates or patterns in the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotope values for the collagen samples and the new  $\delta^{13}\text{C}$  data from the bioapatite in the corresponding dentine serial samples that will be presented in this thesis. The following sub questions formulate this objective:

- 1.1 How does the  $\delta^{13}\text{C}$  data from the dentine bioapatite relate to the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values measured in the dentine collagen?
- 1.2 What are the implications for the analysis of multi-isotopes in incremental dentine bioapatite as a method?

In the second research question the attention zooms out to analysing this case study as a proxy for the potential value of comparative studies such as this in retracing individual life histories. In this line of questioning the advantages and disadvantages of both dentine collagen and dentine bioapatite or a combination thereof will be explored and what the implications are for future multi-isotope research in archaeology, especially in this context. To explore this the subsequent questions will be employed:

- 2.1 Does the comparison of bioapatite data to collagen data provide new/useful insights in the case of SB007?
- 2.2 What are the advantages and disadvantages of using data from bioapatite rather than or in conjunction with data from collagen?
- 2.3 What are situations and/or research questions where collagen and bioapatite used in tandem in incremental dental samples could be useful and what does the future hold for such studies?

### 1.3 Thesis outline

As with any research, context is key, and therefore this thesis will begin with a chapter introducing multi-isotopes and their history and use in archaeology. The third chapter will introduce the case study including the present and archaeological context. Funnelling in on the particular data set belonging to the case study, the subsequent chapter will provide the samples, materials, and methods. The fifth chapter will display the results, followed by the sixth chapter in which these will be discussed, both in the context of the case study this thesis concerns and the broader field of archaeology. Finally, the conclusion will provide a clear summary of all the results, interpretations, and arguments as to how bioapatite and collagen comparisons in incremental dentine sampling will affect the future of the sub-discipline of multi-isotope research in archaeology.

## Chapter 2: Background Isotopes

### 2.1 Underlying principles

There are 118 known chemical elements making up the world we live in, all comprised of atoms and each with a distinct number of protons and electrons, corresponding with their place on the periodic table. Some of these elements have other “versions” of themselves, chemically and functionally virtually identical yet with a different number of neutrons in the nucleus. These are called “isotopes” and though *genuine* stable isotopes are rare, the decay of most nuclides (the term for one specific isotope) is negligible enough to dub them “stable” and these provide, as the name suggests, the foundation for stable isotope research. A small number of isotopes do actively decay and are radioactive or radiogenic, such as one most archaeologists are all too familiar with due to its use in radiocarbon dating:  $^{14}\text{C}$ ; and another element of interest: strontium, due to its radiogenic isotopes (Sharp, 2007, p. 6).

An extra neutron results in a heavier atom and the heavier and lighter isotopes coexist in different ratios within the earth’s lithosphere, atmosphere, and biosphere. This physical difference in mass results in slight differences in behaviour, such as different ways of chemical bonding to other elements and how they undergo physical or chemical reactions and processes in nature or the lab (Sharp, 2007, pp. 5 – 7). The main underlying principle that affords scientists to be able to gain useful information from stable isotopes is that in any multiphase system there is a natural bias in fractionation for heavier or lighter isotopes in any given phase relative to other phases in the system’s process (Sharp, 2007, p. 4). An example of such a multiphase system is the conversion of atmospheric  $\text{CO}_2$  into glucose by plants. Following the basic principle that the difference in mass will result in slightly different reactions: the heavier isotopes will usually react more slowly in the process than lighter isotopes, called the “isotope effect.” The resulting physical change in isotope ratio between plant tissue and the atmosphere is called fractionation (Katzenberg, 2007, pp. 415 - 416).

What is measured for research purposes is the ratio between isotopes with a different mass within a given sample. However, the variations in frequency are proportional to the

differences in mass and, self-evidently, these are very small fluctuations. Therefore, to ensure optimum precision and repeatability, the isotopic composition is usually measured against a universal standard (Ehleringer & Rundel, 1989, p. 3). This differential analysis approach was introduced by McKinney et al. in 1950 along with the standard for expressing isotope ratio measurements with the delta ( $\delta$ ) symbol. The delta value is expressed using the following notation:

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

Multiplying by 1000 expresses the  $\delta$  value in per mil, or parts per thousand, (‰) and allows for clearer data reviewing, as the ratios usually differ at the third decimal point when expressing the  $\delta$  value in percent and therefore the significance can sometimes be obscured by preceding numbers. A negative  $\delta$  value indicates a ratio of heavy to light isotopes lower in the sample than in the standard and a positive  $\delta$  value means a higher ratio of heavy to light isotopes in the sample versus the standard (Sharp, 2007, p. 18; Ehleringer & Rundel, 1989, p. 3).

Isotopes are expressed with its atomic letter preceded by the sum of the total number of protons and neutrons in superscript (not to be confused with its atomic number which refers to its place on the periodic scale or its number of protons and thus always stays the same). For example, the most commonly occurring isotope of carbon has an equal number of protons (6) as neutrons (6) and is therefore expressed as  $^{12}\text{C}$ , as 12 is the sum of 6 and 6. The isotope with seven neutrons becomes  $^{13}\text{C}$ . In light isotopes the heavier isotope is most abundantly found in nature whereas for heavy isotopes the compositions vary greatly and there are generally more isotopes (table 2.1).

Element	Isotope	Abundance (%)
Carbon	$^{12}\text{C}$	98.89
	$^{13}\text{C}$	1.11
Nitrogen	$^{14}\text{N}$	99.63
	$^{15}\text{N}$	0.37

Oxygen	<sup>16</sup> O	99.759
	<sup>17</sup> O	0.037
	<sup>18</sup> O	0.204
Strontium	<sup>84</sup> Sr	0.56
	<sup>86</sup> Sr	9.86
	<sup>87</sup> Sr	7.02
	<sup>88</sup> Sr	82.56

Table 2.1 The abundance of natural isotopes relevant to archaeology and their atomic mass. Adapted from Ehleringer and Rundel (1989).

In the 1970s scientists convened in Vienna at the International Atomic Energy Agency (IAEA) to establish standard protocols for notating data, calibration standards for mass spectrometers, and, perhaps most importantly, a universal international measurement sample for each element for measuring ratios. The original measurement for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  comes from the isotope compositions of a specimen of *Belemnitella americana* originating from the Upper Cretaceous PeeDee in South Carolina. Dubbed the VPBD (Vienna PeeDee Belemnite), this remains the standard for measuring carbon and oxygen composites to this day. Another way to commonly measure oxygen (and hydrogen) composites is against the standard of the VSMOW (Vienna Standard Mean Ocean Water).

The reference standard for nitrogen is noted as AIR (Ambient Inhale Reservoir). There is no Vienna standard like there is for VPBD and VSMOW though there are gas and nitrate samples distributed by the IAEA (Schoeninger & Moore, 1992, p. 253; Sharp, 2007, pp. 24-26).

To measure the ratio of isotopes in a material, the sample is generally converted into a gas (such as  $\text{N}_2$  or  $\text{CO}_2$ ) using either thermal or chemical treatments. The electronic detector in the mass spectrometer then distinguishes the ratio of concentration of masses of the isotope being measured. This is subsequently compared to the in-lab standard sample, which is calibrated to the international standard (Schoeninger & Moore, 1992, pp. 253-254).

## 2.2 History of isotope research

### 2.2.1 Discovering isotopes and making mass spectrometers

The discovery of isotopes stemmed from research into radioactivity in the early 1920s. In 1921 Frederick Soddy won the Nobel Prize in Chemistry for his discovery and definition of isotopes. He built upon the discovery of radioactivity, some 20 years preceding his research, to observe that atoms could exist in numerous forms and still belong to the same elemental category. These variations in the atomic build-up of elemental substances he dubbed 'isotopes' (Sharp, 2018, p. 5; Wilkinson, 2018, p. 57). The following year Francis Aston won the same prize for his invention of the first mass spectrograph with which he was able to identify 212 isotopes (more than half of what has since been discovered), proving Soddy's isotope theory and providing the first instrument able to separate and measure isotopes (Nobel Prize Outreach AB, n.d.).

Aston's apparatus (the mass spectrograph) though ingenious, was still crude compared to the developments that were to come in resolution and efficiency. The Second World War had an impact on the development of the design and construction of new and improved mass spectrometers and in the late 1940s Alfred Nier made important improvements allowing more resolution and sensitivity to be achieved in measurements. He was able to use his new mass spectrometer to separate the two compounds  $\text{CO}_2$  and  $\text{C}_3\text{H}_8$ . This achievement, along with the now more readily available mass spectrometry equipment, promoted the use of stable isotope analysis in other scientific disciplines, outside the traditional arena of physics and physical chemistry (Wilkinson, 2018, p. 64). Mass spectrometry had made its debut in commercial science and throughout the 1950s and 60s the instrument underwent rapid improvements and stable isotope research gained popularity in biology and geochemistry. Although the applications and understandings had boomed during this time, stable isotope analysis was still a long, costly, and specialised process. In the 1980s the spectrometer got another face lift and by 1990 mass spectrometry had become so user-friendly and cost effective that it became almost routine to include multiple stable isotope analysis in archaeological research. (Katzenberg, 2007, p. 414). Since then, only modest improvements have been made in precision or accuracy, but cutting-edge machinery and technology have advanced the field nonetheless, providing scientists with easy and rapid results, needing ever smaller samples sizes and less time and money than ever before. This opens the field up to a myriad of unique analyses and to research requiring bulk data sets, such as in biology. Although this user-friendliness and easy access may seem

like a blessing, many authors now warn that big and fast does not equal better (e.g., Sharp, 2007; Roberts, 2022; Makarewicz & Sealy, 2015).

The tedious, costly work that the likes of Soddy, Aston and Nier put into their research was painstakingly endured to ensure reproducibility but the increase of the quantity of data available now is not necessarily consistent with an increase in quality. The casual inclusion of stable isotope data in archaeological research is something that the founding fathers of stable isotope research could never have foreseen. Patrick Roberts (2022) states that the greatest challenge in isotope research within archaeology today “(...)is to ensure that this “maturity” does not equate to stasis” (p. 1). In the same paper he makes a point of quoting the pioneers of stable isotope archaeology, Vogel and van der Merwe from half a century ago, which will be discussed in the following section. Zachary Sharp (2007) likewise comments on the danger of the potential superfluosity of the hundreds of analyses published every year and sagely advises that older literature is still worth the read, as many of the carefully selected materials and analyses reached similar conclusions to those reappearing in modern publications (p. 4). Sometimes, in order to look forward, you must first look back.

### 2.2.2 Making its way to archaeology

As we have seen above, stable isotope analysis now has broad applications across many disciplines. In archaeology, stable isotope analysis' story begins with the humble ear of corn. In 1977, Vogel and van der Merwe published a revolutionary article using the human skeleton as evidence for maize cultivation in the state of New York. While it was well known that maize was a C<sub>4</sub> plant (more on that in the next section) never before had this concept been applied to measure the ratio of  $\delta^{13}\text{C}$  in skeletal remains as way of studying paleodiet and subsistence strategies (Vogel & van der Merwe, 1977, p. 1). What this meant for archaeology was phenomenal, perhaps even more important than the discovery of radiocarbon dating, as it gave stable isotope research a unique position in its ability to study cultural *and* ecological aspects of the human past, simultaneously. A couple years later, DeNiro and Epstein (1981) published a paper on the application of nitrogen as another isotope useful for reconstructing past human diets. This resulted in a quick succession of



studies into nitrogen on trophic levels (Sealy et al., 1987; Ambrose & DeNiro, 1987) and combined studies on carbon and nitrogen (Schoeninger & DeNiro, 1984).

The remarkable potential of isotope research to unlock both biological and social information in archaeology positioned it in a theoretical paradigm as well. The gaining popularity for this as a research approach in history cannot be isolated from the shift in the scientific agenda in archaeology at the time. A little over a decade after stable isotope research made its debut in archaeology, post-processual archaeologists demanded more regard for individual agency, social dynamics, and subjectivity in archaeological research. Stable isotopes provided a helpful scientific approach, bridging the processual theoretical framework, focussed more on ecological processes, and the budding interpretive archaeology, enabling the new paradigm to pose new and fresh questions pertaining to social and political motivations for subsistence strategies, economy, diet, and mobility while maintaining empirical research methods (Makarewicz & Sealy, 2015, p. 147). However, it is worth noting that science always reflects the time period it takes place in and can never be completely objective in what is on the agenda, how certain analysis methods are put to work and to answer which research questions. The caveat in stable isotope research is that it is easy to become trapped in a narrow narrative and that, along with getting caught up in the over-exuberance of stable isotope analysis, is likely what we are warned against by the authors mentioned above. While this can yield important information and is by no means trivial, it is our scientific responsibility to observe other possible conclusions and interpretations, think beyond the empirical data, and be aware and explicit of our place in history and why our research is important. Pressure from commercial and academic research can result in a desire to portion scientific archaeological data and measurements into defined sections, pre-packaged for neighbouring disciplines to interpret when it is convenient and there is money left over. The narrower the field or research, the cheaper and less people involved. It is important, however, to contextualise all archaeological data. The science in archaeology cannot “stand alone” as quantitative data sets, independent of archaeological interpretation and historical context (Bronk Ramsey, 2008, p. 266).

## 2.3 Applications in archaeology

The most measured isotopes are sulphur, nitrogen, oxygen, carbon, and hydrogen (SNOCH) and these are the most abundant elements in living organism tissues. Also on the rise are stable isotope measurements of metals and one of those is particularly interesting for archaeology, namely, strontium or Sr (Sharp, 2007, p. 7; Slovak & Paytan, 2012). Of the traditional isotope elements, archaeology has benefited most from carbon, nitrogen, and oxygen. The techniques are based on the premise that the food and water consumed during one's lifespan leaves chemical traces in their tissue and therefore stable isotopes are mainly used to study mobility and diet (Katzenberg, 2007, p. 415).

### 2.3.1 Carbon - Diet

Carbon was the first to be commandeered by stable isotope research, stemming from its use by geologists (and subsequently archaeologists) using it for radiocarbon dating methods. So, already familiar with carbon, it was not a huge step to utilize the radioactive isotope element's stable counterparts. These stable isotopes could be used to trace the metabolic fate of proteins within biological systems and therefore the traces of protein consumption left in fossilized bone. The process starts with how plants fix carbon. What determines the plants carbon composition are two phenomena, first the atmospheric composition of CO<sub>2</sub> and, second a particular plant species' photosynthetic or chemosynthetic pathway.

