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Molecular Bioarchaeological Approaches for Identifying Diet and Diagenetic Alteration in a Latte Period Assemblage from Saipan, Northern Mariana Islands

by

Olivia Franklin

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Committee Approval

To the Graduate Faculty:

The members of the committee appointed to examine the thesis of Olivia Franklin find it satisfactory and recommend that it be accepted.

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Dr. John Dudgeon, Major Advisor

_____

Dr. Katherine Reedy, Committee Member

_____

Dr. Bruce Finney, Graduate Faculty Representative

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Thesis Abstract-Idaho State University (2018)

Garapan, a Latte Period (A.D. 1000-1521) archaeological site in Saipan, Northern Mariana Islands, was excavated by Scientific Consultant Services, Hawaii in 2015. The recovery produced over 400 sets of skeletal remains, of which 48 bone elements from 25 individuals were submitted for dietary bioarchaeological analysis in the Center for Archaeology, Materials and Applied Spectroscopy at Idaho State University. This research focuses on the importance of marine versus terrestrial protein sources and introduced plant cultigens to investigate possible cultural affiliation via diet at Garapan. A stepwise methodology is employed to identify dietary patterns, including the evaluation of bone preservation using ATR-FTIR, and extraction and analysis of collagen and carbonate stable isotopes. Using this combined approach, I present our bioarchaeological dietary assessment and offer insight into how this step-wise method for investigating diet in archeological samples provides additional data points for understanding subsistence strategies and patterns in Micronesia. ATR-FTIR data suggests that it is a workable tool for assessing diagenesis in archaeological samples when compared to modern samples. Within these data, Am/P show the largest distinction between the two. Stable isotope analysis revealed a primarily terrestrial diet with a small influence from marine protein sources. When compared to other analysis of Latte Period archeological samples from Garapan (Ambrose et al. 1997), there appears to be a difference in both protein and whole diet content. Additional research is required in order to determine possible causes for these inconsistencies.

Key words: Diet; Diagenesis; FTIR-ATR; Stable Isotope Analysis; Saipan; Micronesia

Chapter 1: Introduction

Saipan is an island in the Mariana archipelago; its indigenous people are the Chamorro. Little is known about the culture and practices of these people. Through time, their stories faded behind the many changes that were taking place in the Marianas (Figure 1). Because of this, the island group has become a place of great opportunities for archaeological research. With stable isotope analysis of bone elements, researchers are able to reconstruct the diet of archaeological populations. This is important because it gives insight to their subsistence strategies. Prior to stable isotope and other analyses, it is important to determine the effect that burial context has had on the biomolecules of bone elements. Diagenetic alteration includes the biomolecular changes that are undergone post mortem, as a result of time as well as burial context (Collins et al., 2002). Determining a method for assessing diagenetic alteration is important for predicting the results of downstream testing of archaeological bone elements. Using FTIR, an assessment and comparison can be made of the organic, inorganic, and crystalline component of bone from both archaeological and modern samples. These data can be used as a range for assessing diagenetic alteration and its effect on future research. When put together, the information gathered from these methods combined will allow further assessment of Latte Period in the Marianas.

Latte Period in the Mariana Islands and Saipan

The Mariana Islands are a group of 15 islands that form an archipelago in the south - western Pacific Ocean. Today the island group is referred to as The Commonwealth of the Northern Mariana Islands (CNMI), and includes all of the islands pictured in Figure 3 with the exception of Guam which is now a territory of the United States. Saipan is one of these islands,

measuring at approximately 120.6 km^2. It is composed of volcanic rocks overlain by younger limestones; its climate is classified as tropical marine with an average temperature of 80°F and average rainfall of ~80in. (Carruth, 2003). Anthropological and archaeological research of the Marianas and their history most often focus on historical instances in Guam, which is the largest island at 549 km^2 (Pietrusewsky et al., 2010). This is due to a significant number of pivotal historical events that took place there.

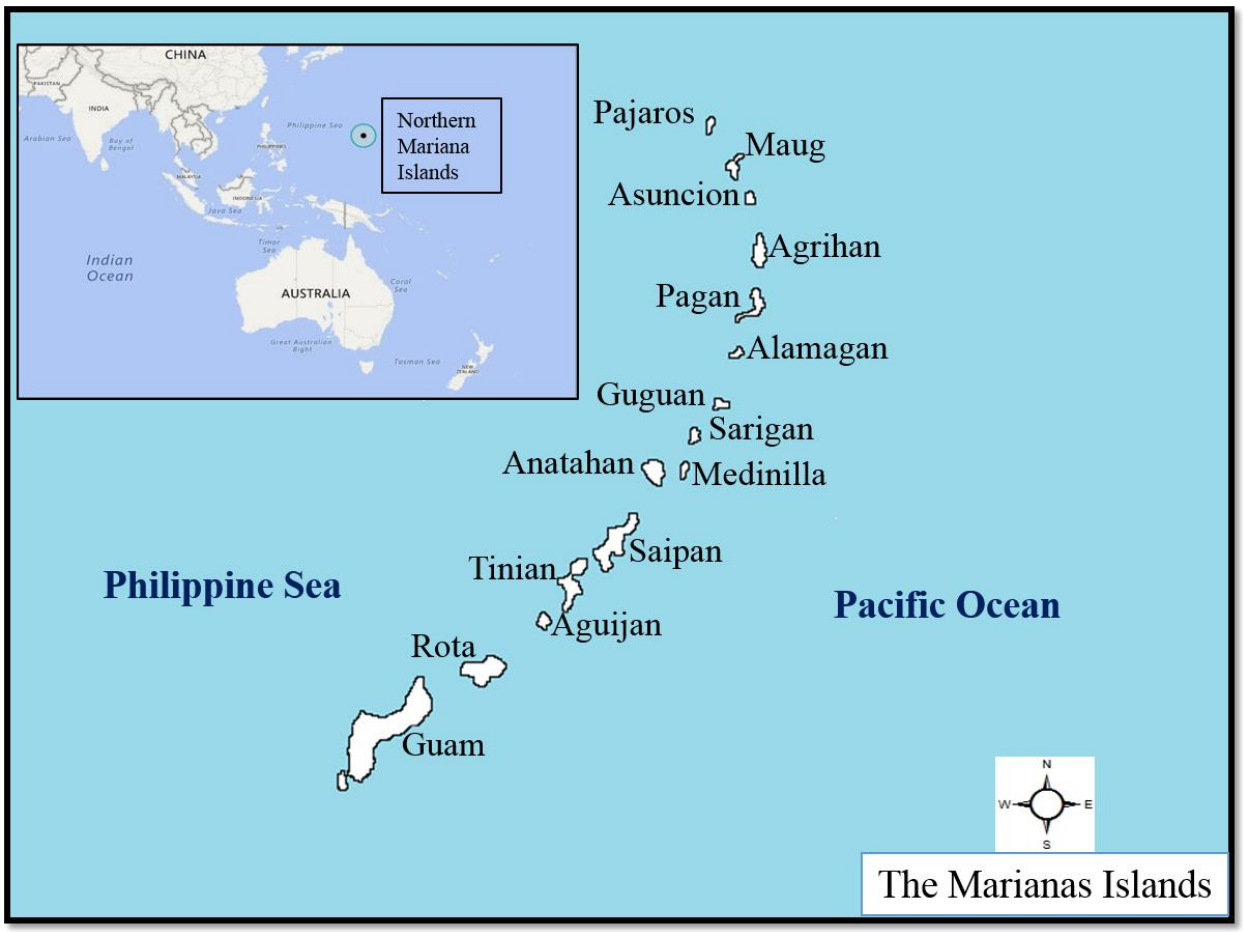

Figure 1: Mariana Islands located in the western Pacific.

The indigenous inhabitants of the Mariana Islands are the Chamorro. The islands were first occupied around 3500 BP (Hunter-Anderson and Butler, 1995). Archaeological as well as

linguistic evidence comparing (Vilar et al., 2013) the languages of the Chamorro to the ones spoken in the islands of Southeast Asia and the Philippines suggests the people who colonized the Marianas are those from the late Neolithic culture (~10,000 BP) of western Asia (Bellwood, 1997). Generally, the pre-contact subsistence strategies of these people included economics consistent with a tropical island; this includes reliance on cultivation of tree and root crops yielding coconut, banana, breadfruit, taro, and yams. Their diet is believed to have been rich in marine resources including reef and deep-water fish and shellfish. Other subsistence strategies of the Chamorro included hunting and gathering of terrestrial fauna including birds, coconut as well as other land crabs, and fruit bats (Pietrusewsky et al., 2010). Daily life consisted of men engaging in most of the physically intense labor including planting, harvesting, and house and canoe building, while women were responsible for collection of marine resources, food preparation, and making baskets, mats and pottery (Alkire, 1977).

Accounts of Chamorro prehistory are typically divided into 3 periods: Pre-Latte, Transitional Pre-Latte, and Latte period (Pietrusewsky et al., 2010). The subsistence strategies aforementioned are most consistent with the Pre- Latte period (1500 B.C.-A.D. 400). The early settlers were composed of small populations housed in coastal villages. Lime impressed decorative motifs distinguish Pre-Latte ceramics from other periods, with fragments suggesting flat bottoms and short vertical sidewalls. This shape is not conducive to boiling food but it is suitable for other types of cooking (e.g. frying, steaming, or roasting), as they are found in areas associated with charcoal and rocks that have been fire altered (Pietrusewsky et al., 2010.) The Transitional Pre-Latte period (A.D. 400-1000) is marked as a turning point in Chamorro pre-history and its subsistence economics. With population growth and subsequent settlement expansions into the inlands came the need for supplemental provisions. This period involved a

time of increased use of terrestrial resources as well as increased crop diversity. Plant food production in the Marianas is believed to have been in the form of informal house hold gardens, as well as managed forests in outlying areas (Carson, 2012). Thicker pottery more suitable for boiling and storage signifies a change in food preparation and storage practices (Pietrusewsky et al., 2010). Weapons used in Chamorro society, for both hunting and during times of war include, sling stones and spears (Cunningham, 1992).

The Latte Period (A.D. 1000-1521) is the pinnacle of indigenous Chamorro culture. Pottery from this period is large and thick, indicating similar food preparation practices as the Transitional period, though on a larger scale. Increased presence of mortars, pestles, and pounders (Hunter-Anderson et al., 1995), suggests more investment in plant processing and agricultural production during this time, while other evidence also suggests an increase in reef and lagoon fishing. The term Latte signifies a time of the usage and presence of latte stone structures, quarried from limestone. Latte stones are large stone pillars (*haligi*) capped with a hemispherical stone (t*asa*) (Figure 2), and are present in large Chamorro villages (Carson, 2014). Throughout time, archaeological research has been drawn to these constructions and the people responsible for them. Archaeological research indicates Latte structures first emerged around A.D. 900-1000 (Carson, 2014). Archaeological evidence as well as written accounts suggests that the pillars were used as foundations for homes for high ranking individuals, but some argue for usages that were more communally based such as storage sheds, men's or women's clubhouses, and community meeting or ceremonial houses (Graves, 1986). Ethnographic accounts suggest that the Chamorro society was a ranked one, divided into descent groups known as clans. Clans comprised the different villages that were present on an island. Within a clan, the rankings included chiefs, whose domain extended only to his village, kin to the chief,

and commoners. The accounts of rank within the Marianas are largely based on the written accounts of Father Luis Diego Sanvitores, who made his observations beginning in 1668. He states that high, low and middle classes were determined based on lineage. While there was little social isolation between ranks, the low people were unable to eat or drink in the noble houses; marriage between ranks was banned. In addition to the rules on etiquette, commoners had differential access to both marine and terrestrial resources, including controlled access to pelagic fish (Cordy, 1983, Graves, 1986). Each clan controlled some coastal as well as some mountain land. It is believed that between the upper and lower castes the distribution of labor was that lower class women collect terrestrial food sources, and lower class men farmed the land, while upper class women collected marine resources, and upper class men fish. Lower class individuals were not permitted to fish (Cunningham, 1992). The clans were also observed as having a system of alliances between clans that controlled access to and redistribution of resources as well as during times of wars between clans over access to resources (Hunter-Anderson and Butler, 1995, Cunningham, 1992). Dental modifications were also present among Chamorro in the Marianas, although it is unclear whether or not it is an indication of rank; there are increased instances of cross hatched modifications in females as a cosmetic enhancement.

Figure 2: Latte Structures built by the Chamorro of the Mariana Islands

Today, the Latte structures can be viewed as windows into ancestral society. This perception of the structures is due in large part to Spanish colonization in the Mariana Islands. There is speculation that end of the Latte period was due to a series of controversial events that took place in its latter years. When Magellan first reached the Mariana Islands in March, 1521, he and several surviving members of his voyage documented their accounts of their journey as well as of the indigenous Chamorro people and their ways of life from their perspectives. A month following their arrival in the Marianas, Magellan and several members of his voyage were killed in the Philippines on their way to the Maluku Islands. While no journals have been found from Magellan or his captains, the accounts were fashioned based on extensive interviews of survivors upon arrival in Spain. Conflicting accounts have caused speculation as to which island Magellan made his first landfall, although they agree that it is one of the islands in the southern-most arc including, Saipan, Rota, or Guam (Rogers and Ballendorf, 1989). Information of the island group was taken back to Spain, leading to over a century and a half of sporadic Spanish contact, including sailors and Roman Catholic priests (Dixon et al,. 2006). The Spanish took

formal possession of the archipelago in 1565. For almost a century, the Marianas served as a provisioning stop for Spanish ships when traveling between Mexico and Manila (Hezel and Driver, 2000). During this time there was no inclination to colonize the island group, as well as minimal resources available to establish a colonial government. However, this changed in 1668 when Spanish troops and missionaries began to occupy and colonize the Marianas with the intent to implement a program of religious and cultural reform (Hezel and Driver, 2000). The population of the Marianas during this time was believed to range between 50,000- 100,000 (Cordy, 1983). Among those first colonizers was Father Luis Diego San Vitores, whose accounts of Chamorro society are used as a basis for interpreting archaeological data. From first contact, the colonizing Spanish were met with resistance from certain Chamorro clans, resulting in a series of violent interactions. In 1972 Father San Vitores was killed in Guam, and this heightened violence on the islands, causing the Spanish military to burn villages and kill their inhabitants (Cordy, 1983). This fighting continued until the beginning Spanish-Chamorro War around 1680. Years of fighting culminated into one last uprising from the Chamorro resistance, with the goal to drive out Spanish forces for the last time. The resistance was met with a force from both the Spanish and the Chamorro allies that had joined them. After months of violence, together they were able to able to overcome the opposing Chamorro people. In 1680 the Spanish forced the people of Guam to move into seven villages, centered around churches, and by1685, and hostilities had subsided. This relocation and effective reduction in group variability increased on a larger scale, affecting the entire Mariana Islands. This time is referred to as *Reduccion*, whereby A.D. 1700 the surviving Chamorro people were forced into consolidated and more controllable villages on the islands of Guam, Rota, and Saipan, leading to the mass abandonment of latte villages and the cultural practices that went along with them (Hezel and Driver, 2000).

