

University of Alberta

After the Earthquake: Dietary Resource Use During the Hellenistic, Roman, and Byzantine Periods at Helike, Greece

by

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Abstract

After a devastating earthquake and tsunami destroyed the Classical city of ancient Helike in 373 BC, the surrounding coastal plain was resettled and was continuously inhabited from the Hellenistic to the Late Byzantine periods. Twenty-eight individuals associated with these post-earthquake periods were analyzed for their bone collagen stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values. The results suggest that diet at Helike was based mainly upon terrestrial C_3 plants and animals, with evidence for varying amounts of marine resource use between the time periods. Temporal differences in the human stable isotope values are attributed to the seismic activity of the Helike region, which resulted in the emergence and disappearance of several lagoons during antiquity, including one that had formed directly as a result of the earthquake in 373 BC.

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Chapter 1: Introduction

Stable carbon and stable nitrogen isotope analysis of bone collagen can successfully distinguish between various types of past dietary resource use, including those involving marine and terrestrial resources. With an appropriate sample, it can also be used to detect changes in resource use over time. Stable isotope dietary reconstruction methods have an advantage over some other sources of information: the analysis of individuals' tissues provides insight into what they were actually consuming in contrast to methods that use indirect evidence, such as archaeological food remains or historical documentation.

This study determines the stable carbon ($\delta^{13}\text{C}$) and stable nitrogen ($\delta^{15}\text{N}$) values of bone collagen from 28 individuals from the archaeological site of Helike, Greece. This site is valuable because the area shows evidence of being continuously inhabited from the Hellenistic to the Late Byzantine period. This continuity allows stable isotope analysis to detect any changes in dietary resource use over time while minimizing the effects of population transitions that might complicate the interpretation of results. By using a sample composed of burials from the same area over a fairly large time span, any changes in resource use become easier to detect as part of an overall trend. Since some of the individuals in this study pertain to the Byzantine period, there is the possibility that the religious dietary fasting rules of the Orthodox Christian church began to affect food choice. If this is the case, we would expect to see an increase in marine resource use during the Byzantine period, as many fishes and other seafood were considered acceptable meat and dairy substitutes on fasting days and would have been available to the people living at coastal Helike. Comparison of the Byzantine individuals to the earlier Hellenistic and Roman period individuals will provide a frame of reference to detect any such changes.

This study also looks at diet and resource use in past Greece as a whole, using this particular site to add more information to existing stable

isotope studies. The typical Greek diet is thought to be based upon the “Mediterranean triad” of foods, which consists of cereals, wine, and oils (Dalby, 1996). The importance of meat and marine resources to the general population is not as clear and there are conflicting ideas about these in the literature. By examining dietary resource use at Helike, this study will add to the existing stable isotope knowledge of diet in past Greece and provide an area- and population- specific perspective on this question.

In order to best interpret the stable isotope values at Helike, it is necessary to examine the broader environmental, historical, and dietary cultural contexts associated with the time periods considered in this study. Chapter One introduces the study. Chapter Two discusses what is currently known about diet in Classical, Hellenistic, Roman, and Byzantine Greece, beginning with an examination of the environment and topography of Greece and followed by the general historical events that were occurring during these periods. Various sources of information about diet in ancient Greece are discussed, including those with historical, literary, zooarchaeological, and stable isotope analysis perspectives. Where available, archaeobotanical information is also provided. A brief discussion of the fasting and feasting practices associated with the Byzantine Orthodox Christian church contributes further information about this aspect of diet.

Chapter Three discusses the basic theory and techniques involved in archaeological stable isotope analysis of carbon and nitrogen, with a focus on the application of these techniques to dietary reconstruction. Some stable isotope values found in terrestrial, marine, freshwater, and lagoon environments are presented as examples of the isotopic patterning that may be present in these types of resources. Additionally, extra-dietary variables that may affect the interpretation of stable isotope results are identified and discussed, as are the archaeological applications of stable isotope analysis to human remains.

Chapter Four describes the archaeological site of Helike, the human individuals that were selected for analysis in this study, and the faunal

samples that were used to establish a local baseline food web value. Faunal samples from Late Roman Corinth were added to this baseline to better reconstruct terrestrial resource values; these are discussed further in section 4.2.2. In order to analyze the bone collagen for its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, it is necessary to separate it from the rest of the bone and to ensure that any extraneous substances or contaminants are removed. The last part of Chapter Four discusses the methods generally used in collagen preparation, as well as commonly used indicators of sample diagenesis, and describes the process used to extract collagen from the Helike and Late Roman Corinth samples in this study.

Chapter Five contains the results of the stable isotope analysis of the Helike and Late Roman Corinth samples. Collagen quality indicators were used to decide which samples were of sufficient quality to consider in the interpretation of the results. The final data set for both the human and the faunal samples is described.

Chapter Six provides the interpretations of the human and faunal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within the context of dietary reconstruction. The Helike human values are compared to the faunal values in order to understand general trophic level relationships at the site. The results indicate that the individuals at Helike subsisted mainly on a C_3 terrestrial diet with the addition of varying amounts of marine protein. The human values are then examined to detect any variation apparent according to the age and sex category of the individual and to detect any temporal variation. There are no significant differences between the age categories or between the sex categories, although the sample sizes associated with these groups are small. The individuals from the Hellenistic period are set apart from those from the Roman and Byzantine periods in that they show significantly higher stable carbon and stable nitrogen isotope values. In section 6.4, the Helike human collagen values are compared to food value reconstructions established for Byzantine Greece by Bourbou et al. (2011); this comparison allows further refinement in the types of resource use that can be interpreted from the

stable isotope values. When considering the range of food resource values, the Hellenistic individuals show evidence of marine resource use consistent with the addition of smaller fishes to the diet. The Roman individuals appear to have consumed a diet based mostly on terrestrial resources with little evidence suggesting the addition of marine resources, while the Byzantine individuals show values intermediate between the other two groups. The results from Helike are compared to the other contemporary Greek and Mediterranean sites discussed in Chapter Two and general patterns of resource use are identified. Finally, the original research questions about dietary resource use and diachronic stable isotope patterns at Helike are addressed.

Chapter Seven provides the conclusions of this study, which include the suggestion that changes in the landscape of Helike contributed to variation in the diets of the individuals, as the availability of local lagoon resources came and went over time.

Chapter 2: Diet in Classical, Hellenistic, Roman, and Byzantine Greece

This chapter provides information about the environment and topography of Greece, a general background of Greek history, and details about food and diet in past Greece according to various sources. The historical, literary, archaeobotanical, zooarchaeological, and stable isotope data are discussed according to each time period. The Classical period is included in this discussion because of the dietary practices and ideas that have stemmed from this time and to provide a related background for the following Hellenistic period. The specific historical, cultural, and geographical factors can be understood as part of the context of the individuals living at Helike during the Hellenistic, Roman, and Byzantine periods. A review of the relevant reconstructions of diet provides evidence about what is known about past Greece from the isotopic literature.

2.1. The Topography and Climate of Greece and the Peloponnese

Understanding of the environment and topography of Greece and the Peloponnese region allows a more accurate interpretation of past dietary resource use. This section focuses on the overall physical layout of the region, the history of seismic activity associated with this area, and the suitability of many parts of Greece for certain types of subsistence.

2.1.1. Topography

Greece, although composed of a diversity of regions and islands, is largely a mountainous country containing small valleys and rivers that fluctuate with the seasons (Palmer, 2010). Modern-day Greece shares its northern borders with Albania to the northwest and Turkey to the northeast, with Macedonia (FYROM) and Bulgaria in between. The majority of the country, however, consists of coastline and thousands of islands along and within the Ionian, Mediterranean, and Aegean seas (Hughes, 2010).

Earthquakes, volcanic eruptions, and tectonic collisions are the major geological processes that have shaped Greek lands and islands (Hughes, 2010). Earthquakes have always been common and some volcanoes are still active today, most famously that of Thira/Santorini (Hughes, 2010). While some soils are volcanic in nature, most are derived from limestone and are prone to erosion which has created challenges for farming and resulted in the use of terracing and other anti-erosion agricultural techniques (Hughes, 2010).

The Peloponnese or “Island of Pelops” is actually not an island but is the southwestern land mass attached to the mainland of Greece by the narrow Isthmus of Corinth (Higgins and Higgins, 1996). It is bordered by the Ionian Sea to the west, the Mediterranean in the south, and the Aegean Sea to the east (Weerakkody, 2010). It has a more Mediterranean climate along the coasts and a continental climate in the mountainous interior (Weerakkody, 2010). The Gulf of Corinth separates the rest of the mainland of Greece from the northern coast of the Peloponnese. It is a mountainous region, with outcroppings surrounding the central district of Arcadia and expanding northwards towards the Gulf (Higgins and Higgins, 1996).

Within the northwestern and central parts of the Peloponnese are the areas of Elis, Achaia, and Arcadia (Higgins and Higgins, 1996). Achaia, the province within which ancient Helike existed, extends along the south shore of the Gulf of Corinth. It consists of a narrow and very fertile coastal plain backed by the Arcadian mountains and is watered by various streams that dry up in the summer and are replenished by the winter rains (Higgins and Higgins, 1996). It has relatively few harbours and since the mountain chains hindered land travel, some communities may have been rather isolated in ancient times (Weerakkody, 2010).

Seismic activity is a common occurrence in Achaia due to the presence of fault lines concentrated in the eastern part of the Gulf of Corinth, along the northern coastline of the Peloponnese, and around the city of Aigion. Many earthquakes and tsunamis have been recorded, including the

major event that destroyed ancient Helike in 373 BC. Similar earthquakes also occurred in 1402 AD and 1881 AD, caused by tectonic movements along the same fault line (Higgins and Higgins, 1996). A total of 6.5 m of geologic movement is estimated to have occurred in the past 5000 years in this area (Higgins and Higgins, 1996), affecting the topography over time.

2.1.2. Climate

Greece lies within the Mediterranean climate zone and experiences relatively short spring and autumn periods and longer winters and summers, which are the two main seasons. Winter is cool and rainy, while droughts and high temperatures occur in summer (Reger, 2005; Mariolopoulous, 1961). The Peloponnese is one of the least cloudy areas in Greece and gets up to 3000 hours of sunshine per year (Mariolopoulous, 1961). While climate in general is described as being fairly uniform across Greece, variability does exist within its regions and microclimates and flora and fauna vary greatly across the country (Hughes, 2010; Weerakkody, 2010).

Due to the generally mountainous nature of Greece, only one-fifth of the land is suitable for cultivation (Hughes, 2010; Palmer, 2010); the soils tend to be mostly poor and water shortages are common. The mountain ranges create variations in the amount of annual precipitation that each region receives; generally, the western and northern parts of the country receive more rain than the eastern and the southern regions (Mariolopoulous, 1961). The annual rainfall ranges from approximately 1400 mm in the mountain regions of western Greece to 600 mm in Athens, with high variability occurring elsewhere in the country (Hughes, 2010). Reger (2005) states that since wheat requires at least 300 mm of precipitation during each growing season and barley at least 200 mm, crop failures caused by unpredictable annual rainfall must have been common occurrences in the ancient world. Crop failures due to drought have been recorded throughout

Classical and Byzantine times in Greece, notably in Athens during 1074 AD, 1180 AD, and 1182 AD (Mariolopoulous, 1961).

2.2. The History of Greece

Food choices are not only influenced by internal factors such as individual preferences, but also by external events that affect food availability and distribution. Political and historical events such as wars, regime changes, and power struggles affect how food shortages and crises emerge and perhaps influence quality of life even more than periodic crop failures, which were easier to prepare for. Exposure to different cultural practices via emigration and trade also can introduce new foods into the dietary repertoire. A brief summary of the history of Greece from the Classical period to the end of the Byzantine period illustrates some of the events that may have influenced resource use for the population residing at Helike.

2.2.1. Classical Greece

The Classical period (480 to 323 BC) is one of the most celebrated eras in Greek history. Famous monuments, such as the Parthenon, were built and playwrights and philosophers like Aeschylus, Sophocles, Euripides, and Aristophanes all contributed to the formation of a Greek identity through their writings (Doumanis, 2010; Rhodes, 2010). External events, including the Persian Wars of 490-479 BC, had also shaped ideas about who was Greek. The *polis* or city-state had already become integral to Athens and Sparta, but in the Classical period, these city-states formed alliances and leagues in order to battle against the Persians. Despite this cooperation, interstate conflict remained commonplace after the Persian Wars as the thirty-one *poleis* competed against each other for power and prestige, most notably Athens against Sparta (Rhodes, 2010).

During the Classical period, the political system in Athens developed into a full democracy. While prominent individuals like Pericles possessed

special skills and were valued for their military leadership, the Athenian citizenry resolved all other matters of public interest (Rhodes, 2010). Democracy was not a permanent political system in Classical Greece, however; after Sparta defeated Athens, handpicked oligarchies replaced the democracies in the Peloponnese (Doumanis, 2010). Anti-Spartan alliances and leagues formed and despite Sparta's attempts to maintain control, the Peloponnese remained politically unstable until Thebes emerged as the *hegemon*, or leader state, in the area (Doumanis, 2010; Rhodes, 2010).

In 359 BC, the democratic system and the city-states suffered another blow when Philip II of Macedon headed across the southern Balkans and into Thessaly in northern Greece. By 338 BC, he had forced most Greek states into an alliance with Macedon and established a system of monarchies that further decreased the independence of the city-states (Doumanis, 2010).

After the assassination of Phillip II in 336 BC, political instability resulted and his son, Alexander the Great, faced challenges to his succession. He eventually overcame his political rivals, quelled the opportunistic Greek revolts, and restored his authority as the Macedonian king (Doumanis, 2010; Rhodes, 2010). Alexander's military prowess led to the conquest of much of the known world at the time and to the spread of the Greek language abroad. His death in 323 BC marks the end of the Classical period in Greece (Doumanis, 2010).

2.2.2. Hellenistic Greece

Although there is some debate in the literature, the start of the Hellenistic period generally dates from Alexander the Great's death and ends with the overthrow of the Egyptian Ptolemaic kingdom by Rome in 30 BC (Erskine, 2005). After Alexander's death, his would-be successors fought amongst each other and struggled for power (Braund, 2005). From 323 to 281 BC, skirmishes and battles were fought in Greece, Asia Minor, the Mediterranean Sea, Egypt, Central Asia, Iran, and India; recurrent alliances and "peaces" were frequently adopted and then abandoned (Braund, 2005).

Eventually, three major dynasties emerged after the Battle of Ipsos in 301 BC, as Alexander's former generals Antigonus Monophthalmus, Seleukos, and Ptolemy established some measure of control (Ager, 2005). Antigonus ended up ruling parts of Greece and Macedon, Seleukos controlled Asia Minor, and Ptolemy took power in Egypt (Braund, 2005).

The Antigonids were the weakest of the three dynasties, but it was the Seleucid dynasty that ended up being shaken the most by events such as the Second and Third Syrian Wars. The third century was a time of turmoil for the Greek cities in Asia Minor (Ager, 2005), and further west, the Achaeans wavered between loyalty to the Macedonians and support of anti-Macedonian factions. Attempts to reunite the Peloponnese in a League under Antigonid leaders was viewed by many Achaean citizens as a political gambit and an unrealistic way for the Greek *poleis* to obtain political power (Braund, 2005).

Eventually, a balance of power between the city-states emerged in the third century BC; however, this balance was based more upon tension between the major players than upon any real cooperation (Ager, 2005). Monarchy, with its roots in Macedonian and local practices, emerged again as the main form of government (Braund, 2005). Even though foreign control and local despots had replaced the democratic *polis* system, the cities seem to have flourished overall and civic duties continued to be a part of a citizen's life.

The spread of Greek language and cultural practices and the establishment of Greek cities continued throughout Alexander's former territories over the next century, but Roman warships and arms also started appearing in Greece in the later part of the Hellenistic period (Derow, 2005). The Romans ended up defeating the Macedonian monarchy in 167 BC and despite promises of freedom, no taxation, and no garrisons (Pobjoy, 2006), Roman control over the city-states ended up increasing so much that the Greeks could not do anything else but 'give heed to the Romans and obey them in their orders', according to the writer Polybius (Derow, 2005). Greek

attempts to resist Roman rule came to a head in 146 BC, when the Achaean League warred against Rome. This ended in defeat for the Greeks, the destruction of Corinth, and the League's dissolution (Derow, 2005). The Romans punished the Achaean League members by destroying their city defenses, imposing harsh tributes, prohibiting rich Greeks from holding property outside of their own city-state, and forcing the payment of indemnities to the pro-Roman Greek cities that the Achaean League had attacked (McGing, 2005). The warring kingdoms of Macedon and Illyria were also dismembered and Greece formally came under Roman rule in 146 BC. Governance changed from the Hellenistic-era monarchies to a system of Roman senatorial commissions and governors (McGing, 2005).

While some of the interactions between Greece and Rome were violent, many were not and cultural diffusion occurred between the two, including the adoption of some Roman religious cults in Greece (McGing, 2005). Over the course of the second century BC, Roman domination appears to have been increasingly accepted by the Greeks (McGing, 2005). This was likely due to the fact that, for most Greeks, little had changed under Roman rule. As long as they paid their taxes and any internal conflicts did not threaten Rome's rule, life went on as usual (McGing, 2005). The risks and consequences of battling the Romans were made even more explicit during the Mithridatic Wars in Asia Minor. After these events in 63 BC, Rome was forced to become much more politically involved in the east in order to maintain its position and interests in the region; it could not longer afford to take a "hands off" approach to governance in these areas. As a result, the Greek East in a political sense disappeared and the foundations of the Eastern Roman Empire were formed (McGing, 2005).

Despite the decline of the democratic city-state during the Hellenistic period, more and larger Greek cities existed than in the Classical period and inter-city contacts and relations remained important to maintain (Billows, 2005). The majority of Greek people during this time are argued to have been

small-scale subsistence farmers who were connected through the *polis* and its market to the wider Hellenistic world (Reger, 2005).

The defeat of the last Ptolemaic dynasty in Egypt in 30 BC by Augustus Octavian is traditionally viewed as the end of the Hellenistic period (Erskine, 2005). Even though the Greeks went through long periods of political instability and ended up as a protectorate of Rome, the Hellenistic period also had more positive outcomes, characterized by the growth of Greek cities and the spread of the Greek-speaking world. These both contributed to the formation of the later Eastern Roman Empire.

2.2.3. Roman Greece

As mentioned above, Roman interests had existed in Greece since the second century BC when Rome replaced Macedon as the main force in the Balkans (Alcock, 1993). The Roman period in Greece is marked by the more formal creation of the Roman province of Achaea in 27 BC. This province was roughly equivalent in area to modern Greece without the inclusion of Crete, Macedonia, and Thessaly (Alcock, 1993). The later centuries of the Roman period are discussed below; this section focuses on some of the characteristics of early Roman rule in Greece.

Once most of the resistance to Roman rule in Greece subsided, Roman officials became more involved in the workings of several of the Greek cities. Although the federal leagues of the Hellenistic period were still in existence, they were of secondary importance to the cities in terms of governance, as provincial administration occurred mainly through the urban centres (Doumanis, 2010; Alcock, 1993). The Roman governor and his staff administered the province of Achaea, while the day-to-day operations of the city-state remained the work of civic leaders (Alcock, 1993). Achaea was considered to be an “unarmed” province and held a mostly non-military role within the empire. The absence of any significant Roman military forces in the area meant that the local elites of Achaea took on the work of enforcing the Roman imperial authority. Maintaining good relations with Rome helped

these individuals retain their positions and status (Alcock, 1993), while *polis* autonomy helped maintain imperial governance through the perpetuation of self-financing and self-governing cities (Doumanis, 2010).

Publicani or tax collectors were present in Greece during the Republican era, but due to the disruptions of that period, the predictability and regularity of tax levies and the effects of taxation on the Greeks are unknown. The provincial taxation systems were probably diverse, but evidence specific to Achaëa is not available (Alcock, 1993). Military levies were infrequent, except under Marcus Aurelius and Caracalla, who both levied for recruitments during their various campaigns. Achaëa's lack of military strategic importance had both economic drawbacks and benefits. While monetary resources, provisions, or troops might occasionally be drained from the province, it was largely sheltered from the economic fluctuations that could result from Rome's military actions (Alcock, 1993). In general, Roman rule created greater stability and reduced interstate conflicts, as the provinces were unified under Roman law and the *Pax Romana*, or "Roman Peace" (Doumanis, 2010).

Settlement patterns during the early Roman period in Greece may have undergone some changes as well. Some evidence suggests that there was a drop in the number of rural sites in Roman Greece as compared to the Classical and Hellenistic periods; this may reflect either the abandonment of rural settlements for the cities or the effects of warfare on mobility patterns (Doumanis, 2010; Alcock, 1993). *Oliganthropia*, or depopulation, was a voiced concern of Greek inhabitants during the first century AD, although likely linked to those individuals initially unhappy with Roman rule (Doumanis, 2010). With the possible increase in urban residence, pastoral activity also appears to have changed during the Roman period as herds were able to travel more freely to distant holdings and were not limited to grazing on the small landowners' fields. There was increased interest in the specialized pastoral production of cattle and horses and greater profitability in engaging in pastoralism, both of which may have encouraged people to

reside in larger communities instead of single-family rural dwellings (Alcock, 1993) and encouraging the growth of large estates (Doumanis, 2010).

Others have argued that the apparent decline in rural settlements, which is based upon archaeological survey studies, did not happen uniformly throughout Greece and may not be directly linked to the effects of Roman rule (Rousset, 2008). Interestingly, crop intensification and specialization do not appear to have been widespread in early Roman Greece, although fertile and productive lands continued to be farmed. This contrasts to the pattern of agricultural intensification reported elsewhere in the Roman Empire (Pobjoy, 2006). It may be due to the fact that many Greek elite urban landowners had sources of income other than farming and were not overly motivated to have their tenants increase agricultural production.

The period of the *Pax Romana* appears to have been fairly uneventful for Greece (Doumanis, 2010). The end of the peace and the ensuing struggles by Rome to maintain and defend its borders did affect Greece, however. Gothic raiders entered the Aegean in the third century AD and sacked Corinth, Argos, Thessalonica, and Sparta and destroyed the region of Thessaly (Doumanis, 2010). The need of the Roman Empire to quickly and efficiently mobilize resources eventually led to the centralization of administration at the expense of *polis* autonomy and rule by the local elites (Doumanis, 2010).

Despite Rome's best attempts, its political and economic power started shifting from the declining Western Roman Empire to the Eastern Roman Empire and became centred on Greek-speaking Byzantium in Asia Minor. Because of this gradual transition, the last centuries of the Roman period are interchangeably referred to as either being Late Roman or Early Byzantine (Treadgold, 1997; Cameron, 1993). In the following section about the general history of the Byzantine period, there are details of what might be considered by others to be Late Roman period events.

2.2.4. *Byzantine Greece*

The Early Byzantine (or Late Roman) period is marked by the transition of power from the Western Roman Empire to the newly created Eastern Roman Empire. In the third century AD, Rome was having difficulties maintaining and securing its eastern provinces. A plague had reduced the number of taxpayers contributing to Rome's coffers, the Parthians were invading in the east, and the Germans were making incursions from the north (Gregory, 2010; Treadgold, 1997). These issues combined to create difficulties in recruiting and paying the Roman army, which was crucial in defending Rome's borders. As its territories began to shrink and its currency became devalued, the Roman Empire entered into a final decline (Treadgold, 1997; Cameron, 1993). In the years after 284 AD, the emperor Diocletian attempted to stabilize the Roman government and the declining empire by introducing major reforms, including an administrative division between the western and eastern halves of the empire (Treadgold, 1997). Many of Diocletian's reforms were kept and reinforced by Constantine (324-337 AD), the Roman Christian emperor who is credited with the creation of the Byzantine Empire (Gregory, 2010). He re-founded the Greek city of Byzantium (later named Constantinople) and it became the capital of the Eastern Roman Empire in 330 AD (Treadgold, 1997).

Within the Byzantine Empire, religion and state were highly intertwined. Christianity was the official religion and Christians were preferentially appointed to civil and military offices as political power and the church became explicitly linked to each other (Gregory, 2010; Treadgold, 1997). The split of eastern orthodoxy from the western Christian tradition also ensured that religious boundaries reflected the geographical ones. Archaeological evidence indicates that the Eastern Empire underwent a population increase and agriculture intensified around this time, supporting the idea of eastern prominence (Cameron, 1993). Under successive emperors and administrations, the Byzantine Empire remained in flux, albeit with more stability and prosperity at some times than others. For the first several

centuries AD, at least, it remained fairly robust and was able to recover several times from territory losses to foreign forces, most notably under Justinian I (527-565 AD) (Treadgold, 1997).

During the Early Byzantine period, taxes in Greece may have been paid in cash for the most part and would have funded the struggling Roman military. From the third century AD onwards, as the Western Empire continued to struggle, Greek taxation levels rose significantly enough that payments were also accepted in kind. This may have induced some urban individuals to move to rural areas where in-kind tax payments were easier to produce than in-cash payments (Alcock, 1993). In addition to this, a type of feudal system started emerging in Greece where people could be “fixed” to the land and have less freedom from the landowners to leave it (Alcock, 1993).

By the mid-fifth century AD, the population of the Byzantine Empire had become much more uniform than that of its western counterpart. Most of its subjects shared at least some major cultural characteristics and the majority followed the official faith of Orthodox Christianity (Treadgold, 1997). Even the economy became more unified within the empire as agricultural expansion reached its peak in several areas (Whitlow, 2008). Revenue sources were primarily land-based, although trade activities contributed a large proportion as well (Treadgold, 1997; Cameron, 1993). Much as in the earlier Roman period, the cities depended on the rural areas for revenue and the majority of the Byzantine population was engaged in agricultural production (Cameron, 1993). Some of the attention the administration paid to its subjects is evident in the taxation structures and tax laws of the time, which greatly affected the peasants and, to a lesser extent, the Byzantine urbanites (Treadgold, 1997). Both head taxes and income taxes were realities of the rural Byzantine world. Most of the peasants owned the land that they worked, but those who were in tenant-landlord relationships paid rent instead and the landlords paid the taxes on their behalf (Laiou, 2008; Treadgold, 1997). Landowners had incentives to

make sure that their tenants did not starve, as they benefitted from being the “middle man” in this arrangement. Not only an important source of taxation income for the administrative and religious branches of the Empire, rural populations also provided most of the food for the cities (Treadgold, 1997; Cameron, 1993).

In contrast, townspeople did not have to pay land or head taxes but certain trade duties were imposed on craftsmen, shopkeepers, laborers, and prostitutes, among others (Treadgold, 1997). Despite the hardships these duties might have created, enough rural-urban migration occurred to suggest that urban people had a slight financial advantage over rural areas (Treadgold, 1997). The Byzantine economy was chiefly agricultural throughout its existence, and as a result, the Empire remained dependent on the rise or fall of the rural population (Treadgold, 1997).

The sixth and seventh centuries AD have been referred to as the Byzantine “Dark Ages” (Gregory, 2010) because of the overall lack of stability and expansion within the Empire. Economic recessions occurred in Anatolia, the Balkans, and in the territories in Greece that still remained under Byzantine control. Urban sites show evidence of shrinking from large, sprawling cities to smaller, more fortified centres (Whitlow, 2008) and from the beginning of the 4th to the end of the 7th centuries AD, there appears to be an increase in dispersed settlements in the Greek countryside (Alcock, 1993). This may have occurred because of declining interest in maintaining a nucleated residence, increased agricultural production, or increased population growth (Alcock, 1993). Cameron (1993) suggests that the increase in rural habitation coincided with population movements to urban refuges, such as fortresses. Plague epidemics, invasions, and economic factors may have encouraged people to seek protection in the more fortified areas above the cities, such as the hilltop fortress of Acrocorinth (Cameron, 1993). Economic and administrative factors in the sixth century also may have played a part, as resource management started shifting from the old Roman-style city administration to the Byzantine church (Cameron, 1993).

Not only warfare ended up shaking the Byzantine Empire's security and stability; plagues and epidemics were common occurrences during this period (Treadgold, 1997). The Justinian Plague, occurring during Justinian I's rule, coincided with the Empire's warfare and expansion attempts. Justinian had decided to weaken the defenses along the eastern borders of the empire in order to have the military resources to expand into Africa and Italy, and the Persian king Khusrau took the opportunity to attack the city of Antioch, forcing its Byzantine defenders to abandon it (Treadgold, 1997). Justinian had to pay Khusrau large ransoms in order for him to spare other nearby cities from the same fate (Treadgold, 1997). In the midst of this, the bubonic plague started appearing in the Mediterranean. Constantinople was hit particularly hard as first its food supply was disrupted and then Justinian himself fell ill. Some contemporaneous estimates state that 230,000 people, or one-third of the city, died (Treadgold, 1997). Despite its weakened state, the Byzantine Empire did not end up being overrun by Persian forces during this time; the Persians also fell victim to the plague, necessitating a quick withdrawal from the Byzantine territories (Treadgold, 1997).

The empire struggled and recovered politically, economically, and militarily several times between the Justinian plague and the Ottoman occupation. The Mediterranean economy in general underwent a major revival in the tenth century AD (Whitlow, 2008), and by 1000 AD, the Byzantine Empire was the richest state in Western Europe. Its borders extended from eastern Turkey to near Capua in Italy, and from the Crimea to the River Yarmuk in Lebanon (Lock, 1995).

The First Crusade, from 1096-1099 AD, brought large numbers of western Europeans to Constantinople, as the Byzantine Empire became the protector of the crusader states in the Holy Land against Islam (Lock, 1995). This reputation collapsed after the Third Crusade (1188-1189 AD) when the Byzantine emperor agreed to help the Islamic forces by slowing down the crusaders (Lock, 1995). After earning the ire of the western Latin powers, the Byzantine Empire entered into a prolonged crisis in the latter part of the 11th

century as its territories and tax revenues were lost to foreign control (Whitlow, 2008). Although there was some economic revival in the 12th century, the Byzantines relied heavily on outside economic and military support to maintain some semblance of authority, which made them vulnerable to foreign influence (Whitlow, 2008). The wealth of Constantinople that was on show during the earlier crusades also tempted Frankish crusaders to capture the city in 1204 AD during the Fourth Crusade (Lock, 1995).

Western European groups had become established within the Byzantine territory for many years prior to the capture of Constantinople (Whitlow, 2008). Venetian and Genoese traders and merchants had been working and settling within the Byzantine Empire for a long time and by the 12th century, a large proportion of Byzantine trade was in Italian hands (Whitlow, 2008). The empire had also made treaties with the Venetians, Genoese, and Pisans that offered commercial privileges in exchange for military support, as there were ongoing attempts made to maintain the empire's now-shrinking borders (Whitlow, 2008). In 1082, 1147, and 1185 AD, Normans coming from South Italy had invaded Greece and the inhabitants were forced to turn for protection to the local powerful landowners and officials (Lock, 1995). This had the effect of further minimizing the authority of the central administration in Constantinople and set the stage for the emergence of regional despots and smaller rival states in Greece.

The year after the capture of Constantinople by the French and Venetian crusaders, the French also conquered Greece and the Peloponnese. Numerous Frankish states were set up in the Aegean and the occupation of some of these areas lasted for 300 years (Lock, 1995). The Venetians were largely concerned with securing the harbours and trade routes that were necessary for their merchant ships, while the Greek islands were left for the sons of Frankish noble families to conquer (Lock, 1995). This Frankish conquest was a fragmented one, composed of different merchant and noble

families and lacking a unified, central ruler over the smaller Aegean states that emerged (Lock, 1995). Eventually, the Greeks were able to re-conquer Constantinople in 1261 AD and the Byzantine government was restored (Lock, 1995).

Under the emperor Michael VIII, most of the Peloponnese was re-acquired in 1261 and became a self-sustaining province, loyal to the Byzantine Empire (Treadgold, 1997). Its main ports were still under Venetian control, but this arrangement was largely beneficial as they acted to provide a market for local goods. Overall, the Peloponnese is thought to have fared quite well economically during the Frankish conquest (Treadgold, 1997) and even after the Black Death reduced the population in the region, the local despots were able to maintain control by bringing in Albanian settlers and mercenaries to re-populate the area (Treadgold, 1997). Even with the influx of new people, the Peloponnese remained predominantly Greek-speaking and relatively politically stable (Treadgold, 1997). During the final decline of the Byzantine Empire, the commercial classes were able to profit and the landholders benefited as peasants became their tenants and paid taxes to them instead of the state (Treadgold, 1997).

In 1453 AD, the Ottoman Turks invaded, conquered, and sacked Constantinople and the rule of the Byzantine Empire came to an end (Gregory, 2010). At this point, the Byzantine Empire was mostly composed of parts of Anatolia and Greece, its borders having shrunk greatly since the 11th century. The loss of the emperor in Constantinople had reduced the Byzantine Greeks to the status of an ethnic group under Ottoman rule, without any special legal or religious considerations (Treadgold, 1997). By 1461, the Turks had eliminated any successor states that could claim descent from Byzantium and consolidated their control of the region. Certain areas of Greece remained under Venetian, Genoan, Hospitaller, or foreign family control during this time, but the majority of Greeks remained subject to the Ottoman sultan until the 19th century (Treadgold, 1997)

2.3. Sources of Information About Diet

Consideration of the range of sources available allows scholars to approach the question of diet and resource use from a variety of viewpoints. Each method and source of information has its strengths and weaknesses, and by combining each, a more complete picture of diet in past Greece can be established. As well, any discrepancies between the different sources illustrate the complexities of food ideals and food realities. Generally, the sources agree that the most important food groups in ancient Greece, those forming the so-called “Mediterranean triad”, are cereals, wines, and oils (Dalby, 1996). However, other foods contributed to the typical Greek diet and merit discussion.

2.3.1. The Classical and Hellenistic Periods

Much of the dietary information about the Hellenistic period is based upon Classical era sources and focuses on Classical food ideals and customs. The information presented here includes a discussion of the Classical period so that diet and food in Hellenistic Greece can be understood as a continuation of the preceding traditions and practices.

2.3.1.a. Historical and literary information

A variety of literature exists that describes the food and drink at Classical feasts and *symposia* and that uses food as a social or political metaphor. Most of these sources focus on the larger city-states, such as Athens, to the exclusion of smaller Greek sites. Contemporaneous philosophers and physicians all had opinions about food and about what constituted the ideal diet for a Greek citizen. While these sources do give some idea of what foods were available, as well as their desirability and status, they do not provide much information about what was actually consumed on a day-to-day basis by the average, non-elite individual. There also can be difficulties in untangling the meanings behind the food metaphors

found in Classical literature and their relationships to other social and political concepts.

Wine and Oils

Wine was typically watered down and drunk after meals, accompanied by sweets, nuts, and fruits (Dalby, 1996). Although alcohol consumption was intended to stimulate conversation between the guests during *symposia* and over-consumption was not encouraged during these occasions, individuals could still frequent tavernas where solitary drinking was acceptable and different social expectations applied (Dalby, 1996). Rural and poorer individuals may have consumed wine vinegar mixed with either water or grape juice, as the prized red wines of Chios and other well-regarded regions would have been unaffordable (Amouretti, 1999; Dalby, 1996). In some places, seawater was added to the must prior to fermentation, as with Coan wine, creating a salty-tasting, yet popular, beverage (Dalby, 1996).

Oils were frequently used in food preparation and cooking and could be considered a staple ingredient in many dishes. Olive oil, followed by other vegetable oils, was the most commonly used (Dalby, 1996). The various grades of olive oil included extra virgin (the oils from the first pressing of black olives), which was reserved for dressings, and ordinary oil, used mainly for cooking other foods (Amouretti, 1999).

Cereals

The most important cereals of Classical and Hellenistic Greece are considered to be wheat and barley, the latter generally eaten in the forms of soups, porridges, or loaves (Amouretti, 1999). Emmer or einkorn wheat was made into flatbreads, leavened breads, and cakes (Amouretti, 1999; Dalby, 1996).

While barley was considered to be an inferior grain compared to wheat, it was very important symbolically; it was awarded to the winners of the Eleusinian Games and was used to pay tributes to Sparta (Megaloudi,

2006). Barley flour could also be combined with mint and made into a drink, which served as both a sacred beverage and also a general refreshment in rural areas (Amouretti, 1999). The area around Sparta is reported to have produced flax and millet crops, which serves as an example of the variations in regional cereal specialties that emerged in the Classical period (Amouretti, 1999). Other cereals available included oats and rye (Garnsey, 1999; Dalby, 1996).

Classical comic writers made fun of rural people for eating more fava beans and chickpeas than grains (Amouretti, 1999). This suggests that some rural Greek farmers were trading their wheat or barley crops for other goods or that legume flours were commonly used as substitutes in bread making in rural areas (Garnsey, 1999). It also is possible that a portion of the cereal crops were being stored for later consumption, while cheaper crops such as lentils and legumes filled in the dietary gap. The absence of food relief programs in Classical Greek cities meant that shortages disproportionately affected the urban poor. It was only later in the Hellenistic period that city benefactors would sell wheat at a lower price or give it away during shortages (Amouretti, 1999). When discussing the resilience of the Mediterranean small farmer against food shortages and famine, Garnsey (1988) states that the traditional practices of mixed-crop farming and domesticated animal husbandry may have allowed some flexibility in dealing with shortages during antiquity. Overall, cereals were considered more desirable foods than lentils or legumes and held higher status in the Classical Greek diet (Dalby, 1996).