Terrestrial plants have three photosynthetic pathways for consuming carbon: the C<sub>3</sub>, C<sub>4</sub> and CAM. The numbers 3 and 4 refer to the number of carbon atoms in the first product of the compound. CAM stands for crassulacean acid metabolism and these plants, mainly succulents, switch between the two pathways depending on the conditions. Plants using the C<sub>3</sub> pathway deplete the <sup>13</sup>C by roughly 19 ‰, resulting in an average δ<sup>13</sup>C content of -26 ‰ (VPDB value vs atmosphere is -7 ‰), whereas C<sub>4</sub> plants have an average value of -12.5 ‰, therefore, C<sub>4</sub> plants have less negative δ<sup>13</sup>C values relative to C<sub>3</sub> plants. Important to note here is that the ranges for C<sub>4</sub> (-9 ‰ to -16 ‰) and C<sub>3</sub> (-20 ‰ to -34 ‰) plants do not overlap, making them of extra interest to archaeologists. Examples of C<sub>4</sub> plants include millet, maize, sorghum, and sugarcane and typically grow closer to the tropics. C<sub>3</sub> plants are

more abundant and can be found at higher latitudes and include cereals such as wheat, barley, oats, and rice but also legumes, and root staples such as potato, manioc, and yam. (Schoeninger & Moore, 1992, pp. 255 - 256; Lee-Thorp, 2008, p. 927; Vogel & van der Merwe, 1977, p. 239). For marine plants the source of carbon undergoes an extra step, namely, the transfer of carbon dioxide from the ocean to the atmosphere which depletes the CO<sub>2</sub> of <sup>13</sup>C in the atmosphere relative to the ocean. Another obvious contemporary factor is the use of fossil fuels, this is reflected in an increase of atmospheric δ<sup>13</sup>C values than in the past (Schoeninger & Moore, 1992, p. 255). Since animals derive their carbon mainly from plants, the carbon isotope composition in bones and teeth can inform us of the carbon source (i.e., C<sub>3</sub> or C<sub>4</sub> plants) at the beginning of their food chain.

Realising the archaeological use of the other elements took place relatively late in comparison with other disciplines, but this meant archaeologists already had a plethora of information from their palaeogeology and biology neighbours (Katzenberg, 2007, p. 415). One additional benefit from stable carbon isotope research is that there are complications in radiocarbon dating when the main carbon source is unknown, especially in marine and coastal areas, and therefore by gaining a better understanding of carbon source in diet, this research has the potential for increasing radiocarbon dating resolution from human tissue as well (Bronk Ramsey, 2008, p. 260).

### 2.3.2 Nitrogen - Diet

Nitrogen was the next isotope to be used by archaeologists and also consists of two stable isotopes, namely, <sup>14</sup>N and <sup>15</sup>N. More than 99 % of nitrogen in the biosphere is found as N<sub>2</sub> gas in the atmosphere or dissolved in the ocean. It is then either absorbed by algae or bacteria on terrestrial plant roots and so it enters the food web. It is also broken down by bacterial processes upon the death of an organism, whereupon vascular plants can use the nitrates that are released. These nitrates have more <sup>15</sup>N than in the atmosphere and therefore vascular plants have a more positive δ<sup>15</sup>N value than N<sub>2</sub>-fixing plants. The atmospheric δ<sup>15</sup>N composition is 0 ‰ and most terrestrial plants, such as C<sub>3</sub> and C<sub>4</sub> grasses, have a δ<sup>15</sup>N value of +3 ‰, although legumes have been observed to have varied values, sometimes much higher (Schoeninger & Moore, 1992, pp. 256 – 258; Richards, 2015, p. 20).

When nitrogen becomes interesting for archaeologists, however, is the change in  $\delta^{15}\text{N}$  values between trophic levels. Whereas for carbon the values vary only  $\sim 1\text{‰}$  between trophic shifts,  $\delta^{15}\text{N}$  values increase consistently with  $+3$  to  $4\text{‰}$  per trophic level. Therefore, the tissues of a herbivore are distinct from those of a carnivore. An issue with measuring  $\delta^{15}\text{N}$  values in marine tissues is the presence of more trophic levels than in terrestrial animals as “there is always a bigger fish” (Schoeninger & Moore, 1992, pp. 256 – 258). The inference can therefore be made that those subsisting off of more marine based diets will have higher  $\delta^{15}\text{N}$  values than those living off of mainly terrestrial diets, although this can be problematic. One issue being that areas with coral reef tend to have lower  $\delta^{15}\text{N}$  values due to the fixing of nitrogen by algae and other bacteria (Varney, 2003, p. 48). Another issue is that some marine species have relatively even *higher*  $\delta^{15}\text{N}$  values, such as those found in pelagic fish (Laffoon et al., 2016, p. 176).

Nitrogen has also been used in research pertaining to climatic shifts, but this has been called into question for its potential unreliability and oversimplification. Oxygen isotopes may provide a more reliable proxy for studying environmental changes in the past and are now often used in conjunction with nitrogen. It is also worth noting that the premise described above that nitrogen is enriched with each increasing trophic shift, ought to be approached with caution for risk of oversimplification and premature conclusions as a great deal of other factors can influence nitrogen variation such as climate and seasons, metabolic structure, stress, and domestication. For these reasons it is important to continue to compare between different isotope values and between different trophic level consumers, but also draw upon multiple paleodiet reconstruction methods used in archaeology and not to rely too heavily on only one model or data source before drawing conclusions about diet (Makarewicz & Sealy, 2015, p. 148).

### 2.3.3 Other uses for carbon and nitrogen isotopes

#### 2.3.3.1 Breastfeeding

Nitrogen and carbon isotope values can be used to track breastfeeding and weaning patterns in infants. During breastfeeding infants will display higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values

than their mother, analogous to those observed in a trophic level shift, i.e., 1 ‰ for carbon and 3 ‰ for nitrogen. The premise for this shift is that infants are essentially consuming their mother's tissue resulting in the enrichment of both isotopes. In the course of weaning, both isotope values generally drop considerably before levelling out to maternal values (assuming infants consume the same diet as their mothers). The  $\delta^{13}\text{C}$  drops more abruptly than the  $\delta^{15}\text{N}$  in the weaning phase and there are several theories to as to why this is, including that the larger enrichment of  $\delta^{15}\text{N}$  means it takes longer to drop, and that  $\delta^{13}\text{C}$  values also reflect the carbohydrates introduced with solid foods, in addition to protein sources (Fuller et al. 2006, pp. 288 – 289).

#### *2.3.3.2 Malnutrition*

Extensive studies have been conducted on the influences of malnutrition and starvation on stable isotope values in hair. Results have shown that in periods of nutritional stress in anorexia patients, the body's reaction is to enter a state of catabolism in which it recycles its own protein manifesting as  $\delta^{15}\text{N}$  enriched tissue and inversely related  $\delta^{13}\text{C}$  values (Mekota et al., 2006, pp. 1608 – 1609). This secondary isotopic fractionation that occurs is gaining interest in archaeology as it can provide evidence for individual and population malnutrition in the past, allowing insight into phenomena such as famine, slavery, and disease (e.g., Beaumont, Geber, et al., 2013; Walter et al., 2020).

#### *2.3.4 Oxygen and Strontium - Mobility*

Another important element for archaeology is strontium. Strontium is a chemically reactive alkaline earth metal and unlike the isotopes already covered, originates in the lithosphere. The parent material of a soil is the strongest influence on the bioavailable strontium, though other processes have also been known to affect the isotopic signature. Geologists have modelled isoscape maps based on samples of the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of local vegetation and soils. Archaeologists can use these isoscapes to map the geographical origin of individuals based on the strontium isotope signature in their tissue compared to any given isoscape region's value (Britton & Guiry, 2020, p. 425).

Oxygen is another element with isotopes used to infer origin. Oxygen isotope ratios reflect the ratios of that in the water consumed by the individual. The  $\delta^{18}\text{O}$  values of the water in a given area are dependent mainly on temperature but also proximity to the coast, precipitation, and altitude (Britton & Guiry, 2020, pp. 425 - 426). Therefore, oxygen isoscapes can be created, similar to those made for strontium, as an individual's isotopic signatures will reflect those of the water in the area in which they live.

## 2.4 Tissues

The tissues used for studying stable isotopes in bioarchaeology are calcified bones and teeth. While both can provide us with a plethora of information they do differ slightly, especially temporally. Here we discuss those differences and the advantages and disadvantages of each tissue and their components.

### 2.4.1 Tooth and bone

The human skeleton is comprised of three main components: an organic phase, an inorganic or mineral phase, and water. The proportions of these components depend on the skeletal element and reflects the rate and pattern of biomineralization and growth. The dominant protein in the organic phase of bone is the robust biomolecule collagen. The inorganic phase consists mainly of bioapatite (Lee-Thorp et al., 1989, p. 586).

Bone tissue can be separated into two sorts: the "spongy" or cancellous bone and the "compact" or cortical bone. The *in vivo* remodelling of bone occurs throughout one's lifespan at approximately 10 % per year but can vary according to which bone and which part of the bone. For example, ribs and epiphyses remodel much faster than the diaphysis of long bones or the cranium and as such it is important to consider which skeletal element and what type of bone is being researched in order to infer the correct phase of life being studied and degree of diagenesis to be expected (Slovak & Paytan, 2012, p. 749). Bone has a

relatively high organic content of approximately 25-30 %, with 9-10 % water and the remaining weight made up of the mineral aspect (Kendall et al., 2018, p. 1).

Living teeth contain four tissues: pulp, enamel, dentine, and cementum, the latter three being mineralized tissues and therefore applicable in archaeological research. Anatomically the tooth has a crown, cervix, and a root. The outside protective layer of the crown is made up of enamel, and the root, cervix, and the inside part of the crown are all composed of dentine. Cementum can coat the whole tooth or just a part of it and has a structure similar to bone. Dentine also has a similar composition to bone with 70 % organic matrix, 20 % protein and the remaining weight in water. Enamel has the least amount of organic matter with only 4 % proteins or lipids and the remaining 96 % composed of the inorganic or mineral phase making it the hardest, densest, and least porous of all human tissue (Ortiz-Ruiz et al., 2018, p. 7; Kendall et al., 2018, p. 6).

The two most important differences between the data that can be compiled by archaeologists from bones versus teeth are: 1) the temporal resolution and position in the individual's life (discussed here), and 2) the diagenesis processes (to be discussed below). Bone constantly remodels throughout the human lifespan whereas teeth form incrementally during childhood and therefore reflect only the juvenile years of one's life, with exception of the third molar which can continue to form into early years of adulthood. However, due to the incremental nature of tooth formation, the temporal resolution of data from teeth is much higher than that from bone (Beaumont & Montgomery, 2015, pp. 407, 409). Therefore, while data from bone will reflect a more average isotope ratio from 5 to upwards of 10 years before death (depending on which bone), teeth have the potential to inform us of specific isotope values throughout childhood. Depending on the research question both can be useful tissues to examine in archaeology (Curtis et al., 2022, p. 1). While the isotopic signatures between different tissues, anatomical elements, and even within an anatomical element itself can differ considerably in the information yield, this is in fact a helpful tool for archaeologists. In comparing these differences, we can make inferences about weaning, diet, mobility, and many other phenomena. It is important however to have basic understandings as to *why* these differences occur in order to make correct assumptions about the aforementioned events. There are advantages and

disadvantages to this as it can be complex when comparing the same isotope ratios in different tissues due to small differences in growth and diagenesis sometimes rendering certain research incomparable.

## 2.4.2 The organic and inorganic components

### 2.4.2.1 Collagen

Collagen is a robust biomolecule that forms over 90 % of the organic component of the human skeleton and is the most prominent protein in mineralized tissue. Collagen can survive for over 100 000 years, which is, on a geological timescale, not very long. Yet, luckily for archaeologists, studies have suggested that even when most of the collagen molecules have degraded, the isotopic composition is still observable. Collagen is sensitive to pH, moisture, and temperature due to the fibrils dissolving relatively swiftly once hydrogen bonds have broken. Contaminated and degraded collagen can alter stable isotope ratios, but standard modern procedures can generally still produce acceptable results (Lee-Thorp, 2008, p. 930).

Collagen yields isotope information for all the elements discussed above and more. Its isotopic integrity and rich information return (also outside of stable isotope studies, for example for C<sup>14</sup> dating methods) make collagen (usually from bone) the most researched tissue of the human skeleton in archaeology (Katzenberg, 2007, p. 415).

### 2.4.2.2 Bioapatite

The inorganic phase of the skeleton is often referred to as apatite, although this is not technically accurate. It is a calcium phosphate that, although structurally similar, does not belong to the apatite mineral family and is therefore better referred to as *bioapatite*. Nevertheless, the terms are frequently used interchangeably (Kreuger, 1991, p. 356). As an information source in archaeology, bioapatite received little attention due to the assumption that it was prone to alteration and contamination. Bioapatite absorbs minerals and ions and must therefore be treated with caution when analysing. The degree of



reliability of information from bioapatite is an ongoing debate within archaeological stable isotope research. Initially there was evidence that with appropriate handling, especially treatment with acetic acid to remove surface contaminants, bioapatite samples are highly reliable. Though it has also been argued that the conclusion from this initial research is untenable as it lies on the premise that carbon isotope values should increase consistently with collagen samples, and this is not a stable assumption. Subsequent studies, however, have assured that with the proper pre-treatment strategies, alterations in bioapatite carbon isotopic signal are insignificant, especially in younger remains (Lee-Thorp et al., 1989, pp. 586-587).

While bioapatite has thus been proved to be a reliable and useful tool for stable isotope research it unfortunately does not retain substantial measurable nitrogen. It is therefore most useful to use a combination of tissues in order to still be able to compare, for example, C:N ratios, a commonly used analysis in archaeological research to assess specimen preservation (Clementz et al., 2009, p. 606).