While resistance had been diminished and this was considered a time of peace, it is believed that the Marianas had lost approximately 70 percent of their population between 1698 and 1720 due to violence and rampant illness (Hezel and Driver, 2000).

Modern Saipan consists of individuals who share lineage from two ethnically distinct groups of people from the Pacific, as well shared lineage as a result of Spanish colonization. The first being the Chamorro, who are believed to be the first inhabitants and the indigenous population of the island of Saipan, and the later Carolinian immigrants who are the indigenous inhabitants of the Caroline Islands just south of Guam. There is significant evidence of contact between the two groups prior to the arrival of the Spanish, suggesting regular trade. Following Spanish contact in the Marianas, the Carolinian peoples ceased all interaction with the Chamorro, keeping their distance as not to incite Spanish curiosity (Hezel and Driver, 2000). It is believed that some Chamorro fled to the Carolines during the war with the Spanish, sparking Spanish interest in locating the islands in search of more indigenous peoples. After many failed attempts, the islands were first located and its people contacted in 1804. The Spanish convinced the Carolinians to resume trading with the Marianas in 1805. Some Carolinians were relocated to Saipan shortly after this time (Pietrusewsky et al., 2010). The mixture of these populations during this time makes determining cultural affiliation of archaeological samples important, as their artifact inventories and characteristic material culture provide insight to the particular temporal interval with which they are associated.

At the time of Spanish contact, Chamorro sailing technology and skills were regarded as among the world's finest. Frequency of fish bones and shellfish remains suggests continued (progressively less) utilization of marine resources during the Latte Period (Carson, 2012). Isotopic analysis of archaeological samples may allow for additional support for ethnological

accounts of subsistence strategies in the Marianas. Information about diet and the timing of the transitions from one subsistence strategy to another can help to determine cultural affiliation.

Stable Isotope Analysis in Archaeology

Stable isotope analysis is currently a commonly employed practice in archaeology as a means of assessing the diet and trophic positions of archaeological samples. For the reconstruction of prehistoric human diet, such data is acquired by measuring isotopic ratios in various bone components. This is due to the fact that consumer tissues (the bones, muscles and organs of people or animals eating plants or other animals) reflect the isotopic composition of their diet, with measurable and somewhat predictable fractionation factors. While diet reconstructions alone are very valuable in understanding subsistence strategies and patterns of past populations, when samples are available for a diachronic perspective they can also be useful in tracking large-scale changes, such as the utilization of resources, environmental degradation, and the emergence and of agriculture, both in its developmental phases and beyond. Using this knowledge, it is possible to determine cultural affiliation of an individual or group of remains. It is widely believed that when first colonizing an island, prehistoric Pacific populations tended to exploit marine resources as they begin to develop an agricultural base, as can be noted by their coastal settlements during early inhabitation (Carson, 2012). Evidence suggests that similar subsistence patterns were present in Saipan and the Marianas (Ambrose et al., 1997). As population expands and agriculture intensifies, terrestrial resources become more important components of the diet (Carson, 2012). If sample size and chronological control are adequate, stable isotopes can provide suitable proxy evidence to identify these transitions.

Stable isotopes in archaeological research are typically examined via bone collagen (organic/protein) and bone apatite or carbonate (inorganic/mineral), that have been extracted

from bone elements. Both tend to be well preserved in archaeological samples, especially in teeth (Ambrose et al., 1997). Bone carbonate analysis provides data for carbon ($\delta^{13}C_{carb}$) in whole diet. Analysis of bone collagen provides data for both carbon ($\delta^{13}C_{coll}$) and nitrogen ($\delta^{15}N$) in the protein dietary contributions. In diet reconstruction, analysis of $\delta^{13}C$ in both carbonate and collagen are used to distinguish the origin energy resources, such as C_3 vs. C_4 plants, or marine vs. terrestrial. The $\delta^{15}N$ isotopes in bone collagen enable researchers to identify the trophic position of the most likely contributors to dietary protein (Ambrose et al., 1997).

The $\delta^{13}C$ ratios are available in both bone carbonate and collagen as they both preferentially integrate carbon from dietary sources. In bone apatite $\delta^{13}C_{carb}$ values in the consumer is dependent on metabolic processes that break down energy substrates that have been consumed. Examples include carbohydrates, lipids, and proteins not required for protein synthesis (Ambrose and Norr, 1993). Carbon molecules from dietary sources are catabolized and integrated into blood bicarbonate (HCO_3^-) which is assumed to be in isotopic equilibrium with bone carbonate. Due to this, bone carbonate represents an average of all dietary components and provides an approximation of carbon values in whole diet. In collagen, $\delta^{13}C$ is characterized mainly by the catabolized dietary protein sources (Ambrose and Norr, 1993) and the direct routing of resulting amino acids towards protein synthesis.

In plants, photosynthesis allows for carbohydrate synthesis from sunlight, water, and atmospheric CO_2; their primary photosynthetic pathways determine $\delta^{13}C$ values. There are two primary processes for photosynthesis, labeled in shorthand as C_3 and C_4 pathways. C_3 plants utilize the Calvin Cycle, producing a 3-carbon molecule at the end (Calvin and Bassham, 1957). C_4 plants which follow the Hatch Slack Cycle which produces a 4-carbon molecule at the end (Hatch and Slack, 1970). The different cycles, as well as elemental fractionation result in large

variances in $\delta^{13}C$; this makes them distinguishable from one another in stable isotopic dietary reconstruction. C_3 plants have a large range of carbon isotope compositions, -27‰ to -35‰ (Kohn, 2010). C_4 plants have higher $\delta^{13}C$, their range is -9‰ to -19‰. This difference in values allows for distinguishing between consumption of primarily one resource over the other. The $\delta^{13}C$ is used to distinguish between C_3 and C_4 as well as marine and terrestrial sourcing. In terrestrial plants, carbon is derived from atmospheric CO_2 sourcing. In marine environments, carbon is derived from dissolved CO_2 (Ambrose et al., 1997). Because of differential carbon availability and their effects on isotopic fractionation, the range for marine plant resources falls in between the C_3 and C_4 ranges. Because of their similarly high $\delta^{13}C$, it can be difficult to distinguish consumer $\delta^{13}C$ values derived from C_4 plants and marine plants without prior ecological information on plant distributions or corresponding $\delta^{15}N$ data.

The metabolic pathways and physiological factors that contribute to bulk collagen $\delta^{15}N$ values make it a viable source for determining protein contributions to the diet (Styring et al, 2009). Trophic level is determined based on the feeding habits of the consumer. There is a stepwise increase in $\delta^{15}N$ values as you move up the food chain, with ~ 3-5‰ enrichment at each trophic level (DeNiro and Epstein, 1981). This is important information for reconstructing diet, as it distinguishes the trophic position of the consumer as it relates to available food sources.

As they are primarily C_3 plants, the $\delta^{13}C$ values derived from terrestrial foods in the Marinas (taro, yams, sweet potato, breadfruit and rice) (Pollock, 1986; Hunter-Anderson et al., 1995) are low compared to those derived from marine protein sources (fish, shellfish) (Ambrose et al., 1997), allowing for estimations of proportion contributions from these distinct sources to diet. This information is consistent with the utilization of stable isotopes in archaeology to better

understand subsistence strategies through the lens of what resources were being consumed. This study incorporates both forms of analysis to allow a more holistic interpretation of diet during the Latte period in the Marianas.

Attenuated Total Reflectance-Fourier Transform InfraRed (ATR-FTIR)

While data obtained from stable isotope analysis and other bio archaeological research concentrated on bone material are currently being utilized to reconstruct past lifeways, due to the time and effort required to obtain these data, under ideal conditions one would assess the preservation of the samples prior to analysis. When bones are buried they begin a process of diagenesis. These taphonomic processes change the biochemical components of bone as a result of both geological and groundwater conditions (Beasley et al., 2014). As bone is made up of collagen and bioapatite, pathways of diagenesis that are undergone include; chemical deterioration of its organic phase (collagen), chemical degradation of the mineral phase (apatite) (Collins et al., 2002). The rate at which protein chemically degrades depends on time, temperature, and pH. At high temperatures, and extremes of both acidic and basic pH the rate of collagen loss is accelerated. The rate of apatite degradation is generally determined by its thermodynamic equilibrium with its environment and precipitation (White and Hannus, 1983). Another form of diagenesis is the degradation to bone caused by microbial activity, optimized at near neutral pH (Collins et al., 2002). In addition to these affects, changes that are undergone biochemically are typically ion uptake/exchange and the changes in the crystalline structure, which can be tracked using vibrational spectroscopy (Hollund et al., 2013). These alterations make it difficult to retrieve information about the living state of the molecules from the individual. In archaeological research, specifically with regard to those requiring molecular analyses of bone material, it is essential to know the level of diagenesis that has occurred after

the individual has died to be able to better predict the outcome of costly and time-consuming laboratory experiments. Being able to track these changes and relate them to the possibility of getting useful data provides the opportunity to avoid spending analytical effort on samples, which are unlikely to yield data of interest to the project.

Attenuated Total Reflectance Fourier Transform InfraRed spectrometry (ATR-FTIR) is a relatively recent improvement on existing vibrational spectroscopic techniques that can be used to investigate post-depositional alterations to archaeological samples with minimal sample preparation or analytical investment. This makes ATR a suitable tool for preliminary assessment of bone preservation when downstream biomolecular analyses are the study goal. This technology provides information about the organic and inorganic chemical composition and level of crystallinity of bone. ATR-FTIR produces spectral data, in the form of peaks across a domain of measured wavenumbers that measure the absorbance at different wavelengths, relating to the chemical structure of bone matrix, notably hydroxyapatite molecules (bone carbonate and collagen) and water bound into the interstitial spaces (Hollund et al., 2013).

In the case of archaeological bone, the peaks that are commonly assessed to investigate diagenetic alterations are the ratio of amide to phosphate (Am/PO_4), the ratio of carbonate to phosphate (CO_3/PO_4), and the splitting factor (SF) (Hollund et al., 2013; see Figure 4). Amides make up a significant portion of proteins, thus the amide to phosphate ratio can be used as a predictor for the survival of different forms of bone collagen, and potentially DNA. Both molecules are susceptible to enzymatic degradation t as a result of, microbial alteration, energetic damage from heat or UV radiation, or hydrolysis in the presence of free water (Collins et al., 2002). Increased Am/PO_4 ratio is indicative of good preservation of bone protein, and by extrapolation to DNA molecules. The carbonate to phosphate ratio can be used to predict if bone

apatite has undergone diagenetic effects. Increased CO_3/PO_4 ratios are an indicator of good bone preservation. When comparing the values derived from FTIR-ATR of bone carbonate and the isotope data derived from bone carbonate, a positive correlation between the two could be an indicator that carbonate isotope values have been affected by diagenesis. The changes in apatite ratios can be traced by FTIR due to the differences in spectra of prehistoric bone with substituents from burial context, and those from modern with none (White and Hannus, 1983). The splitting factor gives information about the level of crystallinity of the bone. In modern samples, the crystalline structure is small and disordered; when an individual is alive, crystal growth inhibitors regulate bone its structure. When a bone undergoes diagenesis, the crystalline structure increases in size as well as order, producing larger, more symmetric crystal lattice matrices. In this case lower SF values, indicative of smaller crystalline structure, are more indicative of a higher degree of bone preservation (Kohn et al., 2002).

While there are other scientific analyses archaeological researchers can use to track and measure levels of bone diagenesis, in one study FTIR-ATR produced more reliable results as opposed to FTIR methods using KBr pellets and DRIFT spectra (Beasley et al., 2014). In the study conducted by Beasley et al, (2014), the three techniques were assessed for correspondence in, and consistency among data. The dataset reflected in their results consist of both faunal and human bone elements, the modern samples span from mid 19thcentury, to present day; the prehistoric data date from 5,000. The three methods can and have all been used to asses diagenesis, because of this it is important to distinguish between the values and data produced by each when determining which method will produce the most consistent results. For a time archaeological research has used Transmission FTIR spectroscopy; the method used previously required a sample be prepared by mixing the sample with potassium bromide (KBr) pellets and

pressing the mixture into a pellet for transmission FTIR analysis. The diffuse reflectance infrared Fourier transform (DRIFT) technique also requires that bone powder be ground with KBr. While effective, these two methods may result in particle size differences that made adversely affect results. The study shows that while the three techniques identify the chemical properties of a sample, the differences in spectral resolution of each result in different values for CO_3/PO_4 and IR-SF. In this experiment ATR was shown to be an effective tool in distinguishing modern bone from prehistoric bone. Its reliability also comes in the form of increased instances of spectral reproducibility. The values indicated in the spectra for modern bone had different ranges than the values from the prehistoric sample set. This result is supported when plotted on a bivariate plot comparing CO_3/PO_4 and IR-SF (Figure 5). In this study, mean value and standard deviation for splitting factor in modern bone is 3.07±0.26, and 3.90±0.37 for prehistoric, when assessed using ATR. Mean value and standard deviation for carbonate/phosphate in modern bone is 0.31±0.08, and 0.16±0.05 for prehistoric, when assessed using ATR (Beasley et al., 2014). These values can be used comparatively in our ATR spectral analysis.