Legumes

Although legumes were less esteemed, lentils and pulses were still considered major components of the Classical diet. Occasionally used to supplement bread flour or as a frequent ingredient in labourers' meals (Flint-Hamilton, 1999; Dalby, 1996), legumes even had their own place in mythology. There was a Greek "God of Beans" called Kyamites and bean

festivals were held in honour of Apollo (Brothwell and Brothwell, 1998). The ancient sources mention consumption of lentils, chickpeas, bitter vetch, broad bean, garden pea, and grass pea (Amouretti, 1999; Flint-Hamilton, 1999). Despite the seeming ubiquity of legumes, it is still important to note that species like bitter vetch were probably only consumed in times of severe famine and only if the other legume options were unavailable; typically, they would have been used as animal fodder (Flint-Hamilton, 1999; Dalby 1996). Other lower status legumes included bird vetch, grass pea, and lupins (Dalby, 1996).

It is not clear if legumes and cereal crops were grown together in the same field or if crop rotation occurred during the Classical and Hellenistic periods, but in any case, legumes provided an essential role in maintaining fertile fields and preventing soil erosion (Flint-Hamilton, 1999). Their nitrogen-fixing capacities enabled cereal crop growth and legume consumption allowed farmers to save their more profitable crops for selling or trading; they were deeply integrated into agricultural production and life, according to several sources (Amouretti, 1999; Flint-Hamilton, 1999).

Fruits and Vegetables

Grapes and olives formed a major part of Classical Greek agriculture, although intensive cultivation activities may have centred around the cereal crops while grape vines and olive trees played a secondary role at the edges of arable land (Amouretti, 1999).

Available fruits in Classical Greece included melons, grapes, figs, pears, quinces, sorb apples, medlars, pomegranates, and almonds (Amouretti, 1999). As fruits could be preserved through drying, they were ideal for travelers, field workers, and the poor (Dalby, 1996). Dates were known in the Classical period, but were imported in their dried form from the east and were likely a more expensive food item (Dalby, 1996). Other fruits mentioned in the literature include plums, sour cherries, watermelons, musk melons, cucumbers (considered a fruit), myrtle berries, mulberries,

blackberries, and nuts, such as almonds (Dalby, 1996). Walnuts, hazelnuts, filbert, chestnuts, and pine kernels are also mentioned as food items (Dalby, 1996).

For vegetables, Classical period gardens may have contained a variety of beets, cabbage, cress, lettuce, orach, purslane, rocket, basil, and hyssop, among other cultivars (Dalby, 1996). Hippocrates lists several vegetables available to the Classical Greeks, such as garlic, onions, leeks, colza, watercress, and turnips (Amouretti, 1999). Various wild mushrooms were collected and consumed as well (Dalby, 1996).

According to the sources, the vegetables utilized mainly by poorer individuals included asphodel, wild chervil, wild spinach, rocket, nightshade, golden thistle, and fennel, which are all wild plants that can be gathered (Dalby, 1996). In addition, rural diets could include not only cultivated agricultural products but also wild herbs and foraged fruits that could also be exchanged for grains (Amouretti, 1999). Some of the poorest individuals may have periodically relied on acorns for food, of which several kinds exist (Dalby, 1996).

Meats and Animal Products

Much attention has been paid to the place of meat in the Greek diet, although the average individual probably rarely consumed larger-sized domesticates such as cattle, sheep, goat, and pig, outside of feasting and religious sacrificial occasions (Amouretti, 1999). Secondary animal products may have been more important to the typical Greek diet than meat itself (Amouretti, 1999).

The majority of information about animal consumption comes from comic literature and plays about *symposia* and gourmet dinners. These tend to focus on some of the more exclusive and expensive food items that would have been uncommon in the typical diet, such as game animals like wild boar, wild goat, ass, fox, red deer, roe deer, bear, and lion.

A variety of birds were also consumed. Domesticates such as chickens, cocks, pigeons, geese, pheasants, mallards, and quail (Dalby, 1996), along with wild birds like moorhens, wild geese, and other marsh birds (Amouretti, 1999) are mentioned in the literature. Songbirds, such as thrushes, larks, starlings, jays, and sparrows, could be purchased either for consumption or as pets from the market in Athens (Dalby, 1996). Consumption of these avian delicacies occurred generally within the symposium environment or as part of an intimate supper, since they were prized more for their taste than for their portion sizes (Dalby, 1996).

There is some evidence to suggest that other smaller animals such as dog, dormouse, turtles, and insects were consumed as delicacies as well (Brothwell and Brothwell, 1998). Land snails and cicadas could also be found in the menu of the gourmet individual, but it is also possible that poorer individuals may have eaten them periodically or sold them at market, making them less exotic as food items (Dalby, 1996).

Since domesticated animal meat was not readily available for the average individual other than during feasts and sacrifices, secondary animal products likely contributed a large amount of protein to the diet. Comic writers frequently associated rural inhabitants with the consumption of large amounts of milk, probably from goats (Amouretti, 1999; Dalby, 1996). Cheese would have been another source of protein for both rural and urban individuals and the numerous cheese stalls that could be found in the market support its prevalence in Classical Athens; it was reported to have been prepared and eaten in numerous ways (Dalby, 1996). Other secondary products had varying levels of importance to the Greek diet. Eggs from quail and domesticated hens would have been available, as would honey, which was the principle sweetener (Amouretti, 1999; Dalby, 1996). A form of yogurt, known as *pyriáte*, also emerged in the Classical period (Dalby, 1996). Despite the relatively infrequent consumption of domesticated meat or game animals, dairy products, along with smaller wild animals and even insects,

would have been widely available as cheap and easily procured protein sources.

Fish and Seafood

Both saltwater and freshwater fish species take on conflicting roles in the Classical Greek literature, co-existing uneasily as both status items and poverty foods. The Greeks preferentially consumed many of the same fish species that are around in modern times: tunny, angler-fish, grey mullet, bluefish, pike, catfish, conger eel, dogfish, skate, ray, carp, sheatfish, sturgeon, swordfish, tuna, sharks, bass, grouper, comber, mackerel, and bream (Dalby, 1996).

Athenaeus states that fish were occasionally sacrificed, much like cattle or sheep were, but this does not appear to have been a normal practice (Dalby, 1996). Overall, fish could be considered *opsos*, or a relish, to a good meal. Certain fish from larger species were expensive, while others that were small and of poor quality were viewed as suitable only for lower status individuals to eat. Given that the majority of the large fish species available in the Aegean and Mediterranean seas tend to avoid congregating in shoals, larger fish required more effort and time expenditure to catch than cheaper, small fish (Gallant, 1985). This extra effort may have contributed to the exclusivity of certain fish species in the Athenian world (Gallant, 1985), although others have challenged the notion of the Aegean and Mediterranean as being relatively fish poor (Lytle, 2010; Mylona, 2008). Similarly, the status of aquatic animals such as cuttlefish, octopus, and squid varied according to their individual size (Dalby, 1996). Smaller fish tended to be sold whole, while larger fish could be sold by the cut, as with tuna belly or with eels (Mylona, 2008). In many cases, the preference for various fishes was not only related to their size, but also to their freshness, method of preservation, taste, and texture (Mylona, 2008). Fishes that were transported to market alive in barrels tended to be more expensive than fishes that were dried or preserved in brine (Mylona, 2008). By the fourth century BC, salting fish was a common

means of preservation throughout the Greek world, but fresh fish and seafood were still considered more desirable and were therefore more costly (Dalby, 1996).

Shellfish, including oysters, cockles, mussels, limpets, scallops, crabs, lobsters, prawns, sea anemones, and sea urchins, were available at markets and for collection along the coast (Dalby, 1996). As with fishes, these animals could be preserved with brine and vinegar and made accessible to those individuals living large distances from the sea (Dalby, 1996).

A particular product, fish sauce (*garos*), was first manufactured in the 4th century BC by Greek colonists in the Black Sea area (Brothwell and Brothwell, 1998; Dalby, 1996). Made using fermented fish, *garos* became a popular condiment, especially later in the Roman period (Garnsey, 1999). Its manufacture involved the use of fish parts not normally consumed otherwise, such as the innards and gills, and possibly fish roe (Curtis, 1991). These were typically fermented in a mixture of herbs, wine, and salt and then strained in order to collect the liquid (Curtis, 1991). *Garos* allowed fishermen to process small fishes or undesirable fish parts that might not have been sellable at market into a preserved trade product (Dalby, 1996). Its popularity beginning in the Classical period and continuing onwards illustrates that certain items in the Greek diet need not have been major dietary components to be frequently consumed.

Therapeutic Foods in the Classical Period

The consumption of foods and the use of medicines frequently overlapped with each other in the Greek world. For example, marine invertebrates were not only eaten but were viewed by some as possessing medicinal and therapeutic properties. Some medical treatises recommended that they be prepared as drinks, suppositories, or compresses and used to treat genitourinary, digestive, and skin disorders (Voultsiadou, 2010). Even the lowly lentil was offered up as a potential remedy for constipation, ulcers, gout, and sore throat among other ailments (Flint-Hamilton, 1999). The

actual efficacy of these treatments is unknown, but if a benefit was perceived it seems likely that these foods could have been preferentially consumed for therapeutic reasons as well as for nourishment.

In general, cereals, oils, and wine all enjoyed an association with the civilized world during the Classical and Hellenistic periods and seem to have been the preferred foods for many individuals (Grimm, 2007; Keenleyside et al., 2006; Dalby, 1996). The longevity and predominance of these items in the Greek diet has led to their reference as the “Mediterranean triad,” although dairy products may have also been important as a main protein source. It is difficult to reconstruct what a general, everyday Greek diet would have been like because much of the literary and historical evidence for food in the Classical period is drawn from Athenian traditions. The focus is on the ideal foods for the Athenian citizen and on plays and orations performed in Athens to the exclusion of other Greek cities. Regional differences were known to exist within Classical and Hellenistic Greece and a “typical” menu would have differed between the wealthy and the poor in both rural and urban settings (Amouretti, 1999).

In contrast to the amount of historical literature about food in Classical Greece, there are markedly fewer sources of archaeological, zooarchaeological, and stable isotope evidence for diet.

2.3.1.b. Archaeobotanical information for the Classical and Hellenistic periods

Sites dating to the Classical period comprise only 5% of the archaeobotanical studies conducted in the Aegean region between 1879 and 2003 (Megaloudi, 2006). Most of the focus appears to be on prehistoric Greece or on the southern regions (Flint-Hamilton, 1999), although efforts to examine later periods are ongoing.

The archaeobotanical evidence for the Classical period in Greece supports the importance of cereal plants to the diet. For instance, barley dominates the botanical samples from the sites of Corinth and Kalapodi (Megaloudi, 2006). Archaeobotanical research at the Greek Classical and

Hellenistic site of Sagalassos, in modern-day Turkey, shows that even outside of Greece, agriculture involved the cultivation of bread wheat with less focus on legumes; no evidence for millet cultivation was found at the site (Fuller et al., 2012). Otherwise, most of the plant specimens that are represented archaeologically are from Greek sanctuaries and are the typical Mediterranean wheat, legume, fruit, and nut species.

The Hellenistic sanctuary at Messene, located in the southwestern part of the Peloponnese, shows similar plant species to those found at Classical period sites, including a large number of carbonized fruits that date to the second century BC (Megaloudi, 2005). At Messene, the cones and seeds from stone pines, olives, grapes, almonds, and chestnuts had been burnt and placed in a hero cult monument, or *heroon*, as a sacrificial offering (Megaloudi, 2005).

Given that the majority of the sites where archaeobotanical remains were recovered are temples and sanctuaries, there is the possibility that these plants were not frequently used outside of religious occasions. Some scholars argue that the archaeobotanical remains found at cemetery sites and temples do actually reflect the foods available and that they should still be considered probable components of the daily diet of many individuals (Megaloudi, 2005, 2006; Prummel, 2003).

2.3.1.c. Classical and Hellenistic zooarchaeological evidence

Zooarchaeological studies about the Classical and Hellenistic periods have contributed information about animal use in ancient Greece and about the prevalence of different domesticated species at various sites. A trend towards increasing terrestrial resource use through pastoralism can be identified in the literature.

At New Halos, a Hellenistic period military town located in the Pagasitic Gulf area, the most numerous domesticates identified were sheep and goats, which are difficult to distinguish from each other osteologically and are usually listed in faunal reports as *Ovis/Capra* or ovicaprines.

Unusually, given their infrequent consumption elsewhere in Greece during the Classical and Hellenistic periods, cattle may have been consumed at New Halos more often than goat meat or mutton (Prummel, 2003). This may have been due to the preferential sacrifice of cattle instead of sheep and goats and their resulting contribution to the diet of the individuals living at New Halos (Prummel, 2003). The remainder of the domesticated faunal material at the site includes pigs, equids, and dogs. Wild animal remains such as hare, fox, badger, red deer, roe deer, and tortoise provide useful reminders that meat sources other than domesticates were available, although these were present in lesser quantities. Shellfish gathering appears to have been important to the individuals who lived at the site and 28 different mollusc species are present in the assemblages; however, only one fish bone was recovered (Prummel, 2003). Given the meticulous nature of the excavation techniques used at the site, this is likely to be caused either by cultural practices in fish consumption or by the differential preservation of fish bone. For example, small fishes may have been consumed whole and their bones digested instead of discarded (Prummel, 2003). Another possibility is that the foddering of domesticated animals, such as dogs or pigs, on food scraps may have caused the fish remains to be completely digested by the other animals at the site (Prummel, 2003). An absence of fish remains also corresponds well with the evidence suggesting increased consumption of cattle; fish simply may not have been eaten to any significant degree. The ongoing use of other dietary reconstruction methods at New Halos may provide some indication of whether this occurred.

An examination of pastoral practices on the island of Delos during the Classical and Hellenistic periods has connected ovicaprine, pig, and cattle remains with contemporaneous epigraphical evidence indicating the importance of sheep and cattle shelters (Leguilloux, 2003). Both these sources of information suggest that animal husbandry, and not fishing, was the main source of income for the islanders. The zooarchaeological assemblage, dating from 100 to 50 BC, is similar to New Halos as it is

dominated by domesticated ovicaprines and cattle, while a small percentage of the assemblage shows evidence for poultry farming. The products of fishing are rarely represented in the archaeological record at this site (Leguilloux, 2003). Whether or not there is a similar pattern of resource use at other Greek Classical or Hellenistic sites is not clear; Delos may be unique in that it is an island and the presence of wild terrestrial faunal species is naturally limited.

At Classical sites outside of Greece, such as Sagalassos, there is a similar trend in the zooarchaeological record towards increased economic reliance on ovicaprines, followed by cattle and pigs (Fuller et al., 2012).

2.3.1.d. Stable isotope analysis of Classical and Hellenistic diets

Despite the relative abundance of stable isotope studies investigating diet in prehistoric Greece, there are only a few dietary reconstructions that cover the Classical and Hellenistic periods found in the published literature. Vika et al. (2009) and Vika (2011) examine Classical and Hellenistic period Thebes, while Keenleyside et al. (2006) and Henneberg and Henneberg (2003) examine Classical Greek colonial populations on the coast of the Black Sea and in Italy, respectively.

Vika et al. (2009) focus specifically on the Classical period individuals from a cemetery located in Thebes. Stable isotope analysis of the bone collagen from these individuals has revealed $\delta^{15}\text{N}$ values that are approximately 5‰, or more than one trophic level, higher than the local terrestrial faunal values. Since the human stable isotope values are otherwise consistent with a primarily C_3 terrestrial diet containing no isotopically detectable contributions from marine protein, Vika et al. (2009) suggest that freshwater fish consumption may have caused the higher $\delta^{15}\text{N}$ values. The use of manure as a fertilizer in agricultural practices is another possibility, as manure is enriched in ^{15}N and has been shown to elevate the ^{15}N content of cereal crops if it is used as a fertilizer (Bogaard et al., 2007).

In Vika (2011), additional Theban individuals dating from 3000 to 300 BC are sampled in order to detect any temporal changes in dietary stable isotope values. As part of this study, stable carbon and stable nitrogen values were obtained from the bone collagen of 29 Hellenistic individuals. The average $\delta^{13}\text{C}$ was $-19.2\text{‰} \pm 0.6\text{‰}$ and the average $\delta^{15}\text{N}$ was $9.6\text{‰} \pm 0.7\text{‰}$. These values differed significantly to those of the burials dating to prehistoric periods (Vika, 2011).

There appears to be a small rise in the stable carbon isotope values, which is interpreted by Vika (2011) as the result of a change in living environment. During the Hellenistic period, Thebans are recorded as having moved away from the hilly city area to the surrounding plains. One male individual with an outlying $\delta^{13}\text{C}$ value of -15.8‰ may provide evidence of the consumption of a more C_4 resource-based diet, perhaps reflecting recent immigration to the area from a place where C_4 crops, such as millet, were regularly consumed.

When examining the stable isotope values according to sex, some differences are apparent in the data. The Classical period male and female individuals were not compared statistically due to the small sub-sample sizes, but a difference between the two groups is not apparent when looking at the raw results. As Vika (2011) notes, lack of statistically significant results does not mean that sex differences in diet did not exist, just that they may not be detectable isotopically. Literary sources do describe different eating practices for males and females in Classical Greece (Dalby, 1996).

For the Hellenistic individuals, even when excluding the outlying male with the higher stable carbon isotope value mentioned above, there appears to be a significant difference in the mean stable carbon isotope results between males (-19.1‰) and females (-19.3‰) in Hellenistic Thebes. The mean stable nitrogen isotope values are not significantly different.

Burial types did yield differences in the stable isotope values in both the Classical and Hellenistic time periods, with “rich” burials showing slightly higher $\delta^{15}\text{N}$ values than the “poor” burials. Interestingly, although the stable

carbon isotope values end up quite similar when averaged out, the “poor” burials showed a wider range in $\delta^{13}\text{C}$ values. This is suggested to be the result of the wealthier individuals having the ability to consume relatively consistent diets, while poorer individuals may have had to adapt to diets based more upon food availability (Vika, 2011). Comparison of the stable isotopic data from Thebes to the historical data shows that during wartime and food shortages in the area, there is no change in the dietary stable isotopic values (Vika, 2011). This suggests that whatever food sources the Theban individuals were consuming during long-term times of strife either did not change or alternate foods were eaten that were isotopically similar.

Both the Vika (2011) and Vika et al. (2009) articles raise interesting questions about the potential causes of outlier values and high $\delta^{15}\text{N}$ values in past Greece. Overall, the stable isotope data appears to confirm that the majority of the Theban individuals subsisted on a predominantly C_3 terrestrial resource diet with the potential inclusion of large amounts of freshwater fish during the Classical period, although this is not the only possibility. The action of manuring fields to improve crops and soil quality is known to increase grain stable nitrogen isotope values enough to overlap with fish consumption values (Bogaard et al., 2007), and this practice coincides with the importance of cereal production and domesticates in the ancient Greece.

Dietary reconstruction at sites inhabited by Greek colonists can also provide insight into past food choices as the resources available at these sites may have differed from those in the founding Greek cities. Keenleyside et al. (2006) analyzed the bone collagen from 54 Classical period individuals from Thracian-founded Apollonia, which dates from 610 BC. Bone carbonate samples were obtained from 29 of these individuals to provide additional data about the bulk stable isotopic values of the diet. Faunal samples from four ovicaprine remains provided an herbivorous baseline for the human values. The archaeological evidence for food at the site consists of plant remains, animal bones, fossilized feces, food utensils, and storage vessels

(Keenleyside et al., 2006). These artifacts suggest that the individuals at Apollonia subsisted largely on a diet of grains, nuts, fish, meat, and shellfish.

The human stable isotope results show that a significant amount of dietary protein was obtained from marine sources, as interpreted from the elevated mean stable carbon and nitrogen isotope values of the collagen (-18.5‰ and 10.1‰, respectively). In comparison, the herbivore faunal samples show an average value of -20.6‰ for $\delta^{13}\text{C}$ and 5.2‰ for $\delta^{15}\text{N}$. Although no marine archaeofauna were sampled in this study, comparisons to modern marine species from the area suggest that the individuals at Apollonia were consuming higher trophic level fish species like tunny or sturgeon (Keenleyside et al., 2006). There is no isotopic evidence that C_4 plants, such as millet, or legumes were consumed. Interestingly, despite Classical literary and historical sources citing differences between the sexes in food consumption, as well as the traditional depiction of fishing as a male activity, no isotopic variation by sex or age was detected in this study. However, the authors note that they cannot rule out the consumption of different foods with similar stable isotope values to each other.

From their results and the differences from what the Classical historical sources have said, Keenleyside and colleagues (2006) have concluded that the geography and availability of resources played a larger part than cultural or historical influences in determining diet at Apollonia. It is almost certain that there are differences between the values of this population and that of the Greek mainland; while the colonists may have been culturally Greek, the ecosystems and underlying water and soil values of the area they lived in were different from the rest of Greece. That makes this study valuable in illustrating some cultural and ecological variables that can affect diet, and in turn, stable isotope dietary reconstructions.

Other than in studies of Mesolithic sites (Cullen, 1995) and the results from a few potentially elite individuals in other older Greek sites (Richards and Hedges, 2008), the Apollonia data provide some of the only indications of marine resources as a major protein contributor to past Greek diets. There is

little evidence for marine resources in the diet of other populations dating to the Classical and Hellenistic periods, with the exception of an investigation of 48 individuals from a rural and an urban cemetery at the Greek colony of Metaponto in Southern Italy (Henneberg and Henneberg, 2003). These individuals date from the 7th to 2nd centuries BC and their dietary stable isotope results are interpreted together with the dental and disease indicators that were recorded during the osteological analysis (Henneberg and Henneberg, 2003). The individuals from the rural cemetery of Pantanello show a mean $\delta^{13}\text{C}$ value of -19.6‰ and a mean $\delta^{15}\text{N}$ value of 10.5‰. The individuals from the urban Crucinla cemetery have a mean $\delta^{13}\text{C}$ value of -19.0‰ and a mean $\delta^{15}\text{N}$ value of 10.7‰. Interestingly, there is a wide range of values found within both cemetery groups, with the urban individuals showing the greatest variability. Despite Metaponto's geographical distance from Apollonia, the general isotopic results are consistent with some of the stable isotope values from that site.

Estimations of the amount of marine protein in the diet of the Metaponto individuals range from 5% to 20% for the individuals buried in the rural Pantanello cemetery and from 5% to 40% for the urban Crucinla individuals (Henneberg and Henneberg, 2003). Females tended to be at the lower end of the proportion of marine protein in the diet for both rural and urban sites, although the wide range of female percentages calculated does encompass the estimated male values. The caries rates and dental wear apparent in the samples are consistent with these values, suggesting that most of the dietary sources of protein were from terrestrial animals and C_3 plants. This is argued to be reflective of the Metapontine economy, which emphasized cereal and domesticated animal use (Henneberg and Henneberg, 2003). These interpretations should be considered carefully, however, given that all the individuals spanning from the 7th to the 2nd centuries BC were included together as one sample group and that the estimates of marine protein in the diet at Metaponto cover a wide temporal range. Unfortunately, Henneberg and Henneberg (2003) did not publish dates for each individual

burial or investigate the possibility of temporal changes in stable isotope values at Metaponto. Since this is unknown, there is the strong possibility that bias has been introduced into the sample selection and more recent individuals are overrepresented as compared to older burials or vice versa. Therefore, any conclusions about rural and urban differences and sex differences at Metaponto should be considered tentative at best.

The stable isotope analysis of bone collagen from four Classical and Hellenistic period individuals from Sagalassos, Turkey shows similar results to those in Vika (2011), with mean $\delta^{13}\text{C}$ values of -19.4‰ and the mean $\delta^{15}\text{N}$ values of 9.7‰ . These are also consistent with a predominantly C_3 terrestrial diet based mainly on domestic animal meat or their secondary products (Fuller et al., 2012). Given that Sagalassos is not located near any major seas or bodies of water, the only readily available local dietary resources would have been terrestrial, making these values useful as indicators of solely terrestrial resource use.

Further studies of well-dated Classical and Hellenistic period individuals may reveal whether site-wide increased marine consumption was specific to Apollonia and perhaps Metaponto. The possibility remains that dietary resource use was more dependent on availability than on commonly accepted cultural preferences during these periods.

2.3.2. The Roman Period

Information about diet in the Roman period tends to focus mainly on Rome itself and less on its provinces and protectorates. While it is likely that Greek dietary practices remained largely unaffected by Roman rule, the typical Roman diet can still be compared to the general Mediterranean pattern of resource use. A discussion of Roman diet therefore facilitates better understanding of the resources that may have been available and the cultural ideas associated with some foods.

2.3.2.a. Historical and literary information

The historical information about food in the Roman period is varied and consists mainly of cookbooks, literature, and travel accounts. Roman historians who visited Greece wrote about their experiences and opinions, not only of the artistic and architectural materials they saw, but also of Greek cuisine (Dalby, 1996). Most of these writings reflect Roman dietary customs and ideals, which were affected by Classical Greek practices. The Romans were strongly influenced by the Greeks and the Etruscans, especially in wine, and are thought to have made improvements to farming methods (Grimm, 2007).

The most famous depiction of food during this period is in the *Deipnosophistae* by Athanaeus, attributed to the late 2nd or early 3rd century AD. This fictional account of a dinner party mentions in great detail the types and amounts of foods consumed (Dalby, 1996). However, as with the Classical comic writers, food consumption is used as a metaphor for social and political issues and may not accurately reflect the foods typically consumed by the average person.

While some similarities existed within the context of the “Mediterranean triad” that the Greek diet tended to be based upon, other foods in the Roman diet were different and were associated with different attitudes. With the exception of the accounts mentioned above, not much was written about food and diet in Greece specifically, although it does seem likely that even with cultural diffusion and exchange between Rome and Greece, the traditional staples remained important to the Greek diet simply because they were available (Dalby, 1996). As a general contrast to the Greek diet, the typical Roman diet is thought to have also been based upon grains, but with increased amounts of meat and fish (Grimm, 2007; Garnsey, 1999).

Wine and Oils

Roman wine drinking followed the customs derived from the Greek *symposia*, with the host mixing the wine with water and distributing it out

among the guests (Faas, 2003). Other, more public, drinking venues continued to exist and many offered food along with beverages. The Romans associated beer drinking with barbarians and foreigners and beer did not enjoy anything close to the popularity and social acceptability of wine (Faas, 2003).

Cereals

The Roman period saw improvements in seed quality and crop techniques within the empire (Grimm, 2007). Porridges were popular in Rome, as was bread in its more traditional forms (Braun, 1995). Varieties of naked wheat, such as bread wheat, came into more frequent use and are notable because they did not require hulling like the traditional wheat and barley varieties (Garnsey, 1988). However, other hulled wheats, such as emmer, continued to be cultivated as a Roman staple food and are mentioned by Herodotus (Braun, 1995). Garnsey (1988) suggests that the naked wheats were likely grown for sale and the traditional varieties were kept for personal consumption. Although both Galen and Columella derided barley in their writings as an inferior grain fit only for animals, in Greece it remained a fairly widespread cultivar even as it declined in overall popularity from the Classical period (Garnsey, 1988).

Legumes

Roman agronomists, as well as Galen, wrote extensively about pulses and lentils and stated that these were some of the main cultivars in parts of Greece and Italy (Garnsey, 1999). Legumes are suggested to have formed part of the typical luncheon snack in the Roman world (Faas, 2003). The relative ease with which lentils and legumes can be dried and stored may have encouraged their use by ordinary farmers and rural peoples (Garnsey, 1988).

Fruits and Vegetables

Olives, raisins, fresh fruit, or nuts formed part of every meal associated with the Romans (Faas, 2003). Vegetables were not imported as

often as cereals were, since personal horticulture was a valued activity in the Roman world (Faas, 2003). Turnips, beets, radishes, carrots, parsnips, taro (colocasia), garlic, onions, asparagus, flower bulbs, artichokes, leeks, cabbages, gourds, and cucumbers are among the items mentioned by Roman writers (Faas, 2003). In Greece, locally available fruit and vegetable varieties would have likely persisted as cultivars from earlier periods.

Meats and Animal Products

Archaeological evidence indicates that the Romans consumed a variety of meats, including pork, mutton, goat, beef, and fowl (Grimm, 2007). Some Romans, especially soldiers, were associated with significant meat consumption (Garnsey, 1999). Pigs have been argued to be more numerous on Italian farms, whereas the Greeks typically reserved them for sacrifices (Garnsey, 1999). Wild game, including boar, roe deer, rabbit and hare, dormouse, and land snails were consumed by all classes within the Roman empire (Faas, 2003; Brothwell and Brothwell, 1998), although big game was also kept in the game reserves and circuses of the wealthy.

For ordinary Greek farmers, it is likely that domesticated animals were reserved for use as sources of traction, dairy, and fibre products, and were only eaten on feast days or during dire food shortages where the farmer was forced to butcher his stock for food. Garnsey (1999) suggests that it was simply more economical for farmers to focus their limited arable land and growing seasons on plant production instead of livestock. Sheep and goats were likely pastured away from the arable land in Greece in order to save the plants and crops for people (Garnsey, 1999). There is some evidence that only the wealthy ate meat regularly and the poor ate mainly vegetarian meals (Garnsey, 1999; Brothwell and Brothwell, 1998), although this is not commonly accepted (Grimm, 2007).

Fish and Seafood

The writer Athanaeus frequently mentions fish as a preferred dish and a luxury food in his writings, but his aesthetic and satirical intentions may

have led to its overemphasis in the typical Roman diet (Garnsey, 1999). Other Roman writers mention the fish ponds of the wealthy, where oysters and moray eels were bred and kept (Faas, 2003). For those who could afford them, oysters, sea urchins, crabs, octopus, and moray eels could be purchased from the market, along with red mullet, tuna, dolphin, shark, anchovies, sardines and mackerel, among others (Faas, 2003). Roman literature, however, tends to use food to express attitudes encouraging generosity in public and frugality in private life (Faas, 2003). This may have skewed the mention of certain foods to those that were visible at events like dinner parties or public feasts.

Despite conflicting evidence, many sources portray fishing as an occupation of the poor but fish breeding itself was a pastime of the wealthy; the cultivation of fish and shellfish was associated with a higher socioeconomic status (Mylona, 2008). According to Pliny, mollusc culturing was started by a Roman named Sergius Orata in the 1st century BC (Brothwell and Brothwell, 1998), and the presence of fishponds and other animal cultivars exists during later periods.

Fish sauce (*garum*, in Roman writings) continued to be a very popular condiment and is particularly associated with the Roman period (Curtis, 1991). Fermented and salted fish products were ubiquitous and traded throughout the Roman empire among urban consumers (Garnsey, 1999), although the basic process of *garum* manufacture remained the same from earlier periods. While Athanaeus mentions that small fishes were frequently sold in Athens, fishes were not likely a large component of the diet for those whose livelihoods depended on agriculture; they may have been more important for providing flavour to the staple Roman foods (Garnsey, 1999). Much of the equipment required to catch fish was not overly complicated and could be used by anyone, depending on the viability of local fish stocks, and agriculture may have been viewed as a more reliable food and economic resource by the average person (Mylona, 2008).

2.3.2.b. Archaeobotanical information for the Roman period

The single study located that mentions Greek archaeobotanical evidence for the Roman period refers to the Early-to-Middle and the Late Imperial Roman periods at the Greek-founded city of Sagalassos. The sediments from this site show that agriculture continued to focus on the production of bread wheat and other cereal crops, with less attention paid to the growth of leguminous crops (Fuller et al., 2012).

2.3.2.c. Zooarchaeological evidence for Roman Greece

Faunal studies at a Late Roman site on Crete have supported the idea that fishing activities were class-based in nature and execution (Mylona, 2003). Fish tanks from the island are numerous and their capacities suggest that only the largest and most expensive fishes were being kept, which is consistent with the higher status these types of fish are thought to have commanded. How these fish were distributed to consumers is not clear, however. As Mylona (2003) states, fish is a resource that can easily be exploited by everyone. Amateur fishing is thought to have occurred in conjunction with a fish-based market economy, at least on Crete during the Roman period (Mylona, 2003).

Whether or not fish consumption became common in daily dietary habits in Roman Greece is not clear but it seems highly likely that terrestrial resources remained important. Archaeological and faunal evidence of fish and fishing activities are found in Roman Corinth, especially in conjunction with the fish market in its Agora, but is also found at other sites in the Peloponnese during this period (Mylona, 2008). Not only are marine fish represented in the few faunal assemblages available, but evidence of freshwater fishes and eels are also found at Sicyon, located on the northern coast of the Peloponnese (Mylona, 2008). It is also possible that fish from Lake Kalydon and other coastal lagoons were exploited during the Roman period (Mylona, 2008).

Outside of Greece, the faunal assemblage at Sagalassos indicates an increased prevalence of pigs beginning in the Middle Imperial period (25 BC–300 AD) as compared to the earlier periods (Fuller et al., 2012). In the Late Imperial period (300–450 AD), the prevalence of pigs declines slightly and cattle appear to have been utilized more. Throughout the Roman period at Sagalassos, however, ovicaprines continued to be present and a major part of the economy (Fuller et al., 2012).

2.3.2.d. Stable isotope analysis of Roman diets

Stable isotope reconstruction of the diets of other Mediterranean Roman populations have indicated that there was significant consumption of marine resources for either the majority of individuals (Keenleyside et al., 2009; Prowse et al., 2004; Prowse et al., 2005) or for at least for some of the individuals at each site (Craig et al., 2009; Killgrove and Tykot, 2013). These sites are useful for comparison to Helike because of their proximity to Mediterranean coastal resources, if not because of any strong cultural similarities. Conversely, at the inland site of Sagalassos, the human stable isotopic values do not show any indication of marine or freshwater resource use (Fuller et al., 2012). As is evident in the following section, the stable isotopic evidence for diet in the Roman period is quite heterogeneous, even within the city of Rome itself (Killgrove and Tykot, 2013).

Keenleyside et al. (2009) analyzed four cemetery populations at Leptiminus, Tunisia that date from the 2nd to 5th centuries AD. The burials showed a mix of higher and lower status individuals, including children, and $\delta^{13}\text{C}$ values ranged from -16.5‰ to -19.2‰ with a mean value of -17.7‰ \pm 0.6‰. One cemetery population with relatively high collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values leads the authors to suggest that there was a shift in resource use, with higher trophic level fish becoming more common in the later diet (Keenleyside et al., 2009). This may have been due to the Vandal invasion and subsequent rule of Leptiminus, resulting in more hardship, decreased

amounts of livestock, and increased reliance on fish as a source of food by the local population (Keenleyside et al., 2009).

The Leptiminus human carbonate $\delta^{13}\text{C}$ values, reflecting the bulk carbon contribution of the diet, ranged from -7.0‰ to -14.5‰ with a mean value of $-11.3\text{‰} \pm 1.6\text{‰}$ (Keenleyside et al., 2009). The lowest of these values corresponds to a strictly herbivorous diet, but the collagen values for the same individual suggest consumption of marine resources. Keenleyside et al. (2009) believe that this carbonate value may be derived from the high consumption of ^{13}C -depleted lipid- and carbohydrate-rich foods, such as olive oil and possibly wine. Contrary to some of the historical data suggesting the importance of legumes and lentils in the diet (Garnsey, 1999), the high $\delta^{15}\text{N}$ values do not support the significant consumption of these foods at Leptiminus.

At Velia, a site in southern Italy founded by Greeks from Asia Minor in 540 BC, Craig et al. (2009) analyzed the bone collagen from 117 adult individuals dating to the Imperial Roman period (1st to 2nd centuries AD). They found that the $\delta^{13}\text{C}$ results range from -20.0‰ to -18.7‰ and the $\delta^{15}\text{N}$ values range from 6.4‰ to 14.1‰ (Craig et al., 2009). Based upon these results, the majority of the individuals appear to have consumed a terrestrial, C_3 cereal-based diet with relatively smaller amounts of meat and fish. The isotopic evidence also points to a high amount of dietary variation within the population at Velia. The lower stable nitrogen value found in some of the individuals is argued to be reflective of legume consumption (Craig et al., 2009). The higher-end values may be from increased consumption of marine foods, but the stable carbon values from these individuals is not consistent with marine resource use (Craig et al., 2009) and may be due to physiological differences in the routing of proteins and stable isotopic values in bone collagen (discussed further in Chapter Three). As Velia was recorded to have been involved in the fish processing and salting industry of the time, the inclusion of some amounts of marine resources in the diet seems likely.

Prowse et al. (2004; 2005) show similar results to Keenleyside et al.

(2009) at Isola Sacra, near Rome. Dating from the 1st to 3rd centuries AD, the largely middle-class population appears to have eaten a diet based on C₃ terrestrial resources, with collagen values ranging from -17.8‰ to -19.7‰ (mean of -18.8‰ ± 0.3‰) for $\delta^{13}\text{C}$ and 7.5‰ to 14.4‰ (mean of 10.8‰ ± 1.2‰) for $\delta^{15}\text{N}$. The bone apatite values ranged from -9.8‰ to -15.6‰ with a mean of -11.4‰ ± 1.2‰. The relatively high collagen and bone apatite values allow Prowse et al. (2004) to interpret them as indications of marine food consumption. Since this is a strong possibility due to the population's access to marine resources, Prowse et al. (2004) compare their results to inland rural dwellers at another Roman site. This comparison supports their interpretation of the Isola Sacra diet as C₃ terrestrial resource-based with the addition of marine foods. Fish sauce alone is unlikely to have caused the higher stable nitrogen and carbon bone collagen values at Isola Sacra, suggesting that higher trophic level marine organisms had some dietary involvement. Interestingly, in Prowse et al. (2005), the only sex differences observed isotopically were found in the higher $\delta^{15}\text{N}$ values of males; older individuals of both sexes also showed higher $\delta^{15}\text{N}$ values. Interpreted in conjunction with the apatite values from these individuals, Prowse et al., (2005) suggest that older individuals were consuming more olive oil and wine than younger individuals. Young children under the age of five years appear to have consumed a purely terrestrial diet, indicating the variability inherent within the population. There is also the possibility that these individuals were experiencing nutritional stress or illness, both of which can result in higher $\delta^{15}\text{N}$ levels unrelated to diet.