#### 2.4.3 Incremental dentine sampling techniques

As previously mentioned, teeth form in increments during childhood and adolescence and are thereafter metabolically inert. In 2003, Fuller et al. conducted an initial study sampling increments in human dentine, inspired by studies conducted on various animal teeth that had yielded promising and informative results. The aim of their study was to detect breastfeeding and weaning patterns (p. 1674). This was an exciting step forward as it provided detail for singular events such as weaning practices, changes in diet, mobility and physiological stresses that was previously unattainable from bulk sampling strategies that were then the norm.

Recent authors have proposed numerous new sequential sampling methodologies, all attempting to refine the temporal resolution and reduce averaging effects, while minimalizing the sample size required. Dentine does not deposit layers in symmetrical parallel lines but mineralizes in S shaped dentinal tubules. The mineralization process occurs in a diurnal rhythm observable under a microscope as differing densities called Andresen

bands. Beaumont, Gledhill, et al. remarked in 2013 that sampling along these lines from a longitudinal section would be ideal and reduce averaging effects significantly but that this would be technically challenging (p. 279). In 2022, Curtis et al. were successful in sampling along the Andersen bands and were able to procure three times as many samples as in the Beaumont method, achieving a resolution of an isotope signature reflecting 3 months in a section (pp. 5 – 6, 8). Cheung et al. (2022) compared micro-punches and micro-slices as potential sampling methods and found that while the former can provide the highest resolution and most increments, the latter is more appropriate when dental elements are poorly preserved and can yield a higher amount of synchronous isotope measurements. It is therefore important to consider the state of the sample being studied and the archaeological research question when choosing which method to employ (p. 13).

A novel study conducted by Stantis et al. in 2021 sought to provide an additional line of evidence by sampling the mineral component of the dentine in the sequential sections, as well as the collagen, enabling a comparison between the two isotopic signatures (p. 2). Why this is momentous for bioarchaeological research will become clear in the upcoming chapters as this thesis implements the same strategy.

#### 2.4.4 Diagenesis and discrimination variations

##### 2.4.4.1 Diagenesis

Diagenesis refers to all processes that affect the quality or isotope ratios of a tissue *post-mortem*. There are two distinct ways in which hard tissues can undergo changes, either an *addition* of material or an *alteration* of the existing matrix (Kreuger, 1991, p. 356). The preservation of isotope signatures and potential contamination are of utmost importance to consider when choosing to research any tissue or component. Between dentine, bone and enamel, the latter is the most resistant to geochemical alteration and is therefore generally the first choice when examining older fossilized remains, especially those older than 0.05 Ma (Clementz et al., 2009, pp. 605). This is due to its high density and low porosity in comparison to bone. Bone and dentine have more complex links between collagen and

bioapatite making them more susceptible to diagenesis due a lower crystallization compared to enamel. There is therefore more room for inclusions and alterations in between particles. Different bones differ in porosity and consequently the degree of alteration will also vary from bone to bone. Dentine, while having a similar composition to bone, has less contact with the vascular system than bone *in vivo*, resulting in less access to soil water post-deposition and reflected in a higher resistance to diagenesis than bone, on average (Kendall et al., 2018, p. 8). The diagenesis of collagen and bioapatite is inherently interlinked and related, though usually collagen is more resistant to diagenesis and will therefore retain a more reliable biogenic isotopic signature than bioapatite (Kendall et al., 2018, p. 25; Chisholm et al., 1982, p. 1131).

Post-depositional environment also affects the taphonomy of human tissue. Soil pH, hydrology, and bioerosion are the main threats to isotopic degradation. Warm environments with cyclical soil saturation make tissues more susceptible to alteration and decay than cooler climates with a stable water table (Kendall et al., 2018, p. 25).

#### 2.4.4.2 Discrimination

The term fractionation is used to describe changes in stable isotope ratios that occur due to or during various physical and chemical processes. A more appropriate term when more than one multiphase process is involved, such as the flow of nutrients in an ecosystem or within an organism, is *discrimination* (Merav & Flaherty, 2012, p. 317). The discrimination between diet and bodily tissue varies depending on what tissue and what component of the tissue is being examined, due to secondary fractionation and synthesis from different dietary constituents. For example, the discrimination between diet and bioapatite is significantly more enriched in  $\delta^{13}\text{C}$  than diet-collagen. In both cases the carbon source in an animal's diet is less enriched than the tissue, with diet-apatite spacing ranging from +12 to +13‰, and diet-collagen spacing sitting between +3 and +6‰. The differences in isotope signature between these two bone phases are due to different biosynthesis processes. Carbon in bioapatite is absorbed from bicarbonate in blood while that of collagen is incorporated mainly from direct protein sources. An implication of this contrast is that carbon values in collagen are biased towards the isotopic signatures of the protein

component of an individual's diet, whereas bioapatite carbon values can inform archaeologists of other macronutrient sources, such as fats and carbohydrates (Lee-Thorp et al., 1989, p. 587).

Next to secondary fractionation and tissue synthesis, other factors influencing intraindividual differences in isotope ratios include tissue composition and turnover time. As mentioned in a previous section, bone, similar to dentine, consists for 30 % out of organic material whereas enamel's organic content is negligible enough that it is generally unused in archaeology. The turnover time has also been mentioned, with bone reflecting the years of an individual's life before death as it continuously remodels, in opposition to enamel and dentine that will both undergo no chemical alterations after formation and therefore grant a window of information limited to juvenile (and sometimes early adulthood) years (Clementz et al., 2009, pp. 605 – 606).

Merav and Flaherty (2012) advise a step-by-step way of analysis when using isotopes in archaeology, first considering the isoscape, i.e., the temperature and climate, latitude, geographical and temporal position. Subsequently, one must consider the compound and product discrimination between trophic levels, often heuristically declared to be 1 ‰ for carbon and 3 ‰ for nitrogen. These assumptions, however, do not consider other factors influencing discrimination or fractionation differences, such as, the above-mentioned factors, and also excretion, assimilation, and tissue variations. Only once this has all been considered can one begin to analyze diets, nutrient flows, and interspecies and trophic interactions (pp. 317 - 319).

## 2.5 Bivariate and multivariate models

### 2.5.1 Bioapatite – collagen spacing.

Comparing the difference between two of the same isotope composition values from different sources is a growing field of study. To elaborate, the comparison between carbon isotope composition of the dentine collagen to the dentine bioapatite, as being done in this thesis, is one example, but other possible contrasts are diet-bioapatite, bioapatite-collagen

in bone or bone collagen-enamel bioapatite (e.g., Passey et al., 2005; Hedges, 2003; Clementz et al., 2009; Codron et al., 2018; Lee-Thorp et al., 1989; DeSantis et al., 2022). As the  $\delta^{13}\text{C}_{\text{collagen}}$  is mainly indicative of the protein component of the diet and  $\delta^{13}\text{C}_{\text{bioapatite}}$  reflects an average of all macronutrients consumed, the spacing between collagen and bioapatite values can provide extra valuable information for the diet consumed. These “spacing” values are expressed with  $\Delta$  and sometimes referred to as “big delta values” or “double D.” One use for this type of analysis is to infer trophic level spacing. Lipids and fats are depleted in  $^{13}\text{C}$  compared to carbohydrates, the main energy sources of carnivores and herbivores, respectively. Therefore, carnivores will have lower  $\Delta$  values due to their higher utilization of  $^{13}\text{C}$  depleted macronutrients. The proposed approximate spacing for increasing trophic levels is  $\Delta^{13}\text{C}_{\text{bioapatite-collagen}} +7\text{‰}$  for herbivores,  $+4.5\text{‰}$  for omnivores; and  $+3\text{‰}$  for carnivores. Therefore, the higher the trophic level the lower the  $\Delta^{13}\text{C}_{\text{bioapatite-collagen}}$  value (Lee-Thorp et al., 1989 pp. 587 – 588). Another use for the comparison of mineral and protein components is to discriminate between  $\text{C}_3$  and  $\text{C}_4$  plant and protein consumers. By plotting the bioapatite and collagen stable isotope data, two linear regression lines can be used to infer  $\text{C}_3$  versus  $\text{C}_4$ /marine protein diets (Kellner & Schoeninger, 2007). While most studies using  $\Delta^{13}\text{C}_{\text{bioapatite-collagen}}$  compare bone-dentine or enamel-bone they therefore reflect an average of the total isotopic composition of the specimen. To date, limited studies have been conducted on  $\Delta^{13}\text{C}$  values using incremental dentine sampling, providing a unique temporal resolution and individual life history.

### 2.5.2 Cluster analysis

The bivariate model mentioned above fails to provide a distinction between  $\text{C}_4$  and marine protein consumption due to both being relatively enriched in  $\delta^{13}\text{C}$  compared to  $\text{C}_3$  plants. Fortunately, the incorporation of  $\delta^{15}\text{N}$  into the analysis potentially helps to remedy this pitfall. Nitrogen stable isotope composition mostly reflects the protein aspect of the diet and is able to distinguish between plant versus animal protein and marine versus terrestrial protein sources. Using this additional proxy can potentially resolve some of the ambiguities encountered when solely using carbon stable isotopes. Marine ecosystems are generally more enriched in  $^{15}\text{N}$  compared to terrestrial food webs and this can therefore aid in

inferring the degree of reliance on marine protein sources. Considering that  $\delta^{13}\text{C}_{\text{collagen}}$  and  $\delta^{13}\text{C}_{\text{bioapatite}}$  values overlap in  $\text{C}_4$  and marine protein sources, this property of nitrogen aids in disentangling the information, especially in areas where a dual reliance on  $\text{C}_4$  plants and marine resources is possible, for example in islands and coastal areas (Froehle et al., 2012, pp. 353 – 354).

In the multivariate or discriminant function model proposed by Froehle et al. (2012) “functions” for carbon and nitrogen are calculated based on controlled feeding experiments using the following algebraic formulae:

Carbon:

$$F1: (0.322 \times \delta^{13}\text{C}_{\text{bioapatite}}) + (0.727 \times \delta^{13}\text{C}_{\text{collagen}}) + (0.219 \times \delta^{15}\text{N}) + 9.354$$

Nitrogen:

$$F2: (-0.393 \times \delta^{13}\text{C}_{\text{bioapatite}}) + (0.133 \times \delta^{13}\text{C}_{\text{collagen}}) + (0.622 \times \delta^{15}\text{N}) - 8.703$$

By plotting these functions, they were able to discriminate between five diet clusters, providing considerably more detail than the original bivariate model by Kellner & Schoeninger (2007). The clusters are as follows: 1) 100%  $\text{C}_3$  diet/protein; 2) 30  $\text{C}_3$ :70  $\text{C}_4$ , >50%  $\text{C}_4$  protein; 3) 50  $\text{C}_3$ :50  $\text{C}_4$  diet, marine protein; 4) 70  $\text{C}_3$ :30  $\text{C}_4$  diet,  $\geq 65\%$   $\text{C}_3$  protein; 5) 30  $\text{C}_3$ :70  $\text{C}_4$  diet,  $\geq 65\%$   $\text{C}_3$  protein.

## Chapter 3: Background case study

The case study being analyzed in this thesis consists simply of five teeth found in a lockbox with no further anatomical context. The fact that, despite this, there is still such a vast amount of data to be acquired and much to be inferred about this individual, is a testament to the ever-growing number of impressive and informative tools and methods available to archaeologists. In this section the historical and archaeological context will provide a backdrop for the analysis of the individual SB007.

### 3.1 Geographical context

#### 3.1.1 Physical environment

Long before modern humans were to frequent the area, more than 500,000 years ago, a volcano formed the island now known as Saba. Lying at 17.38 degrees North, and 63.14 degrees West, Saba is situated at the very north of the Lesser Antilles group in the Caribbean Islands (Espersen, 2017, p. 23). It is a small island, with an area of only thirteen square kilometers, comprising of one main mountain, rising 870 meters above sea level, surrounded by sixteen smaller steep peaks. Due to the steepness of the island both the weather and the vegetation are highly diverse. Average yearly rainfall is 1130 mm and while July to October is usually called “rainy season” and January to April the “dry season,” Saba is so steep and small that this can vary greatly. The mean annual temperature is 25.6 °C with only small variations throughout the year. The lowest recorded temperature is 15 °C but the higher you climb “The Mountain” (the name given to Saba’s main peak) the colder it gets (Espersen, 2017, p. 24). The trade wind blows E-NE, and the island is victim to hurricanes from time to time. Saba has no natural rivers, simply what are referred to as “guts” which are the steep ravines that only fill with water after high rainfall (Romeijn, 1989, p. 46). The island relies therefore on three springs for freshwater, located at Spring Bay, Wells Bay, and Fort Bay (Espersen, 2017, p. 24). Reports vary as to whether Saba is active or not, but most likely the volcano lies dormant, the only signs of activity being the hot springs and unstable

temperatures in abandoned Sulphur mines. The last eruption most likely took place in the 17<sup>th</sup> century, but before colonization (Espersen, 2017, p. 23; Romeijn, 1989, p. 46). The volcanic rock and steep topography make for chemically fertile soils but physically difficult to use for agriculture. Due to the high variation in both precipitation and elevation, the vegetation is incredibly diverse for such a small island. Three main vegetation zones can be identified, changing as one gets higher up the mountain. The lowest level, at sea level, consists of seasonal and dry evergreen forests. These blend into rainforests in the middle, before changing into palm brake and montane ticket on the top of the mountain. These highest growth zones are often surrounded by clouds and receive the most rainfall of the island (figure 3.1) (Romeijn, 1989, pp. 46 - 48).