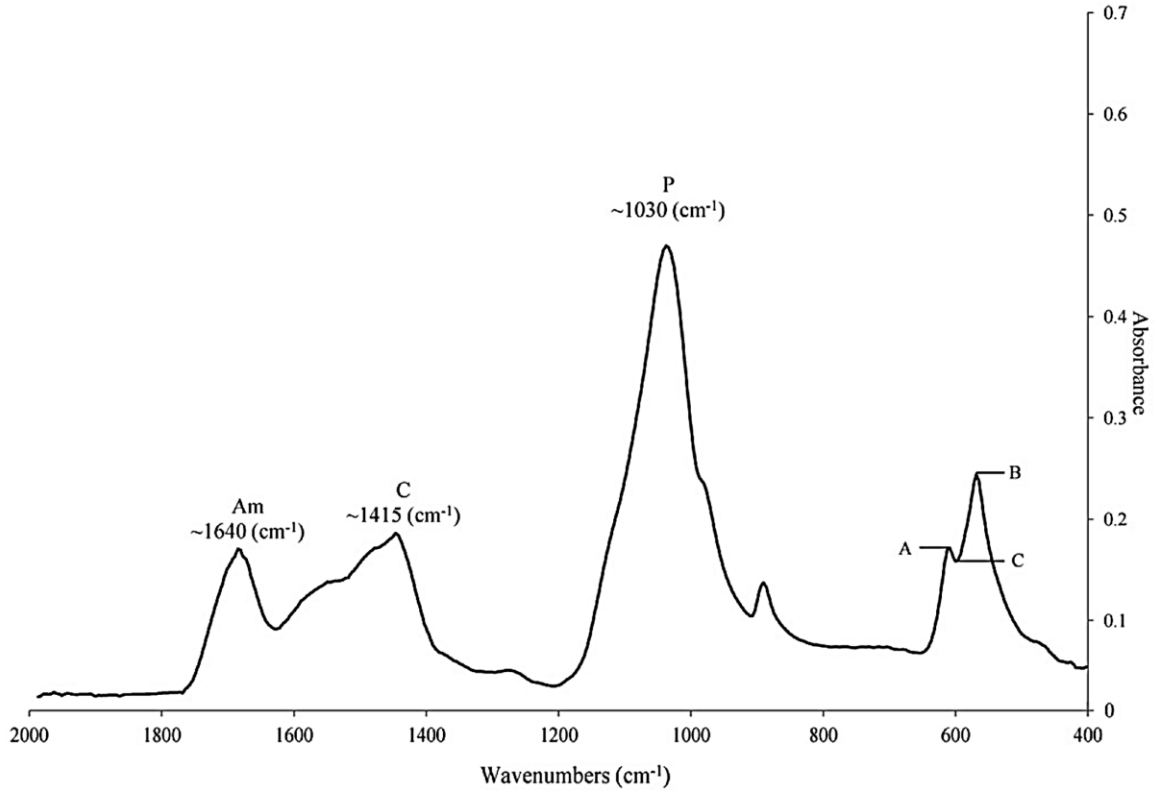

Figure 3: FTIR-ATR spectra and peak locations of the Amide (Am), Carbonate (C), Phosphate (P), and Splitting factor ((A+B)/C). Image adapted from Hollund et al., (2012).

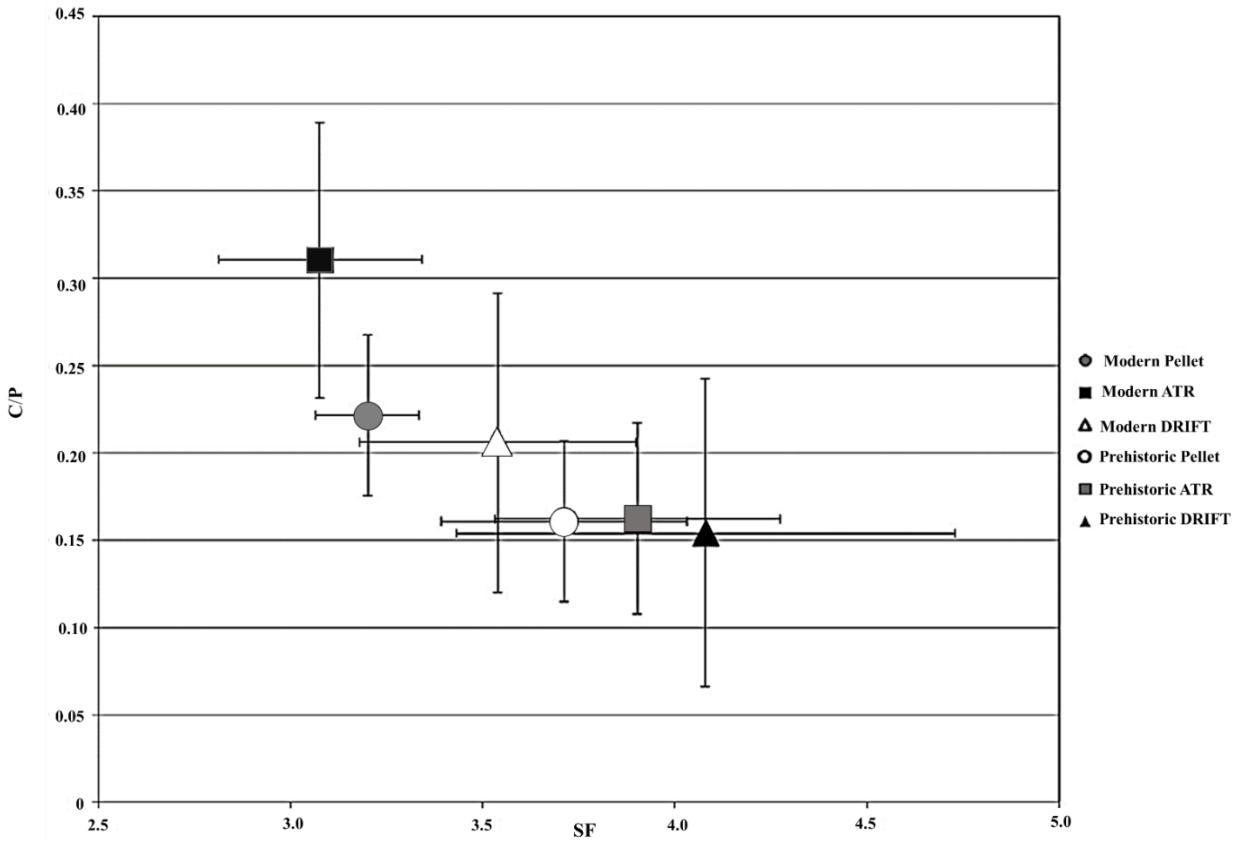

Figure 4: Comparison of C/P and IR-SF values calculated for the three preparation techniques. (Error bars represent one standard deviation). Image adapted from Beasley et al., (2014**)**.

Previous Research

Stable isotope analysis has the ability to shed light onto the lifeways of Chamorro in Saipan. Ambrose et al. (1997) conducted a previous stable isotope analysis of Saipan, Rota, and Guam. The burials assessed on these islands are from contexts associated with AD 700 to European contact. This correlates with the time directly prior to and including the Latte period (AD 1000-1521). The skeletal remains used in this study were collected from Saipan from a site called Garapan, and are directly dated from within the Latte Period AD 1250-1350. Isotopic data for food sources used in their study were collected from Guam. Similarities in both location and time period between the data set in this research and that of Ambrose et al. (1997), allow it to be

used in a comparative analysis. The results of Ambrose's study indicated that the individuals from Garapan, Saipan had a significantly different diet than those from Rota and Guam. In the Saipan dataset, collagen $\delta^{13}C$ and $\delta^{15}N$ indicated primarily terrestrial protein consumption. It also showed the lowest proportions of C_4 and marine plants compared to the other islands. There was a striking contrast from these results when apatite carbonate $\delta^{13}C$ was assessed. The values indicated that the diet of the people of Saipan was constructed mainly of terrestrial plant-based sources (taro, yams, breadfruit, coconut, etc.) with very low protein inclusion, and little to no marine influence. Higher $\delta^{13}C_{carb}$ values in this sample set indicate greater yet minor consumption of C_4 or marine plants, possibly seaweed or sugarcane (Ambrose et al., 1997). The differences when comparing $\delta^{13}C$ from carbonate and collagen allows a snapshot of the whole diet; it reveals a heavy reliance on low protein plants. When compared to the diets of the other islands, the amount of marine protein resource inclusion in the diet on Saipan was significantly less. Ambrose et al. (1997) concluded his study suggesting further data analysis comparing his data to C_4 and marine resources to determine which had an influence on diet.

In an additional study conducted by Froehle et al. (2012), Ambrose et al.'s (1997) data was used to produce a model to allow for integration of all data produced in isotopic analysis. This was under the belief that there was a distinct relationship between $\delta^{13}C$ of bone carbonate and collagen, and $\delta^{15}N$ ratios. Regression lines for this study were produced using data from his experiment in using varying protein and carbohydrate sources. The data for the regression lines in Figure 12 were derived from experiment with swine and rodents; they represent C_3 and C_4/Marine diets. The top represents a C_4/Marine diet, and a C_3 diet on the bottom. Plotted on the y-axis is $\delta^{13}C_{coll}$, which distinguishes protein sources varying from C_3 to C_4/Marine. On the x-axis is $\delta^{13}C_{carb,}$ it differentiates between C_3 and C_4/Marine sources for energy substrates

(carbohydrates, lipids and proteins not required for protein synthesis). Each cluster represented in Figure 12 represents a diet group derived from isotopic analysis of 158 archaeological human samples from contexts where subsistence strategies are well-defined (Froehle et al., 2012). In this analysis, Ambrose et al.'s (1997) data are grouped near cluster 4. While its protein values are consistent with that cluster (≥65% C_3 protein), there is a significant shift that suggests consumption of a low protein C_4 or marine plants. In order to asses this information further, as suggested by Ambrose et al (1997), the data was assessed against C_4 (sugarcane) and C_3 marine (seaweed). This was done by creating a discriminant function that would allow the inclusion of all data derived from isotopic analysis (Froehle et al., 2012). The function combines values for collagen and apatite $\delta^{13}C$, and plots them against $\delta^{15}N$. This allows a clear distinction to be made between the two resources, as Ambrose et al.'s (1997) data revealed a large influence of sugar cane.

An additional isotopic analysis was conducted on ten individuals from Afetna, Saipan, by McGovern-Wilson and Quinn, (1996). These individuals were also believed to be associated with the Latte Period. Collagen $\delta^{13}C$ and $\delta^{15}N$ were assessed and they determined the individuals from Afetna had a diet consistent with mainly terrestrial protein sources, with influence from marine proteins. This is indicated in high $\delta^{15}N$ values for some individuals. McGovern-Wilson and Quinn (1996) had several possible reasons for these high values, including the possibility that these individuals had more access to deep- ocean fish. The collagen $\delta^{13}C$ was also determined to be high in some individuals, but like Ambrose et al., (1997), they also inferred that there is a possibility for influence from some C_4 plants. Isotope values derived in this study also serve as comparison data.

Summary and Hypothesis

Due to the significant amount of changes that occurred in the Marianas during and following Spanish contact, the lifeways and culture of the ancient and protohistoric Chamorro have long been lost. With the consolidation of Latte clans into controlled colonial villages, as well as a significant loss in population, knowledge of the cultural and subsistence practices were effectively diminished. Bioarchaeological research is being conducted to illuminate the lives and practices of the prehistoric Chamorro, from the perspective of diet, health and cultural affiliation/ancestry. This thesis will attempt to answer questions regarding subsistence strategies and cultural affiliation by assessing stable isotope data of the people of Latte period in Saipan. Diagenetic alteration is a large determining factor of what testing can be conducted on archaeological samples that will yield usable results. It is important to look for ways in which the level of diagenetic alteration can be assessed, prior to downstream, more expensive testing. I first hypothesize that I will be able to distinguish a difference between the FTIR-ATR results produced from archaeological samples and those from modern, from which I will be able to determine a pattern for diagenetic alteration of bone organic and inorganic biomolecules as well as crystallinity changes. Secondly, I hypothesize that I will be able to determine the primary mode of subsistence, potential rank in society, as well as possibly determine cultural affiliation for the population represented by the individuals buried in Garapan via stable isotope analysis, and that they will be comparable to the data from Saipan produced by Ambrose et al, (1997).

Chapter 2: Materials and Methods

Garapan Site

The site of Garapan is located off the mid-western coast of the island of Saipan (Figure 3 red). The site was selected for excavation because it had been determined to have high potential for encountering significant archaeological and historical artifacts by the Commonwealth of the Northern Mariana Islands Historic Preservation Office. Scientific Consultant Services, Inc. (SCS) conducted these excavations from April 4–June 30, 2015 (Figure 5), recovering 416 individuals and over 1,000 artifacts dating to the Latte period. The context of the mass burial was a sandy coastal platform within 200 m of the coastline and less than 2 m above present sea level.

Figure 5: Image of Saipan, red circle indicates location of Garapan, to the south in the blue is Afetna

a

b

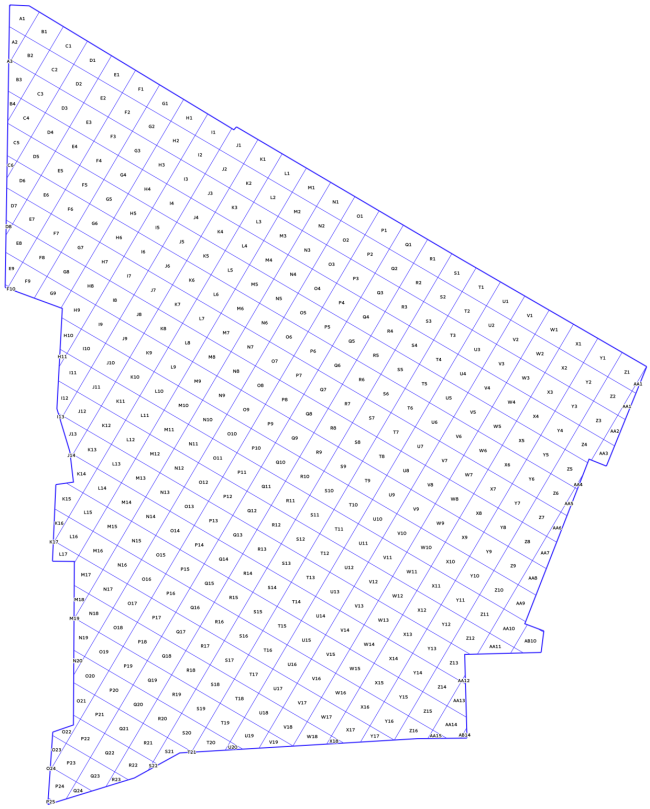

Figure 6: Grid and map of archaeological excavation site Garapan, Saipan. (a) Satellite Map of excavation site. (b) Grid of archaeological site. Grid provided by SCS.