Within Rome and its environs during the Imperial period (1st to 3rd centuries AD), the stable isotope analysis of 48 individuals from an urban cemetery and a suburban cemetery again highlights the variability in diet that can be found within a single population (Killgrove and Tykot, 2013). The bone collagen values show a $\delta^{13}\text{C}$ range of -19.6‰ to -12.5‰ with a mean of -18.2‰, and $\delta^{15}\text{N}$ values range from 7.0‰ to 13.2‰, with a mean of 10.0‰ (Killgrove and Tykot, 2013). The bone carbonate results, which reflect the

bulk stable isotopic value of the diet, range from -13.8‰ to -8.6‰ with a mean of -11.8‰. For both the cemetery groups, the individuals appear to have consumed a diet consisting primarily of C₃ plants and C₃ herbivore protein, with the addition of some aquatic protein or C₄ plants. Killgrove and Tykot (2013) found that the individuals from the cemetery located within Rome showed isotopic evidence of consuming aquatic (either freshwater or marine) resources, while the suburban Romans showed isotopic values consistent with a certain amount of millet, or C₄ plant, consumption. The wide range of values found in this study supports the authors' assertion that while there was a dietary base consisting of cereals, olives, and wine, additional foods were also common in the Roman menu. These may have included terrestrial meats, legumes, fish, and millet, found in differing proportions in the diet and from various sources in the region. There was no significant evidence of sex differences with the dietary stable isotopic values (Killgrove and Tykot, 2013).

The similarities of many of these sites to Helike in regards to their coastal locations and access to resources make their populations useful for comparisons in this study. Keenleyside et al. (2006) found striking similarities between the values at the Classical Greek colony of Apollonia and those of the individuals at Isola Sacra. Although the Helike sample was not analyzed for $\delta^{13}\text{C}$ bone apatite values, these Roman studies show the potential variability that can occur due to the consumption of lipid-rich foods, such as olive oil. This variability serves as a reminder that in omnivorous and mixed-resource diets the contribution of plant resources to tissue stable isotopic values can be significant.

2.3.3. The Byzantine Period

The Byzantine period spans a significant amount of time and involves a large geographical area during the empire's height of power. Diet and food are somewhat better recorded for this period, but much of the information

presented below is biased towards certain groups, such as urban-dwellers or those living a monastic life. What is known about general diet during the Byzantine is supplemented by information about the fasting and dietary edicts, which came in to use through the Byzantine Orthodox church. What is apparent is that for many urban dwellers, a great variety of foods and resources were available to those who could afford them.

2.3.3.a. Historical and literary information

Information about food in the Byzantine period comes from a wide variety of sources such as illustrations, cookbooks, domestic accounts, feast menus, dietetic manuals, accounts of saints' lives, travellers' tales, poetry, and secular satires (Bourbou and Richards, 2007; Koder, 2003; Talbot, 2003; Dalby, 1996). This information generally describes the dietary habits of the upper classes and ecclesiastical institutions, as these groups contained the literate members of Byzantine society (Talbot, 2003; Dalby, 1996). Although much was written about food, there is little direct written evidence about everyday meals (Koder, 2003). Despite this, some general ideas about what constituted everyday foods have emerged in the literature, based upon records of what the market stalls in the cities sold. These records show that olives and olive oil, meat, vegetables, fruits, and dairy products were available for purchase, as well as bread, cheese, butter, oils, olives, legumes and dry vegetables, honey, salted meat, and fish (Demosthenous, 2005; Koder, 2003). Fishmongers and butchers specializing in lamb, mutton, and pork sold the more expensive fishes and meats. The dietary standbys of cereals, pulses, vegetables, olives, wild greens, fruit, oils, and wine likely continued to dominate the food choices in Byzantine Greece (Dalby, 1996). Poorer individuals may have consumed mostly vegetarian meals consisting of fruits and vegetables that were seasoned with vinegar, honey, pepper, cinnamon, cumin, caraway and salt. Cheese, olives, and onions acted as meat substitutes (Dalby, 1996).

Generally, in urban areas, higher status foods consisted of hard-to-find fresh vegetables and meats, which required money to purchase and large amounts of fuel to cook (Woolgar, 2007), although small-scale food cultivation did occur within the city (Kazhdan, 1997). This being the case, hot home-cooked food may not have been regularly achievable for the average urban-dweller due to the high prices of wood (Koder, 2003); eating in taverns appears to have been more affordable (Kislinger, 1999). Not surprisingly, upper class foods could be broader in range and more selective in terms of quality than the lower class foods (Woolgar, 2007).

The role of the ecclesiastical institutions in directing everyday life, including meals, is most obvious with the dietary edicts involving feasting and fasting. These are discussed in further detail in section 2.3.3.b.

Wines and Oils

Wine was the primary drink available within the monastic sphere and was occasionally diluted with heated water (Talbot, 2003). During Lenten periods, it might be replaced by hot water flavoured with cumin, fennel, honey, or grape syrup (Talbot, 2003). In secular life, wine was also offered in taverns, along with simple dishes and soups, and was drunk without the addition of water (Koder, 2003).

Wine flavours available included mastic, aniseed, rose, and absinthe (Dalby, 1996). Poorer individuals may have consumed a mixture of vinegar and water as a substitute for wine (Dalby, 1996).

Olives and olive oils, as mentioned above, remained commonplace food items, although regional differences did exist in the availability of these as well as other foods in Byzantine Greece (Koder, 2003). Linseed and flax oils were also utilized in cooking (Talbot, 2003).

Cereals

Bakers were a favoured trade in Byzantium and enjoyed a range of privileges intended to ensure that the production of bread remained uninterrupted; an exemption from military service was one of these

privileges (Dalby, 1996). Bread appears to have been present at almost every meal in Byzantine Greece, although for the poor it was likely made of inferior kinds of bran or barley and has been referred to as “dirty bread.” “Clean bread” was made from white flour (Koder, 2003; Dalby, 1996). Wheat remained one of the cereals of choice, if there was a choice available. During the earlier periods of the Byzantine Empire, it was imported from Egypt to sustain the population living within the city (Kislinger, 1999). If there were delays or if the cargo of the Egyptian ships was lost, then occasionally the Byzantine rulers would open the imperial reserves and order the barley within them to be distributed to the populace (Kislinger, 1999). This again reinforces the notion of barley as a famine food or a second-choice cereal and suggests that wheat production within the Byzantine Empire was initially not enough to sustain the urban populations. Substitutions for the Egyptian imports were later found through supplies from Thrace, Bithynia, and the Black Sea regions (Kislinger, 1999), although there is some evidence suggesting that grain consumption decreased in the 11th and 12th centuries due to the loss of important grain-exporting territories in the Byzantine Empire (Kazhdan, 1997).

Legumes

Given the potential expense of buying meat or high quality fish at the market and the unfeasibility of regularly butchering and consuming one’s own domesticated animals, legumes may have made up a significant part of the every-day Byzantine menu. Beans, lentils, fresh pulses and lupines are thought to have been staple provisions for all social classes and not just the poor (Koder, 2003), in contrast to earlier historical periods. Lentil flours could also be mixed into breads to stretch out the amount of cereal flour, although porridges may have been the more preferred method of lentil consumption (Dalby, 1996).

Fruits and Vegetables

Koder (2003) draws upon the satirical writings of Ptochoprodemos to obtain a better idea of the kinds of fruits and vegetables were available to the Byzantine citizen. From this source, there is mention of various spices, celery, leeks, green salad, cress, endive, spinach, turnip, aubergine, cabbage, kohlrabi and cauliflower. Fruits included apples, pomegranates, dates, pears, cherries, melons, plums, nectarines, quinces, peaches, and laurel berries (Koder, 2003). Similar to vegetables, fruits could also be consumed dried, which afforded some availability when out of season and when traveling.

In his medical treatise, the physician Hierophilos recommended certain foods to either seek out or avoid consuming during various times of the year. Along with the previously mentioned fruits and vegetables, he lists certain nuts, such as almonds, pistachios, hazelnuts, and “imperial” nuts (Koder, 2003).

Both fresh and preserved fruits and vegetables were probably more affordable for the average urban Byzantine individual than meat or fish. These foods are also thought to have constituted the primary non-cereal portion of the everyday Byzantine meal, in conjunction with low-cost dairy products (Koder, 2003; Dalby, 1996). New foods that emerged in during the Byzantine period in Greece include the aubergine, the orange, imported spices such as nutmeg and cinnamon, and cane sugar, although these would have been expensive. Other spices and seasonings were an integral part of meal preparation, regardless of social status and food resource accessibility (Dalby, 1996).

Meat and Animal Products

If animal flesh was consumed, it was on rare occasions for most people and came from sheep, goats, poultry, pigs and cattle (Koder, 2003). The expense of high quality meats, including bacon and other fatty portions, along with the frequent and extensive fasting periods, prevented their consumption in large amounts (Demosthenous, 2005; Koder, 2003).

Wealthier individuals may have also had access to wild game animals obtained through hunting, such as gazelle, venison, roe deer, boar and hare (Kislinger, 1999; Dalby, 1996.). Sparrows are also mentioned in the literature as a food, similar to the consumption of songbirds by Classical and Hellenistic gourmands (Dalby, 1996).

Meat preservation technology consisted of salting, drying, and smoking processes in Byzantine Greece; Koder (2007) states that by the Middle Byzantine, references in the literature to smoked meat and fish were uncommon, possibly due to the expense associated with obtaining firewood. The most common method of preservation, if the meat was not to be consumed soon after it was butchered, was salting (Koder, 2007). Salted meat (and fish) products appear to have been available at market for most individuals and the demand for salt sustained a robust food processing and trade industry during the Byzantine period (Braund, 1995).

Secondary animal products like dairy and eggs may have been more common in the diet than meat itself due to their availability and relative cheapness. Rural areas continued to be associated with higher dairy and poultry product consumption, although the wealthy in all areas consumed certain quality cheeses and eggs as well (Dalby, 1996). Records of monastic diets mention eggs and Cretan and Vlach cheeses and even though these appear in a satirical context, cheeses were available at many price points, with many affordable enough for the poor to purchase (Talbot, 2003). Feta cheese and yogurt also emerged during the Byzantine period (Garland, 2005; Dalby, 1996).

Fish and Seafood

Fishes and seafood were, as always, available to those living in areas with aquatic resources but it is still not clear from the historical documentation how much of the diet typically consisted of marine foods and if Byzantine religious edicts increased their use (Bourbou et al., 2011). Imported fish products, such as salt roe, caviar, and kippered herrings from

Britain are all known to have found a place in Byzantine cuisine (Dalby, 1996), but were likely expensive due to their scarcity. Although fishes do seem to be associated by some authors with the peasantry more than the wealthy (Kazhdan, 1997), others argue that the opposite was more likely and that the rural peasant diet consisted mostly of dairy and vegetable foods (Koder, 2003; Kislinger, 1999; Dalby, 1996). As in earlier times, there existed a variety of more- and less- desirable fishes, fish cuts, and qualities that affected the market price of fish and their categorization as “poor” food and “rich” food (Mylona, 2008).

Monastic records report that extra servings of fish and shellfish (including oysters, mussels, and scallops) were provided on feast days (Talbot, 2003) and some scholars believe that the consumption of fish was quite large because of the rules of abstinence that governed monastery inhabitants (Louvaris, 2005). In the same 12th century satire mentioned above, Ptochoprodemos exaggerates the differences in eating between the senior and junior monks at the monastery. The senior monks feast on oysters, clams, scallops, crab, squid, lobster, caviar, and on ordinary meal days they consumed mullet, red snapper, striped bass, and flounder. One casserole mentioned includes moray eel, swordfish, carp, small dried mackerel, and eggs (Talbot, 2003). Although this is a satirical representation of the hierarchy found within the 12th century Byzantine monastic communities, it does provide some indication of which fishes and seafood items were available or were considered high quality foods.

The ordering of certain species of fishes as higher or lower status in the 14th century *Opsarologos (Book of Fish)* further supports the argument of larger and rarer fishes being worth more than common smaller fishes, an attitude prevalent centuries earlier (Kislinger, 1999). Using Gallant’s (1985) assertion that the Aegean was a relatively poor source of fish in antiquity, Braund (1995) argues that Byzantium itself was not a major supplier in the fish trade but instead imported much of its fish from the Black Sea area. While fish processing is known to have occurred in the Black Sea area and

trade was an important aspect of the Byzantine economy, others have argued that the Aegean and Mediterranean were not as fish-poor as Gallant (1985) asserts (Lytle, 2010; Mylona, 2008). It is more likely that factors such as where the fish were caught, if they were fresh or preserved, and how they were served influenced the perception of their quality and their place in the food hierarchy (Demosthenous, 2005).

Fish sauce remained in use for the majority of the Byzantine period and was particularly associated with higher status dishes as well as everyday meals (Curtis, 1991). It appears to have declined in popularity after the 11th century AD, although this conjecture is based upon its lack of mention in the historical writings. This trend contrasts with the frequent use of *garos* or *garum* described in earlier time periods, especially the Roman (Koder, 2003; Dalby, 1996).

2.3.3.b. Fasting and feasting in the Orthodox Church

In the fourth century AD, Christianity linked diet and virtue together and promoted abstinence, especially from meats and dairy fats. Early Christian writers often used depictions of Roman excess and gluttony to preach about the importance of fasting and celibacy (Grimm, 2007; Garnsey 1999) and later religious prohibitions against eating foods made with animal blood acted to incorporate and reinforce Judeo-Christian concepts of food purity (Dalby, 1996). With the establishment of the Byzantine monasteries, food decisions and abstinence became even more closely tied to religious practices and fasting was followed to various degrees, some quite extreme in nature (Dalby, 1996). Total fasts involved complete abstinence from food and drink, while partial fasts restricted the consumption of certain foods (Louvaris, 2005).

For individuals in secular life, abstinence from meat was encouraged for at least 195 days out of every year and up to 203 days, according to the Julian calendar (Louvaris, 2005). This range was dependent on which day the

feast day of Easter Sunday was celebrated on (Louvaris, 2005). These 195 to 216 days included the typical weekly fasting days (usually Wednesday and Friday) and four other extended fasting periods including Lent, Pentecost, the Feast of the Virgin, and Christmas. On ordinary, non-fasting days, meat, dairy, and fish consumption were allowed, while on Wednesdays and Fridays they were prohibited, as well as during Lenten fasting periods (Louvaris, 2005). Certain other fasting days when meat was prohibited did permit fish consumption, however (Louvaris, 2005).

Shellfish, possibly due to the perception of them as bloodless and “clean,” escaped religious classification as “meat” and was allowable on meat-free fasting days with calamari, cuttlefish, octopus, crabs, crayfish, lobsters, oysters, mussels, clams, and sea urchins acting as substitutes (Louvaris, 2005). In any case, the majority of the non-elite Byzantine population may have rarely consumed pigs, sheep, and goats as opposed to secondary animal products (Kislinger, 1999). For the non-monastic individual, fish was the food of choice on those particular abstinence days that did not expressly prohibit it, but it was not the only option. Legumes and vegetables were generally always available and were more affordable for the majority of individuals in Byzantine Greece (Woolgar, 2007; Koder, 2003; Kazhdan, 1997).

Most of the information about fasting and abstinence foods comes from monastic settings and provides an idea of what some of these particular fasting practices involved. Individuals following a monastic lifestyle normally ate twice a day during non-fasting days (Talbot, 2003). The staple foods in the monastery were bread, wine, legumes, and green vegetables boiled with olive oil, vinegar, or water. The evening meal consisted of bread, seasonal fruits and vegetables, leftovers from the previous meal, and “dry” foods including nuts, dried fruits, olives, and pickled cabbage (Louvaris, 2005; Talbot, 2003).

During a religious fasting period such as Lent, dietary regulations varied widely between monasteries (Talbot, 2003). Usually they consisted of

one meal per day, with no wine, olive oil, cheese, fish, or eggs allowed. This was also the case for weekly Wednesdays and Fridays, although the regulations usually became more liberal on the weekends (Talbot, 2003). During Lenten periods, wine might be replaced by hot water flavoured with cumin, fennel, honey, or grape syrup (Parry, 2005; Talbot, 2003).

Other sources mention that between Easter and All Saints Day two meals per day were cooked, consisting of vegetables or legumes in olive oil (Parry, 2005). Fish, cheese, and eggs were allowed during this time, along with wine (Parry, 2005). During the fasts of the Holy Apostles and of St. Phillip, no fish, cheese, or eggs were allowed except on days when the hours were not sung. Vegetable and legume dishes, either with or without oil, were acceptable substitutes (Parry, 2005). As is apparent from the brief descriptions above, fasting rules and dietary edicts could be quite elaborate and variable from place to place.

It is still unknown what exactly the effects of Orthodox Christian fasting and abstinence edicts were on the non-elite and non-monastic Byzantine Greek population. There is some suggestion that the Christian fasting requirements made vegetarianism more acceptable as a choice (Parry, 2005) and encouraged the development of an abstinence-compliant cuisine (Louvaris, 2005). Other sources suggest that fasting was not always voluntary but also used as a punishment during ecclesiastical trials, forcing the guilty to become more monastic and “holy” in their eating behaviours (Louvaris, 2005; Kislinger, 1999). Gender differences in observance of fasting and food abstinence are also apparent in the historical record. Women who fasted were praised more highly than men who fasted, as meat was associated with carnality, which was considered a particular weakness in females (Bynum, 2008; Garnsey 1999).

2.3.3.c. Archaeobotanical information for the Byzantine period

Fuller et al. (2012) found millet remains beginning in the Early Byzantine period at Sagalassos and state that the amount of millet appears to

increase during the Middle Byzantine period. The stable isotopic evidence for the faunal remains from this site and these time periods suggests that the domesticated animals were being foddered on this particular grain. Otherwise, the archaeobotanical information from the site shows that wheat and barley continued to play an important agricultural role (Fuller et al., 2012). Interestingly, this evidence also indicates that the cultivation of lentil crops decreased from the Early to the Middle Byzantine period (Fuller et al., 2012).

2.3.3.d. Zooarchaeological evidence for Byzantine Greece

Kroll (2010) presents a review of the current knowledge about faunal use in the Byzantine period, and notes that during the transition from the Late Roman to early Byzantine periods, animal husbandry practices seem to have stayed relatively stable. Sheep and goats were exploited for their use of secondary products, while cattle were used mainly as draught animals. Pig was consumed both at its maximum meat weight and also when young, and evidence in several areas that suckling pig was a delicacy. Overall, while there were slight regional differences, animal husbandry in Byzantine Greece appears to have been relatively homogeneous in nature (Kroll, 2010).

Kroll (2010) also states that pork consumption was common, both as a highly prized food and because pig was a species that could be kept within an urban environment, making it a useful animal protein source in times of siege. The faunal evidence indicates that famines and food shortages were dealt with by increased exploitation of local resources, such as fishes and game meat (Kroll, 2010). In general, the hunting and consumption of wild fowl and fishes appears to have increased during food shortages and also provided some dietary variety during other times.

The faunal remains from the Early Byzantine period (450–600 AD) at Sagalassos indicate that there is a decrease in the proportion of cattle material as compared to the preceding Roman period (Fuller et al., 2012). The proportion of ovicaprines and pigs at the site increased during this time,

as well. The zooarchaeological material dating to the Middle Byzantine period (800 – 1200 AD) show a similar proportion of *Ovis/Capra* material as the Early Byzantine period, although there is a slight increase in the proportion of cattle material and a slight decrease in pig remains (Fuller et al., 2012).

2.3.3.e. Stable isotope analysis of Byzantine diets

Stable isotope reconstruction of diet can become slightly more complicated in the Byzantine time period due to the wide range of dietary sources across the empire and the effects of trade on food selection. Although the application of stable isotopic techniques to this time period in Greece is relatively recent, the available studies that examine diet during the Byzantine period provide data for comparison of results and a wider context within which resource use trends can be placed.

Bourbou et al.(2011) provide a useful multi-site examination of stable carbon and nitrogen isotopes in human collagen from Byzantine Greece, including comparisons of coastal and inland sites to detect marine resource use. Using information from previous stable isotope studies (Garvie-Lok, 2001; Bourbou and Richards, 2007; Bourbou, 2010), general trends involving both human and faunal values and their variations are discussed. The sites involved in this study are the inland sites of Eleutherna, Messene, Sourtara, Stylos, Servia, and Nemea, and the coastal sites of Kastella and Petras. Associated faunal material was only available from Eleutherna, Kastella, and Sourtara and the development of a faunal isotopic baseline was supplemented with values obtained by Garvie-Lok (2001) from Corinth, Athens, and Mytilene.

The results show that the faunal collagen $\delta^{13}\text{C}$ values range from -21.7‰ for a goat at Mytilene to -18.7‰ for both a goat from Kastella and a dog from Sourtara. These indicate animals that subsisted primarily on a C_3 -based diet (Bourbou et al., 2011). Fish stable isotope values are a valuable addition to the information about Byzantine diet, as fish remains are not well

represented in the archaeological record. While modern specimens provide the majority of these values, Pennycook (2008) was able to include a sea bream and a tuna sample recovered from Early Byzantine Stymphalos, located in the Peloponnese region. The $\delta^{13}\text{C}$ value for the sea bream was -21.1‰ and the $\delta^{15}\text{N}$ was 7.5‰ . The tuna's values were -10.4‰ for $\delta^{13}\text{C}$ and 9.6‰ for $\delta^{15}\text{N}$ (Pennycook, 2008). Notably, all the fish values could be separated into higher and lower trophic level categories and could be distinguished isotopically from terrestrial domesticated products, allowing a more refined interpretation of the human isotopic values and resource use.

Somewhat surprisingly, given the purported wide range of foodstuffs available during the Byzantine period and the geographic distances between sites, the human stable isotopic values cited in Bourbou et al. (2011) cluster together fairly tightly. The individuals from the inland sites showed mean $\delta^{13}\text{C}$ values ranging from -18.2‰ to -19.2‰ and mean $\delta^{15}\text{N}$ values ranging from 8.2‰ to 9.5‰ (Bourbou et al., 2011). Individuals from the two coastal sites had mean $\delta^{13}\text{C}$ values of -18.8‰ to -19.2‰ and mean $\delta^{15}\text{N}$ values of 9.1‰ and 9.5‰ . While the inter-site comparisons were statistically significant, the overall results suggest a primary dependence on C_3 -based resources and terrestrial animal proteins, which are argued to be likely dairy products more than meat itself (Bourbou et al., 2011). Evidence of marine resource use was found at Sourtara, an inland site, as well as at the two coastal sites. A few individuals had stable isotope values suggestive of heavy marine resource consumption, which may indicate movement between settlements with differing food strategies prior to death (Bourbou et al., 2011). The presence of individuals with isotopic values indicative of marine resource use is not unknown even from earlier time periods, such as prehistoric Greece, when the consumption of marine foods is thought to have been relatively rare (Bourbou and Richards, 2007). Along with Bronze Age individuals argued to have been high status at Mycenae, later individuals from 1st to 3rd century AD Kenchreai and 4th to 8th century Isthmia show

isotopic values consistent with significant marine resource use (Rife and Garvie-Lok, as cited in Bourbou et al., 2011).

The general isotopic values from the Byzantine period individuals cited in Bourbou et al. (2011) appear consistent with those from another Greek Byzantine site, the coastal settlement of Abdera. The mean human bone collagen values were $-18.8\text{‰} \pm 0.4\text{‰}$ for $\delta^{13}\text{C}$ and $9.1\text{‰} \pm 1.1\text{‰}$ for $\delta^{15}\text{N}$ at this site (Agelarakis, as cited in Bourbou et al., 2011). The values from Late Roman/Early Byzantine Stymphalos also support the evidence for reliance on C_3 dietary resources, with a mean $\delta^{13}\text{C}$ value of -18.7‰ and mean $\delta^{15}\text{N}$ values of 9.0‰ (Pennycook, 2008). The interpretation of these values in the context of the faunal baseline values suggests that they are largely consistent with the predicted values for C_3 meat or dairy-rich diets, perhaps with the addition of lake waterfowl to the diet or the fertilization of crops with manure (Pennycook, 2008).

Although the Middle Byzantine individuals from Sagalassos are from a site located outside of Greece, there is still the same isotopic trend of C_3 terrestrial resource use apparent. The human bone collagen values of these individuals show a mean $\delta^{13}\text{C}$ of -19.0 (with a standard deviation of 0.3‰) and a mean $\delta^{15}\text{N}$ of 9.1‰ (with a standard deviation of 0.9‰) (Fuller et al., 2012). Again, the lack of isotopic evidence for marine resource use is not a surprise given the site's inland location, although it was part of a major trade network and inhabitant may have had access to preserved fish products and other marine items that were transported through the city (Fuller et al., 2012).

The overall lack of variation within these values supports the idea that a generally similar Byzantine Greek diet was consumed regardless of location. Some site-specific and individual variations in diet do appear exist, with coastal settlements showing evidence of dietary supplementation with small amounts of marine resources. While marine consumption did occur in varying amounts, for the majority of the individuals analyzed thus far,

terrestrial sources appear to be the main focus of subsistence in Byzantine Greece.

Overall, the stable isotopic evidence confirms the continued importance of cereals, wine, and oil to the Mediterranean diet and is consistent with the interpretation that dairy product consumption provided the main source of animal protein rather than meat (Bourbou and Garvie-Lok, in press). Leguminous vegetables do not appear to be a major component of diet at these sites since the $\delta^{15}\text{N}$ values are much too high to be congruent with this type of food. This is an intriguing finding, given the emphasis on lentils and legumes in the literature about food and fasting edicts. Small-scale use of C_4 millet is also not visible isotopically, although small amounts in the diet can be overwhelmed by the consumption of more depleted stable carbon resources. While millet was cultivated in Greece, it is found only in certain areas and does not appear to have been a widespread cereal crop (Bourbou et al., 2011).

Although these are not stable isotopic techniques, Papageorgopoulou and Xirotiris (2009) use trace element analysis of strontium, barium, and zinc to examine diet in individuals from Korytiani, Greece (10th to 11th centuries AD). The osteological findings, including dental disease, tooth wear, and muscle markings, lead the authors to conclude the population followed a low-protein diet based primarily upon carbohydrates and vegetables. The results show that the population at Korytiani had relatively high Sr:Ca, which the authors attribute to substantial plant consumption. Ba:Ca ratios suggest no consumption of marine foods, which is consistent with the inland location of the settlement. Zinc values, although of little use on their own, were determined to be moderate in conjunction with the Sr:Ca and Ba:Ca ratios. This was interpreted as the effect of phytate cellulose and hemicellulose in the diet from ingestion of plant foods inhibiting zinc absorption from meat. No sex differences in these trace elements were found. Due to issues with diagenesis and debates over the proper interpretation of values, trace element analysis of bone can be problematic (Burton, 2008). The analysis

leaves some unanswered questions that other methods such as stable isotope analysis may be able to better address.

While certain individuals and several sites appear to show increased marine resource consumption in the Byzantine, these findings are not consistent across Greece or even within sites. Marine resource use appears to have been largely supplementary to the consumption of C₃ terrestrial resources.

Chapter 3: Stable Isotope Methodology and Background

This chapter presents some basic information about stable isotopes, what they are, how they are measured and described, and what is known about their distribution within the biosphere via fractionation processes. More specifically, the stable isotopes of carbon and nitrogen are discussed as these are involved with dietary reconstruction and are also well known in the ecological literature. The chemical properties of each are discussed, in addition to the patterns of each found in autotrophic and heterotrophic organisms. Further discussion of the values associated with terrestrial, marine, freshwater, and lagoon environments is presented. Factors involving the relationship of dietary stable isotopic values to human bone are presented along with the archaeological applications of stable isotope analysis to dietary reconstruction.

3.1. Stable Isotopes

Isotopes are atoms whose nuclei contain the same number of protons but a different number of neutrons (Hoefs, 2009). They are forms of the same element and are not chemically different from each other but they do have different mass because of the variations in their neutron counts. As their name implies, isotopes occupy the “same place” within the periodic table (Fry, 2006).

Isotopes can be divided into stable and unstable species (Hoefs, 2009). Stable isotopes contain a moderate number of neutrons that are equal to or slightly greater than their number of protons. This results in a stable nucleus and a stable form of the element (Fry, 2006). Unlike unstable isotopes such as uranium-238 and carbon-14, stable isotopes do not undergo spontaneous radioactive decay, where the nucleus of an unstable atom releases energy in the form of ionizing radiation (Fry, 2006).

The stable isotopes of carbon (^{13}C and ^{12}C) and nitrogen (^{15}N and ^{14}N) are naturally abundant within both the environment and the body (Fry,

2006); they are present within the food chain, from the smallest bacteria to the highest top-level carnivore. Isotopes such as these have shown to be useful for ecological studies because they can be traced throughout the biosphere in order to determine how they originate and how they circulate (Fry, 2006). The following sections discuss some of the basic information about the stable isotopes of carbon and nitrogen, how they are useful in reconstructing food web structures, and the variables that affect their relative abundance in organisms.

3.1.1. Measurement

Light stable isotope measurements are usually made with an isotope ratio mass spectrometer (Fry, 2006). The main components of this device are the inlet system, the ion source, the mass analyzer, and the ion detector (Hoefs, 2009). To obtain a stable isotopic measurement, the test sample is processed into a form that can be combusted and turned into simple gases. For example, the carbon in a sample is converted into carbon dioxide (CO₂) and the nitrogen is converted into nitrogen gas (N₂). This combustion process can be done with an elemental analyzer, a gas chromatograph, or a laser (Fry, 2006).

Once formed, the gases enter into the source region, or inlet system, where a filament boils off electrons in a violent reaction. The simple gas molecules are ionized, losing an electron and becoming positively charged. They are then accelerated out to the flight tube through electric fields. A magnetic field deflects the positively charged ions by different degrees according to their atomic mass, which separates the isotopes. This creates ion beams that focus into Faraday collectors for conversion into electrical impulses (Hoefs, 2009). Finally, computer software is used to convert the impulses into counts and to calculate the raw δ values (Fry, 2006). For further details about the various types of mass spectrometers available, see Hoefs (2009).

3.1.2. Notation

For light elements such as carbon and nitrogen, the stable isotope content is usually expressed using a notation that reflects the ratio of the heavy to the light stable isotopes of the element (e.g. $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) and relative to the ratio seen in a standard (Hoefs, 2009; Fry, 2006). Each element has an international standard or reference value in order to ensure consistency and comparability of data between laboratories (Hoefs, 2009).

Light stable isotope values are expressed using delta notation:

$$\delta^H X = \frac{R_{\text{SAMPLE}} - R_{\text{STANDARD}}}{R_{\text{STANDARD}}} \times 1000$$

The delta value, or δ value, of a particular stable isotope is always expressed in terms of the heavier stable isotope in question (e.g. $\delta^H X$ would be written as $\delta^{13}\text{C}$ in a stable carbon isotope value). Delta values are, in effect, indicators of the proportion of a heavy isotope that is present in the sample (Fry, 2006).

The δ value is calculated by taking the ratio of the heavy isotope to the light isotope in the sample (R_{SAMPLE}), subtracting the ratio of the heavy isotope to the light isotope in the standard (R_{STANDARD}), and dividing the result by the standard ratio (R_{STANDARD}). Finally, the value is multiplied by 1000 and is expressed as ‰ or per mil. This last calculation amplifies the very small differences that are found between the samples and their respective standards, facilitating reading and mathematical operations (Fry, 2006).

3.1.3. Fractionation

Fractionation and mixing are the two main processes that affect the abundance of heavier and lighter isotopes of an element, with exchange reactions playing a smaller role. Fractionation results from the differential isotope behaviour that occurs in a kinetic, mass-dependent reaction (Hoefs, 2009; Fry, 2006). The lighter isotopes will usually create weaker chemical bonds and react faster than the heavier isotopes (Hoefs, 2009; Fry, 2006). In rare cases, the opposite can occur but this usually does not involve the stable isotopes that are analyzed in this thesis (Fry, 2006).

Mixing can be considered the opposite of fractionation: it reunites isotopes as opposed to separating them out according to mass. In ecological studies, mixing can help explain the various sources of stable carbon that are utilized by plants and animals and again can be very useful to consider in marine and aquatic food web reconstruction (Fry, 2006).

In addition to fractionation and mixing, isotopic exchange reactions can occur. In these exchange reactions, the recombination and recycling of isotopes helps maintain isotopic equilibrium, as with inorganic carbon systems involving calcium carbonate and bicarbonate (Hoefs, 2009). These reactions are of particular importance in marine systems.

Fractionation and mixing processes are both important, but it is fractionation that primarily forms the basis of stable isotope analysis. As each element passes through biochemical reactions at various trophic levels, the differences in the strength of the molecular bonds between the stable isotopes of an element result in fractionation. As mentioned above, the lighter form of an isotope will be preferentially selected and react faster than the heavier form. Fry (2006) notes that at the level of simple compounds, chemical and physical theory can predict the maximum possible fractionation but at the ecosystem level, there are several competing processes involved and understanding the effects of fractionation becomes much more complex.

3.1.4. Stable Isotope Analysis and Ecology

Archaeological use of stable isotope analysis to reconstruct past diet, past environments, and the movements of both objects and people owes a debt to theoretical and methodological work in a variety of disciplines. As a result, most of the information about stable isotopes is spread across a range of literature.

Other disciplines that have laid the groundwork for stable isotope analysis in ecology include plant and animal sciences, geology, and general sciences (Lee-Thorp, 2008). Geochemistry was at the forefront of stable isotope applications, using them to determine the composition of the earth's mantle, volcanic rocks, and ore deposits (Hoefs, 2009). The similarity in $\delta^{13}\text{C}$ values between organisms and plants from the same environment was first noted in 1953 by a geochemist (Hoefs, 2009). Subsequently, stable isotopes began to be used by ecologists to directly trace element cycling, to detect activities such as animal migration patterns, diet and metabolism, and to illustrate ecosystem complexity (Fry, 2006). The relationship between tissue stable isotope values, food values, and isotopic shifts during tissue synthesis forms the basis of dietary reconstruction using stable isotope analysis used today.

3.2. Stable Carbon Isotopes: Characteristics and Measurements

Carbon has sixteen known isotopes, of which two are stable: ^{13}C and ^{12}C . ^{12}C is the most abundant in the atmosphere at nearly 99%, while the majority of the remainder consists of ^{13}C (Smith, 1972). Carbon can be found in a large variety of compounds on Earth, both in the biosphere and in inorganic forms such as atmospheric CO_2 (Hoefs, 2009). The stable carbon isotope value of atmospheric CO_2 has changed over time, most notably in the past 200 years. It ranges close to -7‰ in modern times as the burning of fossil fuels, which are depleted in ^{13}C , has increased (Treydte et al., 2009; Fry, 2006).

The equation for expressing the δ value of stable carbon is as follows:

$$\delta^{13}\text{C} = \frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{SAMPLE}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{STANDARD}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{STANDARD}}} \times 1000$$

The international reference standard for carbon is PDB or PeeDee Belemnite, a Cretaceous limestone fossil material (Craig, 1957). PDB is highly enriched in ^{13}C and, as a result, most biological materials show negative values in comparison, reflecting their relative depletion of ^{13}C . As the original sample has now been exhausted, newer reference samples have come into use (Hoefs, 2009). However, international δ values are still expressed relative to PDB.

3.3. Stable Carbon Isotopes in Autotrophs

Plants are crucial contributors to the carbon cycle on Earth, taking in CO_2 from the atmosphere and releasing oxygen, water, and a small amount of CO_2 in return through respiration. Carbon fixation is done through photosynthesis and, depending on the type of photosynthetic cycle used by the plant, results in differing fractionation between atmospheric carbon and the carbon in plant tissues (Park and Epstein, 1961). As mentioned above, modern atmospheric carbon dioxide has a $\delta^{13}\text{C}$ value of approximately -7‰ (Fry, 2006). Tree ring studies using stable carbon isotope analysis have confirmed a trend towards $\delta^{13}\text{C}$ decrease in atmospheric carbon dioxide since 1800-1850 AD, as well as during several global warming periods in the past (see Treydte et al., 2009 for further tree ring reading). This means that comparisons of archaeological $\delta^{13}\text{C}$ values to modern ones should be done cautiously, as atmospheric $\delta^{13}\text{C}$, and thus typical plant $\delta^{13}\text{C}$, has changed over time. Use of modern plants and other samples for direct stable isotope comparisons to ancient material should take into the account these changes in order to make an accurate interpretation.

This section details the main photosynthetic pathways used by plants and how these pathways affect their stable carbon isotope values. The contributions of the environmental conditions to both the carbon pool and to plant photosynthetic pathways are also discussed.

3.3.1. Photosynthesis

Photosynthesis is the process used by plants to convert light energy into chemical energy; it changes carbon isotope ratios in well-known ways (Fry, 2006). Since the plants generate their own food, they are referred to collectively as autotrophs. The general source of carbon dioxide used by autotrophs is atmospheric, although the kind of environment the plant exists in also affects the type of carbon source used (Smith and Epstein, 1971). The three main photosynthetic pathways in plants are discussed below, including the differences in stable isotope fractionation between them. Previous to Calvin and Benson's 1948 publication, researchers had been aware that terrestrial plants were depleted in ^{13}C as compared to the atmosphere that they drew carbon from (e.g. Craig, 1953; Wickman, 1952) but did not realize that these values were directly related to differences in photosynthetic pathways (Rounick and Winterbourn, 1986).