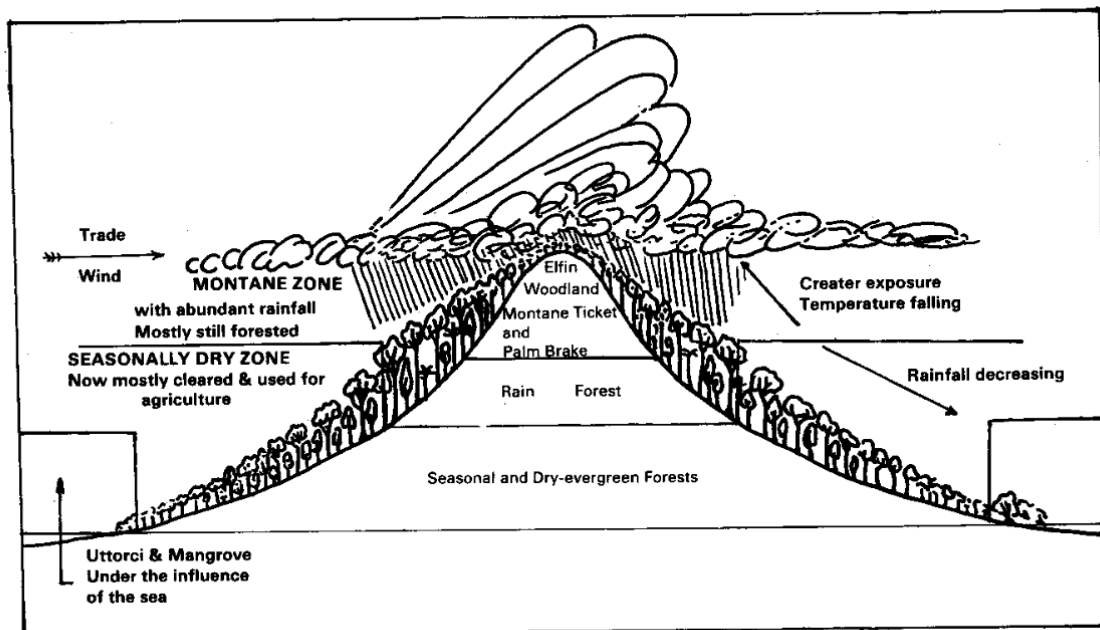


Figure 3.1 An example of Saba's climate and vegetation (Romeijn, 1989, p. 47).

While nowadays small aircraft alight Saba regularly, as one might imagine, the island did not always offer easy access. Finding flat strips of land to land a plane was difficult and before air travel, Saba made those wishing to arrive by sea perhaps even harder. Rocky seabed, strong winds and currents and steep cliffs make Saba an unappealing place to approach by boat. The anchorage surrounding Saba is so egregious that everything had to be rowed



ashore until well into the 20<sup>th</sup> century when the first harbor was built (Espersen, 2017, p. 24; Romeijn, 1989, p. 45).

### 3.1.2 Current political and social climate

The demography of Saba has been relatively stable as population growth is compensated by emigration, with population growing to 1,947 residents in 2016 and generally staying just below the 2,000 inhabitants (Centraal Bureau voor de Statistiek, 2016). Dutch, English, Irish, African, and indigenous peoples have inhabited the island since the colonial era and the ethnic distribution of the modern population reflects this, the ratio of people from (white) European descent to local or African descent always fluctuating around half/half even since during colonial times (Espersen, 2017, p. 14). Due to the obvious geographical challenges, Saba is populated mostly in nooks and crannies around the island, where level land allows for it. Four areas host Saba's inhabitants: The Bottom, St. Johns, Windwardside, and Hell's Gate (Espersen, 2017, p. 23).

When the Netherlands Antilles was dissolved in 2010 due to a referendum, Saba along with neighbours St Eustatius and Bonaire, remained "special" Dutch municipalities. This status as a public entity meant that Saba remained a part of the Kingdom of the Netherlands and therefore also subject to its government's decisions and political landscape. Some have critiqued or questioned the decision to remain under Dutch "power," trading off true political autonomy and national emancipation for participation in the Dutch constitution (Mulder, 2018, p. 11). Mulder (2018) conducted an interesting ethnographic study into the view Sabans have on the current political landscape and concluded that post-colonial resistance on Saba does not take the form of a desire to become an independent nation-state but rather for proficient and engaged governance. The idea that colonies must prefer independence and freedom is absent on Saba, as locals do not feel a lack of autonomy, rather a frustration with the organization of the present bureaucratic structure. While post-colonialism brought with it the assumption that all colonies would have a deep desire for self-governance, Saba is an example of an exception to that narrative, where the complex balance between political autonomy and benefit diverges from the norm (Mulder, 2018, p. 24).

## 3.2 Archaeological context

### 3.2.1 Slavery in archaeology

Caribbean archaeology remains an interesting challenge for researchers due to the complicated colonial past. Archaeologists are not only presented with difficulties in determining “slave sites” but also in attributing identity to physical remains and material culture associated with slavery. Inferring conclusions from the archaeological record such as class, race, social status, and poverty is a meticulous task and ought to be undertaken carefully, lest we make the mistake of confusing, to provide an example, “white poverty” with “free black poverty” based purely on material culture that may have been shared (Espersen, 2017, pp. 16 – 17; 21).

While slavery has been studied extensively historically and there is an ever-growing field of research utilizing quantitative scientific approaches to investigate enslaved populations archaeologically, retracing individual life histories has a special role to play. This is because often these approaches become distanced from the reality of personal experiences and the importance of these studies gets lost in massive data sets of multiple methods on many populations. The power of retracing individual histories and lifeways is to re-humanize the past. As Fricke et al. (2021) eloquently put it: “It is much easier to empathise with one individual than with one thousand, and an emotional connection with slavery in the past can help us to address its lasting modern social, political, and economic effects” (p. 1). Information on diet, especially that of enslaved Africans during the colonial period, contributes to our understanding of the individual experiences of people living through a devastating period in history. A tragic time of stolen identities, systematic racism, slavery, and exploitation that deserves attention in order to reconcile.

### 3.2.2 Spring Bay Flat

Spring Bay Flat has been an area of interest to archaeologists since the 1980s. Initial research conducted in the area focussed on pre-Colonial settlements and revealed an

Amerindian settlement of approximately 50 people from during the Late Saladoid to Ostionoid Periods. However, due to agricultural activities in the area during the colonial era, further research into pre-Columbian occupation at Spring Bay Flat is difficult as the integrity of the archaeological record has been compromised in some areas and completely destroyed in others (Espersen, 2017, p. 168).

From 1650 until 1655 the 120-hectare estate known as “Spring Bay” cultivated sugar at around sea level, until the plantation was destroyed by the British. The plantation was then moved 300 meters south up a ridge to the site of Spring Bay Flat and was in use until 1815. Other smaller cultivated pockets of land provide evidence of coffee and cotton plantations. Spring Bay Flat has an elevation of 220 meters on the eastern side of the island and is accessible only on foot. The site is a small flat area that is covered in boulders making it impractical for agriculture and meant any tillage would have had to have taken place after clearing the area and modifying the terrain. In addition to the sugar plantation, indigo production sites and vats indicate that during the 18<sup>th</sup> and 19<sup>th</sup> centuries indigo was also cultivated in the area (Laffoon et al., 2018, p. 352; Espersen, 2017, pp. 167-168; 2015, pp. 1-2).

The site encompasses not only the sugar and indigo plantations but also several buildings and structures. These include several rock towers built with the cleared boulders (presumably to provide platforms from which to oversee the plantation activities); two cisterns adjoining a small house structure; a cattle mill; an indigo production site; a “Big House;” four huts; and a two-pot Jamaica train boiling house (Espersen, 2017, pp. 167, 170). Most of the materials used to build the structures were re-used to establish the Roman Catholic Church at Windwardside after their dismantling in the 1850s (Espersen, 2017, p. 169).

The area is organized in three distinct areas each with its own function pertaining to the plantation activities conducted and is partitioned by dry stone walls. In the southwestern area lies the cane field, opposite the industrial and inhabited area in the southeast, encompassing the structures of the Big House and the slave huts, but also the mill, boiling house, and indigo vat and, lastly, a largely unusable area in the north (Espersen, 2017, p. 207).

In the domestic section, four huts are deemed to have been belonging to enslaved Africans (figure 3.2). The structures C, D, E and F are positioned close to both the Big House and the rest of the industrial buildings in such a way that suggests a logical placement for convenience for the plantation owner, both in view and close to the working quarters. Another advantage for the owner is that these structures are built on land unsuitable for agriculture but nevertheless resistant enough to erosion to sustain long-term habitation. Further evidence for the use of these structures by enslaved Africans is provided by domestic artifacts and comparisons with other similar structures known to fulfil this function at other sites (Espersen, 2017, p. 177).

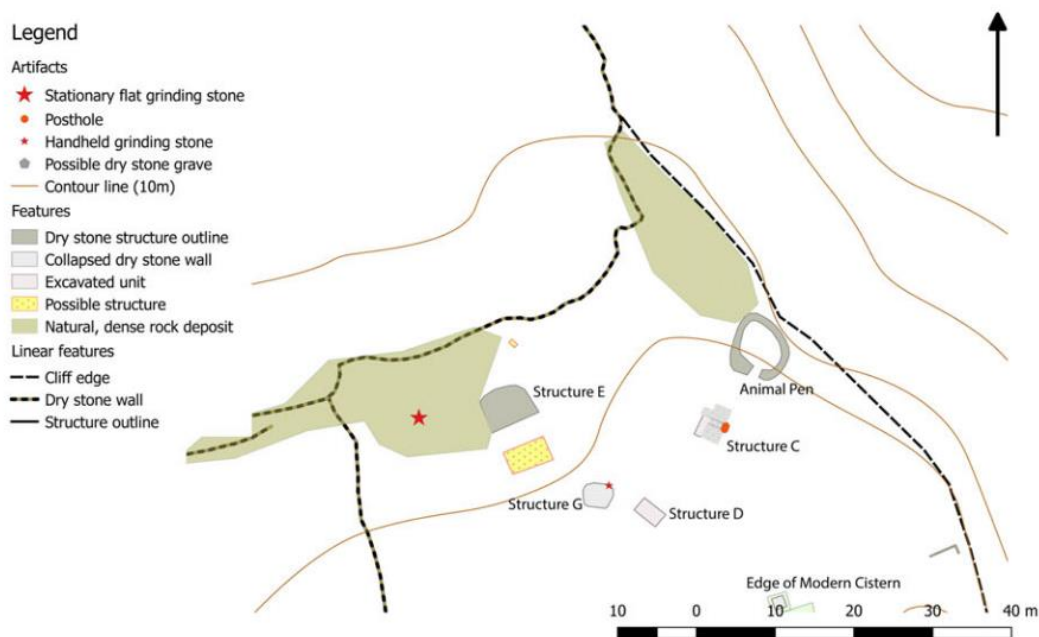


Figure 3.2 Enslaved African Domestic Area at Spring Bay Flat (Laffoon et al., 2018, p. 353).

The building of consequence for this thesis is Structure D. While assumed to be a secondary settlement to Structure C, excavations at Structure D yielded the most finds of all the huts. Most of the finds were ceramics dating to the 18<sup>th</sup> and 19<sup>th</sup> centuries, which corresponds with the previously known dating of the Spring Bay Flat site and with the surrounding structures. Structure D was excavated by trowel in six 1 x 1 m units of 50 cm deep each (Espersen, 2017, p. 182). Most of the artifacts were retrieved from layers 2 and 3, including

those to be discussed in this paper. A ferrous hinge, presumably part of a lock, was found laid on top of an assemblage in layer 3 at the intersection of units 3, 4, 5 and 6. (Espersen, 2017, p. 183). The assemblage consisted of seven nails (two of which were shorter than 8 inches and five of which were longer); a whole *Cittarium pica* shell; a clay pipe stem; faunal remains and five human teeth, which will be discussed below. The assemblage is believed to have been in a wooden lockbox, due to the latch's position resting on top of the artifacts. The context in which these objects were found, namely together and near the wall of the dwelling, indicate a "fetish bag" or "conjure bag," a superstition granting good luck or protection. The placing of a whole shell on a grave was and is a tradition practiced by many Caribbean peoples, locks on coffins are a Jamaican custom to keep away the spirits and the use of iron objects to aid in the transition from the physical to the spiritual realm is a practice often found in West African cultures (Espersen, 2009, pp. 5 -6). Therefore, a second assumption may be made that what is observed here is an interesting and powerful conglomerate of cultures pertaining to afterlife and tradition surrounding death. Further analysis of the context and meaning thereof is beyond the scope of this thesis.

## Chapter 4: SB007

### 4.1 Samples

The five dental elements found in the lockbox are permanent teeth 2.8, 3.5, 4.4, 4.6 and 4.7 (figure 4.1). Reflected in the dataset as elements SB007A (4.7); SB007B (2.8); SB007C (4.6); SB007D (3.5); and SB007E (4.4). Dental wear and morphological analysis indicate that these all belonged to the same individual, referred to as SB007. The presence of the maxillary third molar (2.8) along with the fact that the mandibular second molar (4.7) displays wear facets consistent with the inference that SB007 was also in possession of a third mandibular molar (4.8) enables an age-at-death estimation (Laffoon et al., 2017, p. 352). Based off of AlQahtani et al.'s (2010) atlas of human tooth eruption, the presence of two third molars indicates that this individual was >18 years of age at the time death (p. 485).

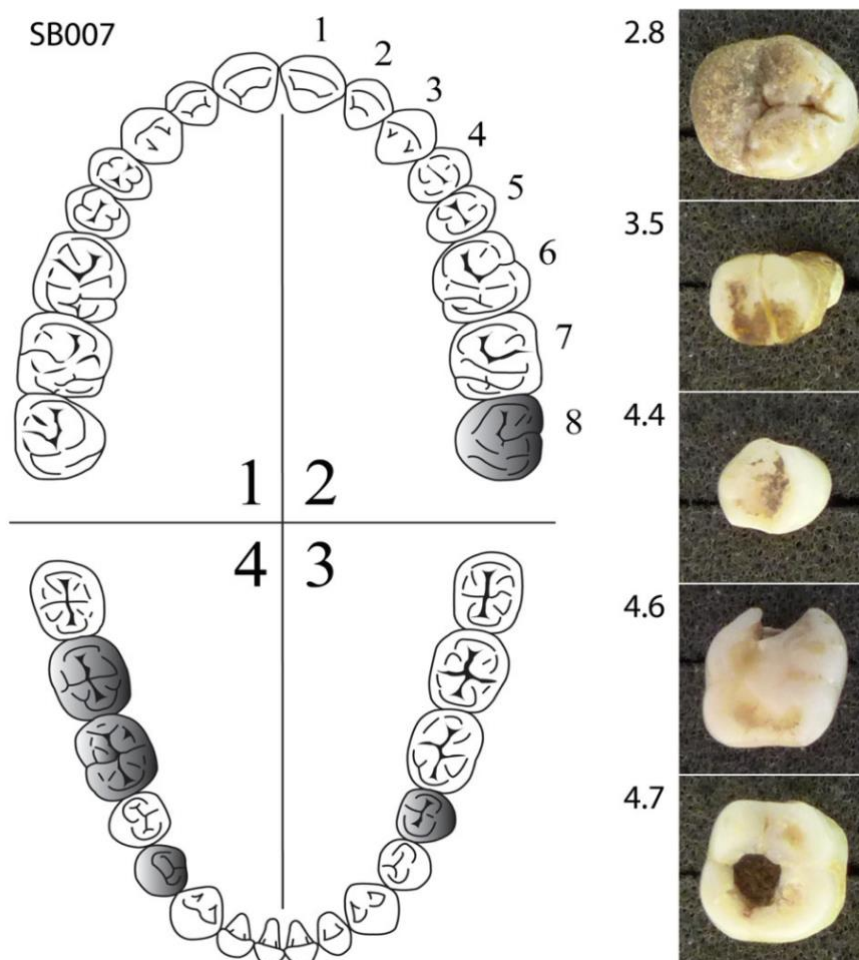


Figure 4.1 The dental elements found in the lockbox at Spring Bay Flat. (Laffoon et al., 2018, p. 354)

## 4.2 Methods and measurements

Sampling was conducted by a student at Leiden University (Anouk van de Ven) as part of an internship in the Laboratory of Archaeological Chemistry at the Faculty of Archaeology, Leiden University. Measurements and analysis took place at the Vrije Universiteit Amsterdam's Faculty of Science in their Stable Isotope Laboratory.