Materials

The dataset for these analyses consists of 25 individuals; among them are 12 males, 7 females, and 1 sub adult and 5 unknown individuals. The age groups represented in this study follow standard paleodemographic classification and include, 4-12 (2 individuals), 21-35 (9 individuals), and 36-39 (4 individuals), and 10 adults with unknown age estimation. Displayed in Figure 7 are examples of teeth that were sampled in 2015. The individuals in the Garapan data set are from two different isotopic analyses, 17 individuals in 2015 and 8 in 2017. In addition to Garapan, in 2018 we received additional samples from the Surfrider Resort in Afetna, Saipan (Figure 5, blue). Sixteen bone elements were assessed for collagen isotope and ATR data. Little is known about the specifics of this site or the burial, but it is a useful comparison as Afetna is located just south of Garapan. Modern human tooth samples were collected from dentists' offices in southeastern Idaho in 2015 to provide a taphonomically-unaltered baseline for collagen and carbonate matrices. An additional dataset used in this study are the collagen isotope data points derived by McGovern-Wilson and Quinn (1996) for 10 individuals also associated with the Latte period from Afetna, Saipan. Stable isotopes were assessed via ThermoElectron Corporation Delta V Plus stable isotope ratio mass spectrometer, ConFlo IV Universal Interface and Gas Bench II (IRMS) at the Center for Archaeology, Materials and Applied Spectroscopy (CAMAS) at Idaho State University. The Bruker Alpha ATR-FTIR system with single bounce diamond crystal (FTIR-ATR) located in CAMAS at ISU was used to produce data for assessing diagenetic alteration (Figure 9)

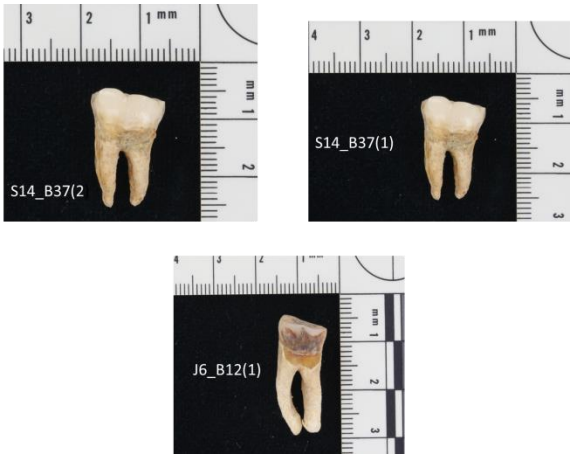

Figure 7: Three of the teeth sampled in this analysis

Methods

Preliminary Bone Processing

Upon receipt, all bone elements used in this thesis were subject to the same treatment as pertains to CAMAS protocol for sample preparation. This includes thoroughly photographing each element prior to any alteration. Once photographed each sample was brushed using a tooth brush and rinsed using 18Ω water. The samples were then placed in 2% bleach for 5 minutes under vacuum with the lids loosely capped (Figure: 8). Each one was then washed three times in 18Ω water in order to remove all bleach. They were then left in weigh boats and allowed to dry overnight. Once dry, bone elements that are relatively large were cut to a smaller size and teeth were cut to separate the crown for later use. This is done using a dremel tool accompanied by a light vacuum. The freshly cut bone elements were ground into a homogenous powder using a SPEX cryomil. Once powdered a multitude of assessments can be conducted.

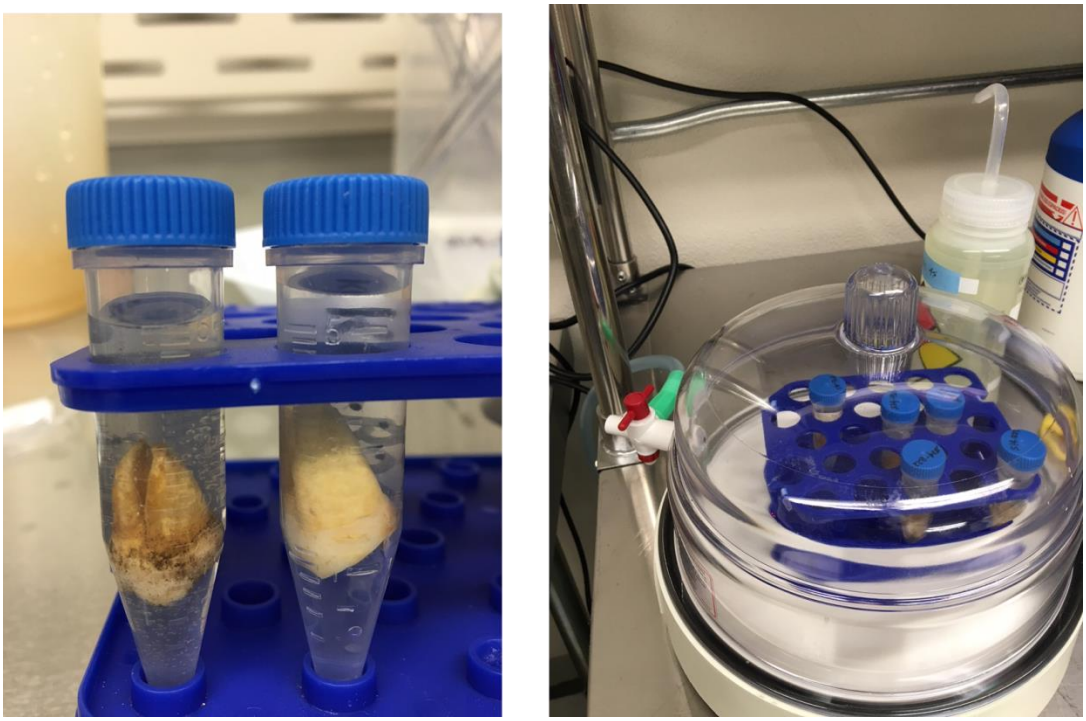

Figure 8: Teeth during wash process (left). Samples soaking under vacuum in 2% bleach

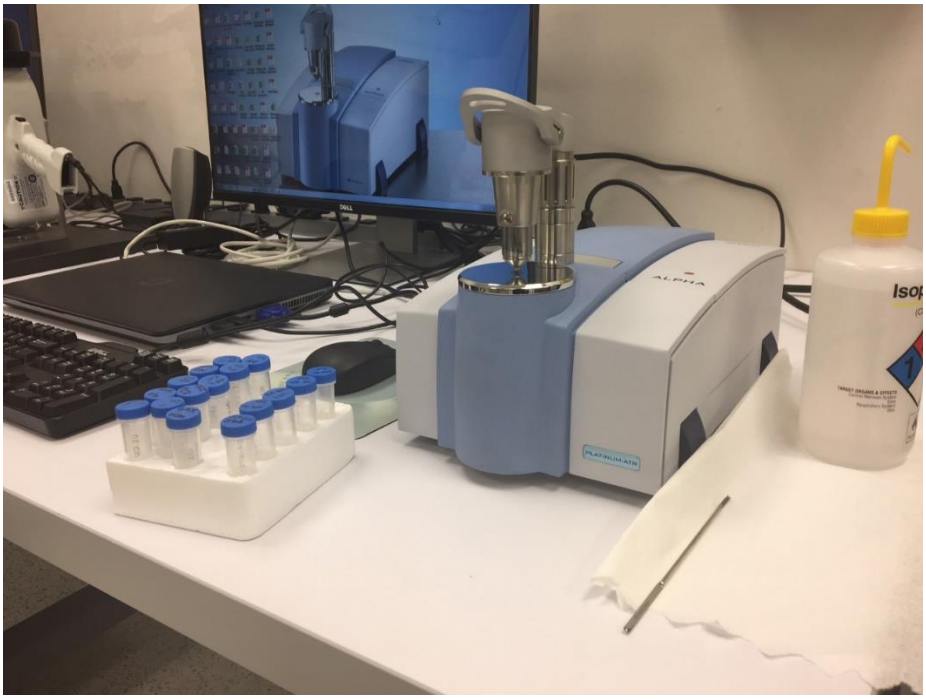

Figure 9: FTIR-ATR located at CAMAS ISU

Collagen Extraction from Bone

In 2015 collagen was extracted from 18 bone elements from Garapan, Saipan, and 3 modern teeth from South-eastern Idaho. To begin, 75mg of each sample was weighed. Once weighed, they were placed on 2:1 chloroform-methanol, for approximately 4-8 hours depending on visual presence of lipids. Chloroform-methanol is used to separate lipids from non-lipids. (Bligh and Dyer, 1959). The 18 archaeological and 3 modern samples were then demineralized in 1.5ml of 1M hydrochloric acid (HCl) for 20 minutes at room temperature. The samples were then centrifuged and decanted and subsequently washed 3 times in 18MΩ water. The samples were then left to soak for 20 hours in potassium (or sodium) hydroxide this is done to remove any soil derived organic acids. The samples were then centrifuged and decanted and washed 3 times in 18Ω water. To gelatinize, the samples were left to soak in 0.01M HCl at 0°C for approximately 24 hours. Scintillation vials were weighed and put into the freezer to chill to prevent loss of collagen due to evaporation. The gelatinized samples were then filtered using Millex-HV 0.45μm filters into the vials and frozen over-night. The samples were then freeze-dried for 48 hours (Commendador et al., 2013). For the 7 samples that yielded collagen, 0.5mg was then weighed and submitted to IRMS for stable isotope analysis. Purified collagen is shown in Figure 10.

Due to low collagen yields caused by potentially harsh methodology, the method for bone collagen extraction as followed was applied to the samples from the Surfrider resort in Afetna in 2018. For bone collagen extraction, initial sample weights were typically targeted between 75 and 150 mg ± 10 mg depending on availability. Once weighed into borosilicate vials they were placed on 2:1 chloroform-methanol, under foil for approximately 4-8 hours depending on visual presence of lipids. The samples were each decanted and allowed to air dry uncapped overnight.

The samples were then placed on 0.5M HCl at 4ºC for 4 hours or until no CO_2 bubbles were observed. They were then centrifuged, decanted and washed three times. Once neutral the samples were allowed to soak on 0.1M NaOH for 20 hours at room temperature to remove soil and humic acid contaminants. They were then decanted and rinsed three times. Once neutral they were gelatinized in 0.01M HCl at 60ºC for 16 to 24 hours. Scintillation vials were weighed and put into the freezer. The gelatinized solution were then plunged through Millex-HV 0.45µm filters into cold scintillation vials and allowed to freeze overnight. The collagen samples were then allowed to freeze dry for 48 hours and weighed in the scintillation vials from which 0.5mg were then submitted to the IRMS for stable isotope analysis.

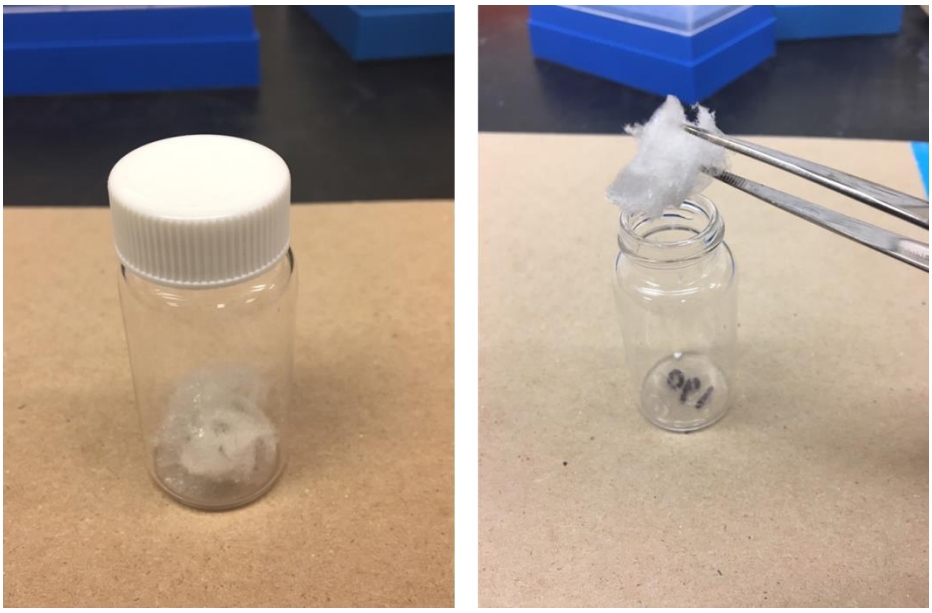

Figure 10: Collagen produced post-extraction

Carbonate Extraction from Bone

For carbonate extractions, our target weight for initial bone powder is between 20-30 mg. The weighed samples are put into 5ml tubes and placed on 2% bleach (sodium hypochlorite, NaOCl) for 3 days at room temperature. Bleach is decanted and replaced with fresh

bleach each day. On the 3rd day each sample is decanted and washed three times with 18MΩ water. They were then soaked in 0.1M acetic acid for 12 hours and washed three times directly after. They were then placed in the drying oven over night at 60ºC.

FTIR-ATR

Once washed, cut and ground, and prior to any chemical alteration, Garapan, Afetna, and modern bone powders were assessed using FTIR-ATR. This was done by applying approximately 2 mg of bone powder to the window of the FTIR. Absorbance spectra were collected at a resolution of 4 cm-1 and were integrated over 24 scans. Three spectra were collected from each sample and the values averaged. The data integration for the various peaks was derived following Hollund et al, (2013). Values for archaeological samples from Garapan and Afetna, and a larger dataset including Fiji, and Taumako from additional studies, were then compared to those derived from modern. CO_3/PO_4 and SF for both were then compared to the data produced by Beasley et al, (2014).

Chapter 3: Results

FTIR-ATR

Spectral analysis was conducted on archaeological samples from Garapan, Afetna, Fiji, Taumako, and modern samples. The sites tested are varying in age and the Fiji dataset is believed to be the oldest. Modern samples were used in order to develop a spectral comparison of a bone that has not yet begun the rigid phase of diagenesis. Assessment of the ratios of Am/PO_4, CO_3/PO_4, and the splitting factor of modern samples should yield different mean values than those produced by archaeological samples. Am/PO_4 ratio is indicative of diagenetic changes that have taken place in the organic matter of bone. In this instance, higher values are indicative of less alteration. The mean ratio and standard deviation for Am/PO_4 for the archaeological samples in this study is 0.059±0.030, and for modern 0.301±0.026. Figure 11 is a bar graph comparing Am/PO_4 of all samples assessed in this study. CO_3/PO_4 ratios also follow this same pathway of decreasing values with diagenetic alteration this is because of differences in and exchange with environmental carbonate. The mean ratio and standard deviation for CO_3/PO_4 for the archaeological samples in this study is 0.177±0.39 and for modern, 0.202±0.019 (Figure 12). Due to an increase in the organization and size of bone crystalline structure with diagenesis, Splitting factor values are increased when a bone has undergone diagenesis. The mean ratio and standard deviation for SF for the archaeological samples in this study is 3.76±0.34 and for modern, 3.474±0.0649 (Figure 13). Figure 14 displays the results of a comparison of Am/PO_4 and CO_3/PO_4 for archaeological samples collected from Garapan, Afetna, Taumako, and Fiji, and modern samples. When plotted together we start to see more isolation of the moderns from the archaeological samples from Fiji, believed to be the oldest of the group tested. Figure 15 shows a

similar separation as Figure 14; it assesses the SF compared to Am/PO_4. Figure 16 displays CO_3/PO_4 vs $\delta\ ^{13}C_{carb}$ linear correlation between these two would suggest that $\delta\ ^{13}C_{carb}$ data were influenced by diagenesis. In Figure 17 is a comparison of Am/PO4 and % yield of collagen extracted. There is distinction between those values for modern as opposed to archaeological samples.