It is also important to note that the $\delta^{13}\text{C}$ values of a plant will vary according to which part is sampled (O'Leary, 1981). In general, leaves appear to be less enriched in ^{13}C than the roots or seeds of a plant (O'Leary, 1981; Deines, 1980). This variation within individual plant values also occurs further up the food chain with consumer tissues and the implications for human dietary reconstruction are discussed further in section 3.8.1.

3.3.1.a. The Calvin cycle

The Calvin-Benson cycle, also known as the Calvin or C_3 pathway, involves incorporation of atmospheric CO_2 by the enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (RUBISCO), which catalyzes the fixation of carbon dioxide into plant sugars, forming an initial three-carbon molecule.

This molecule gives the pathway its C₃ name (Rounick and Winterbourn, 1986; Calvin and Benson, 1948).

C₃ plant $\delta^{13}\text{C}$ values have been documented over the years by various researchers and appear to fall around a mean of -26‰, with a range from approximately -24 to -36‰ (Lee-Thorp, 2008; Deines, 1980). They are relatively depleted in ¹³C as the ¹²C has been preferentially selected and included in the plant tissues. The majority of terrestrial plant species found on the planet, including trees and shrubs, are C₃ plants. These plants tend to be found in areas with adequate water supply and their ability to assimilate larger amounts of carbon dioxide more quickly than other plant types is linked to these conditions (Smith and Epstein, 1971).

3.3.1.b. The Hatch-Slack cycle

The Hatch-Slack cycle, or C₄ photosynthetic pathway, involves the incorporation of atmospheric CO₂ by carboxylation of phosphoenol pyruvate (PEP), producing a four-carbon molecule (Rounick and Winterbourn, 1986; Hatch and Slack, 1970).

Compared to C₃ plants, C₄ plants discriminate less against ¹³C and therefore show relatively high $\delta^{13}\text{C}$ values, with means around -13‰ (Deines, 1980) and a range of -6‰ to -19‰ (Smith and Epstein, 1971). Many C₄ plants are grasses, including maize, millet, and sugarcane.

3.3.1.c. Crassulacean Acid Metabolism

Plants that use the Crassulacean Acid Metabolism (CAM) pathway can fix atmospheric CO₂ by using either the C₃ or C₄ pathway, according to the appropriate option for the plant at the time (O'Leary, 1981). CAM plants are succulents and cacti that live in arid regions; it has been suggested that this combined photosynthetic pathway has evolved as a way to enable these plants to adapt to periods of water shortages and heat. The CAM pathway allows plants to adjust their photosynthetic processes to whichever one is

the most efficient and beneficial in terms of photorespiration and water use (Dawson et al., 2002).

Since the pathway used varies, CAM plants usually show stable carbon values intermediate between those of the exclusively C₃ and C₄ pathway plants, but it is possible for CAM plant values to overlap C₃ or C₄ plant values, as well (Lee-Thorp, 2008; Deines, 1980).

3.3.1.d. $\delta^{13}\text{C}$ variation within plant groups

The variation in stable carbon isotope values that occurs between different plant groups has been identified for some time (Bender, 1968; Wickman, 1952; Craig, 1957; Craig, 1953). Bender (1968) first suggested that the variations in $\delta^{13}\text{C}$ noted in corn and some other grasses were not the result of soil or microclimate factors, but due to something intrinsic to the plants themselves. This intrinsic factor ended up being identified as the difference in photosynthetic pathways between C₃ and C₄ plants, as discussed above.

However, variation has also been noted between similar groups of plants using the same photosynthetic pathway. While a difference in $\delta^{13}\text{C}$ values between CAM plants is likely a reflection of whether the C₃ or C₄ pathway is currently being used, there are other factors that can influence intra-group plant stable carbon isotope values. These include potential metabolic variations within a species, the effects of fertilization on the nutritional health of the plant, temperature, salinity, CO₂ concentration, light intensity, and differential loss of CO₂ through respiration (O'Leary, 1981).

Despite these potential intra-group influences, however, the $\delta^{13}\text{C}$ values of a plant do remain largely reflective of the photosynthetic pathway used (O'Leary, 1981). These $\delta^{13}\text{C}$ differences occur in autotrophs both on land and in marine environments and allow researchers to trace resource use and carbon availability within a given ecosystem (Lee-Thorp, 2008).

3.3.1.e. Marine and freshwater environments

Marine autotrophs (phytoplankton, algae, diatoms, and radiolaria) are enriched in ^{13}C compared to terrestrial autotrophs because their primary source of carbon is dissolved ocean bicarbonate instead of atmospheric carbon dioxide (Lee-Thorp, 2008; Smith and Epstein, 1971). Not only do these different carbon source pools differ from each other in $\delta^{13}\text{C}$, but carbon dioxide also diffuses more slowly in a marine environment (Rubenstein and Hobson, 2004). The ocean carbon reservoir shows a $\delta^{13}\text{C}$ value of approximately +1‰ (Deines, 1980; Smith and Epstein, 1971), but the isotopic composition of dissolved inorganic carbonate (DIC) varies and will therefore be reflected in the plants that use this particular carbon source (Fry and Sherr, 1984). In addition, carbon contributions in aquatic environments can come from particulate organic carbon (POC), which reflects the $\delta^{13}\text{C}$ values of living and dead phytoplankton, bacteria, and other components such as detritus (Fry, 1988; Fry and Sherr, 1984).

Although aquatic autotrophs show a wide range of variation in stable carbon isotope values (Fry and Sherr, 1984), their values usually differ enough from those of land organisms to distinguish marine from terrestrial environments (Rubenstein and Hobson, 2004). However, further variation within marine autotrophs can complicate food web reconstructions as some marine plants use a C_3 pathway and some use a C_4 pathway to fix carbon from either atmospheric or marine sources. Phytoplankton and algae primarily use the dissolved bicarbonate in the ocean water and tend to follow a C_3 cycle (Rounick and Winterbourn, 1986). Consistent with the use of this particular photosynthetic pathway, algal values ranging from -19‰ to -24‰ have been documented (Fry, 2006). Plants such as seaweeds, however, can be more exposed on the surface instead of submerged and may use atmospheric carbon instead of dissolved bicarbonate sources. As well, sea grasses typically use a C_4 pathway and usually show a $\delta^{13}\text{C}$ range of -3‰ to -15‰ (Rounick and Winterbourn, 1986). To further complicate matters, sea grass values as low as -23.8‰ have been reported in the literature (Fry and

Sherr, 1984), indicating the importance of establishing biosphere-specific isotopic baselines when interpreting values.

Whether a marine autotroph is benthic, epiphytic, or pelagic can also affect its stable carbon isotope value. Benthic organisms tend to have higher $\delta^{13}\text{C}$ values than pelagic organisms because of temperature differences between the two areas, surface water carbon dioxide concentrations, and differences in planktonic biosynthesis (Rubenstein and Hobson, 2004). General reported ranges for benthic and epiphytic algae are from -8‰ to -27‰ according to studies cited in Fry and Sherr (1984). The variations in stable carbon isotope values at the lowest level of phytoplankton result in variations all along the food chain and matters are further complicated when two different carbon sources are utilized at the base (Fry, 1988). Marine planktonic blooms are a closed carbon cycling system, where there is no new input of carbon substrates. Therefore, the dissolved carbonate utilized within the bloom has a planktonic origin (Fry, 2006).

Despite these complexities, analysis of $\delta^{13}\text{C}$ in marine environments is still useful, especially with the identification of two isotopically distinct carbon sources at the base of food chains, if not in establishing a trophic level pattern (Fry, 1988).

Freshwater environments also show wide variations in stable carbon values for autotrophs, with some freshwater plants showing values differing by more than 10‰ within the same system (Fry and Sherr, 1984). Physical links between lakes, streams, swamps, and marshes and the terrestrial environment can also affect stable carbon isotope values (Likens and Bormann, 1974).

Stable carbon isotope values for estuarine and riverine particulate organic carbon (POC) are found to range from -24‰ to -30‰ (Fry and Sherr, 1984). The effects of eroding peat beds and soils will affect the $\delta^{13}\text{C}$ values of freshwater environments, depending on the original plant material that is found within these sources (Fry and Sherr, 1984). However, lacustrine plants show more overall ^{13}C depletion as compared to marine plants and

can be differentiated isotopically from most marine and terrestrial plants (Deines, 1980).

3.3.1.f. Lagoon environments

Environments where marine aquatic isolation can occur, such as lagoons, are complex and variable in terms of stable carbon values because of the fluctuations in their structure and composition. Lagoons can be directly connected to the sea, can be separated from it by sand beds or tidal walls, or can vary between both over time. Estuarine and lagoon system food sources are affected by variables such as terrestrial and marine inputs to the system, seasonality, the relative importance of macrophytes, and taxonomic changes in phytoplanktonic populations (Michener and Schell, 1994). Coastal lagoons are restricted environments and species diversity is generally limited within them; benthic diversity is usually low although the overall abundance of organisms is high (Carlier et al., 2007). The isotopic composition of the dissolved inorganic carbonate (DIC) source pool can vary greatly within a given season depending on the physical boundaries of the lagoon, and in situations where ocean water periodically mixes with fresh water and creates a low-salinity environment, this has an important isotopic effect on the carbon sources available for lagoon autotrophs (Fry and Sherr, 1984). Geological sources of bicarbonate in limestone can also contribute to the carbon present in a lagoon system, and lagoons may be used by terrestrial species for hunting or nursery grounds, resulting in increased amounts of terrestrial detritus (Carlier et al., 2007; Likens and Bormann, 1974). Groundwater seepage and rainfall run-off can also contribute to seasonal variations in stable carbon values derived from inorganic carbon sources within lagoons (Vizzini and Mazzola, 2003).

Organic inputs to estuarine systems can include C₃ terrestrial plant material (-23‰ to -30‰), C₄ sea grasses (-3‰ to -15‰, and -26‰ in some species growing in low-salinity reaches), macroalgae (-8‰ to -27‰), C₃ marsh plants (-23‰ to -26‰), C₄ marsh plants (-12‰ to -14‰), benthic

algae (-10‰ to -20‰), and marine phytoplankton (-18‰ to -24 ‰) (Michener and Schell, 1994). Stable carbon isotope values are impacted through changes in living sources that become detritus through decomposition in anaerobic environments (Rounick and Winterbourn, 1986). Generally speaking, most lagoon systems have widely varied $\delta^{13}\text{C}$ values at the autotrophic level, reflecting these multiple inputs.

One of the most significant factors influencing stable carbon isotope value variation in lagoons and estuaries is seasonality and its effects on the inputs discussed above. In summer, increased temperatures, lack of wind, and the decomposition of organic matter all act to reduce oxygen availability, which in turn affects autotroph conditions (Carlier et al., 2007). A study of a French Mediterranean lagoon, where data were collected three times in three different seasons, found that the $\delta^{13}\text{C}$ values of consumers were intermediate between the C_3 and C_4 plants present in the lagoon (Carlier et al., 2007). Another coastal lagoon in western Italy showed similar seasonal variations in both producer and consumer organisms in regards to $\delta^{13}\text{C}$ values (Vizzini and Mazzola, 2003). The highest $\delta^{13}\text{C}$ values were found in summer and the lowest values were found in winter.

Some of the reported values for French Mediterranean lagoons show that the most ^{13}C -depleted inputs into the lagoon systems are freshwater particulate organic matter and the feeder streams from karstic springs and rivers (with mean values of -28.8‰, -27.7‰, and -26.2‰, respectively). Unsurprisingly, C_4 sea grasses were the most ^{13}C enriched, with a mean value of -13.8‰. The values of the macroalgae were highly variable, with means between -28‰ and -16.6‰ (Carlier et al., 2007).

Overall, it is extremely difficult to reconstruct the trophic levels of organisms within a lagoon environment using only $\delta^{13}\text{C}$ values since the variations in the source carbon pools can be quite large. Which source is used is dependent on the type of plants living in the lagoon environment during any given season. For stable carbon, this seems to be generally reflected in

autotroph $\delta^{13}\text{C}$ values that are intermediate to those of exclusively terrestrial or exclusively marine origin.

3.4. Stable Carbon Isotopes in Heterotrophs

Heterotrophs obtain energy for metabolic processes by consuming other organisms as opposed to manufacturing their own energy through photosynthesis. Primary and secondary consumers obtain carbon from their food and their whole body values reflect, with little overall fractionation, the $\delta^{13}\text{C}$ values of their overall diet (Rounick and Winterbourn, 1986). Carbon isotope fractionation in digestion and tissue synthesis result in average whole body heterotroph $\delta^{13}\text{C}$ values that are only about 1‰ above the average value of the diet (DeNiro and Epstein, 1978). This small trophic level effect of ranging from 1‰ to 2‰ can be observed at each level of the food chain, between herbivores, omnivores, and carnivores (Lee-Thorp, 2008). Stable carbon isotope values are particularly useful in differentiating between C_3 and C_4 consumers and between terrestrial and marine consumers (Fry, 2006; Rounick and Winterbourn, 1986).

Stable carbon isotope values for different tissues within the same individual vary, depending on the tissue composition and diet. For example, lipids are depleted in ^{13}C compared to carbohydrates (DeNiro and Epstein, 1978). The type of tissue analyzed will thus determine the stable carbon isotope value obtained (Tieszen et al., 1983; DeNiro and Epstein, 1978). As discussed further below, collagen $\delta^{13}\text{C}$ is generally elevated by about 5‰ relative to dietary protein $\delta^{13}\text{C}$ values (Lee-Thorp et al., 1989; Krueger and Sullivan, 1984).

As with autotrophs, heterotroph stable carbon isotope values have not been shown to be particularly valuable on their own in establishing the trophic level of a consumer within the food chain because of the different pathways and isotopic enrichments that base level organisms can follow (Fry, 1988; Schoeninger and DeNiro, 1984). This section outlines current

knowledge of the behaviour of stable carbon isotopes in heterotrophs living within terrestrial, marine, freshwater, and lagoon environments.

3.4.1. Terrestrial Heterotrophs

Much like plants, terrestrial heterotrophs show a range of stable carbon values dependent on their environment and whether the food they are consuming is from the C₃ or C₄ photosynthetic pathway or a mixture of both. The main determinant of terrestrial heterotroph $\delta^{13}\text{C}$ variation is diet, especially whether the diet was predominantly based on C₃ or C₄ plants or on C₃ or C₄ primary consumers (Rubenstein and Hobson, 2004). Because of this, and because of the differing environmental conditions that favour the establishment of C₃ and C₄ plants and animals within a region, average heterotroph $\delta^{13}\text{C}$ values tend to decrease as latitude increases and increase as altitude increases (Rubenstein and Hobson, 2004). Drier ecosystems also show higher average values than wetter ones (Rubenstein and Hobson, 2004). Since heterotrophs consume autotrophs or the primary consumers of autotrophs, their stable carbon values also tend to be higher because of the effect of fractionation.

DeNiro and Epstein (1978) first discovered the 1‰ whole body $\delta^{13}\text{C}$ elevation above the diet in a study using laboratory-controlled feeding of various animals. Their $\delta^{13}\text{C}$ analysis of diets, whole animals, and several animal tissues found that the whole-body $\delta^{13}\text{C}$ values were minimally altered from the food (DeNiro and Epstein, 1978). This general pattern between diet and an organism's whole-body value has been confirmed in later studies (Lee-Thorp, 2008).

Using data from various sites and animals, van der Merwe and Vogel (1978) determined that a diet consisting totally of C₄ plants should result in average herbivore bone collagen $\delta^{13}\text{C}$ values of about -7.5‰, as calculated from the C₄ plant average of -12.5‰ (Deines, 1980), plus 5‰ from the separation between diet and bone collagen (Lee-Thorp et al., 1989). This estimated endpoint has been utilized in later studies to detect C₄ resource use

(Kellner and Schoeninger, 2007; Finucane et al., 2006; Emery et al., 2000; Cerling et al., 1999).

A diet consisting solely of C₃ plants is expected to result in average herbivore collagen $\delta^{13}\text{C}$ values of -21.5‰ (van der Merwe and Vogel, 1978), as determined from the C₃ plant average of -26.5‰ (Deines, 1980) plus 5‰. This value has been supported in later studies (Emery et al., 2000; Krueger and Sullivan, 1984).

Collagen $\delta^{13}\text{C}$ values falling between the endpoints of -7.5‰ and -21.5‰ would therefore reflect a mixture of C₃ and C₄ terrestrial components in the diet (Krueger and Sullivan, 1984; van der Merwe and Vogel, 1978). Other studies have used this range, or a site-specific modification of it, to confirm the addition of C₄ cultivars such as maize (Emery et al., 2000) or millet (Fuller et al., 2012) to animal diet and to identify feeding differences between C₃ and C₄ browsers and grazers and mixed-pathway ungulates in South Africa (Vogel, 1978). The difficulties in identifying the addition of C₄ terrestrial resources to a C₃ diet are caused by the similarities of mixed C₃/C₄ diet $\delta^{13}\text{C}$ values to the values obtained with the addition of marine resources to the diet (Lee-Thorp, 2008; Koch et al., 1997; Sullivan and Krueger, 1981).

3.4.2. Marine Heterotrophs

As with marine autotrophs, marine heterotroph values are dependent on whether or not the organism is consuming materials or other organisms that utilize the dissolved bicarbonate source pool, the atmospheric carbon pool, or the C₃ or C₄ photosynthetic pathway (Fry and Sherr, 1984). The stable carbon isotope values from mussel and oyster shells tend to be similar to the higher bicarbonate values found in the ocean, indicating that this carbon source contributes more to their shell carbon value than their diet does (Rubenstein and Hobson, 2004). Excluding shell values, which likely approach close to 0‰, marine fauna have an average $\delta^{13}\text{C}$ value of -15‰, with values becoming lower as ocean temperature decreases (van der Merwe

and Vogel, 1978). Most values in marine animals appear to range from -10‰ to -24‰ (Fry and Sherr, 1984).

Chisholm et al. (1982) found that the stable carbon values of marine and terrestrial animals differed by about 7.9‰, reflecting the approximately 7‰ difference between ocean bicarbonate (0‰ to +1‰) and atmospheric carbon source values (-7‰). Marine environments show higher stable carbon isotope values than terrestrial environments because of these differences in carbon sources, in addition to the slower diffusion of carbon dioxide within marine environments; this elevation can be found throughout the marine food chain (Rubenstein and Hobson, 2004). Other factors influencing marine heterotroph stable carbon values include latitude, as northern oceans are also found to have higher $\delta^{13}\text{C}$ values compared to southern oceans, and ocean depth as benthic areas have higher $\delta^{13}\text{C}$ values than pelagic areas (Rubenstein and Hobson, 2004). Bottom-dwelling and bottom-feeding organisms are therefore relatively more enriched in ^{13}C than open water dwelling and feeding organisms.

The development of a site-specific baseline for terrestrial and marine stable carbon values and awareness of the higher $\delta^{13}\text{C}$ values found in marine environments has helped distinguish between the two resource types in several palaeodietary studies (Richards et al., 2005; Little and Schoeninger, 1995; Schoeninger and DeNiro, 1984; Tauber, 1981). However, the use of stable carbon as the sole indicator of a marine or a terrestrial environment is complicated by the overlap in values that occurs with mixed C_3 and C_4 terrestrial diets and those with the addition of marine resources (Little and Schoeninger, 1995).

Schoeninger and DeNiro (1984) found that bone collagen $\delta^{13}\text{C}$ of terrestrial fauna was useful for estimating dependence on marine and terrestrial foods only if there was no possibility of C_4 plant consumption, as the terrestrial values in their study started to overlap those of marine organisms. The authors analyzed bone collagen from more than one hundred modern fish, birds, and mammals from several food webs to test the idea that

stable carbon and nitrogen isotope values in each would reflect marine versus terrestrial feeding. Their results showed that the stable carbon isotope values could separate animals into those that were exclusively marine-feeding and those that were exclusively terrestrial-feeding. However, there was an overlap of about 8‰ in the ranges of the two groups, caused by herbivores that consumed significant amounts of C₄ plants, creating a mixed terrestrial isotopic signature (Schoeninger and DeNiro, 1984). Similar overlaps between marine and mixed C₃ and C₄ terrestrial stable carbon values have been found in other studies (Jones and Quinn, 2009; Little and Schoeninger, 1995). Despite this area of uncertainty, there remains a trend for marine animals to show higher stable carbon isotope values on average than terrestrial animals (Coltrain et al., 2004; Schoeninger and DeNiro, 1984).

As mentioned previously, stable carbon isotopes alone do not appear to be of use, even with a single primary marine food source such as plankton, in distinguishing between trophic levels within a marine environment (Michener and Schell, 1994; Fry, 1988). Although $\delta^{13}\text{C}$ values do increase in organisms higher up on the food chain, the varying food sources, especially at the base of the food chain, show large amounts of $\delta^{13}\text{C}$ diversity.

3.4.3. Freshwater Heterotrophs

Similar to marine ecosystems, a wide range of stable carbon isotope values has been reported at the primary consumer level in many lakes, even in environments where the terrestrial input is minimal (Hecky and Hesslein, 1995). However, as the trophic level of an organism increases, this range becomes much narrower. It is thought that, as with marine systems, preferential consumption of either plankton or benthic algae will affect the stable carbon isotope values of the primary consumers in freshwater environments (Hecky and Hesslein, 1995).

Littoral primary consumers in lake environments are ¹³C-enriched compared to pelagic consumers (France, 1995a), reflecting a similar pattern

found in marine systems. France (1995a) was able to identify two modes of carbon flow in lake organisms: one where POM or phytoplankton is the main mode, resulting in stable carbon isotope values of -33‰ to -34‰, and one where epiphytic algae or terrestrial detritus is the main mode, resulting in higher values of -25‰ to -26‰. The result is a pelagic-littoral difference in carbon flow within the same lake, potentially affected by the differences in algal exposure to water turbulence. In another example of high $\delta^{13}\text{C}$ variation in lake environments, Katzenberg and Weber (1999) reported stable carbon isotope values from Lake Baikal in Siberia for freshwater seals ($-22\text{‰} \pm 0.9\text{‰}$) and three fish species (with means ranging from -15.2‰ to -12.9‰). The fauna in and around Lake Baikal show large variations in their $\delta^{13}\text{C}$ values, attributable to the different stable carbon isotope values present in the deep lake which, again, has multiple carbon sources available at the base of the food chain (Katzenberg and Weber, 1999).

3.4.4. Lagoon Heterotrophs

Establishing clear, discrete $\delta^{13}\text{C}$ ranges for lagoon system consumers is another complicated venture since food sources can include salt marsh plants, sea grasses, macroalgae and phytoplankton, as well as seasonally available migratory animals and birds (Carlier et al., 2007). As mentioned above, autotrophic $\delta^{13}\text{C}$ values are widely variable in lagoon systems and this carries up the food chain. Surprisingly, some studies have found that the $\delta^{13}\text{C}$ range of consumers, including fish and invertebrates, is narrower than those of the potential food sources even when taking seasonality into account (Carlier et al., 2007). The consumer values were all intermediate between the highest and lowest values of potential food sources and varied from -26.2‰ to -16.7‰ in June, -23.7‰ to -15.2‰ in September, and -21.6‰ to -14.8‰ in February (Carlier et al., 2007). These values are broadly similar to those from another Mediterranean coastal lagoon, where the $\delta^{13}\text{C}$ mean for all consumers was $-16.5\text{‰} \pm 2.1\text{‰}$. Most heterotrophic taxa at this lagoon

ranged from -13‰ to -16‰, while a minority ranged from -16‰ to -21‰ (Vizzini and Mazzola, 2003).

Stable carbon isotope variation in higher trophic level fishes in lagoons has been attributed to the consumption of both benthic and pelagic food sources at different times and seasons (Vizzini and Mazzola, 2003). Both resident and migrant fish species in coastal lagoons tend to tolerate fluctuating environmental conditions; flexibility in food source consumption appears to be part of this accommodation (Vizzini and Mazzola, 2003). Schoeninger and DeNiro (1984) found that the bone collagen $\delta^{13}\text{C}$ values for freshwater fish and marine birds that fed in both marine and freshwater systems had stable carbon isotope values that were indistinguishable from terrestrial animals.

In both Carlier et al. (2007) and Vizzini and Mazzola (2003), stable carbon isotopes were particularly useful in distinguishing whether or not a consumer was feeding exclusively on benthic organic matter or phytoplankton and if a shift between the two food sources occurred seasonally. Overall, these studies show the effects of seasonal variability on stable carbon isotope values in lagoon heterotrophs. They also provide some values for comparison and reinforce the importance of obtaining isotopic data specific to each biosphere.

3.5. Stable Nitrogen Isotopes: Characteristics and Measurements

The element of nitrogen has two stable isotopes, ^{14}N and ^{15}N . Most of the earth's surface nitrogen consists of N_2 , in gaseous form in the atmosphere or dissolved in the ocean (Hoefs, 2009). Atmospheric nitrogen (abbreviated as AIR) is the reference standard against which stable nitrogen values are compared, with a defined value of 0‰. Most organic substances have $\delta^{15}\text{N}$ values falling within -10‰ and +20‰ (Hoefs, 2009; Fry, 2006).

The formula for calculating stable nitrogen isotope ratios is:

$$\delta^{15}\text{N} = \frac{{}^{15}\text{N}/{}^{14}\text{N}_{\text{SAMPLE}} - {}^{15}\text{N}/{}^{14}\text{N}_{\text{STANDARD}}}{{}^{15}\text{N}/{}^{14}\text{N}_{\text{STANDARD}}} \times 1000$$

Nitrogen enters the biosphere via conversion from atmospheric or oceanic N_2 (fixation), and is further converted by nitrification and denitrification processes to produce the pool of available nitrogen compounds most autotrophs draw on (Hoefs, 2009). Fixation is done by diazotrophs and involves minimal fractionation (Wada et al., 1975); this process is further discussed in section 3.6.1. Nitrification involves oxidization of nitrogen by several different autotrophs and produces both nitrate (NO_3^-) and various nitrogen oxides (Hoefs, 2009). Denitrification reduces these oxidized forms of nitrogen, again producing various nitrogen oxides as well as replenishing the atmospheric nitrogen used by natural fixation as nitrate is reduced back into N_2 (Wada et al., 1975). The fractionation effects associated with nitrification and denitrification span a large range, depending on factors including overall nitrogen concentration. If the concentration of the nitrogen substrate is low, then fractionation values are close to 0‰ because all available nitrogen is consumed in the reaction (Hoefs, 2009). The complexity of these processes helps to create wide variability in water and soil $\delta^{15}\text{N}$ values (Hoefs, 2009).

The following section discusses autotroph stable nitrogen isotopes in different ecosystems, including the actions of diazotrophs and other autotrophs, and the effects of various environments.

3.6. Stable Nitrogen Isotopes in Ecosystems

The entry of nitrogen into the biosphere occurs first at plant and microorganism levels. Below, the action of nitrogen in diazotrophs (nitrogen fixers) and in symbiotic and non-symbiotic plants is briefly discussed.

Following this, recorded $\delta^{15}\text{N}$ values for terrestrial, marine, freshwater, and lagoon environments are detailed.

3.6.1. Nitrogen in Diazotrophs (Nitrogen Fixers), Soil, and Water

Some bacteria and algae can change inorganic atmospheric nitrogen into forms such as ammonium (NH_4^+), allowing it to enter the biosphere (Hoefs, 2009). The organisms that do this are referred to collectively as diazotrophs, or nitrogen fixers, and live either free in the soil or water or in the roots of plants. Only certain plants, including many legumes species, are capable of hosting diazotrophs in their roots (Delwiche and Steyn, 1970). Nitrogen fixation is associated with little fractionation; the compounds it produces have stable nitrogen isotope values ranging from -3‰ to +1‰, with many remaining close to the standard reference value of AIR or 0‰ (Hoefs, 2009).

Soil $\delta^{15}\text{N}$ values vary according to the bacterial processes in operation, including the occurrence of denitrification during the decomposition of organic matter (Hoefs, 2009). This means that the isotopic composition of soil nitrogen compounds and the cycling of nitrogen between the atmosphere and soil over time are difficult to predict. Determining baseline food web values within a particular ecosystem requires site- and organism- specific values as soil nitrogen values are affected by numerous variables (Delwiche and Steyn, 1970).

Nitrogen compounds in water come primarily from the decomposition of organic matter. Deeper in the ocean, as well as in deeper soil strata, stable nitrogen isotope values tend to become 5‰ to 10‰ higher than in shallower areas, generally due to the faster loss of ^{14}N during particulate decomposition (Fry, 2006). In addition, the value of organic nitrogen in particulate matter depends on the isotopic concentration of dissolved nitrate and on any fractionation that occurs during nitrogen uptake by phytoplankton (Hoefs, 2009). Like soil values, the $\delta^{15}\text{N}$ values of nitrogen

compounds in water are context dependent and highly variable. Seasonality, temperature, salinity, and the oxygen availability within the aqueous environment all affect $\delta^{15}\text{N}$ values, although marine organic matter usually have higher values than terrestrial organic matter (Hoefs, 2009; Wada et al., 1975). Overall, ocean water reservoirs show stable nitrogen isotope values ranging from approximately -4‰ to +13‰ (Hoefs, 2009).

3.6.2. Nitrogen in Plants

Plants obtain their nitrogen either through absorbing the inorganic ammonium and nitrate in the soil or through symbiotic relationships with diazotrophs (Ambrose, 1991, 1993; Shearer and Kohl, 1986). The division of plants into non-symbiotic and symbiotic categories helps to highlight the source and pathway of nitrogen within a given food web.

3.6.2.a. Nitrogen in non-symbiotic plants

Most plants obtain nitrate from soil nitrogen compounds and not through relationships with diazotrophs. The process of denitrification during decomposition ensures that in a closed system, enough nitrate is available for plant growth (Heldt and Piechulla, 2010). Other sources of soil nitrates include lightning strikes and rainwater, the modern values of the latter showing quite high $\delta^{15}\text{N}$ values due to the effects of automobiles and nitrogen inputs from industrial agricultural animal production (Heldt and Piechulla, 2010; Zahran, 1999). Fertilization using synthetic nitrate fertilizers or, in past times, the application of manure, is another way to increase soil nitrogen content and the growth of plants (Zahran, 1999). As is evident from the number of nitrogen sources available, plants that obtain nitrogen non-symbiotically through these means can have a wide range of $\delta^{15}\text{N}$ values, reflecting the values of these inputs (Shearer et al., 1978).

3.6.2.b. Nitrogen in symbiotic plants

Some nitrogen-fixing bacteria live in plant roots, supplying their host plant with organic nitrogen in exchange for the provision of metabolites, such as malate, for their nutrition (Heldt and Piechulla, 2010). Twenty percent of all known legume species have a symbiotic relationship with *rhizobia* bacteria (Heldt and Piechulla, 2010; Zahran, 1999). The *rhizobia* nodules on legumes are found in the soil and grow heterotrophically in the presence of organic compounds, although some species of *rhizobia* can grow autotrophically as well (Heldt and Piechulla, 2010).

N₂ fixation in these root nodules can only occur at very low oxygen concentrations (the enzyme needed for fixation is inactivated by oxygen) and is very energy-inefficient as compared to direct soil nitrate assimilation by plants (Zahran, 1999); nodules are formed only when the soil is nitrogen-deficient (Heldt and Piechulla, 2010).

Symbiotic plants used for food or fodder include soybeans, lentils, peas, clover, and lupines. When their symbiotic bacteria fix nitrogen, the $\delta^{15}\text{N}$ values of these plants generally reflect those of atmospheric N₂ and are close to 0‰ or +1‰. When no fixation is taking place, their tissue values will instead reflect the soil nitrogen values (Heldt and Piechulla, 2010).

3.6.3. $\delta^{15}\text{N}$ Values in Terrestrial Environments

Within a terrestrial environment, factors other than the underlying nitrogen soil values can contribute to organism $\delta^{15}\text{N}$ values. Terrestrial plants show overall stable nitrogen isotope values ranging from -6‰ to +20‰ (Ambrose, 1991), reflecting the effects of these other factors. The presence of forested terrain is associated with more negative stable nitrogen isotope values, with diazotroph and legume values falling closer to -6‰. As heat and aridity increases, these stable nitrogen isotope values can become higher, up to 11‰ (Ambrose, 1991). It is thought that this occurs because the heat and dryness inhibit soil nitrogen fixation and reduce the

contribution of atmospheric nitrogen as a source (Ambrose, 1991).

Conversely, cool and moist soils increase nitrogen fixation, which results in lower stable nitrogen isotope values than for hot, dry, savanna and desert areas. Soil salinity can further increase stable nitrogen isotope values, as much as +18‰ compared to forested areas (Ambrose, 1991).

The contribution of animal waste, such as manure and urine excretion will increase the soil $\delta^{15}\text{N}$ values and thus the values of the plants grown on them (Bogaard et al., 2007; Ambrose, 1991). Animal waste typically has a stable nitrogen isotope value of greater than 5‰ (Hoefs, 2009), with bird guano deposits showing the highest values (Ambrose, 1991). The use of synthetic fertilizers and other anthropogenic nitrogen inputs can also change soil stable nitrogen isotope values from what would occur naturally. Synthetic fertilizers typically have values ranging from -4 to +4‰, which reflect the atmospheric source of the nitrogen incorporated into them during manufacture (Hoefs, 2009).

3.6.4. $\delta^{15}\text{N}$ Values in Marine Environments

The $\delta^{15}\text{N}$ values from ocean water have a wide range of -4‰ to +13‰ (Hoefs, 2009), due to the inherent heterogeneity of biological processes involving nitrogen. Denitrification, nitrification, and nitrate assimilation all contribute to the variation in the natural abundance of $^{15}\text{N}/^{14}\text{N}$ in ocean waters (Wada and Hattori, 1976). Certain patterns do emerge from the various sources of data: reef systems appear to show relatively low $\delta^{15}\text{N}$ values as compared to other parts of the ocean system. Schoeninger and DeNiro (1984) suggest that this may occur because the bulk of nitrogen fixation is done by blue-green algae in the reefs, which results in relatively low $\delta^{15}\text{N}$ values. This algal source then affects the values of consumers higher up the food chain.

Wada and Hattori (1976) investigated the abundance of ^{15}N in the POM (particulate organic matter) from the uppermost aquatic water layers in

the Pacific Ocean. $\delta^{15}\text{N}$ values ranged from -1.7‰ to +9.7‰ with the values in nitrates, phytoplankton and seaweeds measuring 7‰ on average. They also found that the deeper ocean layers mixed vertically with the upper water layers, transporting nitrate upward, where the natural populations of phytoplankton had increased amounts available for use. As a result of the fractionation process occurring in these phytoplankton, their $\delta^{15}\text{N}$ values were lower than the values of the phytoplankton from nitrate-poorer waters (Wada and Hattori, 1976).

In the sea, precipitation and river run-off are the main contributors of nitrogen (Wada et al., 1975). Higher marine $\delta^{15}\text{N}$ values are explained by the occurrence of denitrification in the sea, associated with a high fractionation factor (Wada et al., 1975). As mentioned above, there are detectable $\delta^{15}\text{N}$ differences between terrestrial and marine environments, with marine environments showing consistently higher values over terrestrial plant values (Schoeninger and DeNiro, 1984; Wada et al., 1975).

3.6.5. $\delta^{15}\text{N}$ Values in Freshwater Environments

Freshwater environments also tend to show higher $\delta^{15}\text{N}$ values than terrestrial environments. Freshwater environments may be distinct both at the base of the food chain with the autotrophic organisms (as in Katzenberg and Weber, 1999), and throughout the subsequent trophic levels (Wada et al., 1993). In general, the $\delta^{15}\text{N}$ values within a given river system depend on where samples are collected. For example, Wada et al. (1993) found that the stable nitrogen isotope values of plants, particulate organic matter, and sediments increased as the Nanakita river in Japan flowed closer to the sea. These increases were reflected in the values of the same consumer species studied, depending on where this species was feeding. While anthropogenic nitrogen contributions may have affected the stable nitrogen isotope values, Wada et al. (1993) concluded that the cumulative mixing of terrestrial organic matter, marine phytoplankton, and sea grasses was more responsible

for the isotopic variation that they noted than was the sewage discharge into the river and sea.

3.6.6. $\delta^{15}\text{N}$ Values in Lagoon Environments

The same species of consumer within a lagoon environment may show variations in stable nitrogen isotope values, again reflecting the different nitrogen contributions into the system. These mixed-source environments might receive not only terrestrial inputs, but also marine inputs and the proportions of each depend on the particular lagoon, its location, and its physical characteristics. Wada et al. (1993) found that both land and marine organic matter contributed relatively equally to the molluscs from the lagoon that they analyzed. However, the rest of the organisms they sampled depended exclusively on organic matter of marine origin. The $\delta^{15}\text{N}$ values of the detritus from the reeds in the lagoon were variable and ranged from 3‰ to 7‰, with the average of all the plant materials in the lagoon equaling $8.6\text{‰} \pm 3.6\text{‰}$. To further complicate matters, the stable nitrogen isotope values of the samples varied according to the location within the lagoon where they were collected (Wada et al., 1993).