### 4.2.1 Sampling strategies

Eight incremental samples were taken starting from the cemento-enamel junction (CEJ) to the apex of the root from each of the dental elements (2.8, 3.5, 4.4, 4.6 and 4.7). Each sample weighed approximately 2.5-3 mg. Each sample was soaked in 1 ml 2.5 % bleach solution (NaOCl) for 22 hours before the solution was removed and the samples centrifuged at 3000 rpm for one minute. Centrifuged for another minute with 1 ml of ultra-pure water in a vortex mixer three times cleaned the samples in preparation for the next step. The samples were soaked for four hours in 1 ml of 1 M Ca acetate buffered acetic acid ( $\text{CH}_3\text{CO}_2\text{H}$ ) with a pH value of 4.7. Once again thoroughly cleaned three times, the samples were left to air dry for three days. Approximately 0.4 mg was sent off for analysis, per sample (van de Ven, 2022, pp. 9-10).

### 4.2.2 Analytical parameters

100 % orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ) was added to the 0.4 mg samples and were subsequently placed on a 45 °C block for 24 hours. Measurements were executed on a Delta-Plus IRMS with a GasBench II. All values were normalized to the usual VPDB scale utilizing in-house carbonate references (VICS) and calibrated against certified reference materials (NBS19). Monitoring instrument performance was done by using IAEA CO1 as a cross-check reference (J. Laffoon, personal communication, March 3, 2023).

### 4.2.3 Data selection

Due to no samples being made of the intra-enamel dentine within the crown the information available starts at the formation stage after the CEJ, therefore the first couple of years of this individual's life are absent from the data. Although samples were taken from all five teeth, only the three molars (2.8, 4.6 and 4.7) will be analyzed in the rest of this thesis. This is due to the overlap in chronological tooth formation. The dentine data from the three molars represent approximately the ages 3 to 22 years of age and therefore data from the two premolars (3.5 and 4.4) would be redundant (Beaumont & Montgomery, 2015, p. 409). Owing to potential problematic age approximation, this thesis will refer to the samples by dentine increment number per element (D1-8, e.g., SB007 4.6 D1, SB007 4.6 D2, etc.) and not by estimated age. Additional reasoning behind this decision is that the age approximation is unnecessary for the nature of the research and discussion conducted in this study. The importance here lies in the comparison of temporal trends relating to other multi-isotope data belonging to the individual SB007 and to ascertain the usefulness of dentine bioapatite as a sample source, not in age approximation.



## Chapter 5: Results

The  $\delta^{13}\text{C}_{\text{bioapatite}}$  values range from -5.09 ‰ VPDB to -10.32 ‰ VPDB with an average of -8.40 ‰ VPDB across all teeth and increments.  $\delta^{13}\text{C}_{\text{collagen}}$  has a highest value of -9.56 ‰ and a lowest value of -16.63 ‰ and a mean of -14.46 ‰. The M1 has a highest  $\delta^{13}\text{C}_{\text{bioapatite}}$  value of -5.70 ‰, a lowest of -8.56 ‰ and an average of -7.06 ‰ with a clear increase in value over time. The M2 displays a more stable  $\delta^{13}\text{C}_{\text{bioapatite}}$  profile with a mean of -9.48 ‰ and highest and lowest values of -8.93 ‰ and -10.32 ‰, respectively. The M3 continues this stable trend with very low variation, a mean of -8.66 ‰, a highest of -8.51 ‰ and a lowest of -8.93 ‰.

The  $\delta^{13}\text{C}_{\text{collagen}}$  for the M1 follows a similar increase in value as observed in the  $\delta^{13}\text{C}_{\text{bioapatite}}$  with a highest value of -9.56 ‰ and a lowest of -16.6 ‰ with an average of -13.51 ‰. The M2 has a highest value of -12.75 ‰ and a lowest of -15.89 ‰ and a mean of -14.75 ‰. The M3 has a highest of -13.91 ‰ and a lowest of -16.19 ‰, average of -15.21 ‰.

There is a clear trend between  $\delta^{13}\text{C}_{\text{apatite}}$  and  $\delta^{13}\text{C}_{\text{collagen}}$  values. Values for increments in different dental elements that overlap in time for both measurements of collagen and bioapatite are within error of each other, signifying a reliable measurement and correct matching of adjacent increments. This also provides extra evidence for the teeth belonging to the same individual.

The mean  $\Delta^{13}\text{C}_{\text{bioapatite-collagen}}$  spacing of all dental elements is 6.06 ‰ with a maximum of 9.96 ‰ and a minimum value of 3.82 ‰.

For the M1 the spacing varies greatly, rapidly rising and dropping with no clear increase or decrease, and ranges from 3.86 to 9.69 with a mean offset of 6.44. The M2 averages at 5.27 starting with the smallest difference being 3.82 and the largest, occurring twice, is 5.80. The M3  $\Delta$  also steadily increases, the smallest value being 5.20 and the biggest offset is the last value at 7.68, with an average of 6.54.

The  $\delta^{13}\text{C}_{\text{bioapatite}}$ ,  $\delta^{13}\text{C}_{\text{collagen}}$ , and  $\delta^{15}\text{N}_{\text{collagen}}$  were subjected to the function analysis proposed by Froehle et al. (2012). The results can be found in table 5.1.

Increments	<i>Function scores</i>				
	$\delta^{13}\text{C}_{\text{bioapatite}}$	$\delta^{13}\text{C}_{\text{collagen}}$	$\delta^{15}\text{N}_{\text{collagen}}$	Function 1	Function 2
	‰ (VPBD)	‰ (VPBD)	‰ (AIR)	“Carbon”	“Nitrogen”
<b>SB007 4.6 D1</b>	-5,70	-9,56	10,06	2,770	-1,476
<b>SB007 4.6 D2</b>	-5,09	-10,35	9,92	2,359	-1,908
<b>SB007 4.6 D3</b>	-5,36	-13,33	10,22	0,174	-2,010
<b>SB007 4.6 D4</b>	-6,93	-16,63	11,82	-2,376	-0,835
<b>SB007 4.6 D5</b>	-8,58	-14,64	12,06	-1,409	0,225
<b>SB007 4.6 D6</b>	-8,51	-12,65	12,34	0,120	0,633
<b>SB007 4.6 D7</b>	-7,79	-15,73	13,74	-1,581	0,815
<b>SB007 4.6 D8</b>	-8,55	-15,15	14,22	-1,297	1,489
<b>SB007 4.7 D1</b>	-8,93	-12,75	14,57	-0,266	0,287
<b>SB007 4.7 D2</b>	-9,74	-14,36	14,65	-1,284	1,563
<b>SB007 4.7 D3</b>	-8,66	-14,46	14,79	-0,846	1,576
<b>SB007 4.7 D4</b>	-9,46	-15,09	14,56	-1,497	1,983
<b>SB007 4.7 D5</b>	-10,32	-15,89	14,21	-2,247	2,534
<b>SB007 4.7 D6</b>	-9,82	-15,26	14,36	-1,685	2,272
<b>SB007 4.7 D7</b>	-9,54	-15,34	14,41	-1,712	1,972
<b>SB007 4.7 D8</b>	-9,36	-14,85	14,34	-1,333	1,873
<b>SB007 2.8 D1</b>	-8,65	-15,01	10,06	-1,154	1,762
<b>SB007 2.8 D2</b>	-8,71	-13,91	9,92	-0,355	1,984
<b>SB007 2.8 D3</b>	-8,70	-14,55	10,22	-0,785	1,976
<b>SB007 2.8 D4</b>	-8,81	-15,35	11,82	-1,453	1,774
<b>SB007 2.8 D5</b>	-8,93	-15,73	12,06	-1,843	1,556
<b>SB007 2.8 D6</b>	-8,57	-15,23	12,34	-1,333	1,574
<b>SB007 2.8 D7</b>	-8,43	-15,67	13,74	-1,598	1,486
<b>SB007 2.8 D8</b>	-8,51	-16,19	14,22	-2,017	1,408

Table 5.1 Carbon and nitrogen stable isotope values and function scores (according to the method proposed by Froehle et al., 2012)

## Chapter 6: Discussion

### 6.1 SB007

The  $\delta^{13}\text{C}$  bioapatite and collagen values follow a similar trend when plotted together (figure 6.1). The less negative values of  $\delta^{13}\text{C}_{\text{bioapatite}}$  in the early phases of the M1 are indicative of a diet containing high amounts of  $\text{C}_4$  plants and therefore coincide with the conclusion by Laffoon et al. (2018) that this individual likely had origins in the West African Sahel zone, an area known to have cultivated millet and sorghum, both  $\text{C}_4$  crops (Laffoon et al., 2018, p. 358; Harris, 1976, p. 320). The decline in  $\delta^{13}\text{C}$  enrichment in both tissues suggest less reliance on  $\text{C}_4$  plant proteins, although it should be noted that the values remain intermediate, immediately ruling out a monoisotopic diet.

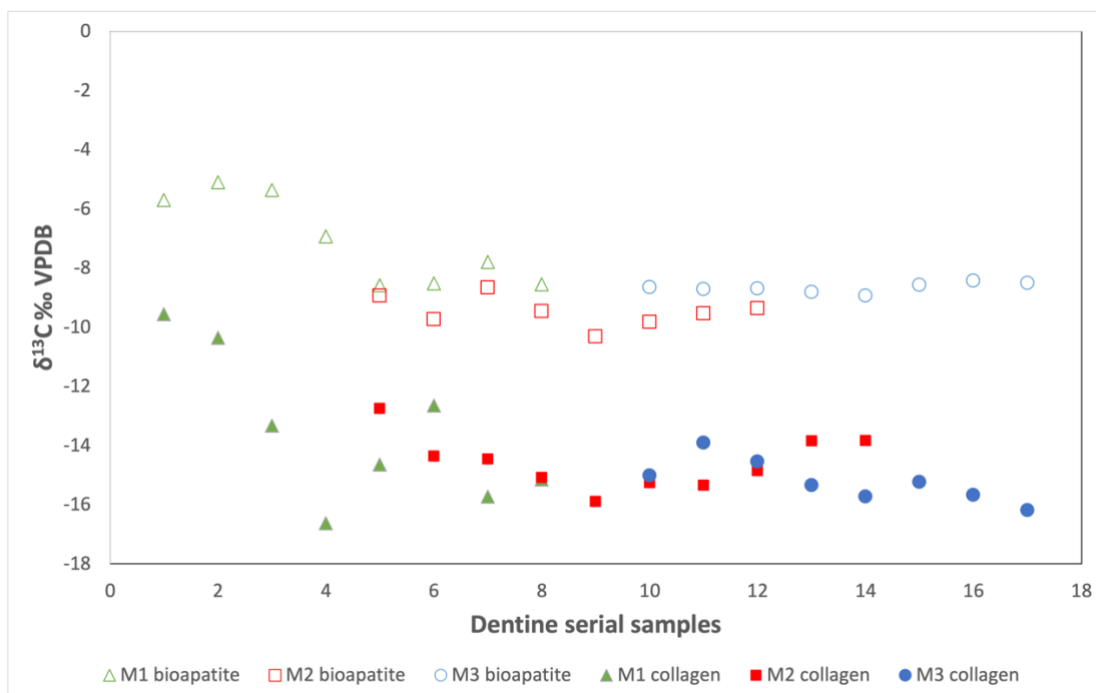


Figure 6.1  $\delta^{13}\text{C}_{\text{bioapatite}}$  and  $\delta^{13}\text{C}_{\text{collagen}}$  values plotted across dentine increments for SB007 from M1, M2 and M3.

The  $\Delta^{13}\text{C}_{\text{bioapatite-collagen}}$  spacing has been studied extensively in large mammals with monoisotopic diets and used to infer trophic levels in inter-species studies but research using this method to study individual diet in incremental analysis in humans is scarce. Having a unique position in the food chain that varies greatly geographically, temporally, and culturally, the implications for offset values are less understood and it can be difficult to

infer whether a shift in  $\Delta$  spacing is due to a change in diet-collagen or diet-bioapatite routing (France & Owsley, 2015, p. 299; Hedges, 2003, p. 67). The  $\Delta$  values suddenly increase in the middle formation stages of the M1 indicating more reliance on lipids compared to carbohydrates for dietary energy (Clementz et al., 2009, p. 613). Carnivores tend to have lower  $\Delta^{13}\text{C}_{\text{bioapatite-collagen}}$  values compared to herbivores, 4.3‰ and 6.8‰, respectively, with omnivores falling somewhere in between with average values of 5.2‰ (Lee-Thorp et al., 1989, p. 598). SB007 displays higher than usual  $\Delta$  values in the later phases of development in the M1, jumping up above the expected range for omnivores and then down again into the lower end of the omnivore range (figure 6.2). This could suggest temporary depleted reliance on protein for energy relative to whole diet, although increased  $\delta^{15}\text{N}$  values oppose this conclusion. It is apparent due to the relative depletion of  $\delta^{13}\text{C}$  values in both collagen and bioapatite and an increase in  $\delta^{15}\text{N}$  values that eventually SB007's diet shifted towards more reliance on  $\text{C}_3$  plants and marine protein, especially towards the end of the formation of the M3 where the  $\delta^{13}\text{C}_{\text{collagen}}$  values drop slightly and the  $\delta^{13}\text{C}_{\text{bioapatite}}$  (see figure 6.1) and  $\delta^{15}\text{N}$  levels (see figure 6.3) stay consistently elevated.