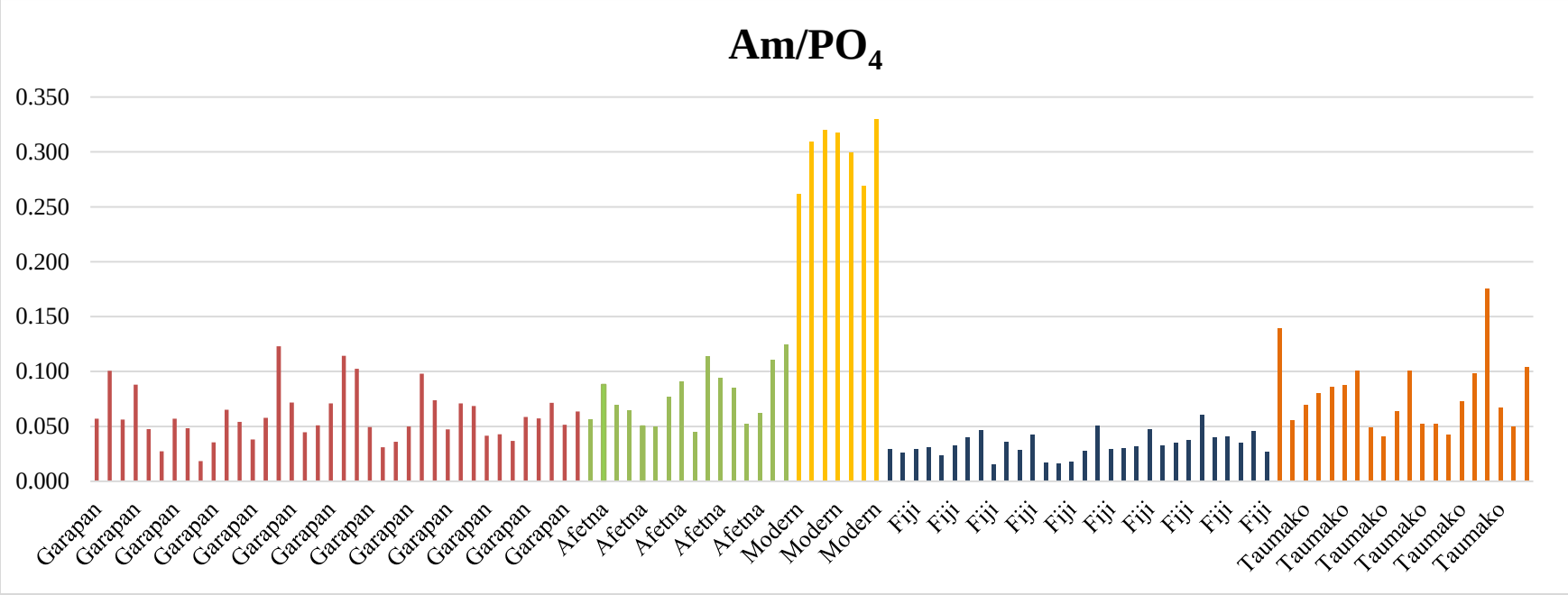

Figure 11: Am/PO_4 ratios for all archaeological and modern bone elements assessed in this study. Color differences are displayed to delineate different datasets from one another.

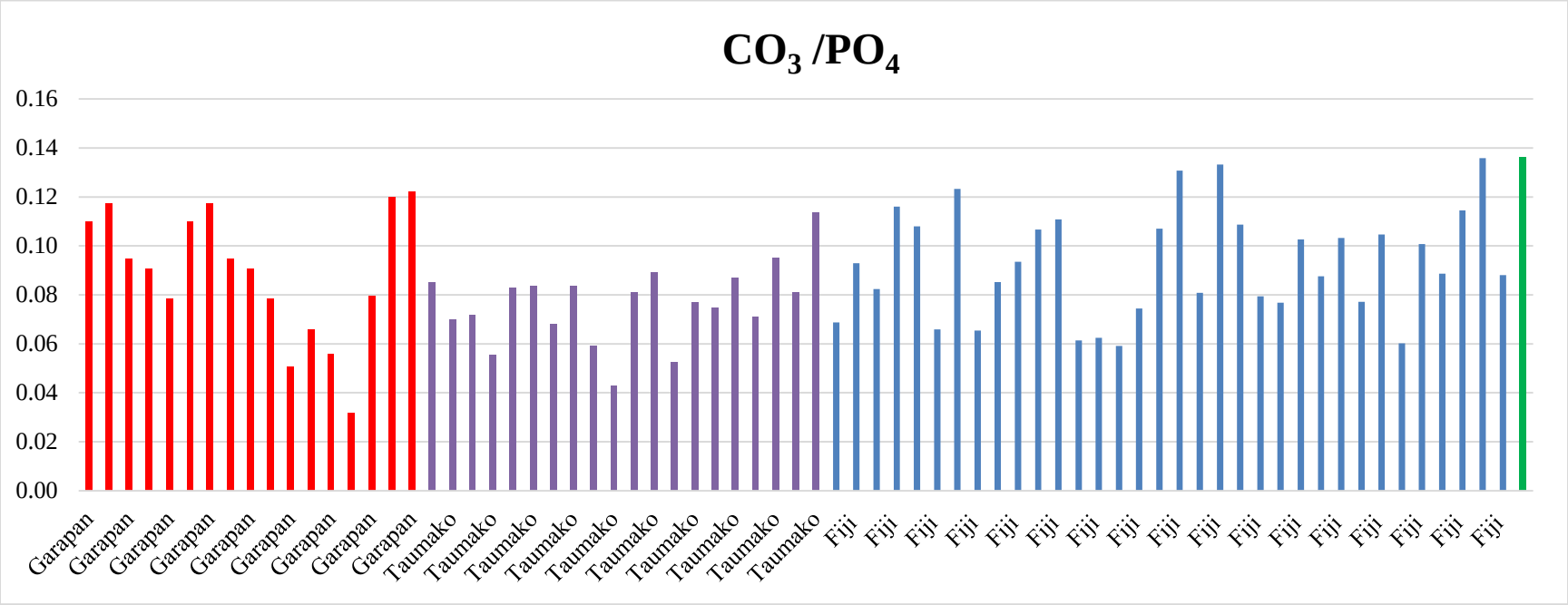

Figure 12: CO_3/ PO_4 ratios from bone carbonate derived from all archaeological and modern samples assessed in this study. Color differences are displayed to delineate different datasets from one another.

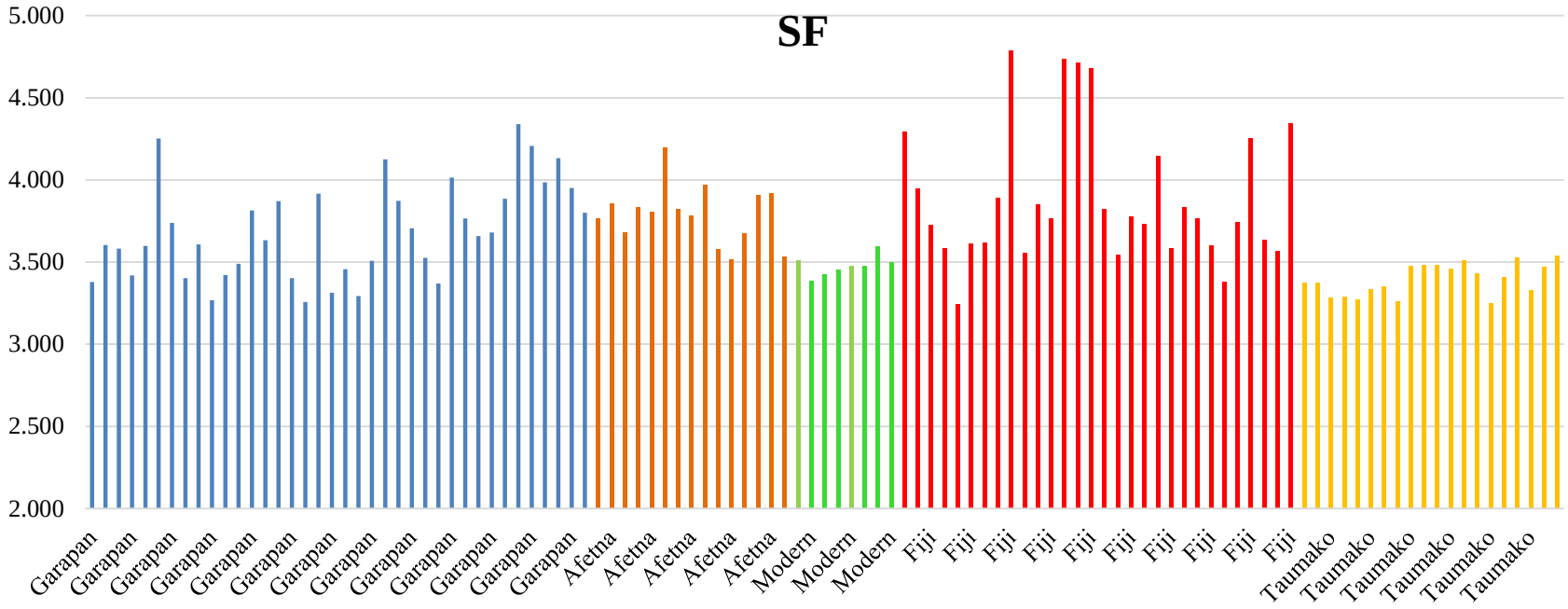

Figure 13 : Splitting Factor for all archaeological and modern samples assessed in this study. Color differences are displayed to delineate different datasets from one another.

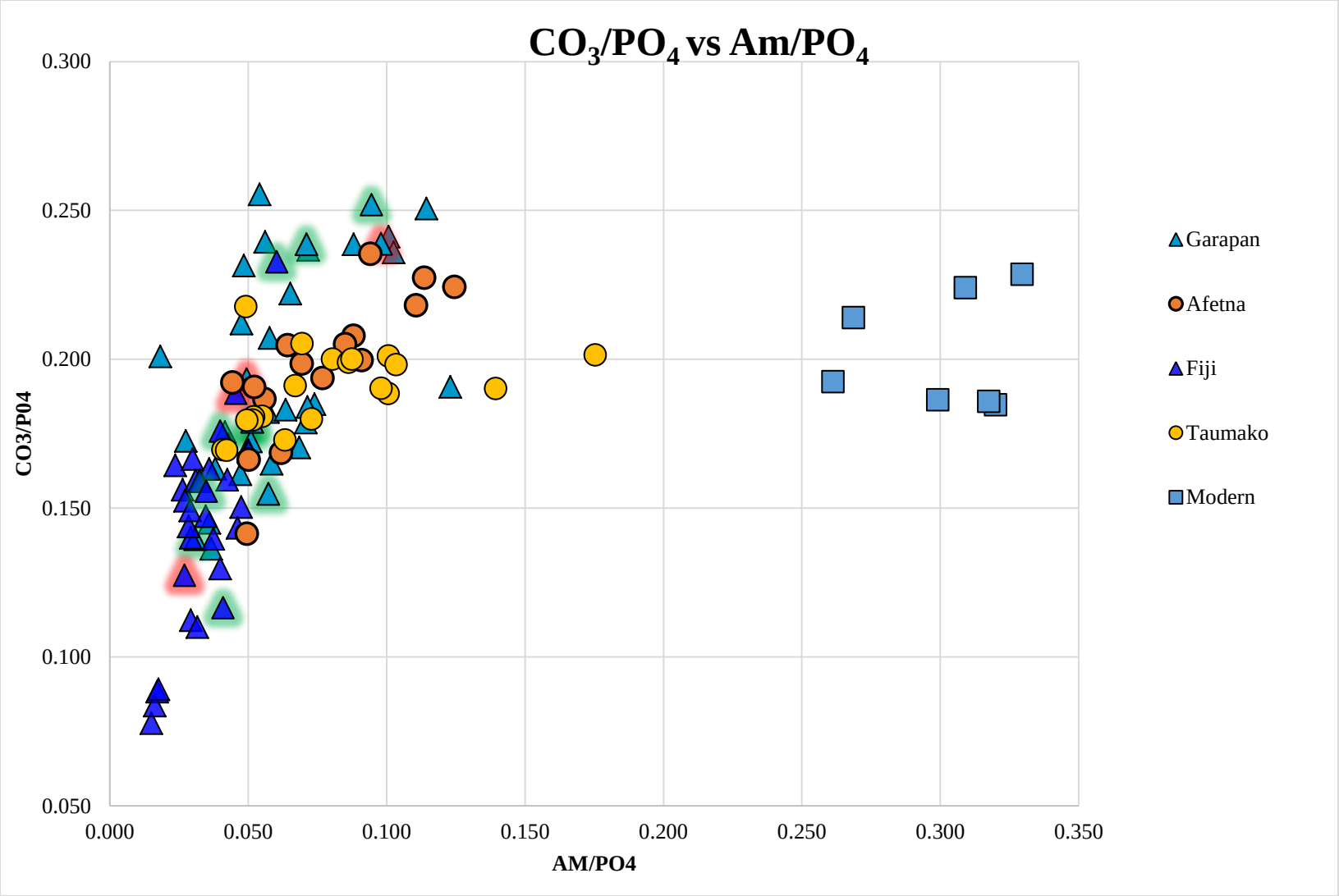

Figure 14 : Bivariate comparrison of CO_3/PO_4 and Am/PO_4 for all archaeological and modern samples in this study. In a separate project, an attempt was made at extracting DNA from some individuals from Fiji and Garapan datasets. Red or green idicates whether or not the sample yielded DNA upon extraction.