Vizzini and Mazzola (2003) found additional sources of variation in stable nitrogen isotope values in a Mediterranean lagoon. Seasonal variations were apparent, but appeared to affect invertebrate species more than the fish species present. These variations were attributed to the use of “new” enriched nitrogen sources in the winter and spring and then the use of recycled ammonia available later in the summer. Despite the variations higher up the food chain, Vizzini and Mazzola (2003) were able to determine that the base of the chain in the lagoon was the particular organic matter (POM), which had a mean $\delta^{15}\text{N}$ value of 6‰. This baseline value compares well to marine and estuarine values from other Mediterranean sites (Vizzini and Mazzola, 2003).

Carlier et al. (2007) found that seasonal activities by humans, namely the application of fertilizer from an upstream vineyard, caused higher $\delta^{15}\text{N}$ values in a French Mediterranean lagoon. The fertilizer underwent denitrification once it was within the lagoon system and was then incorporated into the grasses and marsh plants and upwards in the food chain. Detritus-feeders also contributed a significant amount; there was a wide variation in values, again depending on the location within the lagoon that was sampled. Upland plants showed a mean $\delta^{15}\text{N}$ value of -0.2‰ while the POM that was collected at the outlet of a sewage treatment plant averaged 15.6‰ (Carlier et al., 2007). Clear trophic level systems were difficult to establish due to the changing stable nitrogen isotope values over time and within the lagoon, but overall, Carlier et al. (2007) found that lagoon values could be distinguished from the local terrestrial and marine environments.

3.7. Stable Nitrogen Isotopes in Heterotrophs

The distribution of stable nitrogen isotopes in heterotrophs can be affected by several factors. In this section, the trophic level effect and its usefulness in establishing an organism's position within a specific food web is discussed. The effects of living within an arid environment and water-stress in mammals can cause $\delta^{15}\text{N}$ values otherwise not accountable for by dietary values. Finally, the effects of nursing, growth, and nutritional stress on an individual's $\delta^{15}\text{N}$ values are described as these, too, can result in values not reflective of diet.

3.7.1. The Trophic Level Effect

Reconstructing past diet and the arrangement of a food chain using stable isotope analysis is based upon the idea that the $\delta^{15}\text{N}$ value of animal tissues reflects the $\delta^{15}\text{N}$ value of their diet in a predictable and systematic way (DeNiro and Epstein, 1981). Stable nitrogen isotope values can be used not only to identify diets containing a large proportion of legumes, but also to

differentiate between terrestrial- and marine- based diets. As mentioned previously, understanding of stable nitrogen isotope values and the trophic level effect can also be used to establish the position of a consumer within a given food chain.

Animals kept in laboratory-controlled environments and raised on diets of known $\delta^{15}\text{N}$ value have tissues enriched by about $+3.0\text{‰} \pm 2.6\text{‰}$ (or up to 5‰) relative to the diet (Schoeninger and DeNiro, 1984; DeNiro and Epstein, 1981). This effect has its basis in urea excretion. Laboratory analyses of animals' diet, tissues, and excreted urea show that preferential uptake of ^{14}N occurs in urea synthesis, leaving ^{15}N in the tissues, with the net effect of increasing tissue $\delta^{15}\text{N}$ (Minagawa and Wada, 1984). Observations in the wild are consistent with this, as primary producers show the lowest $\delta^{15}\text{N}$ values and successive consumers show higher values as the enrichment continues up the food chain (Ambrose and Krigebaum, 2003; Minagawa and Wada, 1984). The extent of this trophic level effect varies depending on the type of tissue sampled, but it has been found in both terrestrial and aquatic organisms (Bocherens and Drucker, 2003; Schoeninger, 1985; Schoeninger and DeNiro, 1984; Minagawa and Wada, 1984). While diet and the trophic level effect are the key determinant of heterotroph $\delta^{15}\text{N}$, certain other factors can also affect these values.

3.7.2. Other Factors Influencing Heterotroph $\delta^{15}\text{N}$

Stable nitrogen isotope values in heterotrophs depend not only on their trophic level position and the terrestrial or marine components of their diets, but can also be affected by living in an arid environment, nursing, and other nutritional and physical stressors. When interpreting heterotroph $\delta^{15}\text{N}$ values, these possible factors should be taken into consideration.

3.7.2.a. The arid environment effect

Stable nitrogen isotope values are higher in arid environments, not only in soils and plants, but also in animals (Ambrose, 1991; Sealy et al.,

1987; Heaton et al., 1986). What is apparent is that herbivores from dry environments have higher $\delta^{15}\text{N}$ values and the values of drought-tolerant herbivores are especially high (Ambrose, 1991; Sealy et al., 1987; Heaton et al., 1986). The difference between the $\delta^{15}\text{N}$ values of plants and herbivores increases as rainfall decreases, suggesting that higher soil $\delta^{15}\text{N}$ values are not the sole explanation (Kelly, 2000; Ambrose, 1991). Several reasons for this particular patterning have been suggested, such as the influence of climate on nitrogen metabolism (Heaton et al., 1986) and the differences in urea or ammonia excretion between water-conserving and water-dependent herbivorous mammals (Ambrose and DeNiro, 1987; Schoeninger and DeNiro, 1984).

The model that ascribes the arid environment effect in animals to urea excretion points out that water stress can change the rates of urea excretion and affect nitrogen isotope mass balance (Ambrose, 1991). Most nitrogen is excreted in the form of urea in mammals and, as explained above, this urea is depleted in ^{15}N relative to the animal's diet (Ambrose, 1991). As heat or water deprivation increases, fluids are conserved and the concentration of urea and other solutes in the urine also increases. More ^{15}N becomes available for tissue synthesis in the body's nitrogen pool and this results in increased tissue $\delta^{15}\text{N}$ values. However, animals that are water-independent or drought-tolerant do not need to retain water in this way and would not necessarily show a corresponding increase in body pool ^{15}N and higher tissue values. Browsers are more drought tolerant than grazers due to their ability to concentrate their urine more, as well as their ability to access high-protein dietary sources in order to maintain nitrogen balance (Sealy et al., 1987). Laboratory experiments involving low protein diets and high temperature conditions have failed to replicate the results found in the field, indicating that the actual situation may be more complex than this model suggests (Ambrose, 2000).

Despite the complexity that results from the presence of both water-stressed and drought-tolerant animals in a single environment, nitrogen

isotopes can still be useful indicators of trophic level if the climate and available resources are taken into account.

3.7.2.b. Nursing, growth, and nutritional stress

Breast milk is synthesized from the lactating mother's nitrogen pool and generally shows a 1‰ to 3‰ $\delta^{15}\text{N}$ increase over the mother's diet (see Fuller et al., 2006a for further reading). An infant consuming breast milk is therefore considered to occupy a trophic level above its mother or wet-nurse and will show tissue $\delta^{15}\text{N}$ values elevated above hers (Fuller et al., 2006a; Fuller et al., 2006b; Fogel et al., 1989). This elevation is especially obvious when breast milk is the only food consumed and as weaning and other foods are introduced to the infant's diet, tissue $\delta^{15}\text{N}$ values will gradually decrease (Fuller et al., 2006a). When dealing with archaeological populations, awareness of this effect is important because of the potential for misinterpreting high infant $\delta^{15}\text{N}$ values. In addition, the meat of suckling or nursing animals, such as lamb or suckling pig, will show higher $\delta^{15}\text{N}$ values than the meat of adults from the same species. Many studies have used the nursing effect to investigate weaning in past populations (e.g. Dupras et al., 2001; Fogel et al., 1989).

Growth may affect an individual's tissue $\delta^{15}\text{N}$ values because the relationship between dietary intake and metabolic nitrogen requirements is unbalanced, with a net accumulation of nitrogen taking place. Several studies have found that some tissues with relatively fast growth show differences in the diet-to-tissue spacing of stable isotope values (Trueman et al., 2005; Roth and Hobson, 2000; Ponsard and Averbach, 1999). Growth should theoretically also alter diet-to-tissue $\delta^{15}\text{N}$ spacing in juvenile bones. However, the growing and non-growing areas of bone from older children and adolescents do not appear to show any differences in $\delta^{15}\text{N}$ values, suggesting that this spacing is either absent or negligible in regards to human bone collagen and diet (Waters-Rist and Katzenberg, 2010). Studies of sexually mature but still growing laboratory mice have found that less than

10% of tissue turnover reflects growth (MacAvoy et al., 2005). Although tissue stable isotope values in children may reflect their diet more quickly than adults, a finding noted in juvenile deer mice in laboratory conditions (Miller et al., 2008), these studies do suggest that in older, non-breastfeeding children and in adults, growth is not an additional factor that needs consideration when $\delta^{15}\text{N}$ values are interpreted. The $\delta^{15}\text{N}$ values of their bone collagen can be considered as reflective of their diet rather than as artifacts from past growth processes.

Nutritional stress, however, can have a major influence on tissue $\delta^{15}\text{N}$ values and can cause difficulties in reconstruction of past dietary resource use. The potential effect of this stress on tissue $\delta^{15}\text{N}$ in arid environments has been briefly mentioned in the preceding section. There is evidence to suggest that animals consuming a protein-deficient diet will have to replenish their nitrogen and amino acid pools by breaking down existing tissues in order to obtain the materials needed to manufacture new tissues (Ambrose, 2000). Supporting this model, Hobson and Clark (1992) and Hobson et al. (1993) noted increases in diet-tissue fractionation during nutritional stress in several bird species and suggest that this may have occurred because of the mobilization and re-deposition of proteins from tissues elsewhere in the body. It is also possible that the amino acid composition can change in the tissues, further affecting tissue $\delta^{15}\text{N}$ values because some amino acids have distinctive $\delta^{15}\text{N}$ values (Hobson and Clark, 1992). Under stress, the amino acids for protein synthesis come from catabolism of the existing tissues and not from the diet. Since these tissues are already ^{15}N enriched above the dietary values, their re-use will further increase the $\delta^{15}\text{N}$ value of nitrogen incorporated into newly deposited tissues (Hobson et al., 1993).

The effects of nutritional and physiological stress on tissue $\delta^{15}\text{N}$ values has been observed in humans as well, and appears to be magnified in individuals with wasting diseases. Katzenberg and Lovell (1999) found higher $\delta^{15}\text{N}$ values in newer deposited bone collagen from a modern individual who died from the complications of AIDS. This person, who had a

documented medical history, had also been living with an infectious lung condition that resulted in the formation of osteomyelitis lesions. In this case, this reactive bone had a higher $\delta^{15}\text{N}$ value than the bone tissue that was unaffected; this was likely due to the overall nitrogen imbalance caused by nutritional supply not meeting the demand during the AIDS wasting process (Katzenberg and Lovell, 1999). White and Armelagos (1997) found high $\delta^{15}\text{N}$ bone collagen values in Nubian mummies with osteopenia; this may be another example where nutritional stress has resulted in elevated $\delta^{15}\text{N}$ values in archaeological humans, although the effects of arid environment cannot be ruled out in this case.

The extent of the effect of nutritional stress and protein catabolism on tissue $\delta^{15}\text{N}$ values is still under investigation. Ideally, it should be understood in order to accurately assess an animal's trophic level and it may also be significant for archaeological work. If nutritional stress was an ongoing part of life in past populations or if infants and young juveniles suffered from malnutrition illnesses, the elevated stable nitrogen isotope values may be confused with the effects of breastfeeding or with the consumption of higher trophic level animals.

3.7.3. Literature Values for Heterotrophs

Despite the potential sources of variation in stable nitrogen isotope values discussed above, generally consistent trophic level enrichments do occur in heterotrophic organisms and local heterotroph values remain distinguishable from each other for the most part, regardless of the type of environment. The following sections discuss some of the features of heterotroph stable nitrogen isotope values in terrestrial, marine, freshwater, and lagoon environments with emphasis on the $\delta^{15}\text{N}$ values that can emerge from each.

3.7.3.a. Terrestrial heterotrophs

The following ranges of terrestrial heterotroph values are specific to the areas and resources available to the organisms, and while these values do illustrate the trophic level patterning apparent in each local food chain, they also demonstrate the difficulties in translating values from one geographical and environmental area to another.

DeNiro and Epstein (1981) were able to apply the $\delta^{15}\text{N}$ values they obtained from laboratory animals consuming a terrestrial diet to archaeological human, faunal, and plant remains from the Tehaucan Valley of Mexico and determine dietary resource use in this population. By subtracting the 3.9‰ diet-collagen $\delta^{15}\text{N}$ spacing observed in the laboratory mice from the human values, they identified both legume and maize consumption, which was found to occur much earlier than previously thought. The stable nitrogen isotope values of fossil legumes and non-leguminous plants ranged from 8.4‰ to 13.6‰, although the authors suggest that the plant values became altered post-deposition since they are radically different from the expected value of 0‰.

Ambrose and DeNiro (1986) examined both historic and proto-historic human populations in Kenya, Tanzania, and South Africa and found that with historic documented diets, the $\delta^{15}\text{N}$ values of those most dependent on plant foods ranged from 8 ‰ to 12‰ and the values of those consuming the milk, meat and blood of domestic animals ranged between 12‰ and 18‰. Sealy and van der Merwe (1985, 1986, 1988, as cited in Lee-Thorp, 2008) made use of the regional terrestrial and marine food web values to test models of hunter-gatherer seasonal mobility in the Holocene period in South Africa. By using local values instead of global averages, they showed that the terrestrial/marine dividing point of 10‰ suggested by Schoeninger and DeNiro (1984) was not applicable to all environments. The highest mean stable nitrogen isotope value for an herbivore species was 10.6‰ for elephants and Grant's gazelle, reflecting perhaps the effects of aridity of the environment within which these species are found (Sealy et al., 1987).

Heaton et al. (1986) also found wide variation in the $\delta^{15}\text{N}$ values of specific trophic levels of prehistoric human and modern faunal samples from Africa. The humans from the interior, who were consuming a diet based upon C_3 terrestrial resources, showed values similar to those individuals who were exploiting coastal marine resources; these overlapping food resource values could not be attributed to diet since the inland group did not have access to the coast. The authors suggest that accounting for specific climatic habitats is key to understanding the relationships between producers and consumers when $\delta^{15}\text{N}$ values are not entirely reflective of dietary values. Animal tissues do show consistently elevated $\delta^{15}\text{N}$ values relative to their diets, but this spacing is not constant between different ecosystems or even within the same trophic level (Sponheimer et al., 2003; Ambrose, 1991).

Other reviews have reported the general known $\delta^{15}\text{N}$ ranges for terrestrial heterotrophs from modern samples (Ambrose, 1991). Herbivore obligate drinkers and water-conserving animals range from 2‰ to 20‰, carnivores of various trophic levels range from 6‰ to 20‰, and both modern and prehistoric humans with mostly non-marine diets range from 6‰ to 18‰.

Ambrose's 1991 review agrees with the idea that generalized relationships between trophic levels are not particularly helpful when applied to specific ecosystems, as these generalizations are based upon global averages. Regional and contextual isotope studies are more useful, as baseline $\delta^{15}\text{N}$ values are reflective of herbivore dependence on leguminous or non-leguminous plant foods, as well as the environmental conditions described above (Lee-Thorp, 2008).

3.7.3.b. Marine heterotrophs

Reported values for marine heterotrophs include those from Schoeninger and DeNiro's review (1984) of more than 100 marine and terrestrial animals. The average $\delta^{15}\text{N}$ value for marine organisms was 14.8‰ \pm 2.5‰, with a range of 9.4‰ to 23‰. Tertiary consumers such as sea lions

showed the highest values, consistent with their trophic level position. In contrast, terrestrial values in the same study averaged out to $5.9\text{‰} \pm 2.2\text{‰}$, with a range from 1.9‰ to 10‰ . Sealy et al. (1987) reported stable nitrogen isotope values from marine animals from South Africa and found agreement with Schoeninger and DeNiro's (1984) values, although there was some overlap with terrestrial heterotrophic values from other ecosystems. Animals higher on the food chain, such as seals, whales, piscivorous fish, and sea birds showed values ranging from 12.7‰ to 18‰ , while lower-level feeders, such as mussels and other shellfish, had values from 7.1‰ to 8.7‰ .

Minagawa and Wada (1984) also found a stepwise trophic level enrichment in consumers in marine food chains. Primary consumers $\delta^{15}\text{N}$ values (mussels, shore crabs, sand worms, and starfish) ranged from 8.4‰ to 9.5‰ and were about 2‰ to 3‰ higher than plant values. Secondary consumers (sea anemones, octopuses and fish) ranged from 10.6‰ to 12.7‰ , with $\delta^{15}\text{N}$ values 3‰ above the primary consumers. Tertiary consumers (sea gulls) were 15.6‰ to 16.8‰ , with enrichment of 4.4‰ above the secondary consumers. Zooplankton from the Bering Sea showed wide variation in values, from 5.6‰ to 14‰ ; these values overlapped with zooplankton from a freshwater lake, discussed further in the next section.

As with marine autotrophs, the $\delta^{15}\text{N}$ values of marine heterotrophs are dependent on whether they live in benthic, pelagic, or littoral waters. Therefore, a range of values from each area and type of feeder, as well as knowledge about the types of marine resources consumed by various heterotrophs, is helpful in reconstructing a specific marine food web (France, 1995b). As mentioned previously, marine heterotrophs tend to show higher $\delta^{15}\text{N}$ values than terrestrial heterotrophs mainly due to the longer food chains and increased number of consumer levels found in marine ecosystems. In addition, the baseline sources of nitrogen in marine systems tend to be more enriched than those of terrestrial systems.

3.7.3.c. Freshwater heterotrophs

Freshwater bottom-dwelling feeders show different values than open-water and coastline feeders. Zooplankton collected from Lake Ashinoko in Japan by Minagawa and Wada (1984) showed average $\delta^{15}\text{N}$ values of 8.1‰, while fish averaged 11.1‰. Schoeninger and DeNiro's 1984 collection of data shows freshwater fish with somewhat lower stable nitrogen isotope values averaging 8.0‰ and ranging from 6.6‰ to 9.5‰.

Katzenberg et al. (2011) provide an extensive list of fauna, as well as human stable isotope values, from the Lake Baikal region in Siberia. Although Baikal is the deepest freshwater lake in the world, and contains a variety of systems with varying baseline $\delta^{15}\text{N}$ values, the stable nitrogen isotope values of the fish still reflect their overall trophic level position. The terrestrial fauna from the area remained distinguishable from the freshwater for the most part, although distinctions became clearer when $\delta^{13}\text{C}$ values were considered. A single northern pike sample was 20.6‰; the fish samples were all of modern origin and modern fertilizer or runoffs, as well as differences in riverine and lake sampling sites within the area may have affected some of them. Otherwise the highest $\delta^{15}\text{N}$ values were seen in the Baikal seals, a freshwater seal species, which reached 15.5‰, a value that falls within Schoeninger and DeNiro's (1984) range for marine-dwelling seals and is consistent with tertiary consumer values for the area. Other than the single pike, the highest freshwater species fish value was in the Baikal sturgeon, a carnivorous tertiary feeder, which showed stable nitrogen isotope values of 14.1‰ to 14.6‰. The range of terrestrial $\delta^{15}\text{N}$ values was from 1.1‰ up to 15.2‰, with an average of 12.1‰. In contrast, the lacustrine samples ranged from 6.2‰ to 20.6‰ (including the northern pike mentioned above), illustrating the differences between terrestrial and freshwater food webs.

As with marine and terrestrial heterotrophs, general freshwater $\delta^{15}\text{N}$ value guidelines should not be applied to specific sites and regions in order to determine trophic level since similar species living in different ecosystems will show different $\delta^{15}\text{N}$ values.

3.7.3.d. Lagoon heterotrophs

Heterotrophs in a lagoon environment can be as complex in regards to their $\delta^{15}\text{N}$ values and trophic level reconstructions as those in freshwater environments. In addition to the seasonal variations discussed previously, organisms can go between feeding and being fed upon in a lagoon environment or move between terrestrial and marine areas, as with birds. Some of the reported values in the literature for various lagoon sites in Japan and the Mediterranean illustrate this complexity and act as a further reminder that generalizations should not be applied to different ecosystems and environments.

Wada et al. (1993) measured faunal stable nitrogen isotope values of 9.4‰ to 16.7‰ in various lagoon environments in Japan, with fish species and an amphipod species showing the highest values. Compared to this, *Amphipodae* in the western Mediterranean coastal lagoon of Lake of Sabaudia in Italy (Vizzini and Mazzola, 2003) showed lower $\delta^{15}\text{N}$ values ranging from 6.0‰ to 8.9‰, depending on the season. Fish values at Sabaudia ranged from 9.2‰ to 15‰, again varying with the seasons. As the samples were of modern origin, fertilizer runoff and human inputs into the lagoon possibly affected the nitrogen source pools at various times.

A bivalve Mediterranean coastal lagoon species (*C. glaucum*) showed $\delta^{15}\text{N}$ values ranging from 4.5‰ to 9.0‰. An amphipod species (*G. aequicauda*) ranged from 6.3‰ to 10.5‰, and the means for *Pomatoschistus* spp. fishes ranged from 10.5‰ to 15‰. Again, seasonal variation affected the values of these heterotrophs. However, trophic level enrichments were apparent throughout the seasons despite fluctuations in individual species' stable nitrogen isotope values (Carlier et al., 2007).

The lagoon water environment, its openness or restrictiveness to other input sources, and the type of feeder belonging to a particular heterotrophic species are all factors that affect $\delta^{15}\text{N}$ values. Food webs can therefore be harder to disentangle and understand in lagoon environments, and like most aquatic ecosystems, require understanding of specific

situations more than generalizations about terrestrial/marine cut-off points for stable isotope values. As shown above, lower trophic level organisms from lagoon environments can have stable nitrogen isotope values approaching those of marine organisms or, conversely, may have values that are closer to terrestrial organisms.

3.8. Stable Isotopes from Diet to Bone

While the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in organisms reflect overall dietary values as well as environment, there are also individual factors that affect the incorporation of stable carbon and nitrogen isotopes into various tissues. Differences in stable isotope values between an individual's tissues are reflective of the synthetic pathways involved and of how quickly a given tissue turns over, or is replaced, during life. This section discusses current knowledge about diet-tissue fractionation and tissue turnover rates and the impact of these processes on the stable isotope values of bone.

3.8.1. Fractionation Values Between Diet and Bone

As noted in the preceding sections, the trophic level difference between a consumer and its diet is fairly predictable when examining bone collagen $\delta^{15}\text{N}$ values; each trophic level generally reflects a 3‰ to 4‰ increase over the dietary value (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984). However, some studies have found a larger range of enrichment extending from 1.7‰ to 6.9‰ above the diet (Bocherens and Drucker, 2003; Adams and Sterner, 2000), suggesting that the spacing between trophic levels can vary between environments.

Carbohydrates and fats do not contain nitrogen and therefore do not contribute to a consumer's tissue $\delta^{15}\text{N}$ values (Lee-Thorp, 2008; Ambrose et al., 1997). This makes nutrient-to-tissue $\delta^{15}\text{N}$ spacing relatively simple in that essentially all tissue nitrogen comes from dietary protein. With stable carbon isotope values, however, feeding studies have demonstrated large differences

in $\delta^{13}\text{C}$, not only between diet and tissue (see Ambrose and Norr, 1993; Schwarcz, 1991), but also between bone collagen and bone carbonate values, with the latter showing the highest values (Lee-Thorp et al., 1989). The observation that stable carbon isotopes are not incorporated into all components of bone in a constant manner led in the 1980s to the development of a linear mixing model (see Schwarcz, 1991) and a routing model (Chisholm et al., 1982). The linear mixing model assumes that the carbon atoms from fat, carbohydrate, and protein dietary components have equal chances of being incorporated into consumer tissues and explains the relationship between the $\delta^{13}\text{C}$ value of the diet and the $\delta^{13}\text{C}$ value of consumer bone apatite quite well. There may be differences in this relationship in some herbivores as compared to carnivores, perhaps due to the increased use of fats as energy sources in carnivorous diets or differences in ruminant herbivore methane production in the gut (Ambrose et al., 1997).

While the linear mixing model explains the relationship between stable carbon isotope values of the diet and bone apatite, it does not explain the relationship between diet and bone collagen $\delta^{13}\text{C}$ values. The routing model explains this by stating that while all nutrients contribute some carbon to collagen, dietary protein is preferentially routed to tissue protein. Bone collagen $\delta^{13}\text{C}$ therefore does not reflect the isotopic composition of the whole diet but instead is biased toward protein $\delta^{13}\text{C}$, with the degree of bias depending on the diet involved (Ambrose et al., 1997). This model has been confirmed through controlled feeding experiments with laboratory animals (Ambrose and Norr, 1993; Tieszen and Fagre, 1993) and has also been noted in archaeological populations (Lee-Thorp, 2008). As a result of this protein routing, the spacing between human dietary protein and bone collagen $\delta^{13}\text{C}$ values appears to range from 3.7‰ to 6‰ (Bocherens and Drucker, 2003), although a difference around 5‰ is commonly cited in the literature (Ambrose and Krigbaum, 2003; Ambrose et al., 1997). When considering the bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of archaeological populations, it is important to realize that the $\delta^{15}\text{N}$ values reflect only the protein components

of the diet, and the $\delta^{13}\text{C}$ values are biased toward protein and away from carbohydrate and fat values. This has implications for dietary resource interpretation since some important foods may not be well-reflected in collagen values.

3.8.2. Tissue Turnover

The rate at which a tissue's cells and matrix turn over and are replaced with newer material can affect its stable isotope value, as newly deposited tissue will reflect the current dietary value. However, the exact timing and how long a given tissue takes to turn over are not well known. In ecological studies, there is much variation in turnover rates between tissue types even within a given species (Crawford et al., 2008). In controlled laboratory experiments, measurement of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values indicates that in the soft tissues, liver tissue turns over the quickest, while blood has the slowest turnover time for carbon and muscle is the slowest in incorporating nitrogen (Miller et al., 2008). Hair, composed of keratin, has values that are established after growth and does not turn over once formed. In general, tissues that grow or turn over more rapidly will incorporate dietary signals more quickly than those that are slower. This can be seen with blood plasma stable isotope values, which will reflect diet within two hours of food consumption, while bone has been found to take years to fully equilibrate with the diet, provided the diet remains consistent. Skin is thought to reflect diet within a few weeks to a few months (Tieszen et al., 1983).

Stable isotope studies using mummified individuals provide some more information about tissue turnover in humans. White and Schwarcz (1994) analyzed the bone collagen, muscle, and skin tissue values for a sample of Sudanese Nubian mummies dating from 350 BC to 1400 AD. They found that their results were consistent with the theoretical lifetimes of carbon and nitrogen in the tissues they analyzed; hair, skin, and muscles reflected diet within the last year of life, while bone collagen reflected diet up to 25 years prior to death.

Within system differences in stable isotope values are apparent in the literature, as well. For example, different bones in the same individual have different turnover rates, resulting in differences in stable isotope values of the collagen if diet changes over time. Hedges et al. (2007) analyzed radiocarbon from 67 human femora to determine adult collagen turnover times. They found that the rate of collagen turnover decreases after 20 years of age in females to a greater degree than it decreases in similarly-aged male individuals. As is consistent with what is known about the effects of growth on bone, adolescents between the ages of 10-15 years showed increased bone collagen turnover activity, especially in males. Most applicable to dietary reconstruction using stable isotope analysis, the femoral bone collagen analyzed in this study reflected diet for over 10 years prior to death. In femora that had a slower turnover rate, Hedges et al. (2007) were able to detect the dietary signature formed during adolescence in some of the older adults.

There are archaeological implications for the rates of tissue turnover and replacement. As some bones will equilibrate with diet faster than others, the choice of element sampled for stable isotope analysis can affect the dietary time frame that is visible isotopically. When investigating the average diet in past populations, intra-individual differences in bone values are less important than detecting the overall trend in resource use. When dealing with archaeological material, the availability and the quality of the skeletal samples also need to be considered.

3.9. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Analysis in Archaeology

The following section briefly discusses some of the current applications of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis within the discipline of archaeology, with a specific focus on dietary reconstruction.

3.9.1. *Applications*

The archaeological applications of stable carbon and nitrogen isotope analysis to dietary reconstruction owe their beginnings to the fields of geochemistry and ecology. More than 35 years ago, researchers demonstrated the possibility of distinguishing C_3 versus C_4 plant consumption in archaeological human and animal remains (Lee-Thorp, 2008; van der Merwe and Vogel, 1978; Vogel and van der Merwe, 1977). From that beginning, the use of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis expanded to answer questions about the adoption of agriculture in various regions, marine-based diets and marine consumption intensity in hunter-gatherers, potential dietary differences between prehistoric populations, and early hominin diet in Africa (Lee-Thorp, 2008). More recent work has also applied stable isotopic techniques in other novel ways.

One of the first dietary questions that stable carbon isotope analysis was used to answer was the timing of the introduction of maize, a C_4 cultivar, into Woodlands North American cultures (van der Merwe and Vogel, 1978; Vogel and van der Merwe, 1977). By measuring the stable carbon isotope values of individuals and comparing these values to the food web baseline, this application not only identified the approximate timing of the onset of maize agriculture in the region, but also detected increasing reliance on maize as a staple food source (van der Merwe and Vogel, 1978; Vogel and van der Merwe, 1977), proving that stable carbon isotope analysis is a useful indicator of C_4 plant consumption in a predominantly C_3 environment. Later, other studies used the isotopic distinctions between C_3 and C_4 plants as a basis for detecting the onset of millet and rice cultivation in prehistoric Asia (e.g. Schoeninger and Moore, 1992).

Detection of marine resource use was also an early focus of archaeological stable isotope analysis. As discussed in the sections on nitrogen and trophic level, consumers in a marine-based food web consistently show higher stable carbon isotope and nitrogen values than those in terrestrial-based food webs. This enrichment has been used to answer questions about marine resource use in the European Mesolithic and Neolithic time periods in studies using both human and faunal remains (e.g. Fischer et al., 2007; Schulting and Richards, 2002; Chisholm et al., 1982) and has contributed to the knowledge about the Mesolithic-Neolithic transition from marine- to terrestrial-based diets, as well as intensity of marine resource use during these time periods.

Dietary stable isotope analysis has been used with bone collagen up to 200,000 years old, allowing researchers to examine the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of both early modern human and Neanderthal populations (e.g. Jones et al., 2001; Richards and Hedges, 1999). This information has allowed archaeologists to detect differences in trophic level between modern humans and Neanderthals possibly caused by differences in ecological niche use. Stable carbon isotope values have also been obtained from the bone mineral and tooth enamel of early hominins in Africa (Lee-Thorp and Sponheimer, 2006; Schoeninger and Moore, 1992), providing insight into environment and early hominin dietary adaptations.

Other research questions have used stable carbon isotope and nitrogen analysis to detect sex and status differences in diet (Ambrose et al., 2003), breastfeeding and weaning practices in various archaeological populations (Fuller et al., 2006b; Schurr, 1998), and to detect changes in individual diet over the lifetime. This last question has been answered in a variety of ways using intra-individual sampling techniques. This has involved analysis of different layers of enamel and dentine in the teeth (Burt and Garvie-Lok, 2013; Eerkens et al., 2011) of different teeth that form at different times (Dupras and Tocheri, 2007), as well as the analysis of soft

tissues such as hair or mummified tissues (Finucane, 2007; White and Schwarcz, 1994).

Dietary reconstruction has been applied to understudied populations, including individuals from Rome's early Christian population (Rutgers et al., 2009) and tropical regions where bone and tooth tissues may not be as well preserved as elsewhere (Ambrose and Butler, 1997). Future research using stable carbon and nitrogen isotopes will likely involve refinement of intra-individual sampling techniques, such as the use of laser ablation to section serial tooth samples (Lee-Thorp, 2008), as well as developing a better understanding of maternal and fetal metabolism and how it affects the stable isotopic values of newborn infants and their mothers.

3.10. Summary

Stable carbon and nitrogen isotopes show patterned variation in any given ecosystem, with an individual's tissue values primarily reflecting their dietary values. These dietary values themselves reflect the resource they represent, whether it is terrestrial, marine, freshwater, or lagoon-based. Other factors may influence $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, however, including environment, water stress, nursing, growth, and nutritional stress. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of a consumer's tissues vary due to the effects of fractionation and rates of tissue turnover, but these values have been used effectively in other studies to address questions involving diet, resource use, and weaning behaviour in archaeological populations.

Chapter 4: Site, Sample, and Methods

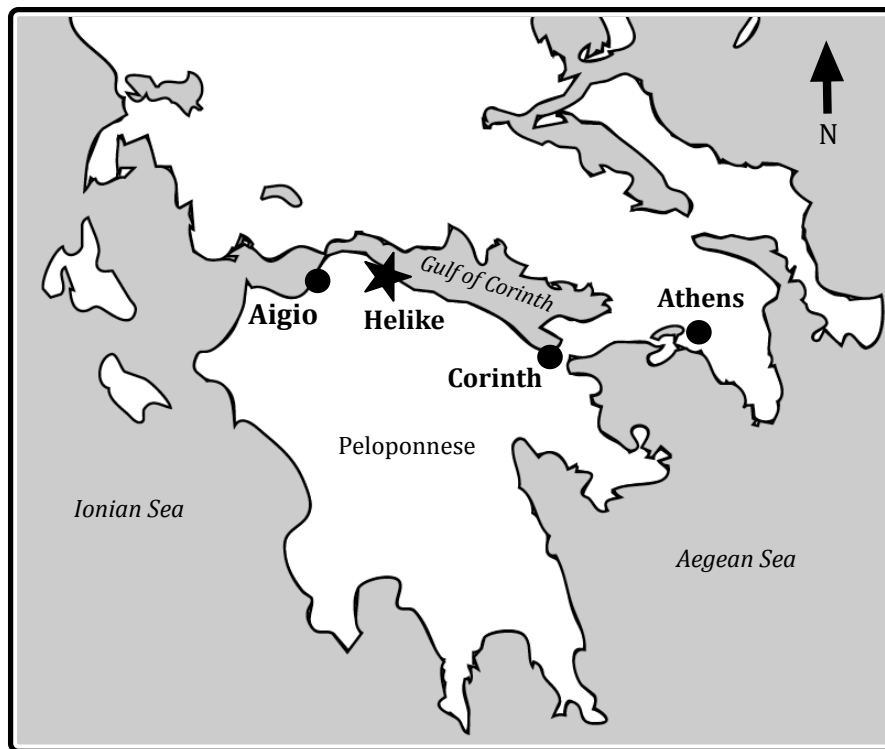
This chapter discusses the archaeological site of Helike, Greece with the purpose of outlining the context of the skeletal samples used in this study, including the general excavation history and associated finds. The human and faunal samples from Helike are described by the element selected, individual age and sex (where applicable), and the time period associated with each burial. Also described are additional faunal samples from Late Roman Corinth included in the analysis in order to better establish an isotopic baseline. There are several methods available for processing and preparing bone collagen for stable isotope analysis; discussion of the most commonly used methods is followed by the descriptions of the indicators used to determine sample quality and to detect diagenesis. The process used to extract the bone collagen from the Helike and Late Roman Corinth samples in this study is outlined.

4.1. Site

For dietary reconstruction and resource use purposes, it is more practical to think of the archaeological site of Helike as covering an area rather than as a single, defined settlement. Ancient Helike and its surroundings are located on the Helike Delta, a coastal plain on the southwestern shores of the Gulf of Corinth in the Peloponnese region of Greece (Figure 4.1). Today, the modern villages of Eliki, Rizomylos, and Nikolaiika are located within confines of the delta. They are roughly contiguous with each other, with Eliki being the most western community and Nikolaiika being the most eastern. The Gulf of Corinth is found within a seismically active rift valley and the Helike Fault line runs from east to west through the Helike Delta. This particular fault line is quite visible in the topography of the area, as it forms the border between the coastal plain and the mountains immediately to the south (Soter and Katsonopoulou, 2011).

The general area shows evidence of both gradual uplifting and periodic seismic events, with almost two meters of uplifting occurring between 450 BC and 150 AD (Soter, 1998). Earthquakes have also changed the courses of the rivers located in the delta, notably the Selinous and Kerynites rivers (see Figure 4.2; Soter and Katsonopoulou, 2011). Although the modern sea level, coastline, and topographical features have changed since antiquity, the coastal plain itself has remained suitable for agriculture. The soil in this region is alluvial and rich, and mountain springs provide the water supply (Marinatos, 1960). These advantages likely encouraged agricultural subsistence in earlier times and supported the numerous settlements that populated the delta region.

Figure 4.1 - Map of Greece*



* Approximate locations

4.1.1. Helike

Helike is first mentioned by ancient writers living after the Classical period. According to these sources, the city of ancient Helike was founded during the Bronze Age, “forty stades” or seven kilometers east of Aigion and two kilometers from the sea (Pausanias, 7.24.5). The Classical city was the political and religious capital of the Achaean League, had its own Poseidon cult, and established several colonies, including Priene in Asia Minor and Sybaris in Italy (Courakis, 1998; Katsonopoulou, 1998). Despite its political and religious importance, ancient Helike is probably best known for its dramatic destruction in 373 BC during a major earthquake and subsequent tsunami. Pausanias, writing between 143-176 AD, states that:

“This was the type of earthquake, they say, that on the occasion referred to leveled Helice to the ground, and that it was accompanied by another disaster in the season of winter. The sea flooded a great part of the land, and covered up the whole of Helice all round. Moreover, the tide was so deep in the grove of Poseidon that only the tops of the trees remained visible. What with the sudden earthquake, and the invasion of the sea that accompanied it, the tidal wave swallowed up Helice and every man in it.” (Pausanias, 7.24.12).