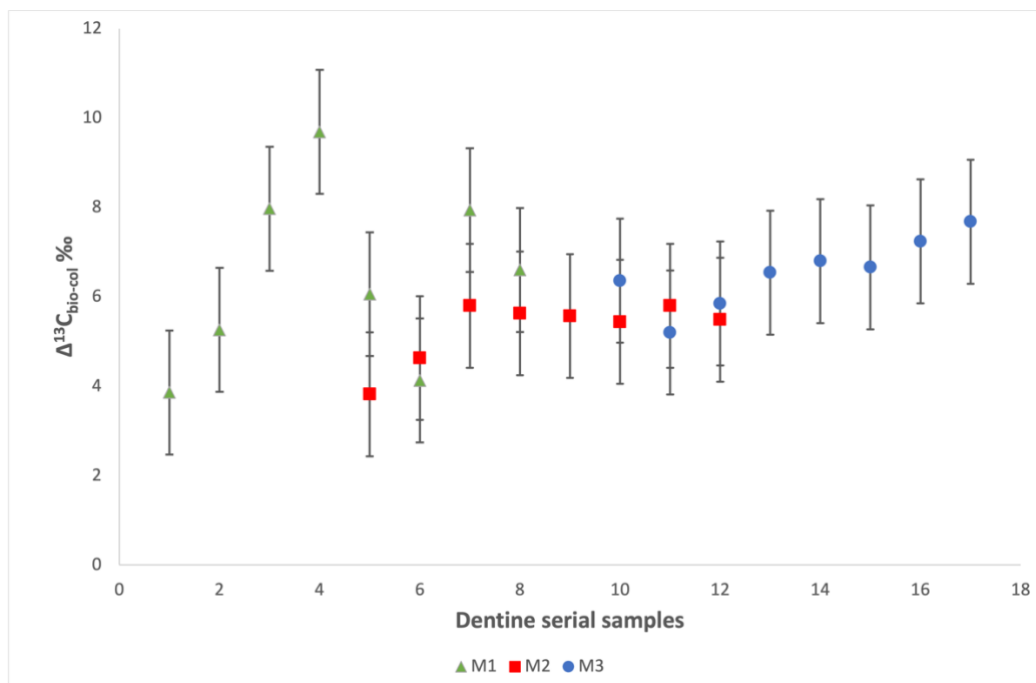


Figure 6.2  $\Delta^{13}\text{C}$  values for SB007 across dentine increments from M1, M2 and M3.

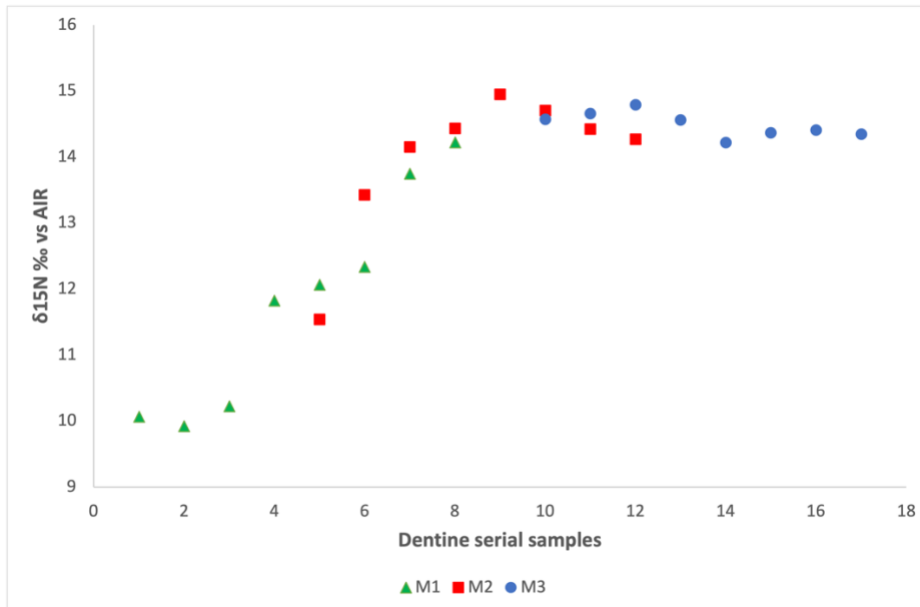


Figure 6.3  $\delta^{15}\text{N}$  values for SB007 across dentine increments from M1, M2 and M3.

On a bivariate plot (figure 6.4), the first increments of the M1 fall into the 95 % confidence interval for  $C_4$  subsistence (consistent with the prior conclusions) before shifting into the 95 % confidence interval for  $C_3$  protein sourcing and then jumping up into the  $C_4$  range again at the beginning of the samples for the M2. The remainder of the samples then stay in between the two intervals with the exception of the last increment of the M3 that falls back into the  $C_3$  interval again. There are several potential explanations for this jumping around. The first possibility is that this individual migrated more than once and therefore consumed a different diet again during the later formation phases of the M1 and earliest phases of the M2. This is highly plausible as the radiogenic strontium isotope analysis conducted by Laffoon et al. (2018) was also suggestive of a possible second migration. Considering the possibility that this individual was forced to migrate during the formation period of the M1, it is also feasible that the apparent shift into  $C_3$  protein reliance on the bivariate graph is reflective of the moving period itself, the so-called “Middle Passage.” Historical evidence shows that enslaved people were fed rations of mashes made from wheat flour, oats, cornmeal, barley, and root vegetables on the ships during transportation (Varney, 2003, p. 23). Therefore, it is entirely plausible that SB007 was fed rations dominated by  $C_3$  foodstuffs and that this is reflected in the dramatic depletion of  $\delta^{13}\text{C}$  and increase in  $\Delta^{13}\text{C}$  across all models. Another explanation is that this individual’s diet changed while on Saba. It is likely that enslaved people did not have much control over their diet and were dependent on

what was provisioned, or what they were allowed to grow by the plantation owner. This could mean that the diet varied depending on what was made available to them and possibly suspect to irregular change. Slaves were known to have eaten salted and pickled fish and a wide variety of staple grains and root vegetables. Along this same line of reasoning, seasonality is also a major contributor to food availability in the islands and things such as hurricanes and changes in import due to weather and political and social unrest during the colonial period could also have led to fluctuations in food sources (Varney, 2003, pp. 23 – 25). A third tenable interpretation is that a period of severe malnutrition or an adjustment period to the local diet has influenced the biogenic  $\delta^{13}\text{C}$  signature. It has been observed that nutritional stress can cause a decrease in  $\delta^{13}\text{C}_{\text{collagen}}$  due to catabolic wasting, the body's metabolic response to insufficient food intake in which it breaks down tissue in an attempt to continue producing energy (Neuberger, 2013, p. 22). SB007 shows a dramatic decrease in  $\delta^{13}\text{C}$  values that does not return back to the original values, thus not indicating a recovery. Pertaining to the conclusion of a potential period of malnutrition this suggests one of three things: that this individual did not experience a period of undernutrition but that the change was only due to diet; or the subsequent diet had similar values to those reflected by a bout of nutritional stress; or the nutritional stress extended into adulthood and a recovery period is not reflected in this data set. As the human body cannot maintain a long-term catabolic state without eventually resulting in death, and historical sources confirm the common occurrence of malnutrition amongst enslaved peoples, it is most likely that the recovery period is obscured by a concurrent or immediately subsequent dietary shift (Handler, 2006, p. 177). This conclusion is further supported by the negative covariance in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values.

A final explanation for the erratic pattern of the  $\delta^{13}\text{C}$  values in figure 6.4 is purely the limitations of the model. The bivariate model has received criticism in the past and as we can see this model leaves room for much ambiguity and guess work in regions with various possible protein and plant sources and the effects of childhood malnutrition.

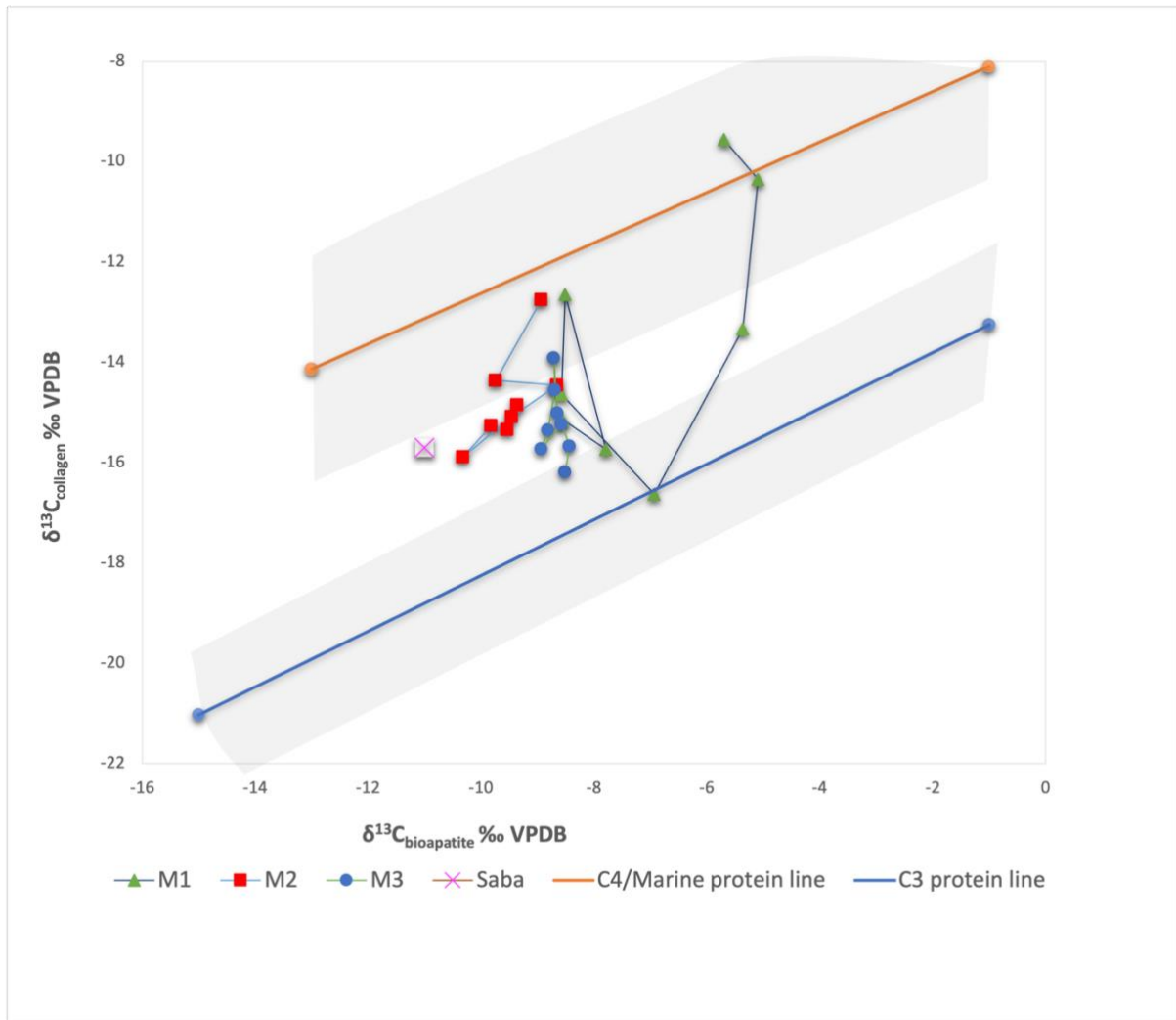


Figure 6.4  $\delta^{13}\text{C}_{\text{collagen}}$  and  $\delta^{13}\text{C}_{\text{bioapatite}}$  values from SB007 M1, M2 and M3 across all dentine increments plotted on against a bivariate model as proposed by Kellner and Schoeninger (2007). The bottom shaded linearity represents a 95 % confidence interval of a  $\text{C}_3$  protein dominated diet and the top shaded area a 95 % confidence interval of  $\text{C}_4$ /marine protein dominated diet.

Discriminate function analysis such as that proposed by Froehle et al. (2012) and the inclusion of nitrogen data into the analysis can potentially help provide more resolution. The formation of the M1 falls into Cluster 2 with a high reliance on  $\text{C}_4$  diet and protein, consistent with the observation of the individual likely living in the Sahel crop zone during the formation of this tooth (table 6.1). However, upon migration the values all fall outside of the proposed cluster areas on the graph. This, while problematic for using this model for inferring specific diet composition, is similar to previously observed data on enslaved Africans, also generally falling outside the parameters proposed by this model (figure 6.5). This model has been known to face challenges in areas where marine sources could be reef fish, which are less enriched in  $\delta^{15}\text{N}$  (Rand et al., 2015, p. 403). This is unlikely the case for SB007 as high nitrogen levels are demonstrated throughout the later samples of the dentine

sequence. It is therefore more likely that either nitrogen *enriched* marine resources were consumed, such as pelagic fish; or that malnutrition contributed to higher-than-average  $\delta^{15}\text{N}$  and  $\Delta^{13}\text{C}$  values; or that plants with higher  $\delta^{15}\text{N}$  values such as legumes were consumed, all of which could result in discriminate function results that are not yet studied well enough to be properly represented in the model.

What is clear is that the diet consumed by SB007 was neither the same as that based on the data available for Saba in general nor the averages for enslaved Africans or pre-colonial Lesser Antilles but falls somewhere in between the three (figure 6.5).