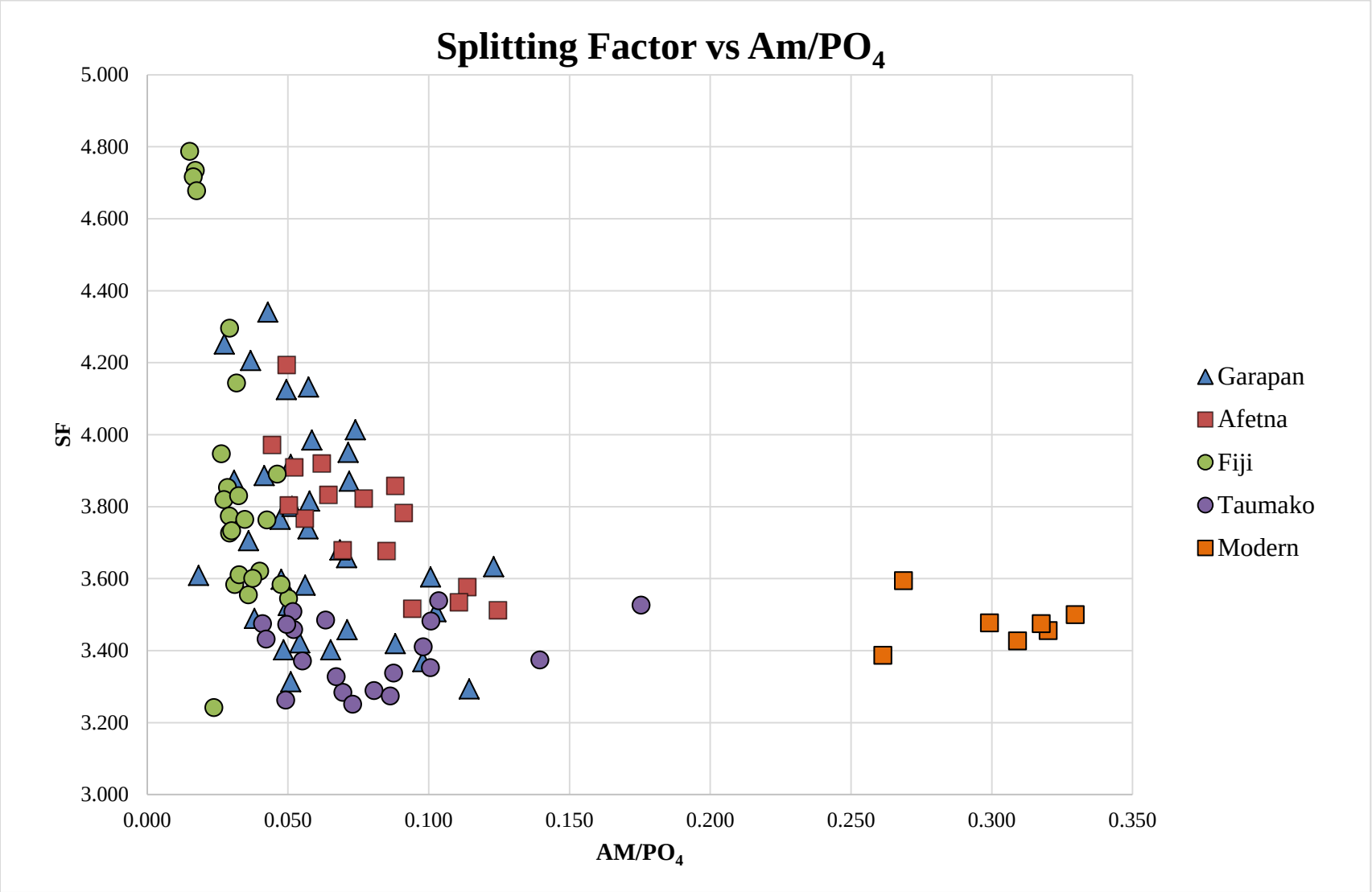

Figure 15: Bivariate analysis of Splitting Factor and Am/PO_4 for all archaeological and modern samples in this study.

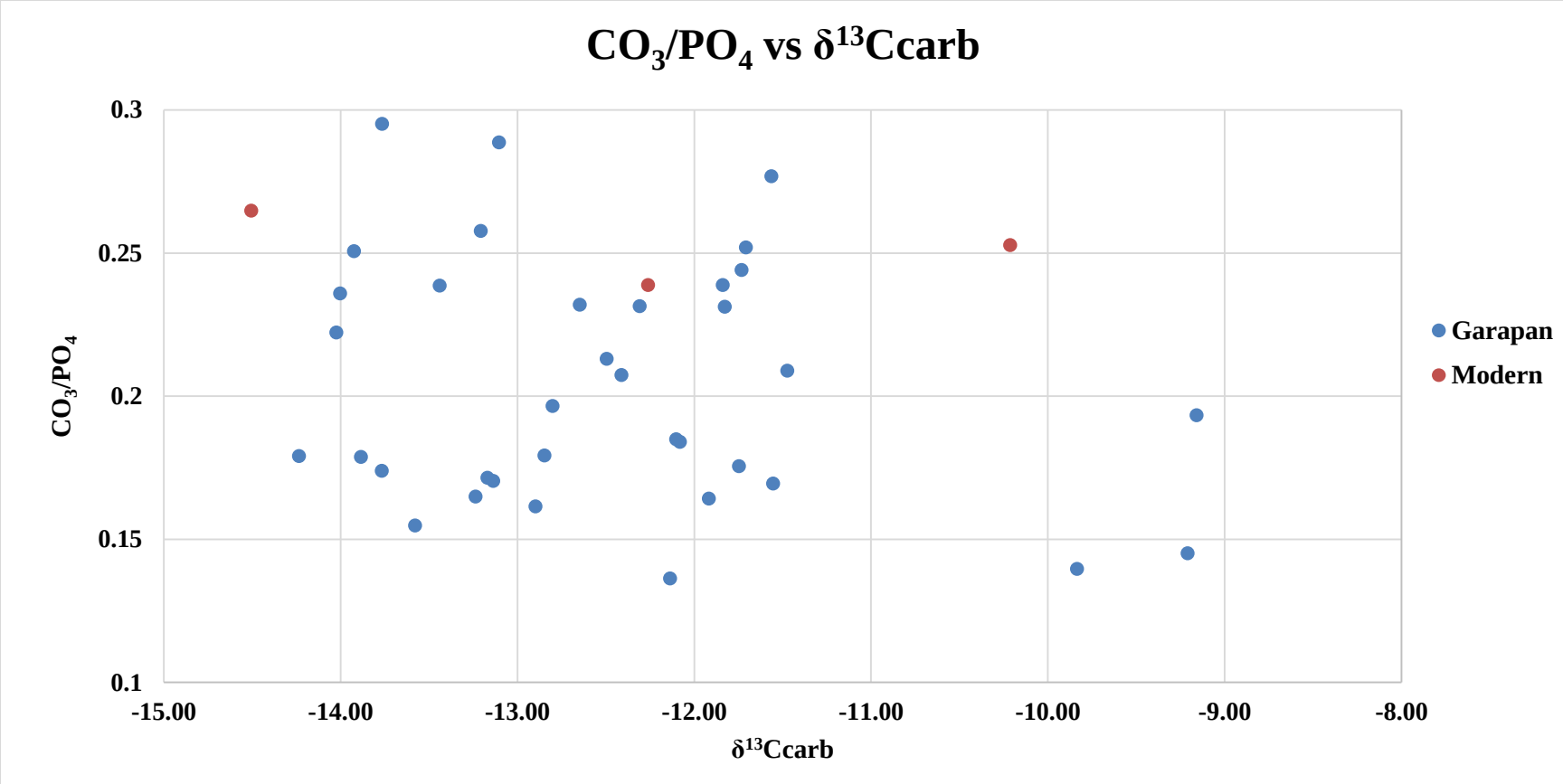

Figure 16: CO_3/PO_4 vs $\delta\ ^{13}C_{carb}$ linear correlation between these two would suggest that $\delta\ ^{13}C_{carb}$ data was influenced by diagenesis

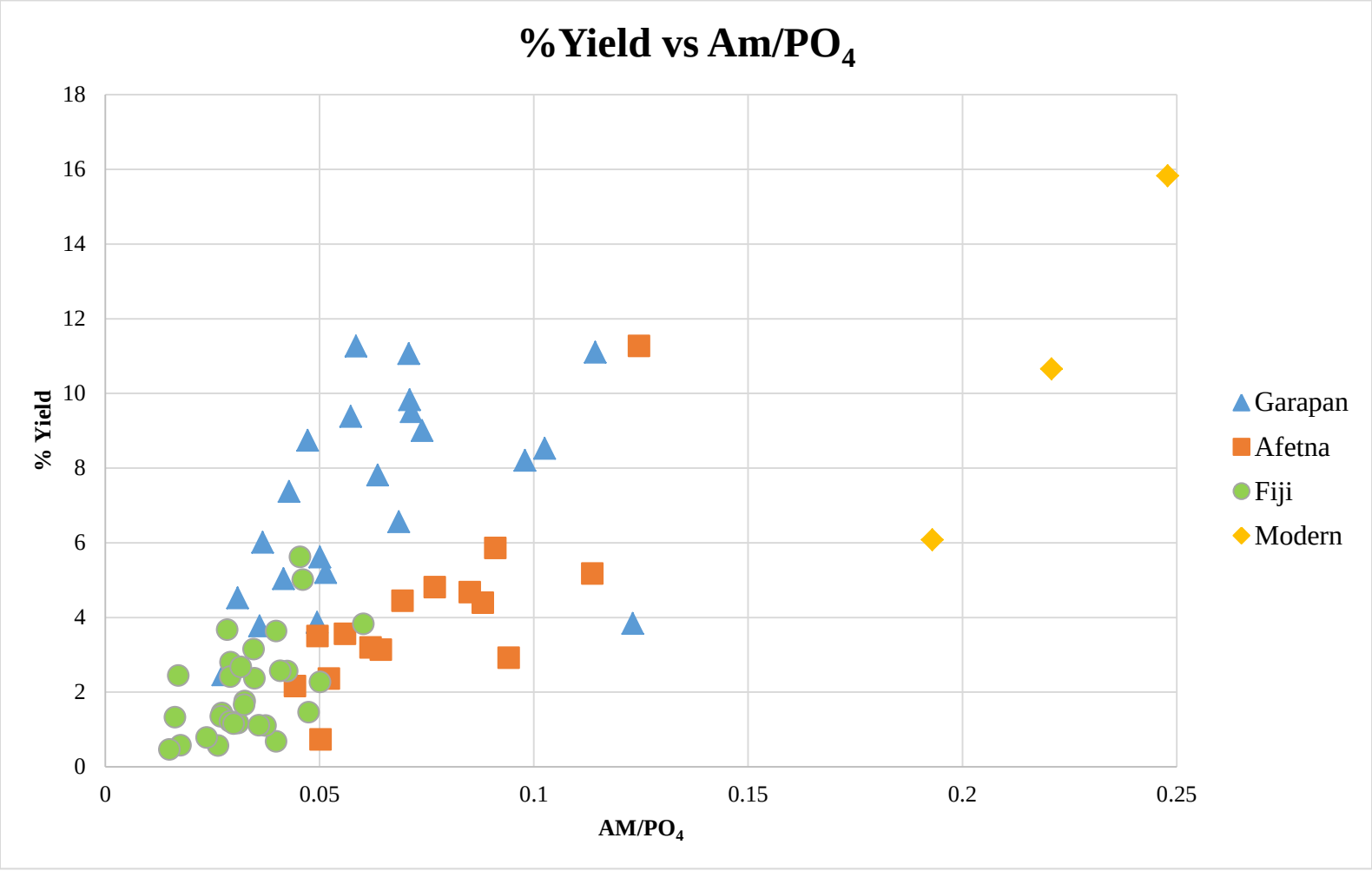

Figure 17: A comparison of Am/PO_4 and % yield of collagen extracted. Note a clear distinction between archaeological samples and modern

Stable Isotope Analysis

Isotope analysis of this dataset from Garapan, Saipan, began in 2015. This initial analysis allowed assessment of methods to develop changes in protocol to produce more favorable outcomes. In 2015 the original dataset assessed consisted of 18 individuals, of those only 7 yielded enough collagen for IRMS analysis. When run in 2017 and 2018, the new protocol changed was changed in order to have less harsh demineralization phase, the molarity was adjusted from 1M to .5M and the temperature was adjusted from room temperature to 4°C and all elements produced weighable collagen. The list of isotope data for the Garapan and Afetna sites are located in Appendix A. All data produced good C/N range between 2.9–3.6, as well as good carbon (15.3–47%) and nitrogen concentrations (5.5–17.3%) (Ambrose et al, 1997). The mean value and standard deviation for $\delta^{13}C_{carb}$ for the Garapan dataset is -12.40±1.28, for $\delta^{13}C_{coll}$ -17.90±0.87, and for $\delta^{15}N$ 9.23±0.67. The mean and standard deviation for the Afetna $\delta^{13}C_{coll}$ is -18.92±0.63, and for $\delta^{15}N$, 8.92±0.50. The mean values for males and females, (with data) in the Garapan dataset showed little distinction and there was little variance in $\delta^{15}N$ within the dataset (Table 2 Appendix A).

Isotopic analysis includes comparison of consumer isotope ratios to those of food sources. This is done by comparison of $\delta^{13}C$ and $\delta^{15}N$ values of consumer collagen with those obtained from various food sources. Data provided in Ambrose et al. (1997) reflected a wide range of food sources collected from the Marianas (Figure 17). Represented are C_3 plants including breadfruit, taro, yams, coconut, and rice, C_4 plants included sugarcane and seagrass. Terrestrial animals represented are fruit bat, coconut crab, and land crab. Marine animals represented include isotope data from the giant clam, marlin, spiny lobster, dolphin fish, octopus, and a variety of both deep and lagoon fish. All carbon isotope data food sources have been

adjusted due to diet tissue fractionation (+5‰). Food $\delta^{15}N$ values were also adjusted due to trophic level (+3.5‰). Food sources have been adjusted for Seuss effect in their $\delta^{13}C$ (+1.3‰) as the samples were obtained in 1997 (Ambrose et al, 1997). When the collagen data from this study were incorporated into the food web, it plotted with the range that suggests consumption of primarily terrestrial animals, as well as C_3 plants. The range in which our $\delta^{13}C_{coll}$ fell is more than those produced by Ambrose et al. (1997). The Ambrose et al (1997) dataset displays lower in $\delta^{15}N$ values than the samples processed in this study. This suggest more consumption of lower level terrestrial items than our dataset. These differences in values suggest the need for additional isotope analysis.

Figure 18 is a bivariate graph of the $\delta^{13}C$ values in carbonate versus collagen. In this figure, **cluster 1** represents a 100% C_3 diet/protein, **cluster 2** represents 30% C_3 and 70% C_4 diet and >50% C_4 protein, **cluster 3** is consistent with a 50% C_3 and 50% C_4 diet, and exclusively marine protein, **cluster 4** reflects 70% C_3 and 30% C_4 diet, and ≥ 65% C_3 protein, and **cluster 5** shows 30% C_3 and 70% C_4 diet and ≥ 65% C_3 protein. (Froehle et al., 2010 & 2012). When plotted following this model, the archaeological samples from this study as well as those from Ambrose et al., (1997) produced values consistent mostly with that of cluster 4. Cluster 4 represents 70% C_3 and 30% C_4 diet, and mostly C_3 protein (≥65%) (Froehle et al., 2012). In this model, a large shift in $\delta^{13}C_{carb}$ (towards C_4 non-protein) in Ambrose et al.'s (1997) data was observed.