As a result of this seismic event, the waters of the Gulf of Corinth appear to have covered the area containing the city *chora* (territory) and part of the area surrounding the city. These remained submerged for quite some time within the body of water that had formed. Strabo (64 BC-23 AD), in his *Geography*, writes that:

“And Eratosthenes says that he himself saw the place, and that the ferrymen say that there was a bronze Poseidon in the strait, standing erect, holding a hippo-campus in his hand, which was perilous for those who fished with nets.” (Strabo, 8.7.2).

Eventually, the exact location of Classical Helike was forgotten until the Helike Project began sonar surveys in the Gulf of Corinth in 1988 with the

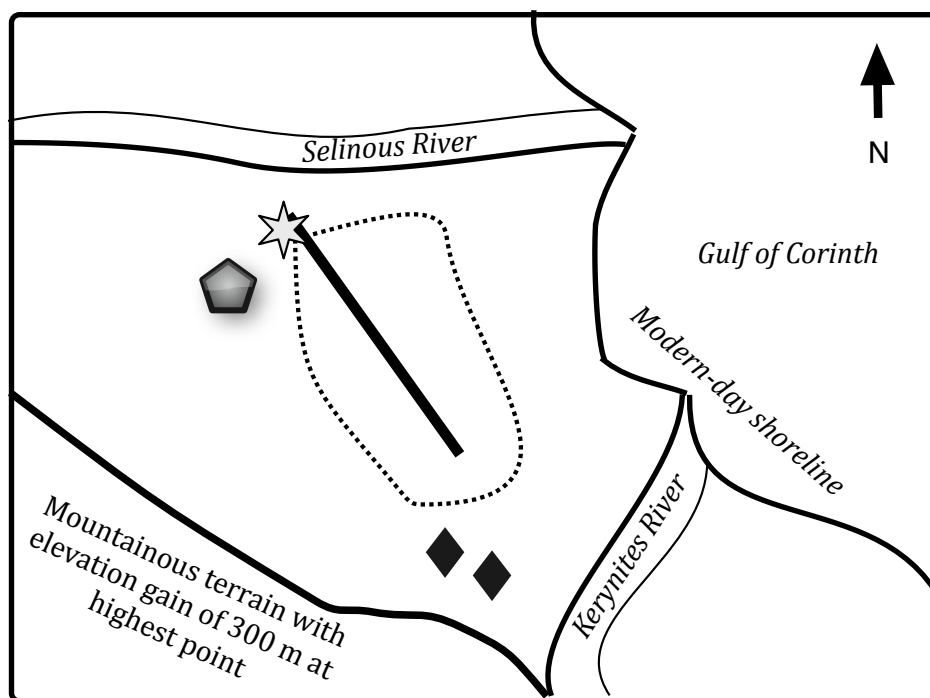
intention of locating the ancient city. Scans of the sea floor did not reveal any conclusive evidence of the city, so attention was directed next to the coastal plain of the Helike Delta (Soter and Katsonopoulou, 2011). In total, 99 boreholes were drilled into a two kilometer square area of the delta between 1991 and 2002. These samples came up with occupational layers dating from the Early Bronze Age, Mycenaean, Geometric, Classical, Hellenistic, Roman, and Late Byzantine periods (Soter and Katsonopoulou, 2011), indicating the extensive human habitation of the area over time. In 2000, the Helike Project and Helike Society excavated ten test trenches in the areas between the villages of Eliki, Rizomylos, and Nikolaiika for the purpose of fully identifying and describing some of these occupation horizons (Katsonopoulou, 2005b). Annual excavations of the area continued until 2008 and these have recovered the majority of the burials used in this study. It is interesting to briefly note that the borehole evidence pertaining to the Classical period strongly suggests that these occupations were affected by a major earthquake and tsunami, consistent with the historical reports of the 373 BC earthquake. These Classical occupations are outside the presumed location of the *chora* of ancient Helike, which is currently at a depth below sea level (Soter and Katsonopoulou, 2011).

Katsonopoulou (1998) suspected that the *chora* of ancient Helike had turned into a lagoon after the earthquake and tsunami, based upon the literary evidence cited above. Geoarchaeological surveys and borehole sampling have found sediments consistent with a brackish lagoon environment overlying some of the Classical layers within the central area of the Delta (Soter and Katsonopoulou, 2011). The presence of Hellenistic remains demonstrates reoccupation of the region, which appears to have occurred around the shores of the lagoon. This lagoon gradually vanished as a combination of tectonic uplift and silting returned the area to dry land. By the Byzantine period, a widespread black clay deposit suggests the presence of a marsh (Soter and Katsonopoulou, 2011; Alvarez-Zarikian et al., 2008; Katsonopoulou, 2005a).






Both freshwater and brackish lagoons and lakes appear to have both formed and disappeared at various points in the past; georadar and core sampling throughout several square kilometers of the delta have provided evidence of these, although their importance as a resource to the people living in the area is not clear (Soter and Katsonopoulou, 2011). Some sediment samples associated with the various occupational layers in the delta contain shells from edible marine molluscs; these are argued to be evidence of food remains rather than indicators of a past marine environment (Alvarez-Zarikian et al., 2005).

For the purposes of this study, the archaeological finds related to the Hellenistic, Roman, and Byzantine periods provide the focus of the following descriptions, despite the evidence of settlements from much earlier periods within the same trenches. For further information about the complex stratigraphy and the occupational layers not discussed here, Soter and Katsonopoulou (2011) and Katsonopoulou (2005b) provide more comprehensive excavation histories. Figure 4.2 (adapted from Soter and Katsonopoulou, 2011) shows the locations of the burials and the site features most relevant to this study.

Figure 4.2 - Site Schematic With Location of Burials*



*Approximate locations. Not drawn to scale

-  Hellenistic burials - Papamichalopoulou area
-  Roman burials - Karelis and Dimopoulou areas
-  Byzantine burials - Romanos area
-  Roman road
-  Deposit of black clay

The Hellenistic period individuals included in this study were excavated in 2002 and 2003 from trenches H25 and H33 in the Papamichalopoulou area. These trenches also contained a Roman period destruction layer with wall stones, roof tiles, pottery, and glass and metal objects at the upper levels (Soter and Katsonopoulou, 2011). Seven tile-covered graves of the late 4th to early 3rd centuries BC (based upon grave style and stratigraphy) were located at the lower levels, from which the Hellenistic individuals were excavated. A bronze ring was found in association with individual H25.4 from these graves. Only six of these individuals were sampled in this study; the individual from H25.5 is estimated to have been an infant and the bone fragments present were unsuitable for stable isotope analysis.

The Hellenistic faunal remains sampled in this study are from trench H7, located in the Giannakopoulou field in Rizomylos. These were found in association with walls, pottery, and cooking vessels and are dated based upon these objects. Some of the animal bones from this trench showed evidence of burning (Katsonopoulou, 2005b), which is consistent with the food processing activities that likely occurred within this structure.

The Roman period individuals are from two different areas within the Helike Delta. Five graves, covered in massive tiles, were excavated in 2000 from trench H5 in the Karelis vineyard in modern Nikolaiika. These burials date to the 2nd or 3rd centuries AD (Katsonopoulou, 2005b) according to the grave goods, which included clay vases, lamps, and a bronze coin minted with the image of the Roman emperor Hadrian (117-135 AD).

The eight individuals from trench H58, located on the Dimopoulou property in Nikolaiika, were excavated in 2006. These individuals were recovered from seven tile-covered graves, of which several had shown indications of reuse resulting in more individuals than graves. These burials contained “rich” goods (Soter and Katsonopoulou, 2011), including clay and glass vases, a coin with the image of Constantine (306-337 AD), iron knives,

and bronze and ivory objects. Based upon these items, this burial group appears to date to at least the 4th century AD.

The majority of the Byzantine individuals in this study were excavated from a cemetery on the Romanos property. Seven individuals were excavated from trench H4 and individual H4.3.2 from this group was recovered with bronze earrings. These burials appear to date to the 14th to 15th centuries AD based upon association with nearby architectural remains and stratigraphy (Katsonopoulou, 2005b). Two individuals, designated T13.1 and T27.1, were excavated from a test trench in 2000 about which there is no further information. These burials were dated based upon stratigraphic information.

The archaeological evidence supports the idea that the Helike Delta was resettled after the earthquake of 373 BC sometime in the Early Hellenistic period, and remained continuously inhabited up until the Late Byzantine. In addition to the burials, it is important to be aware of some of the other settlement features from the various time periods. Evidence of Hellenistic economic activity is found within the remains of a large textile workshop. Floor cisterns, vats, storage rooms, and workshop areas were found in conjunction with vases, a terracotta figure, lamps, loom weights, and numerous bronze coins (Soter and Katsonopoulou, 2011).

In addition to the Byzantine cemetery mentioned above, the Romanos field contains evidence of earlier occupational layers, including part of a Roman road that extends for 1300 meters (Soter and Katsonopoulou, 2011). This would have provided a major transportation route through the area and also gives some indication of how this particular part of the landscape might have looked during the Roman period. A nearby Roman-era building containing fragments from glass vases, large quantities of pottery, bone and bronze objects, as well as marble decoration and bronze coins, shows evidence of destruction by a late fifth century AD earthquake (Katsonopoulou, 1998). This acts as a reminder that earthquakes and seismic activity were not events limited to the Classical period.

Perhaps the most pertinent geoarchaeological evidence of landform change is found with the variation in sea level and resulting bodies of water that were formed within the Helike Delta. Both freshwater and brackish lagoons appear to have come and gone in various locations over the time periods in question as a result of tectonic uplift and subsidence (Soter and Katsonopoulou, 2011). Stratigraphic data from H7, H8, and H10 suggest that an ancient inland freshwater lagoon in what is now the modern community of Rizomylos transformed into a marsh sometime after the Hellenistic period (Katsonopoulou, 2005b). Although this is not the same body of water that is thought to have formed over the *chora* of ancient Helike, it is highly likely that several lagoons of varying sizes and characteristics existed and may have provided additional resources to the individuals of the time.

4.2. Sample

This section describes the characteristics of the human samples from Helike, including age and sex estimations. The faunal samples from Helike and from Late Roman Corinth are described according to genera and selected element.

4.2.1. Human

As mentioned above, the human skeletal remains analyzed in this study had been excavated during previous field seasons up until 2008. Since then, they had been stored in the Helike Society laboratory, located in the modern village of Eliki. Cleaning and osteological analysis of these remains occurred over two field seasons in 2009 and 2011. Bone samples for isotopic analysis were selected preferentially from ribs, as well as from cranial bones and long bones when the ribs could not be assigned to a specific individual.

Table 4.1 presents the sex and age estimations for the 28 individuals that were selected for stable isotope analysis. Six individuals date to the Hellenistic period, 13 to the Roman period, and nine to the Byzantine period.

Sex and age estimations were done using the methods outlined in *Standards for Data Collection from Human Skeletal Remains* (Buikstra and Ubelaker, 1994) and were based upon both pelvic and cranial morphology when available. One individual was estimated as female, eight as probable females, four as male, six as probable males, and nine were of indeterminate or unknown sex. For the purposes of data interpretation, the probable sex estimation categories were combined with the more certain sex estimation. This created a final sample consisting of nine females, ten males, and nine unknown or indeterminate sex individuals.

Age estimations used the following general categories: Juvenile (under 18 years of age), Young Adult (18-35 years), Middle Adult (35-50 years), and Older Adult (50 or more years of age). Several individuals, although clearly adult, were not able to have their age estimate refined enough to fall within these categories and are described as Young to Middle Adults (n=2) and Middle to Older Adults (n=3). The rest of the individuals in the sample consist of one juvenile (approximately 3-4 years of age, according to dental estimations), seven Young Adults, three Middle Adults, and two Older Adults in addition to the above intermediate categories. Ten skeletally mature individuals could not be reliably assigned to any age category and are classified as "Unknown" age. The uncertainties in the assignment of sex and age categories reflect the highly fragmented bone material and complex stratigraphy found within many of these burials. Many indeterminate sex and unknown age individuals were represented only by bone fragments and lacked intact skeletal elements on which to base further estimations.

Table 4.1 - Human Samples from Helike

Sample Number	Time Period	Sex Category	Age Category
H25.1	Hellenistic	Male	Young Adult (18-35 years)
H25.2	Hellenistic	Probable Female	Young Adult (18-35 years)
H25.3	Hellenistic	Probable Male	Young Adult (18-35 years)
H25.4	Hellenistic	Male	Older Adult (50+ years)
H25.6	Hellenistic	Unknown	Juvenile (3-4 years)
H33.1	Hellenistic	Probable Female	Young to Middle Adult (18-50 years)
H5.1	Roman	Probable Male	Young to Middle Adult (18-50 years)
H5.2	Roman	Probable Male	Unknown
H5.3	Roman	Probable Male	Middle Adult (35-50 years)
H5.4	Roman	Unknown	Unknown
H5.5	Roman	Probable Male	Middle to Older Adult (35-50+ years)
H58.2	Roman	Unknown	Older Adult (50+ years)
H58.4	Roman	Probable Female	Middle to Older Adult (35-50+ years)
H58.5.1	Roman	Probable Female	Young Adult (18-35 years)
H58.5.A	Roman	Probable Female	Middle Adult (35-50 years)
H58.5.B	Roman	Unknown	Unknown
H58.5.C	Roman	Probable Female	Unknown
H58.6	Roman	Unknown	Unknown
H58.7	Roman	Unknown	Young Adult (18-35 years)
H4.1.1	Byzantine	Unknown	Unknown
H4.1.2	Byzantine	Probable Male	Unknown
H4.1.3	Byzantine	Unknown	Unknown
H4.1.4	Byzantine	Probable Male	Unknown
H4.2	Byzantine	Male	Middle Adult (35-50 years)
H4.3.1	Byzantine	Male	Older Adult (50+ years)
H4.3.2	Byzantine	Female	Young Adult (18-35 years)
T13.1	Byzantine	Unknown	Unknown
T27.1	Byzantine	Probable Female	Young Adult (18-35 years)

4.2.2. Faunal

Food webs are complex and the use of local faunal material for sample helps establish baseline values for the interpretation of the human values. Fifteen faunal elements, all associated with the Hellenistic period, were selected from previously excavated materials at the laboratory in Eliki. These bones had been excavated from middens and likely underwent food processing activities prior to their deposition, although there was no evidence of burning on any of the selected samples. Care was taken to select elements excavated from different deposits, that were of different sizes, or that were the same element from the same side of a particular genus (e.g. left scapulae from two different *Ovis/Capra*) where possible, in order to minimize the chance of sampling from the same animal twice.

There were no usable marine fauna from Helike, but the sampling of domesticate animals does help provide local terrestrial values. Genus-level identification was possible for most of the samples and was confirmed using comparative faunal material from the University of Alberta Zooarchaeology Reference Collection. One sample was not identifiable and was excluded from isotopic analysis.

The genera represented by the 14 samples are *Ovis/Capra* (n=3, including one older juvenile), *Bos* (n=5, including one older juvenile), *Sus* (n=5), and *Cervus* (n=1), most likely roe deer. With the exception of the ovicaprine and *Bos* samples mentioned above, all appeared to be from skeletally mature (i.e. adult) animals. A summary of the faunal individuals and elements used for isotopic analysis is found in Table 4.2.

Table 4.2 - Faunal Samples from Helike

Sample	Genus	Maturity	Element sampled
HF-01	<i>Bos</i>	Mature	Right distal metacarpal
HF-02	<i>Bos</i>	Mature	Proximal phalanx
HF-03	<i>Bos</i>	Mature	Proximal phalanx
HF-04	<i>Bos</i>	Mature	Intermediate phalanx
HF-05	<i>Bos</i>	Immature	Right distal tibia
HF-06	<i>Sus</i>	Mature	Intermediate manual phalanx
HF-07	<i>Sus</i>	Mature	Right proximal manual phalanx
HF-08	<i>Sus</i>	Mature	Left talus
HF-09	<i>Sus</i>	Mature	Right proximal ulna
HF-10	<i>Sus</i>	Mature	Right mandibular ramus fragment
HF-11	<i>Ovis/Capra</i>	Mature	Glenoid from left scapula
HF-12	<i>Ovis/Capra</i>	Mature	Glenoid from left scapula
HF-13	<i>Ovis/Capra</i>	Immature	Left proximal ulna
HF-14	<i>Cervus</i>	Mature	Right calcaneus

Since there were few faunal samples from Helike, additional material from Late Roman Corinth was consulted for this study. Corinth is located approximately 90 kilometers east from Helike and shares the same coastline, making the faunal samples from this site quite comparable to Helike and useful in developing local isotopic food web values. The samples in question were brought to the laboratory for another ongoing study and were prepared by this author during practical training activities.

The Late Roman Corinth faunal bones had been excavated over several field seasons by the American School of Classical Studies at Athens and comprise part of an ongoing isotopic study of the site. The archaeological site of Corinth has been studied since 1896 and is both well known and extensively documented (see Williams and Bookidis, 2003 for further information). The faunal samples in this study date to the Late Roman period, which overlaps temporally with many of the human burials studied. The genera represented include ovicaprids (n= 5, including one skeletally immature individual), *Sus* (n=6, including five immature individuals), *Bos* (n=4, including one immature individual), and *Canis* (n=5). While dog meat

was not likely part of the diet, the *Canis* samples had been selected in part to address the questions of the ongoing Corinth study and the results are reported here for interest. Table 4.3 lists the sample identifications, genus, skeletal maturity, and the elements sampled from the Corinth fauna used in this study. All identifications were confirmed using the Zooarchaeology Reference Collection at the University of Alberta. The estimated ages listed for some of the immature individuals below were derived from available dental development and eruption data for the species.

Table 4.3 – Faunal Samples from Late Roman Corinth

Faunal Identification	Genus	Maturity	Element sampled
CRF-01	<i>Ovis/Capra</i>	Mature	Left mandible
CRF-02	<i>Ovis/Capra</i>	Mature	Left mandible
CRF-03	<i>Ovis/Capra</i>	Immature 1-2.5 years	Left mandible
CRF-04	<i>Ovis/Capra</i>	Mature	Left mandible
CRF-05	<i>Ovis/Capra</i>	Mature	Left mandible
CRF-06	<i>Canis</i>	Mature	Right ulna
CRF-07	<i>Canis</i>	Mature	Right ulna
CRF-08	<i>Canis</i>	Mature	Left ulna
CRF-09	<i>Canis</i>	Mature	Left ulna
CRF-10	<i>Canis</i>	Mature	Left ulna
CRF-11	<i>Sus</i>	Immature	Left metacarpal
CRF-12	<i>Sus</i>	Immature	Left metacarpal
CRF-13	<i>Sus</i>	Immature	Left scapula
CRF-14	<i>Sus</i>	Immature	Left scapula
CRF-15	<i>Sus</i>	Mature	Right scapula
CRF-16	<i>Sus</i>	Immature	Right scapula
CRF-17	<i>Bos</i>	Immature ~30 months	Right mandible
CRF-18	<i>Bos</i>	Mature	Right mandible
CRF-19	<i>Bos</i>	Mature	Right radius
CRF-20	<i>Bos</i>	Mature	Right metacarpal

4.3. Methods

The following section discusses the main methods of collagen preparation that have been developed for stable isotope analysis and the indicators of diagenesis commonly cited in the literature. The process used to prepare the Helike and Corinth bone collagen samples is described.

4.3.1. *Collagen Preparation*

In order to analyze bone collagen for its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, it must be separated from the bone mineral and any lipids that are still present. The various components of bone tissue reflect different aspects of the diet and lipids have much lower $\delta^{13}\text{C}$ values than bone collagen, which can drastically affect the values obtained if these are not removed (Jørkov et al., 2007; Liden et al., 1995; Ambrose, 1993). Additionally, archaeological bone may contain contaminants such as diagenetic carbonates, carbon and nitrogen from the surrounding soils, and humic and fulvic acids from the burial matrix (Ambrose, 1993). The inclusion of any of these substances in the collagen samples will affect the stable isotope values obtained and provide erroneous results. Therefore, the goals of collagen preparation for stable isotope analysis are to separate the collagen from the other bone components, remove contaminants, and maximize the amount extracted while minimizing damage to the collagen itself (Jørkov et al., 2007; Brown et al., 1988). Several methods have been developed to address these issues and the main differences found between the most commonly used methods today appear to be the use of powdered versus whole bone chunks, the use of NaOH to remove humic acid contaminants, and the use of ultrafiltration/gelatinization as a final action to remove contaminants. With all methods, the bone requires demineralization to separate the bone collagen from the bone mineral, whether through hydrolysis or chemical means.

The Longin (1971) method is one of the earliest and stemmed from the need for more accurate radiocarbon dates from extracted bone collagen. This method involves crushing the bone sample and soaking it in an 8%

hydrochloric acid (HCl) solution in order to break the hydrogen bonds of the collagen and to make it soluble in hot water. The actual collagen extraction occurs when residue from the HCl soak is placed in slightly acidic water at 90 degrees Celsius for several hours. The end substance obtained through this process is a gelatin intended to be “relatively free of contaminants”, which remain in the residue (Longin, 1971).

Unfortunately, several researchers using the Longin method were still finding contaminants in the collagen gelatin, and a modification to the original process was needed in order to address this issue. Brown et al. (1988) published a modified Longin method based upon the idea that the contaminants in bone are likely to be of low molecular weight while the collagen consists mainly of higher molecular weight peptides. The gelatin from this process is filtered, lyophilized, and then ultrafiltered, with this last step added in order to separate the contaminants from the collagen (Brown et al., 1988). Similar to Longin, the original ultrafiltration method also uses powdered bone samples that are demineralized in HCl.

Other methods deal with contaminants by soaking the demineralized collagen sample in a sodium hydroxide (NaOH) solution to remove humic acids. This step was added after the realization that the HCl soak and demineralization steps did not completely remove these contaminants on their own, even with a longer soaking time (Ambrose, 1993, 1990; Schoeninger and DeNiro, 1984; DeNiro and Epstein, 1981). While some studies have found that the use of NaOH decreases the collagen yield (Liden et al., 1995; Ambrose 1990; Chisholm et al., 1983), this has not been consistently shown across the literature (Jørkov et al., 2007; Katzenberg and Weber, 1999; Ambrose, 1990). The quality of the collagen after undergoing a NaOH soak also appears to remain the same, even in lower yield samples, as measured through C:N atomic ratios and %C and %N indicators (Jørkov et al., 2007; Liden et al., 1995; Ambrose, 1990). Different methods can result in varying amino acid composition of the end product, but the overall stable carbon isotope values are not always affected (Liden et al., 1995). A

suggested way to further decrease the risk of collagen destruction while using a NaOH soak is to shorten the treatment time (Kennedy, 1988). Most methods that use NaOH as a decontaminant recommend a maximum of 20 hours treatment time in order to minimize collagen deterioration (Ambrose, 1993).

Much attention has been paid to the costs and benefits of the various collagen extraction methods (Jørkov et al., 2007; Liden et al., 1995; Chisholm et al., 1983), but the general consensus is that since burial environments and bone preservation vary according to time and place, there is no single method that is best for all samples. Demineralization methods using HCl generally recommend using a concentration of 1% to 5%, with the idea that a longer, slower demineralization process will produce more collagen with higher C and N concentrations than a process that uses a stronger HCl solution for a shorter time (Katzenberg, 2008; Ambrose, 1993). Methods involving ultrafiltration or gelatinization may be better for poorly preserved bone in order to maximize the collagen yield, but methods that use NaOH remain in common use as this is a relatively easy step that deals with the issue of contaminants (Katzenberg, 2008; Jørkov et al., 2007).

Several researchers have argued for the inclusion of a lipid removal step during the collagen extraction process (Liden et al., 1995; Chisholm et al., 1983), while others have argued that the presence of lipids in archaeological bone is uncommon and that the NaOH soak should remove any trace amounts left (Ambrose 1993). Some have included a specific step involving soaking the sample in a mixture of chloroform, methanol, and water or similar solutions to remove lipids (Chisholm et al., 1983) or recommend ultrafiltration, especially with modern bone samples, as NaOH does not appear to be sufficient enough to remove all lipids (Liden et al., 1995).

Jørkov et al. (2007) recommend using chunks of whole bone for collagen extraction instead of powdered bone, especially in samples that are poorly preserved; grinding already poorly preserved bones may damage the

remaining collagen. With methods that use a whole chunk demineralization process (e.g. Katzenberg and Weber, 1999; Richards and Hedges, 1999; Sealy, 1986), compact bone is preferred over trabecular, as the amount of surface area on trabecular bone increases the likelihood of contaminants being present (Jørkov et al., 2007; Ambrose, 1993). In at least one case, this has resulted in an altered stable nitrogen isotope value as well as a stable carbon isotope value (Jørkov et al., 2007).

Generally, the most commonly used methods involve mechanical cleaning of the bone sample, demineralization in a HCl solution, and the removal of contaminants using either solubilization or gelatinization through heating or a NaOH soak (Ambrose, 1993). The resulting product is freeze-dried and then analyzed (Katzenberg, 2008; Sealy 1986). Other less common methods include the use of EDTA (ethylenediaminetetraacetic acid) instead of HCl to demineralize the bone sample (Bocherens et al., 1995; Tuross et al., 1988) or the use of enzymes such as collagenase to extract purified collagen from poorly preserved bones (DeNiro and Weiner, 1988). The decision of the researcher on which method to use should be based upon the burial environment, the condition of the bone, and the availability of laboratory resources.

4.3.2. Diagenesis

Accurate interpretation of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bone collagen requires confirmation that the substance analyzed is actually collagen; the sample should not appear to be contaminated or so degraded that the stable isotope values obtained are not reflective of actual dietary values. There are three common criteria used to assess collagen quality and preservation: yield, carbon-to-nitrogen ratio (C:N), and the carbon and nitrogen content of the collagen (%C and %N). Used in conjunction with each other, these criteria can help the researcher determine if collagen sample in question should be accepted or rejected in the overall results interpretation, although there is some suggestion that these criteria will identify only “the worst of the worst”

samples consistently (van Klinken, 1999). Most samples showing evidence of smaller diagenetic changes will end up being assessed on a case-by-case basis (van Klinken, 1999). The final assessment of collagen quality and preservation should still take all these indicators into account as degraded, low-yield collagen samples tend to show variable and lower %C, variable and higher C:N, and mostly lower $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ (van Klinken, 1999). Contaminated collagen usually has higher %C and C:N ratios and lower $\delta^{13}\text{C}$ values (van Klinken, 1999).

4.3.2.a. Collagen yield

The percentage weight of collagenous material extracted from dry bone for stable isotope analysis is one indicator of quality and preservation. The collagen yield of a sample is expressed as a percentage, where the weight of the extracted material is divided by the total weight of the dry, pre-processed bone sample. Modern bone contains about 22% collagen (van Klinken, 1999), but archaeological bone can show wide variations in this amount after spending many years in the burial environment (Pfeiffer and Varney, 2000; van Klinken, 1999).

Although the method used to extract the collagen can impact the final collagen yield (Schoeninger et al., 1989; Tuross et al. 1988), the main concern about low yield samples is that the collagen obtained is more likely to be of poor quality and will provide erroneous stable isotope values. The collagen's amino acid profile may indicate preferentially lost individual amino acids of differing isotopic values (Schwarcz and Schoeninger, 1991). How low yield collagen samples are defined varies from researcher to researcher and according to the study.

Ambrose (1990, 1993) and DeNiro and Weiner (1988) both suggest that a collagen yield of less than 2% marks the threshold for accepting or rejecting samples, a value supported by Lee-Thorp (2008) as indicative of collagen that has likely maintained its isotopic integrity. Schwarcz and Schoeninger (1991), Schoeninger et al. (1989), and Tuross et al. (1988) have

advocated for a cut-off point of between 5% and 6%. Depending on the yield obtained, some researchers have recommended amino acid profiling to confirm that lower yield samples are still reflecting intact collagen. Schwarcz and Schoeninger (1991) recommend at least a 5% collagen yield, with anything less than 10% requiring amino acid profiling. However, profiling is expensive and time-consuming and not available at all laboratories.

Other, lower cut-off points for low collagen yield are found throughout the literature. While Schoeninger and Moore (1992) state that samples are acceptable with a 1% or greater yield, anything less than 1% is considered to run the risk of having artificially enriched $\delta^{15}\text{N}$ values, even as much as 15‰ over the original collagen values (Schoeninger and Moore, 1992). van Klinken (1999) also states that samples with less than 1% collagen yield should be rejected.

van Klinken (1999) distinguishes between collagen contamination and degradation issues when discussing collagen yield as a quality indicator. Collagen yield is more sensitive in detecting the loss of collagen from the bone than it is in detecting contamination, since contamination is not likely to cause a low yield value on its own. Schoeninger et al. (1989) found that samples that were not completely demineralized showed “collagen” yields of greater than 25%, which are obvious outliers given that the collagen content of modern bone is about 22% (van Klinken, 1999).

Collagen yield values should not be the only indicator of sample quality used because higher yield samples are not guaranteed to be free from contamination or degradation (van Klinken, 1999; Schoeninger and Moore, 1992). Other indicators, such as C:N and %C and %N, may provide more important information about collagen quality. Considering that most archaeological bone normally shows some sort of collagen loss, the burial context and environmental variables should also be taken into account when deciding on low yield acceptance or rejection values (van Klinken, 1999; Ambrose, 1990). Although there is a wide variation in the standards used to identify low yield samples, researchers agree that these should be rejected.

4.3.2.b. Carbon to nitrogen ratio

Another collagen quality indicator is the ratio of carbon to nitrogen (C:N) contained in the sample; this is measured during mass spectrometry isotopic analysis. The C:N value is the cumulative ratio of carbon to nitrogen atoms in each molecule of the amino acids in the collagen sample (Schoeninger et al., 1989). Each amino acid contained in collagen is present in various proportions depending on what it is. A single collagen chain has approximately 1000 amino acid residues of which every third one is glycine (Schoeninger and Moore, 1992), which results in a C:N atomic ratio of 3:1 in intact collagen.

DeNiro (1985) first proposed the C:N ratio as an indicator of collagen quality. His analysis of C:N values for collagen in fresh bones from 69 animal species and 40 modern human bones showed that C:N ratios ranged from 2.9 to 3.6. He proposed that the magnitude of the change in stable isotope values is related to the size of the shift in the C:N ratio; this makes sense given that the stable carbon and stable nitrogen isotopes are derived from the total carbon and nitrogen present in the collagen sample. A significant relationship between C:N and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ may then indicate a major problem with sample quality. Katzenberg (2008) notes that DeNiro's ranges are for atomic ratios and some modern mass spectrometry machines provide weight ratios; the researcher needs to be aware of which ratio is being provided and convert the measurements if necessary.

When using both collagen yield and C:N to assess sample quality, van Klinken (1999) notes that extreme ranges are more often found with low yield samples (between 0.5% to 1%) than in samples that are merely contaminated. This criterion may better identify those samples that are affected by degradation. It is still possible to have a contaminated sample with a C:N ratio between 2.9 and 3.6 if the carbon contributing to the ratio is non-collagenous in origin. Schoeninger and Moore (1992) found that with a collagen yield of greater than 10%, the C:N assessment was a "superfluous" measure (see also Schoeninger et al., 1989) and a yield of less than 5% with a

good C:N was ambiguous (although a bad C:N indicated rejection). In samples with less than 1% collagen yield, C:N may not be useful as a quality indicator (Schoeninger and Moore, 1992) and this should be taken into account. As with collagen yield, the C:N does not appear to be a very sensitive tool when used on its own (van Klinken, 1999), although some argue that it is still a robust indicator overall (Lee-Thorp, 2008).

4.3.2.c. Carbon and nitrogen content

Another indicator of collagen quality is the percentage of carbon and nitrogen contained in the sample and expressed as a percentage. As with C:N ratios, the %C and %N values are measured during mass spectrometry (van Klinken, 1999).

van Klinken (1999) has suggested that the %C of intact bone collagens is around $35\% \pm 8.8\%$, while the %N is between 11% and 16%. Ambrose (1990) suggests that a %C range of 15% to 47% and a %N ranging from 5% to 17% are acceptable. While these ranges do overlap somewhat, both Ambrose (1990) and van Klinken (1999) emphasize that these quality indicators need to be interpreted in conjunction with other data when deciding on exact cut-off points. Ambrose (1990, 1993) has also stated that well-preserved collagen in archaeological bone has at least 3% carbon and 1% nitrogen, and these values have been used as guidelines when assessing collagen quality in studies with poorly preserved archaeological samples (Bourbou et al., 2011; Pfeiffer and Varney, 2000).

4.3.3. Bone Collagen Preparation

The human and faunal bone samples from Helike were prepared for stable isotope analysis according to the following steps:

Depending on the size and type of element available for sampling, amounts of dry bone ranging from 200 mg to 1000 mg were cut from the main sample using a Dremel power tool with a rotary head attachment. The outer surfaces and any areas of trabecular bone present on the bone chunk

were sanded to remove any superficial contaminants (Ambrose, 1993). Each bone chunk was then rinsed with tap water, brushed with a toothbrush, and placed in a 50 ml Pyrex flask filled with distilled water. All sample pieces were cleaned ultrasonically in separate flasks for at least 10 minutes. Once the distilled water in each flask appeared to be clean, the samples were air-dried for a minimum of 24 hours in petri dishes and then weighed to obtain the dry, pre-processed weight for each.

In order to remove the bone mineral and any remaining superficial contaminants that may have been adhering to the bone mineral structure, each sample piece was demineralized in 50 ml of 1% HCl solution (Sealy, 1986). This solution was changed every two days and the progression of demineralization for each sample was documented with every change. Recorded indicators of demineralization included the presence of bubbles, the colour of the solution, and the pliability and translucency of the samples (Ambrose, 1990; Schoeninger et al., 1989).

Once demineralization was complete and the collagen component was isolated, the sample was rinsed at least twice with distilled water, with a minimum of one day between rinses. This removed all traces of the hydrochloric acid, ensuring that each sample returned to a neutral pH.

Humic acids from soil contaminants were removed by soaking the collagen samples in 50 ml of 0.125 mol NaOH for 20 hours (Katzenberg and Weber, 1999; Ambrose, 1993). They were then rinsed with distilled water at least twice, with one day between rinses. Any signs of sample deterioration apparent after the NaOH soak were documented. After the rinses, the pH of the distilled water in the sample vials was tested to ensure that all traces of the basic NaOH were gone and that the sample pH was approaching neutrality. The water was drained, each collagen sample was placed into a previously weighed scintillation vial, and the vials stored in the lab freezer until they were freeze-dried at the Biogeochemical Analytical Service Laboratory at the University of Alberta.

Final extracted collagen weights were obtained from the freeze-dried samples using the same scale that had been used to obtain the initial pre-processed bone weights. Amounts of collagen ranging from 0.9 mg to 1.1 mg were packaged into tin foil capsules and submitted for stable isotope analysis to the Biogeochemical Analytical Service laboratory.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, as well as the percentages of carbon and nitrogen present in each sample and C:N, were obtained using a EuroVector EuroEA3028-HT elemental analyzer coupled to a GV Instruments IsoPrime continuous-flow isotope ratio mass spectrometer. The standard error associated with these measurements is 0.2‰ for $\delta^{15}\text{N}$ and 0.1‰ for $\delta^{13}\text{C}$.

4.4. Summary

This section discussed the archaeological site of Helike, the human and fauna samples used in this study, and the various methods available for preparing bone collagen for stable isotope analysis. Helike is located in a seismically active region along the Gulf of Corinth, which has potential implications for the landscape during the Hellenistic, Roman, and Byzantine periods. Fluctuating sea levels and land subsidence appear to have been factors that resulted in the formation of both long-standing and short-term lagoons in the area. The human and samples from Helike were described in terms of their age and sex categories and their associated time periods. The Helike faunal samples and the Late Roman Corinth faunal samples were described in terms of the genera represented, the maturity of the animal, and the element sampled. Commonly used methods of collagen extraction and assessment of preservation provided a background to the method and indicators used in this study.

Chapter 5: Results and Assessment of Sample Preservation

In this chapter, the $\delta^{13}\text{C}$ values, $\delta^{15}\text{N}$ values, and the collagen preservation indicators of the Helike human and the Helike and Corinth faunal samples are presented. The samples are all described in terms of their collagen yields, C:N, %C, and %N. Those samples that failed to meet acceptable quality indicator values as indicated in Chapter Four were rejected from the final interpretation. All samples included in the final data set were further investigated to identify any strong correlations between the quality indicators.

5.1. Bone Collagen Preservation

Helike Human and Faunal Samples

The results of the bone collagen preservation assessments are presented in Table 5.1. Preservation ranged from very poor to good, with eight of the faunal samples (HF-01, HF-02, HF-06, HF-07, HF-08, HF-09, HF-11, and HF-12) reported to be non-conforming by the biogeochemical analysis lab. These samples contain carbon and nitrogen in amounts that were too low for the mass spectrometer to analyze accurately, which suggests that they had degraded to the point where the substance undergoing analysis was not collagen. The majority of these non-conforming samples had yields greater than 2% but failed to meet the criteria for inclusion based upon the other quality indicators. The results from these rejected faunal samples are described in the text below but are excluded from the figures.