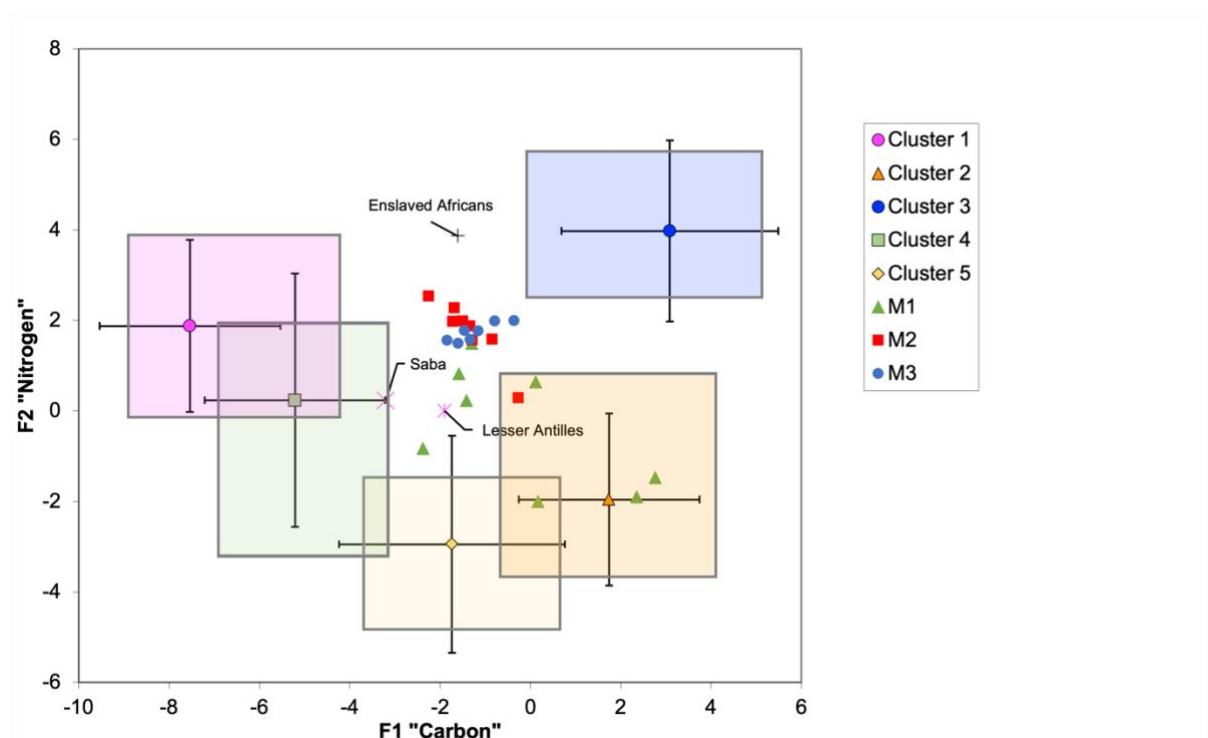


Figure 6.5 SB007 dentine increments of M1, M2 and M3 plotted using the discriminate function analysis model by Froehle et al. (2012) (For clusters see table 6.1). Data for Enslaved Africans, Saba and precolonial Lesser Antilles obtained from personal communication with J. Laffoon on April 14, 2023.

Clusters	Diet
<b>Cluster 1</b>	100% C <sub>3</sub> diet/protein
<b>Cluster 2</b>	30 C <sub>3</sub> :70 C <sub>4</sub> , >50% C <sub>4</sub> protein
<b>Cluster 3</b>	50 C <sub>3</sub> :50 C <sub>4</sub> diet, marine protein
<b>Cluster 4</b>	70 C <sub>3</sub> :30 C <sub>4</sub> diet, ≥65% C <sub>3</sub> protein
<b>Cluster 5</b>	30 C <sub>3</sub> :70 C <sub>4</sub> diet, ≥65% C <sub>3</sub> protein

Table 6.1 Discriminate function clusters as proposed by Froehle et al. (2012).



In Laffoon et al.'s (2018) previous analysis of dentine collagen and enamel stable isotope values of the same teeth the authors observed, based on  $^{87}\text{Sr}/^{86}\text{Sr}$  results, that this individual experienced a forced migration during the formation of the M3 enamel with an age estimation of 8-15 years. This estimation coincides with the formation of the root of the M2 (keeping in mind that the samples here do not include intra-coronal dentine) (Beaumont & Montgomery, 2015, Figure 2). However, the large dietary changes during the formation stages of the M1 observed in all the above analyses indicate that this (initial) migration may have occurred earlier.

## 6.2 Advantages and disadvantages of analyses

The sole use of  $\delta^{13}\text{C}_{\text{collagen}}$  data independent of  $\delta^{13}\text{C}_{\text{bioapatite}}$  is not a reliable indicator of whole diet. The fractionation process from diet-collagen has been proven to be biased towards the protein portion of the diet and can therefore provide false conclusions neglecting carbohydrate and fat intake sources. The combination of data from both tissues helps to gain more confidence in the form of spacing and offset values, though this study provides an example of the limitations this can experience as a method of analysis as well. While the method seems to work well to infer trophic level spacing in some cases (Lee-Thorp et al., 1989), results can remain ambiguous when trying to diagnose whole diet. Bivariate plotting of  $\delta^{13}\text{C}_{\text{bioapatite}}$  against  $\delta^{13}\text{C}_{\text{collagen}}$  attempt to resolve this matter and would do so quite elegantly if not for the overlap between  $\text{C}_4$  and marine protein. This makes it a useful model in places where one does not expect to find marine sources or migrants from a coastal area but can experience difficulties in disentangling protein sources in areas with high variability and mixed diet/food sources. A way to resolve this issue is by introducing nitrogen data into the analysis and making use of multivariate models such the one proposed by Froehle et al. (2012). Where  $\delta^{13}\text{C}$  values fail to discriminate between  $\text{C}_4$  and marine protein sources  $\delta^{15}\text{N}$  data can help, although further research into areas where coral reef, pelagic fish marine ecosystems are present, or bouts of nutritional stress are likely is needed to refine the model. This is exhibited in the analysis of SB007's diet.

Therefore, the combination of different tissues and methods can be helpful in tracking individual life histories though they should always be approached with caution due to small data sets and potential limitations in models and diagenesis in bioapatite. There are certainly ways to limit the risks of incorrect conclusions based on both the aforementioned limitations, but it remains something to keep in mind when using incremental dentine multi-isotope and tissue comparisons for intra-individual analysis. Nevertheless, this has proved a very promising method that could open doors for unprecedented temporal resolution in retracing life histories with bioarchaeology.

### b6.3 Context in archaeological research

The use of multiple stable isotope research in colonial archaeology has important implications for how we study slavery. Espersen (2009) cautions against reaching hasty conclusions surrounding poverty based purely on material culture (p. 341). By using stable isotope methods extra insight can be gained into forced migration, captivity, and individual experiences as a slave during the Transatlantic slave period at a resolution impossible to gather from other archaeological methods alone. Slaves tend to be underrepresented in the historical record and results from studies such as these can help contribute to information about the forced African diaspora, shedding more light on the origins and roles of people with a unique place in history, having endured a morally reprehensible era (Price et al., 2012, p. 396). This case study is one example of a successful application of this method. By analysing these teeth in this way, even outside of anatomical context, detailed and varied information has been obtained that can then provide extra context for research into cultural and social practices, dietary behaviour, and agency. The investigation into burial contexts such as this one in a lockbox can be conducted with more certainty about this individual's origins, status as a slave and individual life history. The reconstruction of individual lifeways in this manner holds promise for the present and future ways of conducting archaeological research in an empathetic way.

## Chapter 7: Conclusion

### 7.1 Summary of findings

Based on the data it is not possible to make general assumptions about the diet consumed by SB007 during the whole childhood. Due to major fluctuations in subsistence during this individual's juvenile years, it is, however, possible to track an interesting life history with great resolution. This makes it an ideal case study for testing the method of incremental dentine sampling and especially comparing multiple isotopes in the organic and inorganic phases.

The findings in this study have reinforced the conclusions drawn by Laffoon et al. (2018) that this individual has natal origins in the African Sahel and primarily subsisted on C<sub>4</sub> foodstuffs, likely millet, or sorghum, for at least the first years of their childhood. A dramatic shift in stable isotope values across the board indicates a migration period and dietary shift. The difference in the corresponding first increments of the M2 and increments of the M1 compared to the rest of the dentine sequence suggests that during the formation period this individual had a temporary dietary shift associated with either a separate migration or a transportation period in which reliance on C<sub>3</sub> rations was high and a likely concurrent bout of severe malnutrition occurred, evidenced by the dramatic increase in  $\delta^{15}\text{N}$  values in the fourth dentine sample of the M1. The dentine profiles seem to stabilize towards the end of the juvenile years and the diet was likely highly mixed possibly comprising C<sub>3</sub> and nitrogen-rich marine and plant resources, such as off-shore fish and leguminous plants. This is credible as historical and other isotopic evidence suggest enslaved African diets were variable. The conclusion is further supported by the natural and geographical constraints and allowances afforded by the island pertaining to food and import availability. More research needs to be done to include data from individuals that have suffered from nutritional stress to provide models such as those used in this study with more resolution about how malnutrition affects multi-isotopic signatures in different tissues.

## 7.2 Implications for further research

As briefly touched upon in previous sections, though isotope research is incredibly valuable, it is not without its pitfalls and can certainly provide false promises when one is not careful. Most important when dealing with stable isotope data is to draw conclusions from multiple data sources, compare studies and be aware of potential fallacious interpretations. That is one obvious reason for conducting comparative studies, both within a given sample and its context.

### 7.2.1 Multiple isotopes from incremental dentine samples

Carbon isotope composition in collagen and bioapatite reconstruct different aspects of the diet and are not interchangeable data sources, nor predictive of each other. This makes a comparison useful for specific dietary resource reconstruction. Applying this concept to incremental dentine samples then provides a higher temporal resolution of individual diet during childhood, the combination leading to detailed analyses important in areas with various possible diets. This can also be important in studying migration due to shifts in diet containing different plant proteins and meat protein sources that may cancel each other out, such as marine and C<sub>4</sub> resources. Nitrogen isotopes provide additional dietary evidence to resolve these challenges.

The use of dentine increments is a promising way to recreate individual life histories from small sample sizes with limited context. However, more work needs to be done into the effects of malnutrition on the nitrogen and carbon functions in multivariate discrimination function analysis. Especially in slave trade, detailed osteobiographies such as this one can help observe historical and socio-political phenomena which is important for the position of multiple-stable isotope research in a broader aspect both within the discipline and archaeology's extended place in the world.

I suggest that studying bioapatite and collagen in dentine increments for dietary studies is highly valuable in contexts and eras in which diasporas, famine, and/or wide scale slavery

and exploitation occurred, as it can help to retrace personal circumstances within times of tragedy. This method is also particularly beneficial in geographical and climatic areas where multiple food resources converge.

### 7.2.2 Isotopes in archaeology

Isotope analysis has a unique place in archaeology as it provides a method for simultaneous social and biological lines of evidence. We have seen the potential issues with mindlessly collecting massive amounts of data as being treacherous for the future and 'maturity' of stable isotope research within archaeology. Conversely, we have also borne witness to how narrow focus on one isotope value or reliance on heuristic devices can lead to premature conclusions and false information. It is therefore, keeping both these things in mind, of paramount importance that all data be considered within the available context, and compared and analysed carefully and mindfully in order to achieve high resolution in data and useful interpretation.

The study of isotopes is a powerful tool in archaeology and the future looks bright for this ever-growing field of study. The individual resolution afforded by multi-isotope and multi-tissue analysis helps us connect with the past and tell the stories of enslaved humans in a personal and impactful manner.

## Abstract

This thesis examines a new way of tracing individual life histories in the context of slavery in the Caribbean during the colonial period. The carbon stable isotopes in bioapatite were compared to carbon and nitrogen stable isotopes in collagen of serial dentine samples in the case study of an enslaved African found on the island of Saba. Five dental elements were found out of anatomical context in a lockbox at Spring Bay Flat plantation. The use of bioapatite and collagen in tandem has been proved to provide extra valuable information in dietary studies and doing so in dentine increments yields an individual and high temporal resolution new to bioarchaeology. The individual studied here forcibly migrated from the African Sahel during the formation of the first molar, suffering from a period of nutritional stress before experiencing a dramatic dietary change. Multi-isotope analysis of both organic and inorganic matrixes in dentine increments is proven to be a valuable and innovative method of reconstructing individual life histories in unprecedented detail. The production of osteobiographies such as this one helps us to understand individual experiences during periods of slavery, captivity, and exploitation on a level that we can empathise with and therefore offers important and profound contributions to discussions on slavery and colonialism in a broader sense.