Figure 19 shows a multivariate analysis of the Saipan data used in this study and those from Ambrose et al (1997). This graph uses functions determined by Froehle et al (2012) to include all isotope data collected. Doing this allows analysis of $\delta^{13}C$ from both protein and energy sources to be compared against protein $\delta^{15}N$ values. When assessed using this method

we are able to see the cause for the differences in $\delta^{13}C$ in our dataset and that of Ambrose. The location of Ambrose's data on this is indicative of consumption of sugarcane as opposed to seaweed. It appears that our dataset had little to no consumption of either of these plants.

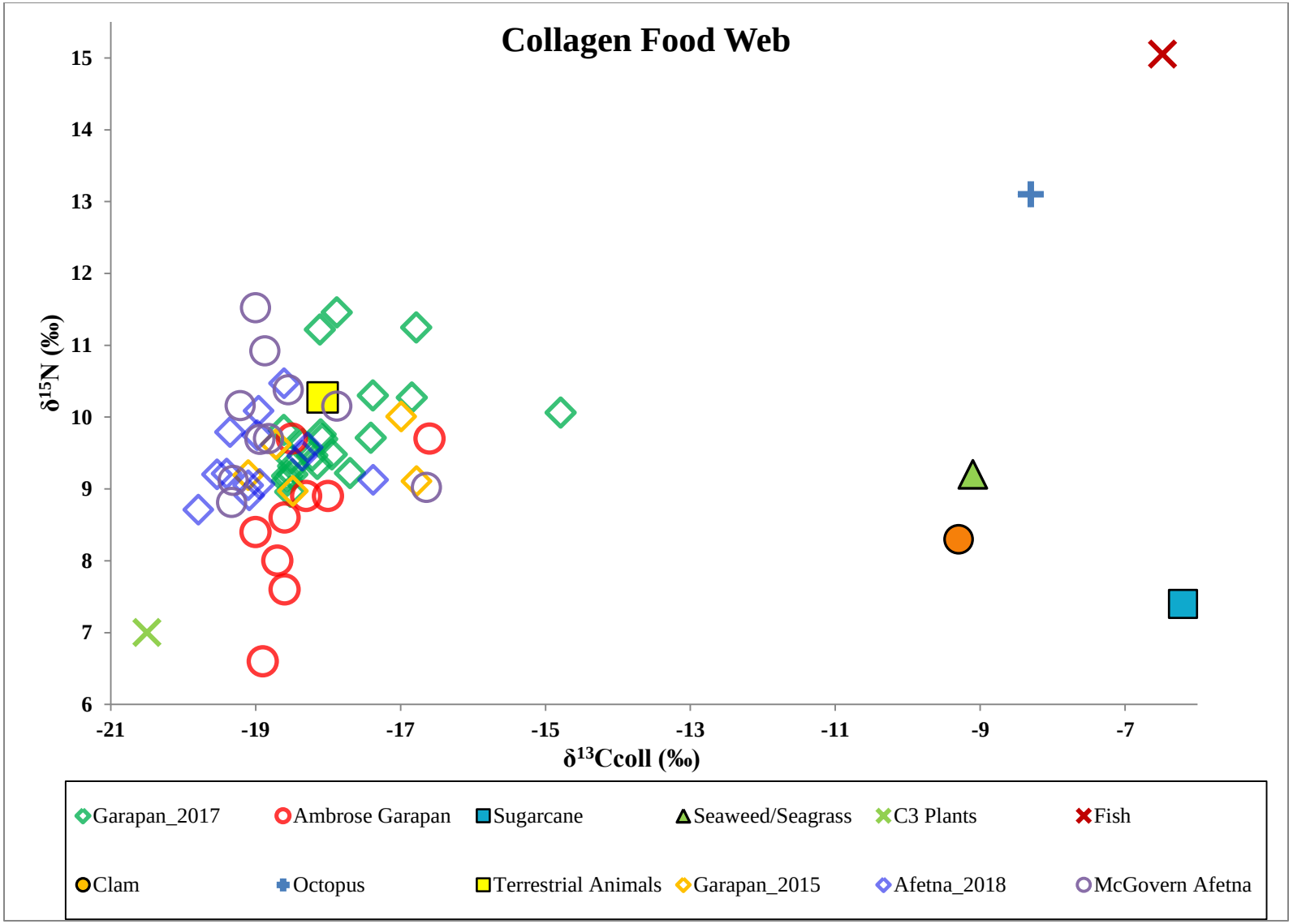

Figure 18: Collagen data food web for the archaeological samples used in this report from both 2015 and 2017, with comparison to Ambrose et al., (1997) data on archaeological samples from Rota, Guam and Saipan. Food source data was provided by Ambrose et al., (1997) Food sources have been adjusted due to diet tissue fractionation (+5‰). Food sources have been adjusted for Seuss effect (+1.3‰).

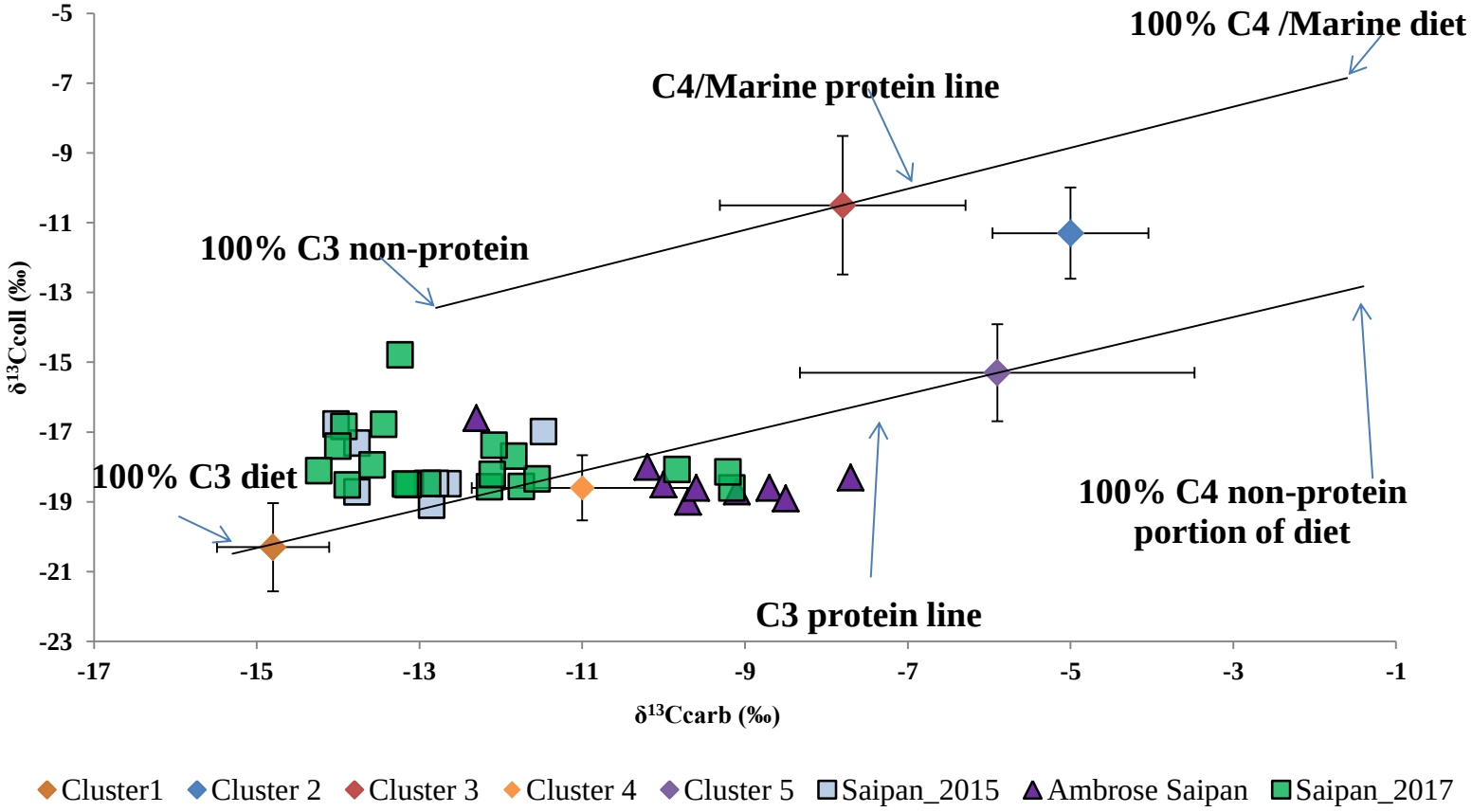

Figure 19: Bivariate Comparison of $\delta^{13}C$ of Carbonate vs. Collagen of archaeological samples from this study with comparison to Ambrose et al, (1997). Regression lines and clusters (Froehle et al., 2010 &2012).

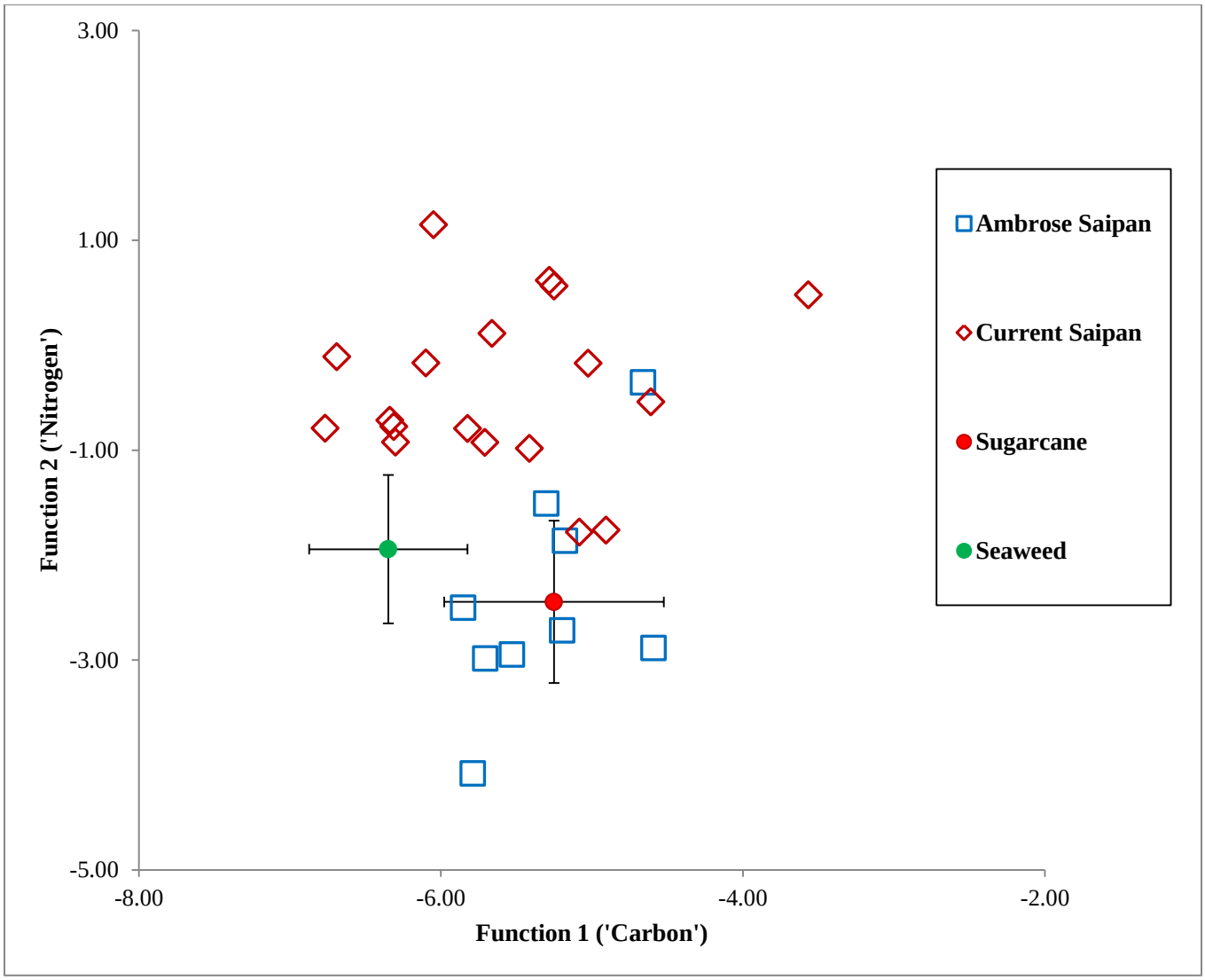

Figure 20: Multivariate analysis of $\delta^{15}N$ and $\delta^{13}C_{carb\text{-}coll}$ of both our dataset and those produced by Ambrose et al., Model and functions provided by Froehle et al., (2012)

Chapter 4: Discussion

While stable isotope analysis is a common practice in the various sciences, preferential methods for extraction of both collagen and carbonate are employed based on the discretion and experiences of the institution. While different methods for collagen and carbonate extraction are very similar, it is important to develop a method that suits the particular circumstances. As in this study, the methods we use today are a result of a series of both trials and errors. Through this experience, I developed a better understanding about the functionality of each step in the protocols; this allowed us to determine what situations could be done differently to produce more favorable outcomes. All data were subject to proper adjustments due to fractionation during analysis. The same can be said about the methods used in order to integrate the data produced via FTIR-ATR.

Bone Diagenesis

The first projected outcome was to determine a method of assessing bone diagenesis using the FTIR-ATR. This was done using a comparison of spectra derived from modern bone elements collected in Eastern Idaho in 2015 and those from samples that have been subjected to diagenetic alteration. Preliminarily we have determined that there are spectral differences between the two, using method for determining area and height of the Amide, Carbonate, Phosphate and Splitting factor ((SF1+SF2/SFV)). Differences were observed in Amide/Phosphate, Carbonate/Phosphate, and Splitting factors between the modern and archaeological in the dataset as shown in the various bar graphs. The mean for each were

different between archaeological and modern. The Am/PO_4 showed the largest distinction in mean value between the two sets.