Table 5.1 - Helike Human and Faunal Collagen Data

Sample	Collagen Data					
	Yield (%)	$\delta^{13}\text{C}$ (‰PDB)	$\delta^{15}\text{N}$ (‰AIR)	C:N (atomic)	%C	%N
<i>Helike Human</i>						
H25.1	13.3	-18.1	9.8	3.17	45.0	16.6
H25.2	7.5	-18.3	9.5	3.20	41.6	15.2
H25.3	9.7	-16.9	9.0	3.21	44.6	16.2
H25.4	11.0	-18.5	8.8	3.19	45.0	16.4
H25.6	6.6	-16.9	9.1	3.23	44.2	15.9
H33.1	14.0	-17.5	9.5	3.18	43.0	15.8
H5.1	7.5	-19.4	8.4	3.27	45.6	16.3
H5.2	3.9	-19.8	8.4	3.25	42.9	15.4
H5.3	16.9	-19.4	8.8	3.20	45.2	16.5
H5.4	8.6	-17.1	8.9	3.24	44.7	16.1
H5.5	12.1	-19.3	9.0	3.21	44.5	16.2
H58.2	11.5	-19.4	7.1	3.19	44.5	16.3
H58.4	6.9	-19.9	8.4	3.28	32.6	11.6
H58.5.A	2.9	-19.4	8.8	3.21	36.1	13.1
H58.5.B	5.4	-19.5	8.5	3.20	49.2	17.9
H58.5.C	6.5	-19.3	8.9	3.22	38.6	14.0
H58.5.1	2.0	-19.8	8.0	3.63	19.0	6.1
H58.6	1.2	-19.7	8.5	3.63	22.2	7.1
H58.7	1.4	-19.9	7.4	3.45	22.9	7.7
H4.1.1	9.2	-17.5	9.1	3.34	31.8	11.1
H4.1.2	5.8	-19.6	9.8	3.34	36.1	12.6
H4.1.3	8.7	-19.1	10.2	3.27	41.0	14.6
H4.1.4	6.2	-19.1	10.0	3.35	32.9	11.4
H4.2	10.9	-17.8	9.0	3.21	45.6	16.6
H4.3.1	8.5	-19.2	8.5	3.23	44.8	16.2
H4.3.2	7.6	-18.3	8.5	3.21	45.5	16.5
T13.1	18.1	-19.2	8.8	3.19	44.8	16.4
T27.1	1.6	-21.4	10.1	4.22	28.3	7.8
<i>Helike Faunal</i>						
HF-01	1.3	-22.8	5.3	5.53	8.1	1.7
HF-02	1.5	-22.8	4.4	6.00	8.4	1.6
HF-03	3.5	-20.5	7.0	3.30	29.5	10.4
HF-04	4.4	-21.2	3.9	3.24	28.7	10.3
HF-05	3.3	-21.8	5.1	3.85	9.0	2.7
HF-06	2.3	-25.8	3.3	14.64	1.1	0.1
HF-07	4.6	-23.9	6.2	5.67	7.2	1.5
HF-08	2.3	-25.4	7.4	10.50	1.3	0.1
HF-09	1.6	-24.9	11.8	11.30	2.5	0.3
HF-10	8.7	-21.0	4.4	3.31	26.1	9.2
HF-11	2.2	-24.6	6.9	15.38	2.1	0.2
HF-12	2.6	-24.1	6.4	6.20	3.8	0.7
HF-13	7.2	-20.4	3.8	3.23	34.8	12.6
HF-14	4.0	-21.3	4.8	3.34	25.0	8.8

The quality indicators of collagen yield, C:N, %C, and %N were considered for each sample with the goal of identifying samples that were out of the ranges recommended by Ambrose (1990) and van Klinken (1999). A few samples had %C or %N values that were just slightly out of range; inclusion or exclusion of these was based upon individual consideration of their other quality indicators.

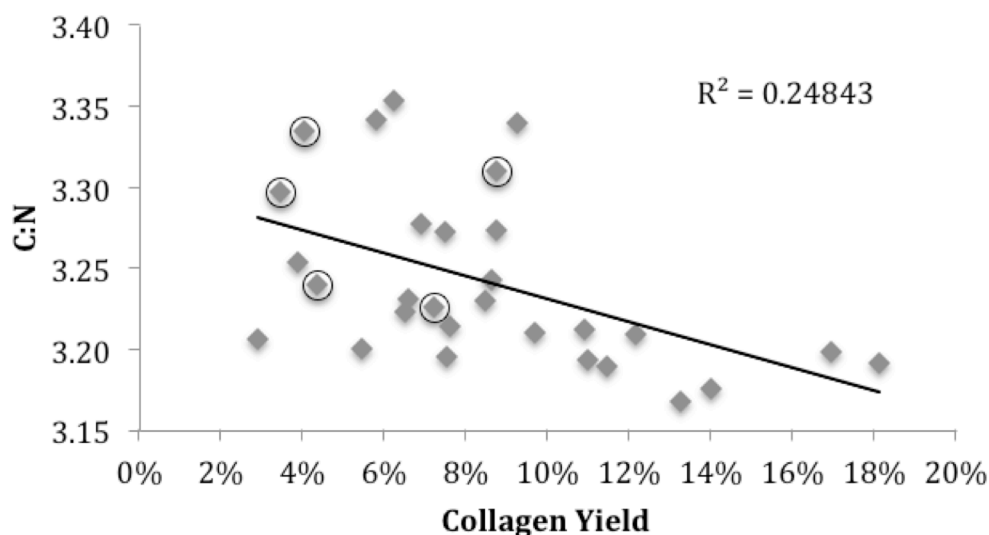
Collagen yields ranged from 1.2% to 18.2% with a mean of 6.5%. Six samples with yields of less than 2% were considered to be low yield; these samples were also out of range for the other quality indicators and were rejected.

The C:N for the Helike samples ranged from 3.1 to 15.2 with a mean of 4.0. Four of the highest ratios were found in non-conforming faunal samples. When the samples with out of range ratios are removed, the C:N ranges from 3.1 to 3.6 with a mean of 3.2. In total, 12 samples were rejected for having a C:N greater than 3.6, with nine of these samples being faunal specimens.

After rejecting the samples with unacceptable quality indicators, the remaining sample set was tested to determine if there was a relationship between collagen yield and C:N. As is apparent in Figure 5.1, an increase in collagen yield is found with a decrease in C:N. This finding is consistent with the relationship that van Klinken (1999) observed between poorly preserved collagen, lower yield, and C:N. As collagen degrades and the yield decreases, the C:N starts to move away from its original, *in vivo* value. The variability in C:N observed in the Helike samples is most apparent between the 3.5% and 8.7% yield amounts but as all of these samples fall within the range for modern collagen of 3.1 to 3.4, they are included in this study.

The effect of the faunal sample values on the overall regressions is also apparent when looking at the following tables. A circle around the corresponding plot marker identifies the faunal values.

**Figure 5.1- C:N versus Collagen Yield,
Helike Final Data Set**

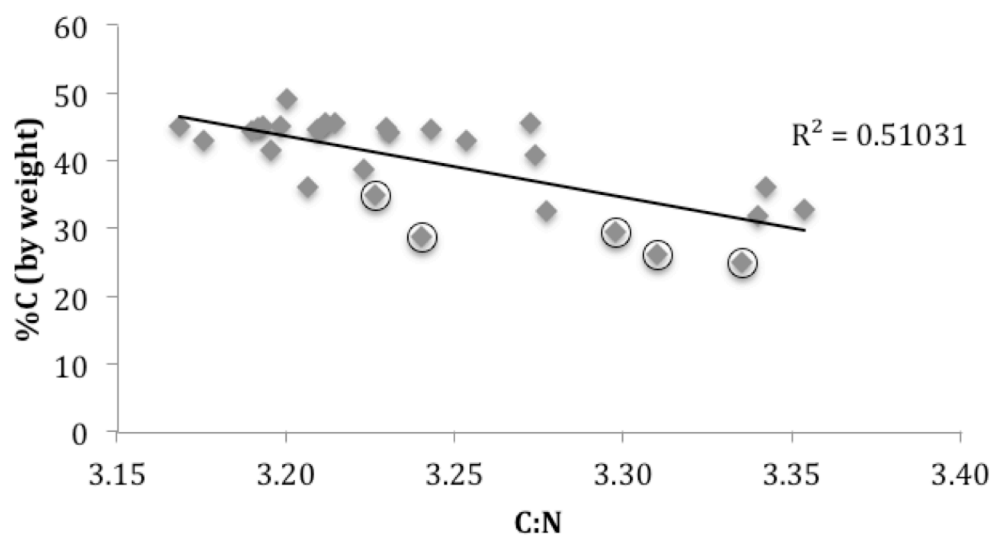


%C and %N show a wide range of values, with the most extreme values found in the faunal sample data. %C ranges from 1.1% to 49.2% with a mean of 35.3%. Samples with values outside of van Klinken's (1999) and Ambrose's (1990) recommendations were rejected, as were some samples with borderline values, in conjunction with other poor quality indicator values.

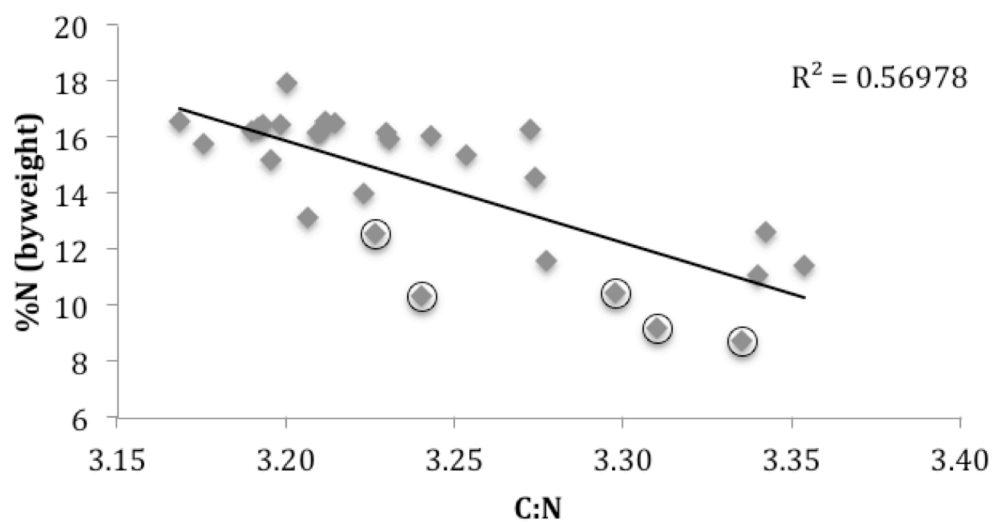
%N ranges from 0.1% to 17.9% with a mean of 10.8%. Samples with values less than 11% but above 5% were considered suspect unless their other quality indicators were acceptable. A sample with a slightly high %N value of 17.9% was also kept as its other indicators were satisfactory. As with %C, the majority of samples with unacceptable %N also had other unacceptable quality indicator values; these were rejected.

There is a relationship between collagen C:N as compared to %C and %N (Figures 5.2 and 5.3). With the Helike samples, as %C and %N decrease, C:N increases slightly. This suggests that the amounts of carbon and nitrogen in the lower yield samples were affected by collagen degradation and the

**Figure 5.2 - Collagen %C versus C:N,
Helike Final Data Set**



**Figure 5.3 - Collagen %N versus C:N,
Helike Final Data Set**



resulting loss of the carbon and nitrogen from the samples. There are no unusual departures from this trend that could indicate that the C:N values are significantly affected by contamination from the burial environment.

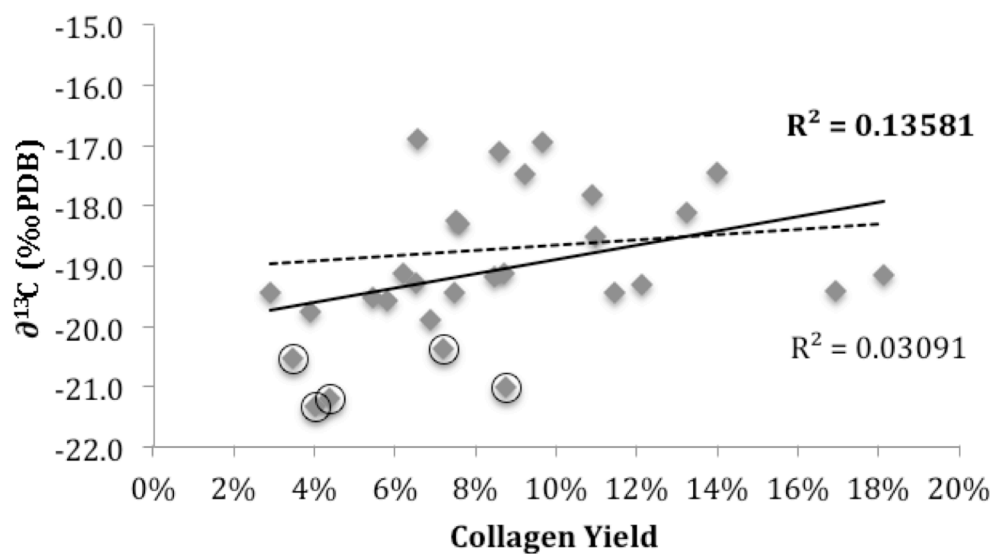
All of the thirteen samples rejected from this study had C:N, %C, and %N values outside of the recommended values (see Table 5.2), indicating that these quality indicators were able to detect the worst-preserved samples and that unacceptable values in one indicator were reflected in unacceptable values of the other indicators. Some samples that were borderline, with %C or %N slightly above or below the recommended amounts (HF-03, HF-04, HF-10, HF-11, HF-13, HF-14), were included in the final data set because they also had good C:N and acceptable yields. These borderline samples were all faunal bones from the Hellenistic refuse pit at Helike, and likely were buried in an environment that was more challenging to collagen preservation than the burial environments for the Helike humans.

Table 5.2 – Helike Human and Faunal Samples Rejected from Study

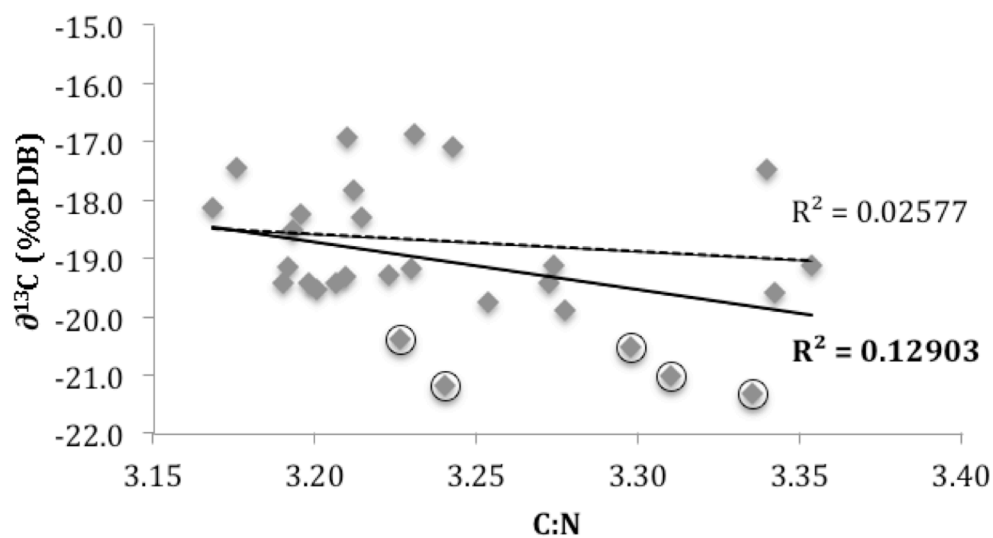
Sample	Collagen Data				<i>Rationale</i>
	<i>Yield %</i>	<i>C:N</i>	<i>%C</i>	<i>%N</i>	
H58.5.1	2.0	3.63	19.0	6.1	C:N, %C, %N
H58.6	1.2	3.63	22.2	7.1	Yield, C:N, %C, %N
H58.7	1.4	3.45	22.9	7.7	Yield, C:N, %C, %N
T27.1	1.6	4.22	28.3	7.8	Yield, C:N, %C, %N
HF-01	1.3	5.53	8.1	1.7	Yield, C:N, %C, %N
HF-02	1.5	6.00	8.4	1.6	Yield, C:N, %C, %N
HF-05	3.3	3.85	9.0	2.7	C:N, %C, %N
HF-06	2.3	14.64	1.1	0.1	C:N, %C, %N
HF-07	4.6	5.67	7.2	1.5	C:N, %C, %N
HF-08	2.3	10.50	1.3	0.1	C:N, %C, %N
HF-09	1.6	11.30	2.5	0.3	Yield, C:N, %C, %N
HF-11	2.2	15.38	2.1	0.2	C:N, %C, %N
HF-12	2.6	6.20	3.8	0.7	C:N, %C, %N

There does not appear to be a strong relationship between $\delta^{13}\text{C}$ and yield (Figure 5.4). There is also not a strong relationship between $\delta^{13}\text{C}$ and C:N (Figure 5.5), $\delta^{13}\text{C}$ to %C (Figure 5.6), $\delta^{15}\text{N}$ and C:N (Figure 5.7), $\delta^{15}\text{N}$ and yield (Figure 5.8), and $\delta^{15}\text{N}$ to %N (Figure 5.9). Again, the effect of the Helike faunal values on the regression relationships is apparent in Figures 5.3 through 5.9. Two regression lines were plotted, one representing all the data (the solid line with bolded R^2 value) and one representing only the human data (the dotted line with unbolded R^2 value). The Helike faunal samples are easy to spot on the various plots relating to $\delta^{15}\text{N}$ and %N, as the values associated with these fall markedly below the all data trend line. This pattern is likely related to the systematically poorer collagen preservation of these samples coupled with their lower, typically herbivorous $\delta^{15}\text{N}$ values as compared to the human samples. This creates the impression of $\delta^{15}\text{N}$ correlation with preservation indicators in the overall sample set. When the humans are considered alone, though, it becomes clear that no real relationship exists. The lack of correlation between the quality indicator values and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ indicates that the preservation state of the collagen is not creating values with systematic stable isotope offsets.

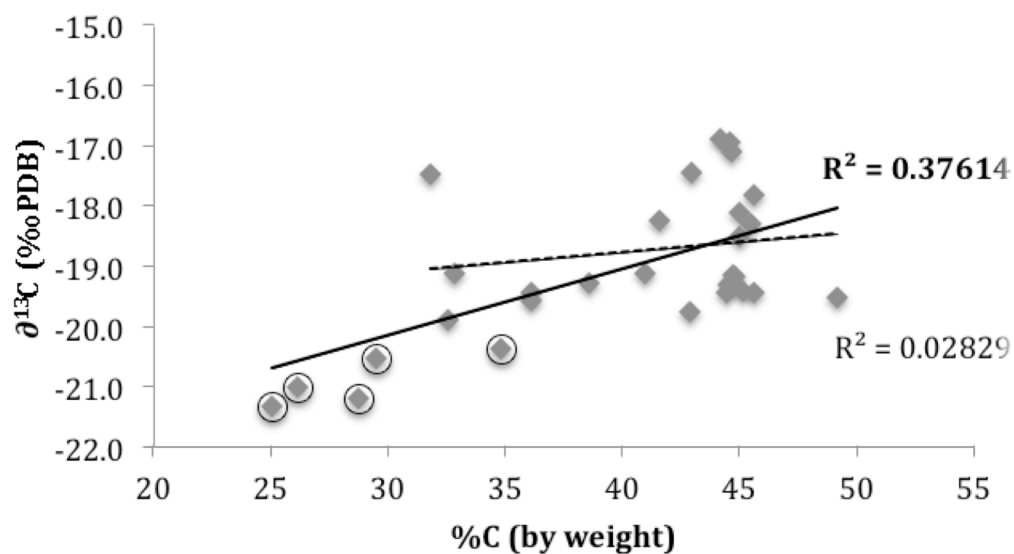
**Figure 5.4 - $\delta^{13}\text{C}$ versus Yield,
Helike Final Data Set**



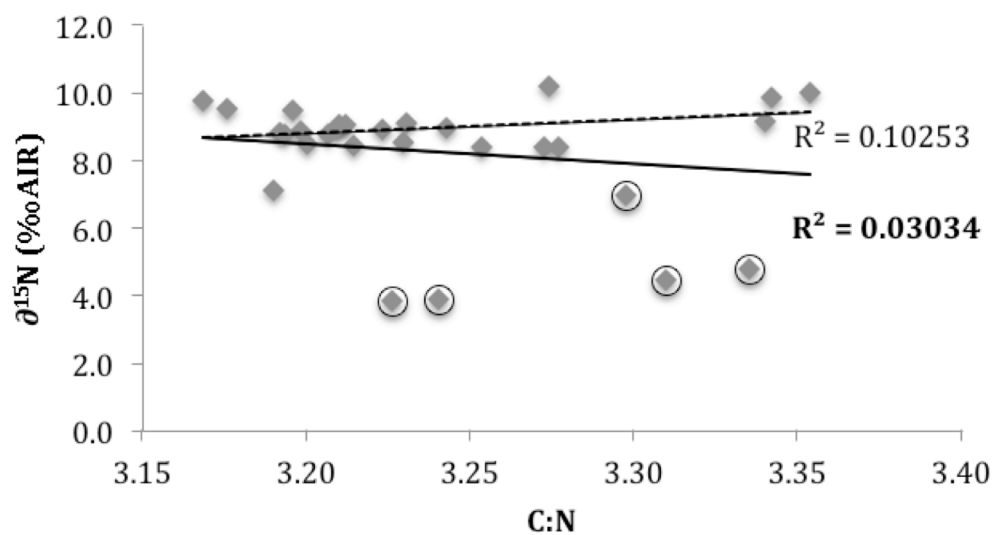
**Figure 5.5 - $\delta^{13}\text{C}$ versus C:N,
Helike Final Data Set**



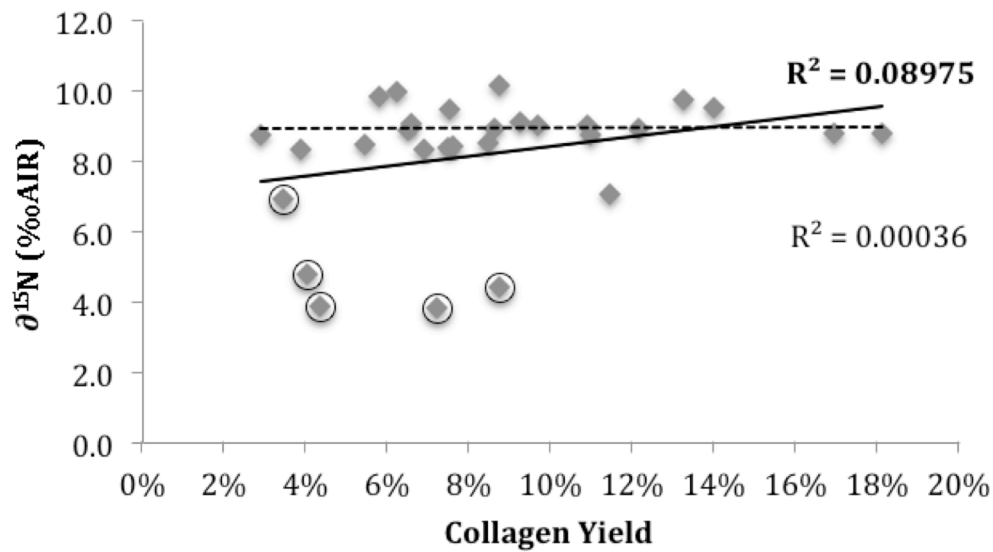
**Figure 5.6 - $\delta^{13}\text{C}$ versus %C,
Helike Final Data Set**



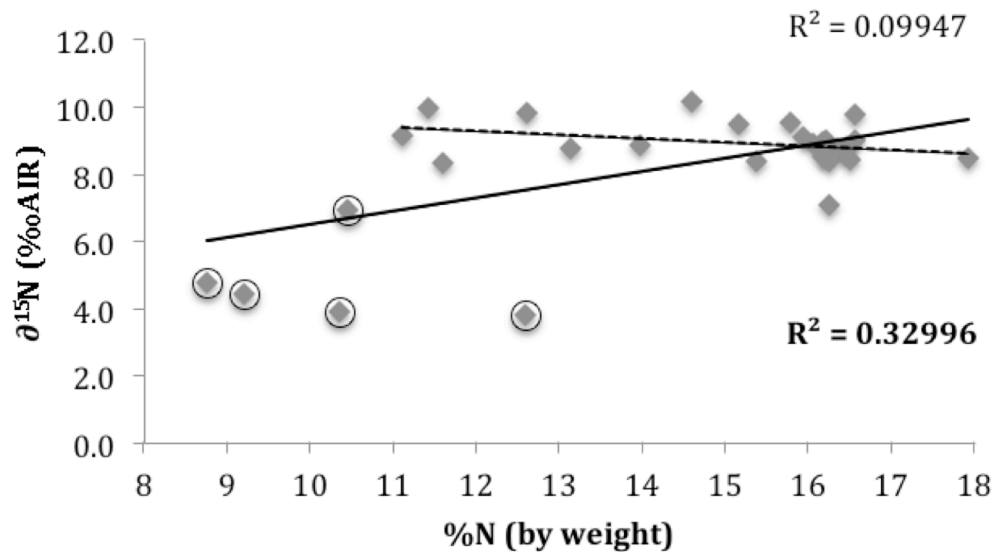
**Figure 5.7 - $\delta^{15}\text{N}$ versus C:N,
Helike Final Data Set**



**Figure 5.8 - $\delta^{15}\text{N}$ versus Yield,
Helike Final Data Set**



**Figure 5.9 - $\delta^{15}\text{N}$ versus %N,
Helike Final Data Set**



Late Roman Corinth Faunal Samples

The Corinth faunal samples had collagen yields ranging from 2.4% to 16.9% with a mean of 6.6%. Three samples produced yields of less than 2% (CRF-06, CRF-14, and CRF-15) and were excluded from the final data set (Table 5.3). With all samples, including CRF-06, CRF-14, and CRF-15, the C:N ranged from 3.10 to 3.24 with a mean of 3.17, indicating that all the faunal samples were within an acceptable range for this indicator (DeNiro, 1985). Figure 5.4 shows the relationship between yield and C:N for the Corinth materials.

Table 5.3 – Corinth Faunal Samples Rejected from Study

Sample	Collagen Data				<i>Rationale</i>
	<i>Yield %</i>	<i>C:N</i>	<i>%C</i>	<i>%N</i>	
CRF-06	1.3	3.18	44.5	16.3	Yield
CRF-14	1.3	3.23	44.1	15.9	Yield
CRF-15	0.5	3.24	29.1	10.5	Yield, %C, %N

%C and %N were within acceptable ranges for all but one faunal sample, CRF-15, and for 5 samples with %N values of 17.1% or 17.2%. Given the low yield of 0.5% associated with CRF-15, it had already been excluded on this basis. The samples with slightly high %N were included as their other collagen preservation indicators were good.

In total, 17 faunal samples from Late Roman Corinth were accepted for inclusion and their collagen data are listed in Table 5.4. As with the Helike samples, further tests were done to determine the relationships between each of the collagen preservation variables and between these variables and the stable isotopic values of the samples.

Table 5.4 – Corinth Faunal Collagen Data

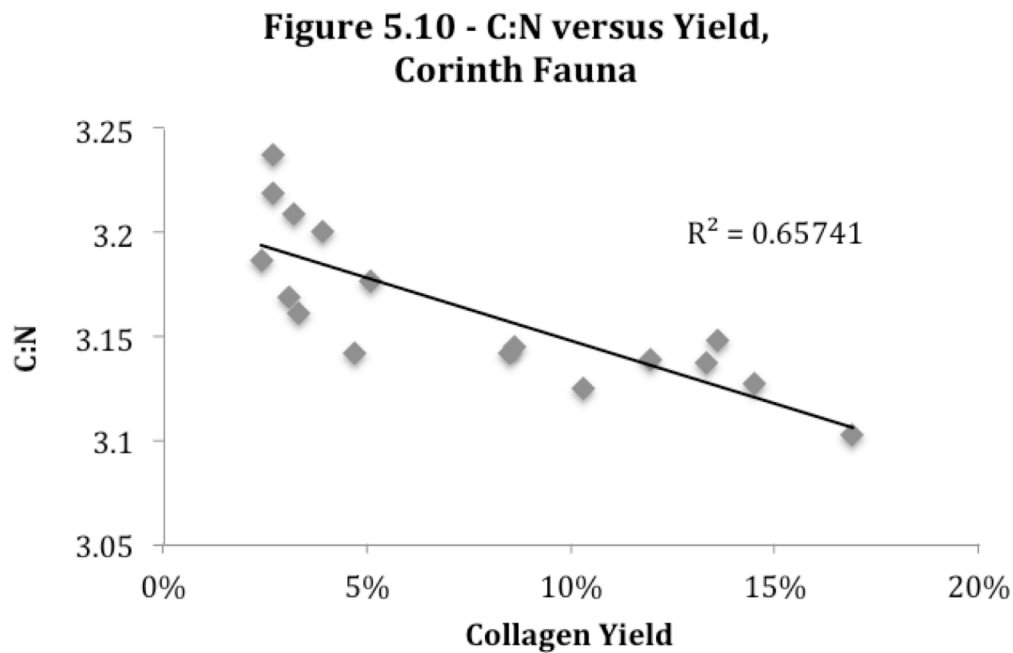
Sample	Collagen Data					
	Yield (%)	$\delta^{13}\text{C}$ (‰PDB)	$\delta^{15}\text{N}$ (‰AIR)	C:N (atomic)	%C	%N
CRF-01	2.4	-19.9	3.0	3.19	45.3	16.6
CRF-02	8.6	-19.6	3.1	3.15	45.7	16.9
CRF-03	2.7	-20.0	3.1	3.22	45.3	16.4
CRF-04	3.2	-20.2	3.8	3.21	45.8	16.6
CRF-05	3.1	-20.3	4.6	3.17	46.2	17.0
CRF-06	1.3	-18.9	8.4	3.18	44.5	16.3
CRF-07	5.1	-19.2	8.6	3.18	46.4	17.0
CRF-08	8.5	-19.5	10.3	3.14	45.6	16.9
CRF-09	3.9	-19.6	8.8	3.20	45.6	16.6
CRF-10	3.3	-18.9	8.4	3.16	45.6	16.8
CRF-11	14.5	-21.6	4.6	3.13	45.7	17.1
CRF-12	12.0	-21.6	6.1	3.14	46.1	17.1
CRF-13	13.6	-20.7	0.8	3.15	46.0	17.0
CRF-14	1.3	-21.4	5.6	3.23	44.1	15.9
CRF-15	0.5	-20.9	7.7	3.24	29.1	10.5
CRF-16	4.7	-21.4	3.2	3.14	45.5	16.9
CRF-17	10.3	-21.5	3.6	3.12	45.8	17.1
CRF-18	16.9	-21.4	4.1	3.10	45.9	17.2
CRF-19	2.7	-21.5	2.7	3.24	45.6	16.4
CRF-20	13.3	-17.9	5.3	3.14	46.3	17.2

The Corinth faunal samples show a decrease in C:N related to an increase in collagen yield (Figure 5.10), similar to the Helike samples. This relationship is not strong and is expected with the varying yields that were obtained. As the C:N remains within 3.1 and 3.2, indicating quite good preservation, all samples were kept and included in interpretations.

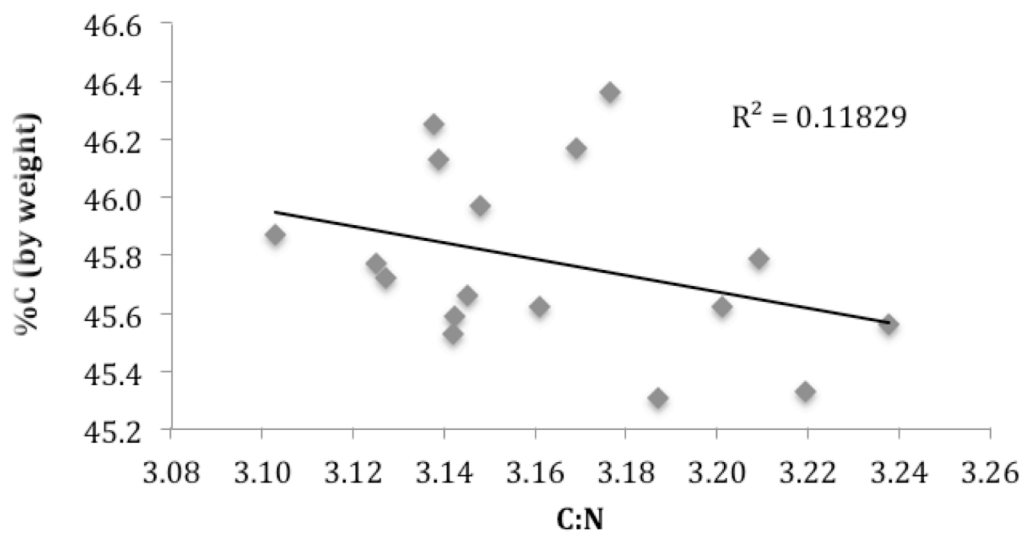
There is no strong relationship apparent between %C and C:N (Figure 5.11), $\delta^{13}\text{C}$ and yield (5.13), $\delta^{13}\text{C}$ and C:N (Figure 5.14), $\delta^{13}\text{C}$ and %C (Figure 5.15), $\delta^{15}\text{N}$ and C:N (Figure 5.16), $\delta^{15}\text{N}$ and yield (Figure 5.17), or $\delta^{15}\text{N}$ and %N (Figure 5.18).

There is a strong correlation between %N and C:N evident (Figure 5.12), with a $R^2 = 0.83237$, showing that as %N decreases, the C:N also decreases. The sample with the highest %N and corresponding C:N also yielded 13.3% collagen, while the sample with the lowest %N and C:N yielded 2.7%. This indicates that there is a relationship between the C:N and

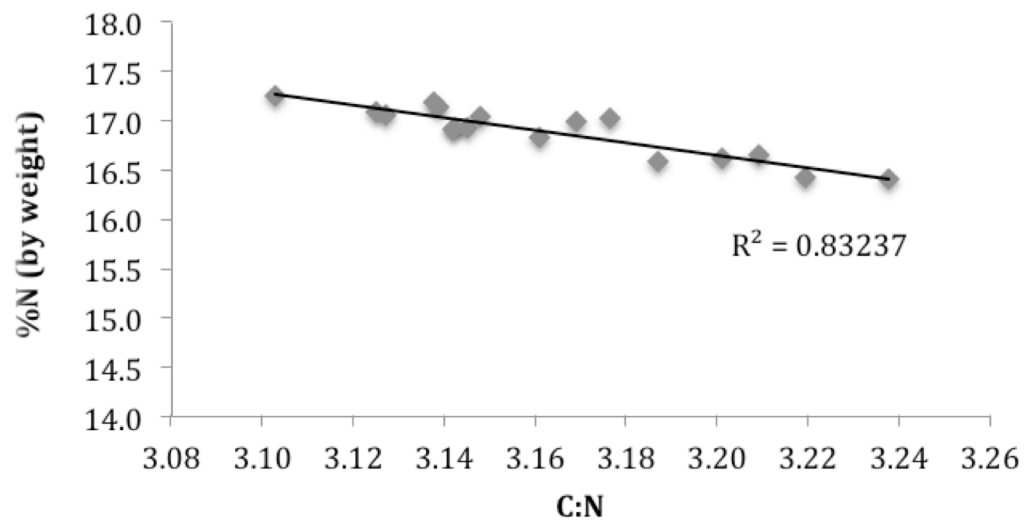
%N related to yield, with sample nitrogen content decreasing as yield falls. The weak correlation of the $\delta^{15}\text{N}$ values with %N (Figure 5.18) shows that the stable isotope values of the samples are not systematically offset because of this relationship. The Corinth faunal samples included are all of quite good preservation and collagen quality as compared to the Helike faunal samples and all the indicator values for this data set are within acceptable ranges.