## References

- AlQahtani, S. J., Hector, M. P., & Liversidge, H. M. (2010). Brief communication: The London atlas of human tooth development and eruption. *American Journal of Physical Anthropology*, 142(3), 481–490. <https://doi.org/10.1002/ajpa.21258>
- Ambrose, S. H., & DeNiro, M. J. (1987). Bone nitrogen isotope composition and climate. *Nature*, 325(6101), 201–201. <https://doi.org/10.1038/325201a0>
- Beaumont, J., Geber, J., Powers, N., Wilson, A., Lee-Thorp, J., & Montgomery, J. (2013). Victims and survivors: Stable isotopes used to identify migrants from the Great Irish Famine to 19th century London. *American Journal of Physical Anthropology*, 150(1), 87–98. <https://doi.org/10.1002/ajpa.22179>
- Beaumont, J., Gledhill, A., Lee-Thorp, J., & Montgomery, J. (2013). Childhood diet: a closer examination of the evidence from dental tissues using stable isotope analysis of incremental human dentine. *Archaeometry*, 55(2), 277–295. <https://doi.org/10.1111/j.1475-4754.2012.00682.x>
- Beaumont, J., & Montgomery, J. (2015). Oral histories: A simple method of assigning chronological age to isotopic values from human dentine collagen. *Annals of Human Biology*, 42(4), 407–414. <https://doi.org/10.3109/03014460.2015.1045027>
- Britton, K., & Guiry, E. (2020). Isotope bioarchaeology in historical archaeology. In C. E. Orser, A. Zarankin, S. Lawrence, & J. Symonds (Eds.), *The Routledge Handbook of Global Historical Archaeology* (1st ed., pp. 423–442). Routledge. <https://doi.org/10.4324/9781315202846-23>
- Bronk Ramsey, C. (2008), Radiocarbon dating: revolutions in understanding. *Archaeometry*, 50, 249-275. <https://doi.org/10.1111/j.1475-4754.2008.00394.x>

- Centraal Bureau voor de Statistiek (2016, July 21) *Population Caribbean Netherlands stable*.  
<https://www.cbs.nl/en-gb/news/2016/29/population-caribbean-netherlands-stable>
- Clementz, M. T., Fox-Dobbs, K., Wheatley, P. V., Koch, P. L., & Doak, D. F. (2009). Revisiting old bones: Coupled carbon isotope analysis of bioapatite and collagen as an ecological and palaeoecological tool. *Geological Journal*, 44(5), 605–620. <https://doi.org/10.1002/gj.1173>
- Codron, D., Clauss, M., Codron, J., & Tütken, T. (2018). Within trophic level shifts in collagen-carbonate stable carbon isotope spacing are propagated by diet and digestive physiology in large mammal herbivores. *Ecology and Evolution*, 8(8), 3983–3995.  
<https://doi.org/10.1002/ece3.3786>
- Curtis, M. J., Beaumont, J., Elamin, F., Wilson, A. S., & Koon, H. E. C. (2022). Method of micro-sampling human dentine collagen for stable isotope analysis. *Rapid Communications in Mass Spectrometry*, 36(13). <https://doi.org/10.1002/rcm.9305>
- DeNiro M.J. and Epstein S. (1976). You are what you eat (plus a few permil): the carbon isotope cycle in food chains. *Geological Society of America Abstracts with Programs* 8, 834-835.
- Deniro, M. J., & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, 45(3), 341–351. [https://doi.org/10.1016/0016-7037\(81\)90244-1](https://doi.org/10.1016/0016-7037(81)90244-1)
- DeSantis, L. R. G., Feranec, R. S., Southon, J., Cerling, T. E., Harris, J., Binder, W. J., Cohen, J. E., Farrell, A. B., Lindsey, E. L., Meachen, J., Robin O’Keefe, F., & Takeuchi, G. T. (2022). On the relationship between collagen- and carbonate-derived carbon isotopes with implications for the inference of carnivore dietary behavior. *Frontiers in Ecology and Evolution*, 10.  
<https://doi.org/10.3389/fevo.2022.1031383>
- Ehleringer, J. R., & Rundel, P. W. (1989). Stable Isotopes: History, Units, and Instrumentation. In P. W. Rundel, J. R. Ehleringer, & K. A. Nagy (Eds.), *Stable Isotopes in Ecological Research* (Vol. 68, pp. 1–15). Springer New York. [https://doi.org/10.1007/978-1-4612-3498-2\\_1](https://doi.org/10.1007/978-1-4612-3498-2_1)



- Espersen, R. (2015). *Identifying Dry Stone Enslaved African Housing at the Spring Bay Flat Plantation, Saba, Dutch Caribbean*. [Symposium] International Association of Caribbean Archaeologists 26th Congress, St. Maarten.
- France, C. A. M., & Owsley, D. W. (2015). Stable Carbon and Oxygen Isotope Spacing Between Bone and Tooth Collagen and Hydroxyapatite in Human Archaeological Remains: Stable Carbon and Oxygen Isotope Spacing in Humans. *International Journal of Osteoarchaeology*, 25(3), 299–312. <https://doi.org/10.1002/oa.2300>
- Fricke, F., Laffoon, J., & Espersen, R. (2021). Unforgotten: The osteobiography of an enslaved woman and child from 18th century Saba. *Journal of Archaeological Science: Reports*, 36, 102838. <https://doi.org/10.1016/j.jasrep.2021.102838>
- Froehle, A. W., Kellner, C. M., & Schoeninger, M. J. (2012). Multivariate carbon and nitrogen stable isotope model for the reconstruction of prehistoric human diet. *American Journal of Physical Anthropology*, 147(3), 352–369. <https://doi.org/10.1002/ajpa.21651>
- Fuller, B. T., Fuller, J. L., Harris, D. A., & Hedges, R. E. M. (2006). Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios. *American Journal of Physical Anthropology*, 129(2), 279–293. <https://doi.org/10.1002/ajpa.20249>
- Fuller, B. T., Richards, M. P., & Mays, S. A. (2003). Stable carbon and nitrogen isotope variations in tooth dentine serial sections from Wharram Percy. *Journal of Archaeological Science*, 30(12), 1673–1684. [https://doi.org/10.1016/S0305-4403\(03\)00073-6](https://doi.org/10.1016/S0305-4403(03)00073-6)
- Handler, J. S. (2006). Diseases and Medical Disabilities of Enslaved Barbadians, From the Seventeenth Century to around 1838: Part 2. *Journal of Caribbean History* 40(2), 177–214.
- Harris, D. R. (1976). Traditional systems of plant food production and the origins of agriculture in West Africa. In J. R. Harlan, J. M. J. De Wet, and A. B. L. Stemler (Eds.), *Origins of African plant domestication*, 311–56. Mouton.

- Hatch, K. A., Crawford, M. A., Kunz, A. W., Thomsen, S. R., Eggett, D. L., Nelson, S. T., & Roeder, B. L. (2006). An objective means of diagnosing anorexia nervosa and bulimia nervosa using  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios in hair. *Rapid Communications in Mass Spectrometry*, 20(22), 3367–3373. <https://doi.org/10.1002/rcm.2740>
- Hedges, R. E. M. (2003). On bone collagen - apatite-carbonate isotopic relationships. *International Journal of Osteoarchaeology*, 13(1–2), 66–79. <https://doi.org/10.1002/oa.660>
- Katzenberg, M. A. (2007). Stable isotope analysis: a tool for studying past diet, demography, and life history. In M. A. Katzenberg & S. R. Saunders (Eds.), *Biological Anthropology of the Human Skeleton* (2nd ed., pp. 413–441). John Wiley & Sons, Inc.
- Kellner, C. M., & Schoeninger, M. J. (2007). A simple carbon isotope model for reconstructing prehistoric human diet. *American Journal of Physical Anthropology*, 133(4), 1112–1127. <https://doi.org/10.1002/ajpa.20618>
- Kendall, C., Eriksen, A. M. H., Kontopoulos, I., Collins, M. J., & Turner-Walker, G. (2018). Diagenesis of archaeological bone and tooth. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 491, 21–37. <https://doi.org/10.1016/j.palaeo.2017.11.041>
- Krueger, H. W. (1991). Exchange of carbon with biological apatite. *Journal of Archaeological Science*, 18(3), 355–361. [https://doi.org/10.1016/0305-4403\(91\)90071-V](https://doi.org/10.1016/0305-4403(91)90071-V)
- Laffoon, J. E., Espersen, R., & Mickleburgh, H. L. (2018). The Life History of an Enslaved African: Multiple Isotope Evidence for Forced Childhood Migration from Africa to the Caribbean and Associated Dietary Change: The life history of an enslaved African. *Archaeometry*, 60(2), 350–365. <https://doi.org/10.1111/arcm.12354>
- Laffoon, J. E., Hoogland, M. L. P., Davies, G. R., & Hofman, C. L. (2016). Human dietary assessment in the Pre-colonial Lesser Antilles: New stable isotope evidence from Lavoutte, Saint Lucia.

*Journal of Archaeological Science: Reports*, 5, 168–180.

<https://doi.org/10.1016/j.iasrep.2015.11.020>

Lee-Thorp, J. A. (2008). On isotopes and old bones. *Archaeometry*, 50(6), 925–950.

<https://doi.org/10.1111/j.1475-4754.2008.00441.x>

Lee-Thorp, J. A., Sealy, J. C., & van der Merwe, N. J. (1989). Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. *Journal of Archaeological Science*, 16(6), 585–599. [https://doi.org/10.1016/0305-4403\(89\)90024-1](https://doi.org/10.1016/0305-4403(89)90024-1)

Makarewicz, C. A., & Sealy, J. (2015). Dietary reconstruction, mobility, and the analysis of ancient skeletal tissues: Expanding the prospects of stable isotope research in archaeology. *Journal of Archaeological Science*, 56, 146–158. <https://doi.org/10.1016/j.jas.2015.02.035>

McKinney, C. R., McCrea, J. M., Epstein, S., Allen, H. A., & Urey, H. C. (1950). Improvements in Mass Spectrometers for the Measurement of Small Differences in Isotope Abundance Ratios. *Review of Scientific Instruments*, 21(8), 724–730. <https://doi.org/10.1063/1.1745698>

Mekota, A.-M., Grupe, G., Ufer, S., & Cuntz, U. (2006). Serial analysis of stable nitrogen and carbon isotopes in hair: Monitoring starvation and recovery phases of patients suffering from anorexia nervosa. *Rapid Communications in Mass Spectrometry*, 20(10), 1604–1610. <https://doi.org/10.1002/rcm.2477>

Mulder, N. (2018). Stories of Autonomy on Non-Sovereign Saba: Flipping the Script of Postcolonial Resistance. *Etnofoor*, 30(1), 11–28.

Nobel Prize Outreach AB. (n.d.) *Francis W. Aston – Biographical*. Retrieved March 3, 2023.

<https://www.nobelprize.org/prizes/chemistry/1922/aston/biographical/>

Neuberger, F. M., Jopp, E., Graw, M., Püschel, K., & Grupe, G. (2013). Signs of malnutrition and starvation—Reconstruction of nutritional life histories by serial isotopic analyses of hair.

*Forensic Science International*, 226(1–3), 22–32.

<https://doi.org/10.1016/j.forsciint.2012.10.037>

Ortiz-Ruiz, A. J., Teruel-Fernández, J. de D., Alcolea-Rubio, L. A., Hernández-Fernández, A., Martínez-Beneyto, Y., & Gispert-Guirado, F. (2018). Structural differences in enamel and dentin in human, bovine, porcine, and ovine teeth. *Annals of Anatomy - Anatomischer Anzeiger*, 218, 7–17. <https://doi.org/10.1016/j.aanat.2017.12.012>

Passey, B. H., Robinson, T. F., Ayliffe, L. K., Cerling, T. E., Sponheimer, M., Dearing, M. D., Roeder, B. L., & Ehleringer, J. R. (2005). Carbon isotope fractionation between diet, breath CO<sub>2</sub>, and bioapatite in different mammals. *Journal of Archaeological Science*, 32(10), 1459–1470.

<https://doi.org/10.1016/j.jas.2005.03.015>

Price, T. D., Burton, J. H., Cucina, A., Zabala, P., Frei, R., Tykot, R. H., & Tiesler, V. (2012). Isotopic Studies of Human Skeletal Remains from a Sixteenth to Seventeenth Century AD Churchyard in Campeche, Mexico: Diet, Place of Origin, and Age. *Current Anthropology*, 53(4), 396–433.

<https://doi.org/10.1086/666492>

Rand, A. J., Healy, P. F., & Awe, J. J. (2015). Stable Isotopic Evidence of Ancient Maya Diet at Caledonia, Cayo District, Belize: Isotopic Evidence of Maya Diet at Caledonia, Belize.

*International Journal of Osteoarchaeology*, 25(4), 401–413. <https://doi.org/10.1002/oa.2308>

Richards, M. P. (2015). Stable Isotope Analysis of Bone and Teeth as a Means for Reconstructing Past Human Diets in Greece. *Hesperia Supplements*, 49, 15–23.

Roberts, P. (2022). Isotope analysis in archaeology grand challenge. *Frontiers in Environmental*

*Archaeology*, 1, 988656. <https://doi.org/10.3389/fearc.2022.988656>

Romeijn, P. (1989). The Forest of Saba. *BOS NiEuWSLETTER*, 8(19), 44–51.

- Schoeninger, M. J., & DeNiro, M. J. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta*, 48(4), 625–639. [https://doi.org/10.1016/0016-7037\(84\)90091-7](https://doi.org/10.1016/0016-7037(84)90091-7)
- Schoeninger, M. J., & Moore, K. (1992). Bone stable isotope studies in archaeology. *Journal of World Prehistory*, 6(2), 247–296. <https://doi.org/10.1007/BF00975551>
- Sealy, J. C., van der Merwe, N. J., Thorp, J. A. L., & Lanham, J. L. (1987). Nitrogen isotopic ecology in southern Africa: Implications for environmental and dietary tracing. *Geochimica et Cosmochimica Acta*, 51(10), 2707–2717. [https://doi.org/10.1016/0016-7037\(87\)90151-7](https://doi.org/10.1016/0016-7037(87)90151-7)
- Sharp, Z. (2007). *Principles of Stable Isotope Geochemistry* (1st ed.) Pearson Prentice Hall.
- Slovak, N. M., & Paytan, A. (2012). Applications of Sr Isotopes in Archaeology. In M. Baskaran (Ed.), *Handbook of Environmental Isotope Geochemistry* (pp. 743–768). Springer Berlin Heidelberg. [https://doi.org/10.1007/978-3-642-10637-8\\_35](https://doi.org/10.1007/978-3-642-10637-8_35)
- Stantis, C., Buckley, H. R., Commendador, A., & Dudgeon, J. V. (2021). Expanding on incremental dentin methodology to investigate childhood and infant feeding practices on Taumako (southeast Solomon Islands). *Journal of Archaeological Science*, 126, 105294. <https://doi.org/10.1016/j.jas.2020.105294>
- van de Ven, A. (2022). *The archaeological stories behind teeth: isotope laboratory internship report* [Unpublished Internship Report, Leiden University].
- Varney, T. L. (2003). *Reconstructing diet and tracing life histories in colonial populations of the Northeastern Caribbean using stable carbon and nitrogen isotopes*. [Doctoral dissertation, University of Calgary].
- Vogel, J. C., & Van Der Merwe, N. J. (1977). Isotopic Evidence for Early Maize Cultivation in New York State. *American Antiquity*, 42(2), 238–242. <https://doi.org/10.2307/278984>

Walter, B. S., DeWitte, S. N., Dupras, T., & Beaumont, J. (2020). Assessment of nutritional stress in famine burials using stable isotope analysis. *American Journal of Physical Anthropology*, 172(2), 214–226. <https://doi.org/10.1002/ajpa.24054>

Wilkinson, D. J. (2018). Historical and contemporary stable isotope tracer approaches to studying mammalian protein metabolism: A history of stable isotope tracers in protein metabolism. *Mass Spectrometry Reviews*, 37(1), 57–80. <https://doi.org/10.1002/mas.21507>