In the study conducted by Beasley et al, (2014) the mean values and standard deviation determined for CO_3/PO_4 and Splitting factor respectively were 0.16±0.05 and 3.90± .037 for archaeological samples, and 0.31±0.08 and 3.07±0.026 for modern. From our data set the mean and standard deviation for CO_3/PO_4 and splitting factor were 0.177±0.039 and 3.76±.034 respectively for archaeological samples and for modern 0.202±0.019 and 3.474±0.069. While not identical, these two both show shifts in mean values between the archaeological and modern data sets. Similarities in mean values for archaeological data versus modern suggest that we have a method for assessing diagenesis that is similar to those presented in literature. Analysis of CO_3/PO_4 in bone elements allows us to determine the effect diagenesis has had on bone carbonate. When plotted together the two show no correlation, this indicates that our carbonate data has not been affected by diagenesis and only by diet (Figure 16).

As mentioned before, the largest distinction between modern and archaeological data was present when assessing the peak ratio of Am/PO_4. As there is no direct comparison in the literature for this ratio that assesses archaeological and modern data, it is difficult to have the same clarity on data validity. When compared to collagen percent yield the dataset showed clear separation between the archaeological and modern samples (Figure 17). There was a visible correlation between the two, as samples with low Am/PO_4 also had low percentage yield. From this, it appears as though this peak would most consistently show the largest distinction between modern and archaeological samples. I believe this is due to the fact that organic and inorganic bone material degrade at different rates, and that the organic components of bone are much more susceptible to diagenetic alteration and therefore values derived via Am/PO_4 are good indicators

of diagenetic alteration. Preliminarily we can report that in order to produce a usable amount of collagen, 5-6%, Am/PO_4 ratio must be over 0.0500. In order to better identify samples that have the potential to yield suitable collagen prior to the time consuming process of extraction, it is possible to incorporate them into our current plot and compare them to those that we have already assessed. While a direct comparison to archaeological elements that have failed to yield collagen is useful, a larger sample size is required to assess thresholds one can use to make decisions on sample quality.

Diet during Latte Saipan

The second goal of this thesis was to understand more about cultural and subsistence practices during the Latte period in Saipan via analysis of stable isotopes in bone elements. Extraction of bone carbonate and collagen produced data via IRMS analysis. When compared to the collagen food web (Figure 18) produced by Ambrose et al. (1997), our dataset falls within the range of many terrestrial protein resources including fruit bats and coconut crabs. On the same food web, our data also fell within the values consistent with consumption of C_3 plants including breadfruit, taro, yams, and coconuts. The $\delta^{15}N$ values between our dataset and those from Ambrose et al., (1997) suggests individuals in our dataset either consumed proteins from a higher trophic level or small amounts of marine protein. The latter suggests more equilibrium in protein and energy input into the diet of those in our dataset vs. Ambrose et al., (1997). An assessment of these values indicates a lower $\delta^{13}C_{coll}$ in our dataset. In order to get to the cause of this shift, further analysis of $\delta^{13}C$ of both whole diet and the protein portion of the diet was necessary. This was done via bivariate analysis of $\delta^{13}C$ in both carbonate and collagen in order to validate these claims. It showed alignment with cluster 4 (Figure 19). The diet reflecting in cluster 4 is consumption of 70% C_3, 30% C_4 non protein, and primarily terrestrial protein sources. In order

to investigate this further, a multivariate analysis was conducted (Figure 19). As mentioned before, inclusion of all isotope data in analysis is the most effective way to distinguish information that may not be visible in any one alone. Our data plotted against those produced by Ambrose et al, (1997) using the functions produced by Froehle et al (2012) showed no evidence of either sugar cane or seaweed consumption (Figure 20) and suggests nitrogen sources from higher trophic levels. This is in contrast to Ambrose's data, which cluster consistent with sugarcane consumption

I hypothesized initially that I would be able to determine the primary mode of subsistence and possibly cultural affiliation for the population represented by the individuals buried in Garapan via stable isotope analysis. Revisiting the accounts of Chamorro subsistence strategies, the data produced in this study is consistent. This analysis revealed that the diet of the individuals in this group consisted of primarily terrestrial resources, with a small influence from marine protein sources. This is indicative of the Latte period dietary habits that are known today. With known differential access to marine resources by lower classes, it is expected to see fluctuating $\delta^{15}N$ values within a population as a result. Individuals of higher class or with more access to marine resources would display higher values. Another potential indicator of rank in Chamorro society is dental modifications, the individuals assessed in this study that were recorded as having dental modifications showed no differences in isotope data from the ones without. Assessment of differences between males and females within the Garapan dataset revealed little differences in the diets between the two at this site (Table 2 Appendix A). The isotope data produced in this study suggests the individuals in the burial at Garapan had very similar diets as one another. This suggests the individuals could all belong to the same class.

The isotope data derived in this study from Garapan and Afetna fell into similar ranges, with reliance on primarily terrestrial animals with little influence from Marine resources. Lower $\delta^{15}N$ in Ambrose et al's (1997) data set suggests the individuals had less influence from marine resources. A possible reason for these differences is that the individuals assessed are from two different classes, with similar utilization of terrestrial resources, and varying usage of marine protein sources. As class effects utilization, also does availability, another possibility is that the individuals assessed in this study are from a time where environment favored the utilization of Marine resources. As these times of availability fluctuate, it is difficult to determine a chronological relationship between the different datasets. When compared, McGovern-Wilson and Quinn Afetna (1996) appear to have similar utilization of marine resources although higher in some individuals. This could be due to differences in protocol, as $\delta^{15}N$ values in that study were derived bone powder as opposed to collagen.

Chapter 5: Conclusions and Future Directions

Conclusion

The goal of this thesis was to gain more insight to the lives of the Chamorro during Latte Period in Saipan (AD1000-1521). Due to the large-scale changes that have taken place in the Marianas from European contact, it is important to begin to restore the knowledge that has since been lost. In order to accomplish this goal, I used stable isotope analysis in order to determine what the individuals excavated from Garapan, Saipan were eating. Knowing a person's diet speaks to what they individually may have been eating, as well as changes in food sources of a population. Isotope data allows for determining the primary mode of subsistence of a population. When compared to the food web and human isotope data produced by Ambrose et al. (1997) it was determined that the individuals in our dataset were almost exclusively consuming terrestrial plants and animals. Similar $\delta^{13}C_{coll}$ to Ambrose et al, Garapan (1997) suggests a potential for influence from a low protein C_4 or marine plant. Differences in the $\delta^{15}N$ values of the dataset suggest more protein consumption in our dataset. The shift in $\delta^{13}C_{carb}$ in the Ambrose dataset was proven to be due to sugar cane consumption. Our dataset showed no consumption of sugarcane or seaweed. Although our data revealed no consumption, it is possible that sugar cane is still a part of the diet in our Garapan dataset in low amounts, as low protein consumption could have caused the strong signature in the Ambrose dataset. During this time, it seems as though the primary mode of subsistence has been shifted to an agricultural base on a large enough scale for it to be their primary strategy, as well as a small reliance on marine proteins. Our data suggests a few things, all of which could and should be considered. 1. Individuals in our dataset could be from a higher rank than Ambrose et al., (1997) 2. The individuals excavated by SCS and used in this study are possibly from a different time in Garapan than the Ambrose et al., (1997).It is

unclear whether or not they are from before or after, but varying protein consumption and by extension availability as well as increased consumption of sugar cane suggests they were not consuming the same diet. Sugar cane was present in the Marianas from inhabitation, brought to the islands by the people from the ISEA (Dixon et al, 2010). This makes it difficult to discern possible reasoning for the increased presence of sugar cane in one dataset vs. the other. 3. There is also a possibility that the samples in this study are from the end of, or directly following, the Latte Period, this could be interpreted from the inclusion of primarily terrestrial food sources in the diet. As the Latte Period ended, the Chamorro had a small-scale agricultural base, but were supposedly still avid fisherman; this evidence is not supported in our isotopic data. The large influence of terrestrial resources on diet in this dataset as well as others in the Marianas suggests a different understanding of life ways and subsistence strategies in the Marianas during the Latte Period. These results indicate how isotope data outside of a diachronic perspective are difficult to interpret, as the influences of resource utilization such as environmental changes and resource availability are difficult to determine. My goal of shedding additional light on the story of Saipan and the Marianas is one that will be ongoing.

As the data in my thesis pertains to FTIR-ATR, I can say that we are moving in the right direction with understanding and interpreting the data it produces. I have successfully gotten consistent data throughout our datasets that reflect different values for archaeological samples than moderns in most aspects. When compared to results from Beasley et al., 2014, our data produced comparable results. Am/PO_4 comparison in modern and archaeological data suggests that it will reflect the most numerical changes with different levels of diagenesis. This allows me to say with a bit more comfort that we are able to see diagenetic alteration in human bone via FTIR-ATR.

Future Directions

When it comes to our goal of knowing more about the Chamorro of Saipan and the Marianas, additional data received from Afetna Saipan will undergo the sample stable isotope analysis as the Garapan, and used as additional data points moving forward. In the future, it would prove useful to submit the Garapan as well as those sampled from Afetna for radiocarbon dating. This will allow a better understanding of the chronology of these datasets as it pertains to major or minor shifts in modes of subsistence. An additional array of individuals from Latte Period burials would be useful in determining more similarities and differences within the Chamorro groups. In addition to this, I would also like to gain more data of food sources in the Marianas to determine any influences on diet that may not be represented. Without isotope data available for all of the food sources that were present on the islands, it is difficult to determine exact influences of each on diet.

As far as FTIR-ATR, our future directions are to be able to determine a threshold for each ratio that would determine whether a sample would yield usable data. This threshold would be determined after extensive additional modern and archaeological data collection. This will allow for a larger pool of assessment and to assess a wider range of diagenetic alteration. Developing this threshold, will allow for comparison of archaeological bone elements in the future, determining if they will produce a usable amount of data. This will effectively save on cost and time consumption processing unviable samples.

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Appendix A

Table 1: Stable Isotope Data

Sample ID	Sex	δ^{13}Ccarb	δ^{18}O	δ^{15}N	δ^{13}Ccoll	Coll N%	Coll C%	C/N
Garapan								
J6B13A	M	-11.92	-6.87					
J6B13B	M	-11.47	-7.83	9.51	-16.99	12.85	36.34	3.30
K6B1		-12.31	-5.64					
K6B3		-11.73	-5.48					
K6B30	M	-12.85	-6.70	8.69	-19.10	13.61	38.82	3.33
K6B5	F	-13.76	-6.40	9.12	-18.72	13.12	36.37	3.23
K6B6A	F	-12.65	-5.69	8.47	-18.48	13.86	38.69	3.25
K6B6B		-12.49	-6.47					
M8B4A		-10.21	-6.61					
M8B4B		-12.41	-7.49					
S14B8A	M	-13.10	-5.99					
S14B8B	M	-13.77	-11.90	9.18	-17.32	13.33	37.01	3.24
S15B15	M	-11.83	-9.79					
S15B22	F	-14.02	-8.24	9.62	-16.78	15.02	40.69	3.16
T14B10	M	-12.80	-6.32	8.61	-18.47	13.87	38.66	3.25
Z11B17		-11.56	-6.30					
Z11B3A.1	SA	-11.71	-6.19					
Z11B3A.2	SA	-13.21	-5.90					
J6B12(1)	M	-13.44	-7.04	10.75	-16.78	13.22	36.32	3.20
J6B12(2)	M	-13.92	-7.31	9.77	-16.84	15.46	43.06	3.25
J6B12(3)	M	-14.00	-9.88	9.21	-17.41	15.37	42.98	3.26
K6B18(1)	M	-12.90	-6.32	8.82	-18.48	15.45	42.99	3.24
K6B18(2)	M	-13.88	-7.08	8.46	-18.52	15.33	42.61	3.24
K6B18(3)	M	-13.14	-6.51	8.92	-18.51	14.87	41.66	3.27
K6B18(4)	M	-11.75	-6.54	8.68	-18.56	14.29	40.09	3.27
K6B18(5)	M	-13.17	-6.57	8.70	-18.49	15.10	41.86	3.23
K6B18(6)	M	-12.14	-7.16	8.61	-18.57	15.61	43.06	3.22
K6B8A	M	-9.16	-5.34	9.32	-18.61	14.69	40.86	3.24
M8B2(1)		-9.83	-6.86	9.19	-18.08	14.80	41.52	3.27
M8B2(2)		-9.21	-8.93	8.84	-18.15	15.30	42.76	3.26
M95A	F	-11.55	-6.51	9.12	-18.35	15.67	43.38	3.23
Q14B2	F	-11.84	-7.01	8.72	-17.70	12.38	33.58	3.16
Q14B2				9.26	-18.10	13.36	38.00	3.32

S14B37(1)	F	-13.24	-8.63	9.56	-14.79	13.41	38.15	3.32
S14B37(2)	F	-13.58	-7.02	8.98	-17.95	15.22	42.52	3.26
S14B37(3)	F	-12.08	-6.81	9.80	-17.38	14.65	40.76	3.25
S14B37(4)	F	-12.10	-6.67	8.96	-18.22	14.73	39.74	3.15
Z11B3A(1)	SA	-14.23	-4.73	10.72	-18.11	14.12	38.35	3.17
Z11B3A(2)		*	*	10.96	-17.88	13.81	38.71	3.27
Modern1		-10.21	-6.19	12.41	-18.16			
Modern2		-12.26	-7.79	11.45	-16.81			
Modern3		-14.50	-11.50	11.24	-17.86			
Afetna								
ND8				9.59	-18.96	13.26	37.23	3.27
ND9				8.96	-18.35	12.95	36.77	3.31
ND37				9.25	-18.98	13.62	39.03	3.34
ND37-2				8.71	-19.40	12.55	36.43	3.38
JE005				8.57	-18.94	13.41	37.97	3.30
JE06				8.55	-19.10	17.84	52.84	3.45
JE53				8.21	-19.79	12.89	37.63	3.40
JE72				8.63	-17.38	13.33	37.85	3.31
JE73				9.97	-18.60	12.45	36.36	3.41
JE73-2				8.70	-19.53	12.97	37.73	3.39
JE81-2				8.41	-19.08	13.06	37.42	3.34
BC14				9.29	-19.35	13.12	37.89	3.37
AL6				9.08	-18.28	13.31	38.28	3.35

Table 2: Male vs Female Mean and Standard Deviation

Garapan	δ^{13}Ccarb	δ^{15}N	δ^{13}Ccoll	Coll N%	Coll C%
Males	-12.66±0.745	9.09±0.613	-18.05±0.811	14.50±0.960	40.45±2.580
Females	-12.87±0.907	9.21±0.434	-17.58±1.293	14.46±0.904	40.04±2.307