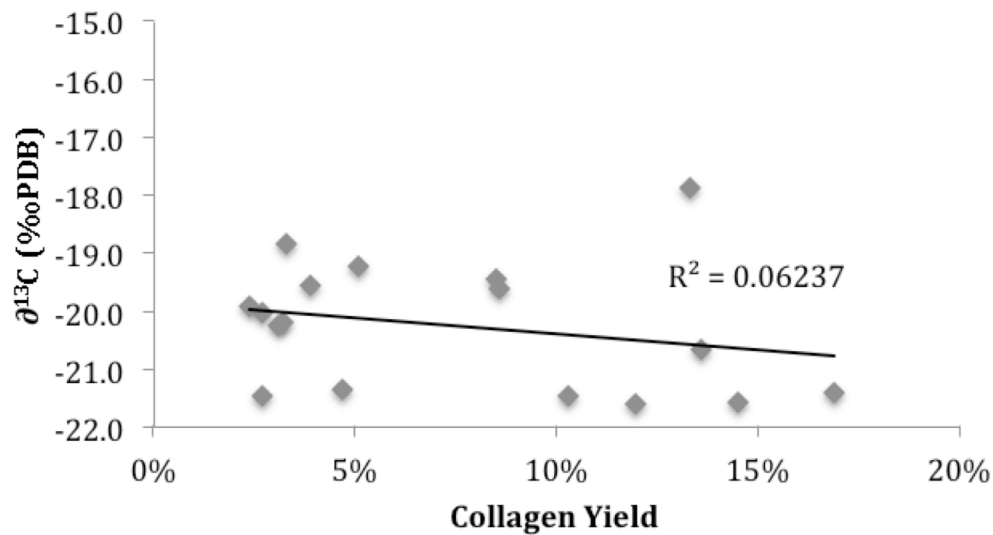
**Figure 5.11 - Collagen %C versus C:N,
Corinth Fauna**



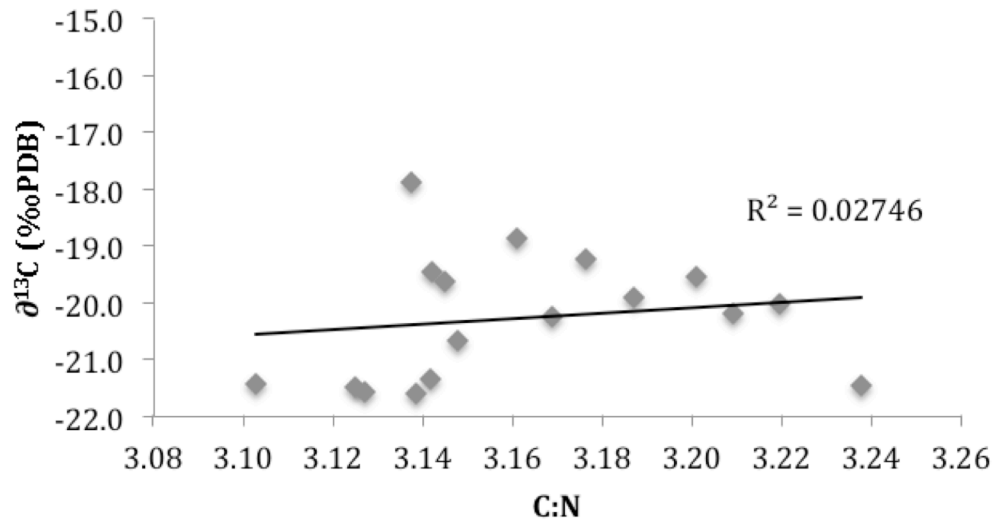
**Figure 5.12 - Collagen %N versus C:N,
Corinth Fauna**



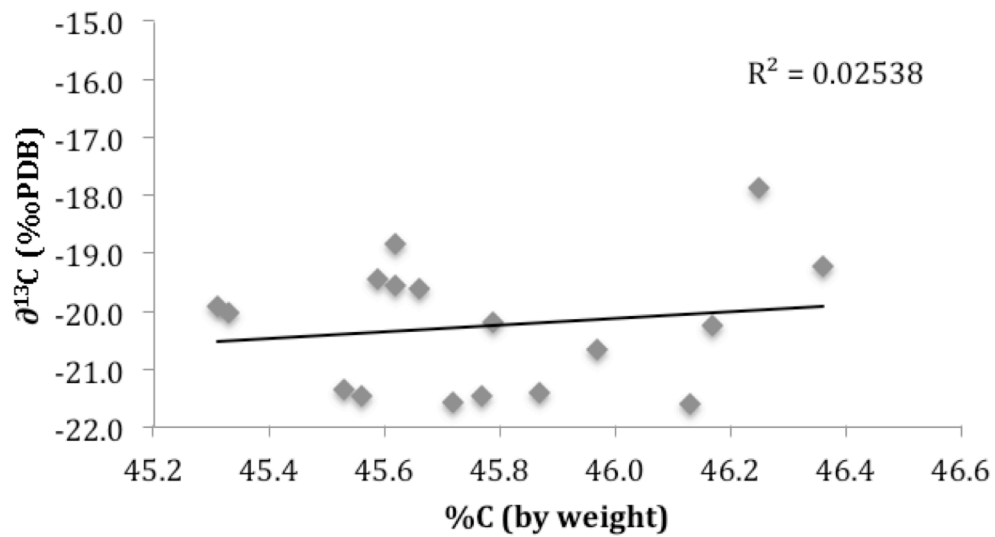
**Figure 5.13 - $\delta^{13}\text{C}$ versus Yield,
Corinth Fauna**



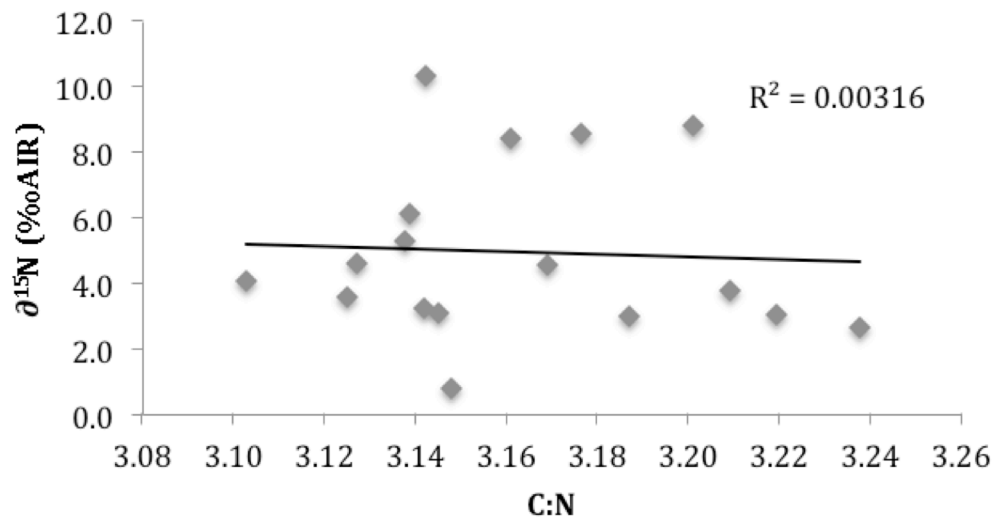
**Figure 5.14 - $\delta^{13}\text{C}$ versus C:N,
Corinth Fauna**



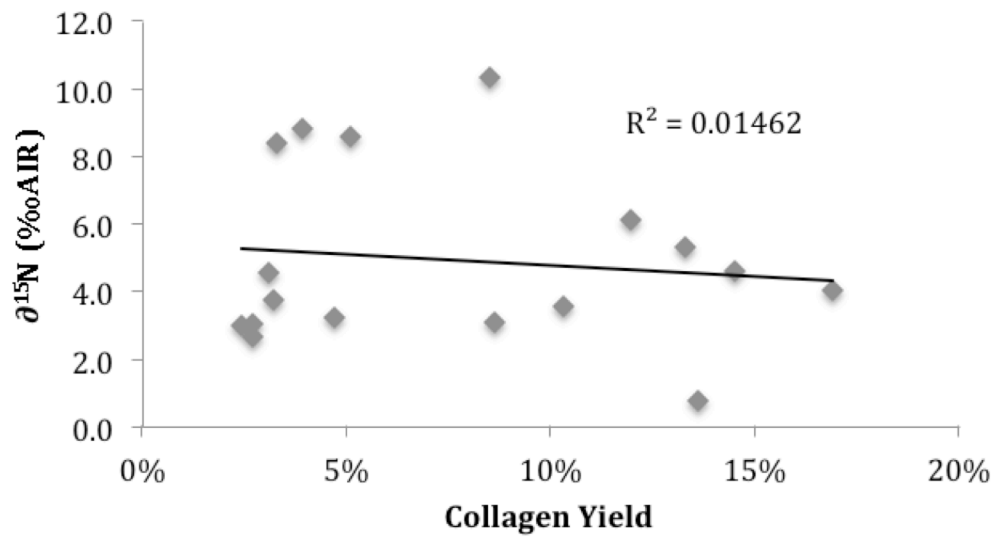
**Figure 5.15 - $\delta^{13}\text{C}$ versus %C,
Corinth Fauna**



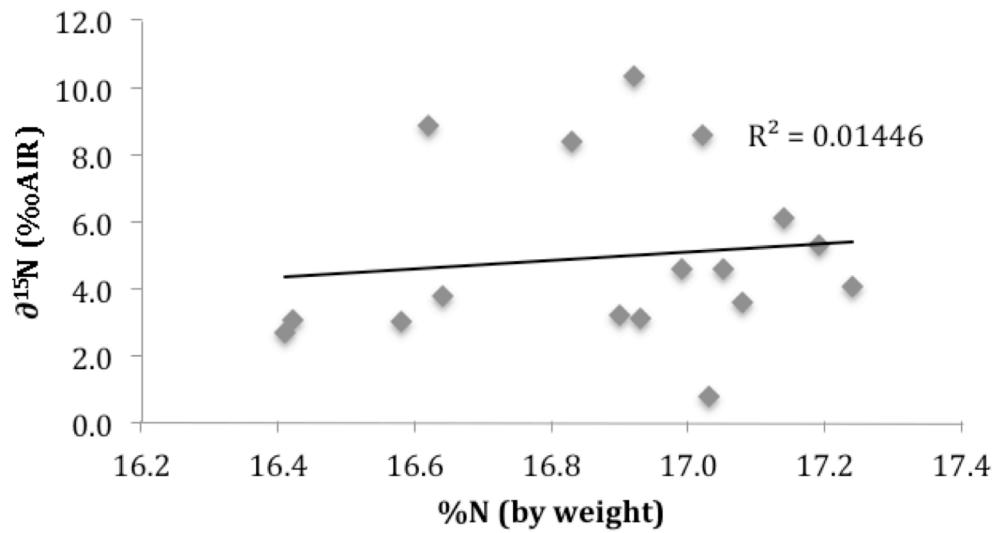
**Figure 5.16 - $\delta^{15}\text{N}$ versus C:N,
Corinth Fauna**



**Figure 5.17 - $\delta^{15}\text{N}$ versus Yield,
Corinth Fauna**



**Figure 5.18 - $\delta^{15}\text{N}$ versus %N,
Corinth Fauna**



5.2. Summary

Using the collagen quality indicators described in chapter 4, the bone collagen from the Helike human and faunal samples was assessed for contamination and degradation. Twenty-four of the 28 human samples were within acceptable ranges for yield, C:N, %C, and %N, while six of the 13 faunal samples were accepted based upon slightly low %C or %N in conjunction with acceptable C:N and yield. In order to provide additional data with which to construct a local isotopic food web, faunal samples from Late Roman Corinth were considered. The majority of these 20 additional samples met collagen preservation and quality indicator guidelines and were included for comparison.

The final data set for this study consists of 24 human samples from Helike, five faunal samples from Helike, and 17 faunal samples from Late Roman Corinth.

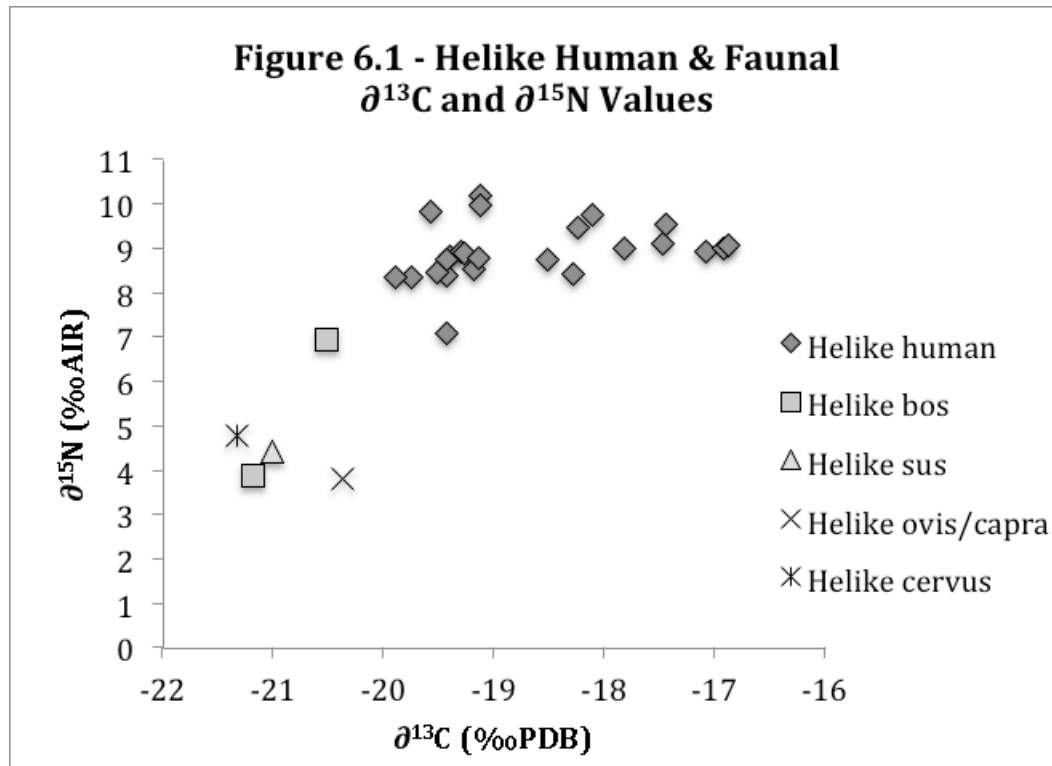
Chapter 6: Interpretation

This chapter discusses the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the Helike human, Helike faunal, and the Corinth faunal bone collagen samples. For the human values, within-sample patterning is described with specific attention paid to the age and sex categories outlined in section 4.2.1. The effect of time period on the results is also examined and some possible reasons for temporal variation are discussed. In order to best interpret the Helike human values, it is necessary to compare them to both reconstructed food resource values (Bourbou et al., 2011) and to the results of the stable isotope studies reviewed in Chapter Two. These comparisons allows the human values to be understood within a broader context and provide an additional frame of reference for reconstructing past diet at Helike. Finally, the original research questions of this thesis are addressed.

6.1. Helike Human Values Compared to the Helike Faunal Values

Figure 6.1 shows the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the Helike human samples in relation to the Helike faunal values. A clear trophic level effect of the humans above the faunal values is apparent. The two *Bos* samples, HF-03 and HF-04, both have values consistent with an herbivorous diet of C_3 plants (mean $\delta^{13}\text{C} = -20.9\text{‰}$). The mean $\delta^{15}\text{N}$ value is 5.4‰ , although HF-03 is approximately 3‰ higher than HF-04. Even though both samples were estimated to have been from skeletally mature animals, there is the possibility that HF-03 was young enough to have retained an isotopic signature reflecting a juvenile diet that included milk. The size of the element sampled from HF-03 would suggest that it was mature at time of death, but bone collagen turnover may not have completely erased the juvenile dietary signature from its bone tissue, as this can take several years to occur. Another possible reason for the $\delta^{15}\text{N}$ difference between the two *Bos* samples is the consumption of plants that had been fertilized by manure. As mentioned previously, manuring would increase the $\delta^{15}\text{N}$ value of cereal

plants and in turn, result in a higher stable nitrogen isotope value of the consumer of these plants.



The Helike *Sus* (HF-10) stable carbon isotope value is also consistent with an animal consuming a predominantly C_3 diet ($\delta^{13}\text{C} = -21.0\text{‰}$). Its $\delta^{15}\text{N}$ value of 4.4‰ falls within the range of the *Bos*, the *Ovis/Capra*, and the *Cervus* values, suggesting that, like these herbivores, this particular pig was being foddered on plants and not animal products.

The ovicaprine shows values consistent with a C_3 diet, with no apparent addition of C_4 plants ($\delta^{13}\text{C} = -20.4\text{‰}$, $\delta^{15}\text{N} = 3.8\text{‰}$). The *Cervus* also shows values typically associated with an herbivorous browser of C_3 plants with a $\delta^{13}\text{C}$ of -21.3‰ and $\delta^{15}\text{N}$ of 4.8‰ . As most of the Helike faunal samples are the single representative of their genus, the addition of more faunal data is needed to fully understand the relationships between the faunal and human values. This is partly addressed with the use of the Corinth faunal data described below in section 6.1.1.

Following the domesticate patterning, the Helike human values are mostly consistent with a predominantly C₃ terrestrial diet ($\delta^{13}\text{C} = -19.8\text{‰}$ to -16.1‰ and $\delta^{15}\text{N} = 7.1\text{‰}$ to 10.2‰). They are a full trophic level above the fauna, which is consistent with the consumption of cattle, pig, sheep or goat, or deer. Frequent consumption of animal meat is considered to have been uncommon throughout the Hellenistic, Roman, and Byzantine periods in general, but opinions on this have varied in the literature. As C₃ cereals were probably the main crops cultivated at Helike, animals such as cattle would have been more useful as draught animals and dairy sources than for their meat. It is more likely that consumption of secondary animal products, such as dairy, account for these higher $\delta^{15}\text{N}$ values.

Overall, the Helike human values are fairly homogeneous in terms of stable nitrogen isotope values ($8.9\text{‰} \pm 0.66$). The $\delta^{13}\text{C}$ values, however, are more variable ($-18.7\text{‰} \pm 0.96$). As depicted in Figure 6.1, the data points show a clear horizontal spread. Some possible reasons for this patterning are discussed further below in section 6.3.

6.1.1. Helike Human Values Compared to Both Corinth and Helike Fauna

When the Corinth faunal values are considered, the context for the Helike faunal values becomes clearer (Figure 6.2).

The Corinth *Bos* samples (n=4) show $\delta^{13}\text{C}$ values ranging from -17.9‰ to -21.5‰ with a mean of -20.6‰ . Like the Helike *Bos*, these values are generally consistent with C₃ terrestrial herbivore values. However, sample CRF-20 has a $\delta^{13}\text{C}$ value of -17.9‰ , suggesting that this animal was consuming a small amount of C₄ plants as well. It is possible that this cow was being seasonally pastured and consumed millet in addition to C₃ plant fodder. Another possibility is that this animal was not local to the area, was foddered on a different diet than the other *Bos*, and was brought to Corinth for either trade or for sacrifice.

The Corinth *Bos* $\delta^{15}\text{N}$ values range from 2.7‰ to 5.3‰ with a mean of 3.9‰ . Again, the upper stable nitrogen isotope value is from sample CRF-

20, causing it to stand apart from the others isotopically. The sample from this animal appears to be skeletally mature and although past consumption of milk was raised as a possible reason for the higher values in the Helike *Bos* sample, given the $\delta^{13}\text{C}$ value of CRF-20, it seems more reasonable to suggest that a non-local origin may be the reason for the differences in this animal.

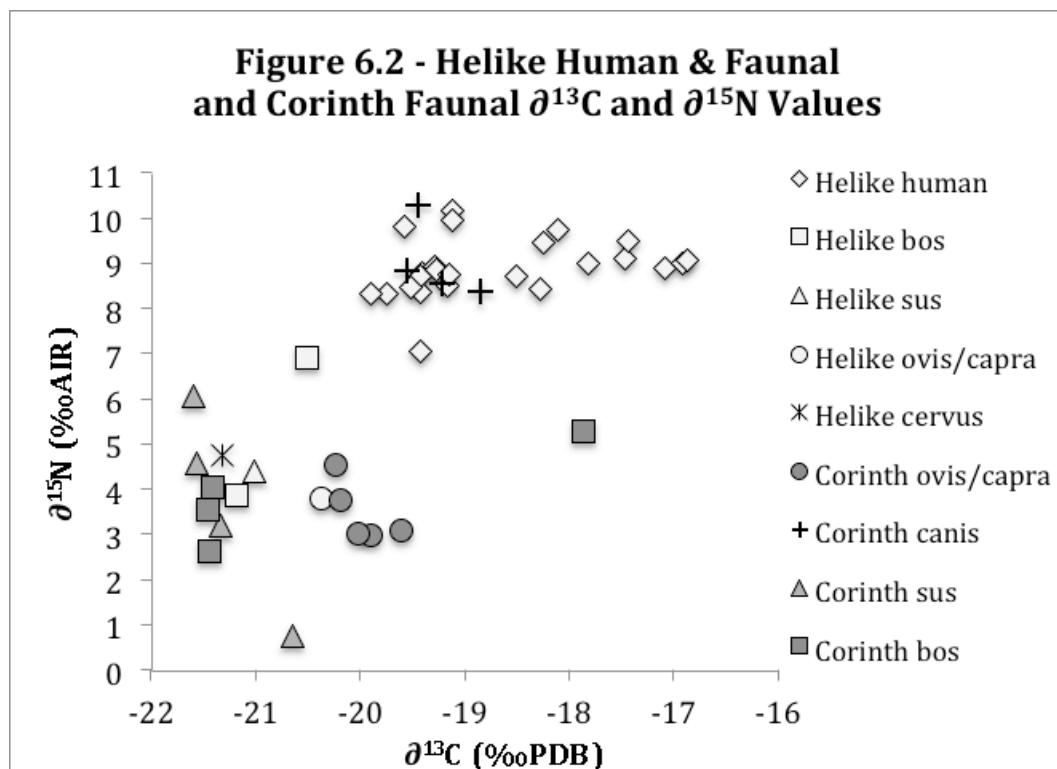
The Corinth *Sus* $\delta^{13}\text{C}$ values (n=3) range from -20.6‰ to -20.7‰ with a mean of -21.3‰. *Sus* $\delta^{15}\text{N}$ values range from 0.8‰ to 6.1‰ with a mean of 3.7‰. The wide range of stable nitrogen isotope values associated with this group of animals is not unexpected, as pigs are omnivorous and can be foddered on a wide variety of foodstuffs that may differ between households. Sample CRF-13, which has a low $\delta^{15}\text{N}$ value of 0.8‰, may be evidence of a pig that was foddered on almost an entirely leguminous diet. As legumes generally have a stable nitrogen isotope value of close to 0‰, a pig consuming a diet based upon legumes would also be expected to show a very low $\delta^{15}\text{N}$ value. A number of leguminous plants, including bitter vetch, were used as animal fodder in the ancient world (Dalby, 1996).

The Corinth *Ovis/Capra* samples (n=5) show a $\delta^{13}\text{C}$ ranging from -20.3‰ to -19.2‰ with a mean of -20.0‰. Their $\delta^{15}\text{N}$ values range from 3.0‰ to 4.6‰ with a mean of 3.5‰. The Helike *Ovis/Capra* falls within this range and all values are consistent with this species' apparent consumption of a C_3 diet.

The Corinth *Canis* samples (n=4) have stable isotope values overlapping with the Helike human values. The $\delta^{13}\text{C}$ values range from -19.6‰ to -18.9‰ with a mean of -19.3‰ and the $\delta^{15}\text{N}$ values range from 8.4‰ to 10.3‰ with a mean of 9.0‰. Similar overlaps of *Canis* and human values have been found in other studies (Losey et al., 2011; Choy and Richards, 2009) and result from the consumption of human food, either through purposeful feeding or through scavenging of leftovers and scraps. The dietary isotopic similarities found between humans and domesticated dogs are well established in the literature and have been used to reconstruct

diet in the absence of human samples by using the dogs as proxies (Guiry, 2012; Cannon et al., 1999).

Overall, the Corinth and Helike faunal samples show similar isotopic values within the species and between the two sites. For those samples that appear to be outliers, there is the possibility that animal mobility, coupled with different local feeding practices such as legume foddering, is causing these samples to stand apart from the others of their species. Even when considering these outlying values, the human values reflect trophic level enrichment above the fauna consistent with predominant consumption of C_3 plants and terrestrial animals or animal products. If marine resources had comprised a large part of the human diet, we would expect to see higher human stable carbon isotope and stable nitrogen isotope values reflective of the different marine carbon and nitrogen signatures. Similarly, if C_4 plants like millet were being consumed or provided in large amounts to domesticated animals as fodder, the human stable carbon isotope values would generally be much higher.



6.2. Human Variation by Age and Sex

Figure 6.3 shows the Helike human $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values according to age category. Individuals that were assigned to more than one category (e.g. as a “Young-Middle Adult”) were kept as separate groups. A look at Figure 6.3 shows no apparent relationship between age category and isotopic values. This is not surprising given the multiple age groups represented and the large number of adults for whom no age estimate was possible. As a result of the small category sizes, no statistical tests were performed to otherwise detect differences according to age.

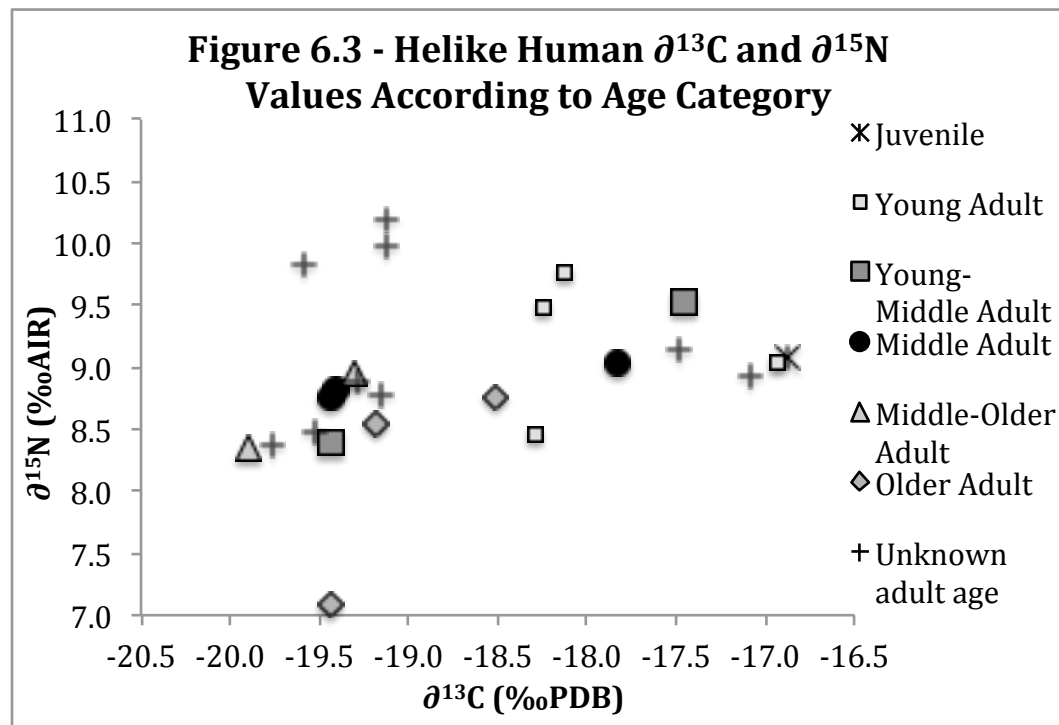
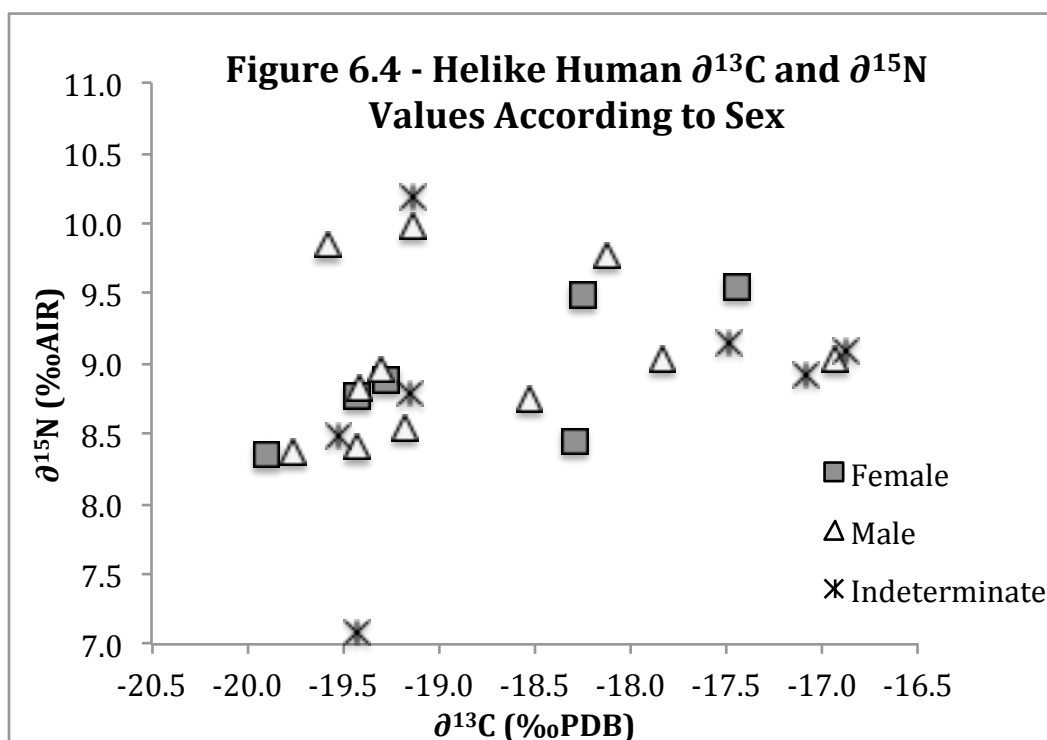


Figure 6.4 shows the Helike human collagen values according to sex. As some of the adult skeletons could not be assigned a sex category (n=7), the extent of interpretation of the male (n=11) and female (n=6) values in relation to each other is somewhat limited. The $\delta^{13}\text{C}$ for males ranges from -19.8‰ to -16.9‰ with a mean of -18.8‰, while for females, it ranges from -19.9‰ to -17.5‰ with a mean of -18.7‰. A comparison of these two

means shows that there is no significant difference in $\delta^{13}\text{C}$ values between males and females at Helike ($t = 0.1395$, $p\text{-value} = 0.89$). Statistical comparisons in this study were made using the unequal variance t -test, which is recommended when combined sample sizes are below 30 and sample variances are unequal (Ruxton, 2006).

The $\delta^{15}\text{N}$ values for males at Helike range from 8.4‰ to 10.0‰ with a mean of 9.0‰. Female $\delta^{15}\text{N}$ values range from 8.4‰ to 9.5‰ with a mean of 8.9‰. Again, a comparison of these means shows no significant difference between male and female $\delta^{15}\text{N}$ values at Helike ($t = 0.4954$, $p\text{-value} = 0.63$).

There are many references in the literature to dietary differences between males and females in ancient Greece (Garnsey, 1999; Dalby, 1996). However, isotopic similarity does not preclude sex-based food consumption differences, such as females consuming different parts of animals than males. The small sample sizes for males and females, the presence of a high proportion of indeterminate sex individuals, and the span of these samples across the various time periods may also obscure any sex differences in isotopic values. The lack of evidence for age or sex differences does facilitate the comparison of the samples according to time period, which is discussed in the next section.



6.3. Patterning Between Time Periods

As mentioned in section 6.1, there is horizontal spread of $\delta^{13}\text{C}$ values on the charts for the human collagen data. Although there are no detectable differences between age or sex categories, another variable may be associated with this data distribution. When the collagen values for each human individual are separated into their respective time periods, an interesting pattern emerges (Figure 6.5). There is a clear separation between the majority of the Roman period individuals and the Hellenistic individuals, with the Byzantine data points falling in between the two groups. Tables 6.1 and 6.2 list the descriptive statistics for each time period according to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

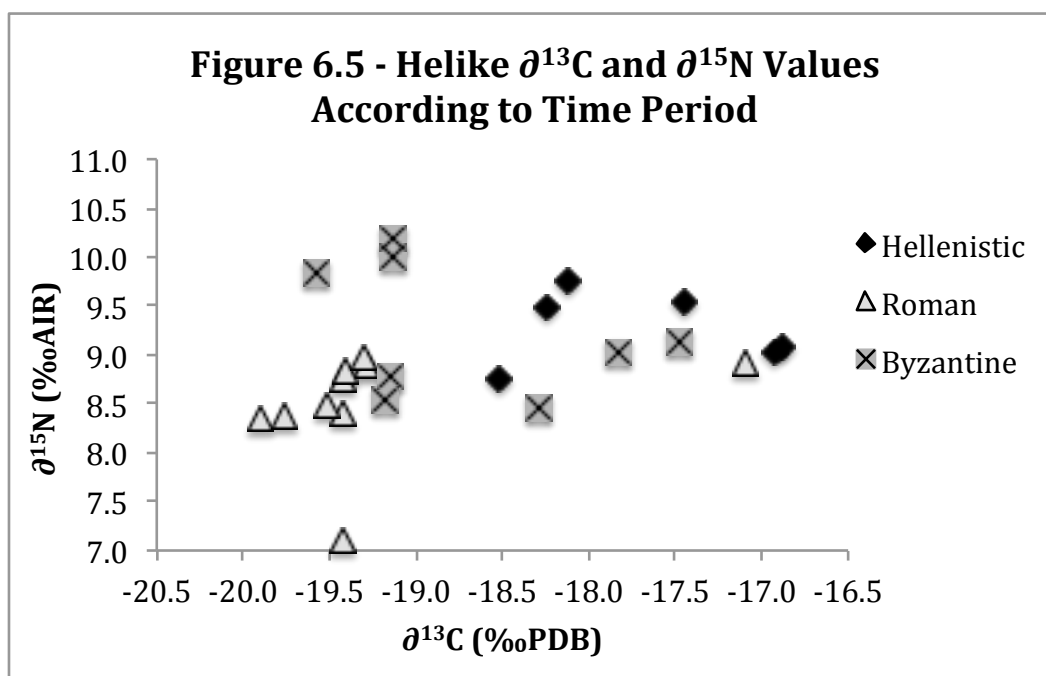


Table 6.1 – Descriptive Statistics for Each Time Period, $\delta^{13}\text{C}$

Time Period	Count	Minimum ‰	Maximum ‰	Mean ‰	Standard Deviation	Standard Error
Hellenistic	6	-18.5	-16.9	-17.7	0.704	0.287
Roman	10	-19.9	-17.1	-19.3	0.785	0.248
Byzantine	8	-19.6	-17.5	-18.7	0.755	0.267

Table 6.2 – Descriptive Statistics for Each Time Period, $\delta^{15}\text{N}$

Time Period	Count	Minimum ‰	Maximum ‰	Mean ‰	Standard Deviation	Standard Error
Hellenistic	6	8.8	9.8	9.3	0.381	0.156
Roman	10	7.1	9.0	8.5	0.553	0.175
Byzantine	8	8.5	10.2	9.2	0.676	0.234

When comparing the mean $\delta^{13}\text{C}$ of the groups (Table 6.3), there is a significant difference between the Hellenistic and Roman periods ($t = 4.116$, $p\text{-value} = 0.001$) and between the Hellenistic and Byzantine periods ($t = 2.625$, $p\text{-value} = 0.024$). There is no significant difference between the Roman and Byzantine periods ($t = 1.464$, $p\text{-value} = 0.164$).

With the mean $\delta^{15}\text{N}$ values (Table 6.4), there is a significant difference between the Hellenistic and Roman periods ($t = 3.281$, $p\text{-value} = 0.005$) and between the Roman and Byzantine periods ($t = 2.493$, $p\text{-value} = 0.026$), but no significant difference between the Hellenistic and Byzantine periods ($t = 0.107$, $p\text{-value} = 0.917$).

In general, mean $\delta^{13}\text{C}$ values in the Hellenistic period are higher than in the Roman period and Byzantine period, while the mean $\delta^{15}\text{N}$ values are roughly similar between the Hellenistic and Byzantine periods. The Roman period shows the lowest mean $\delta^{15}\text{N}$ value of all three periods. The differences between some of these mean values and time periods suggests that individuals during the Hellenistic period, although still basing much of their diet upon terrestrial C_3 resources, were also consuming significant amounts of isotopically distinct foods that were different from those used in the Roman period and possibly from the Byzantine period. Identifying the resources that might have been involved requires establishing a range of expected values for various diets specific to Greece. For this study, this is best achieved by comparing Helike to the dietary resource values established by Bourbou et al. (2011) using data gathered from a range of Byzantine Greek sites.

**Table 6.3 – Comparison of Mean $\delta^{13}\text{C}$ Values for Each Time Period:
Unequal Variance *t*-test and p-values ($\alpha = 0.05$)**

	Hellenistic	Roman	Byzantine
Hellenistic		t = 4.116 p = 0.001	t = 2.625 p = 0.024
Roman	t = 4.116 p = 0.001		t = 1.464 p = 0.164
Byzantine	t = 2.625 p = 0.024	t = 1.464 p = 0.164	

**Table 6.4 – Comparison of Mean $\delta^{15}\text{N}$ Values for Each Time Period:
Unequal Variance *t*-test and p-values ($\alpha = 0.05$)**

	Hellenistic	Roman	Byzantine
Hellenistic		t = 3.281 p = 0.005	t = 0.107 p = 0.917
Roman	t = 3.281 p = 0.005		t = 2.493 p = 0.026
Byzantine	t = 0.107 p = 0.917	t = 2.493 p = 0.026	

6.4. Comparison of Helike Collagen Values to Food Value Reconstructions

Even within a given time and culture, variation in underlying nitrogen and carbon baseline values will cause site-specific $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in resources. This variation can affect the accuracy of dietary reconstruction and could result in some dietary resources going undetected. Using a broad range of sites and samples for each reconstructed food value group reduces the likelihood of this and is more likely to accurately reflect the stable isotope values of available resources. Bourbou et al. (2011) reconstructed a set of resource values for Byzantine period Greece using multi-site faunal data and estimated plant group values. By applying stable isotopic ranges to these various categories, they created a chart where the human collagen values could be visually interpreted according to dietary resource use. Since only two archaeological fish and no archaeological plant stable isotope values were available, values for these were determined using modern Aegean fish and plant data from the general isotopic literature. The addition of the Hellenistic Helike and Late Roman Corinth faunal data from this study further refines the estimated resource values.

As shown in Figure 6.6, Bourbou et al. (2011) used reconstructed $\delta^{13}\text{C}$ values for C_3 plants that range from -24‰ to -28‰, based upon a mean of -26.5‰. Reconstructed $\delta^{15}\text{N}$ values range from 0‰ to 4‰, based around a mean of 2‰. The $\delta^{13}\text{C}$ range for C_4 plants is -15‰ to -11‰ with a mean of -13‰, while the $\delta^{15}\text{N}$ is from 0‰ to 4‰ with a mean of 2‰. Legume stable carbon isotope values were estimated to be the same as other C_3 plants, but with a $\delta^{15}\text{N}$ value of 0‰. These values are consistent with what has been reported in the literature for these plant groups and the ranges account for differences in soil nitrogen values at the various sites.

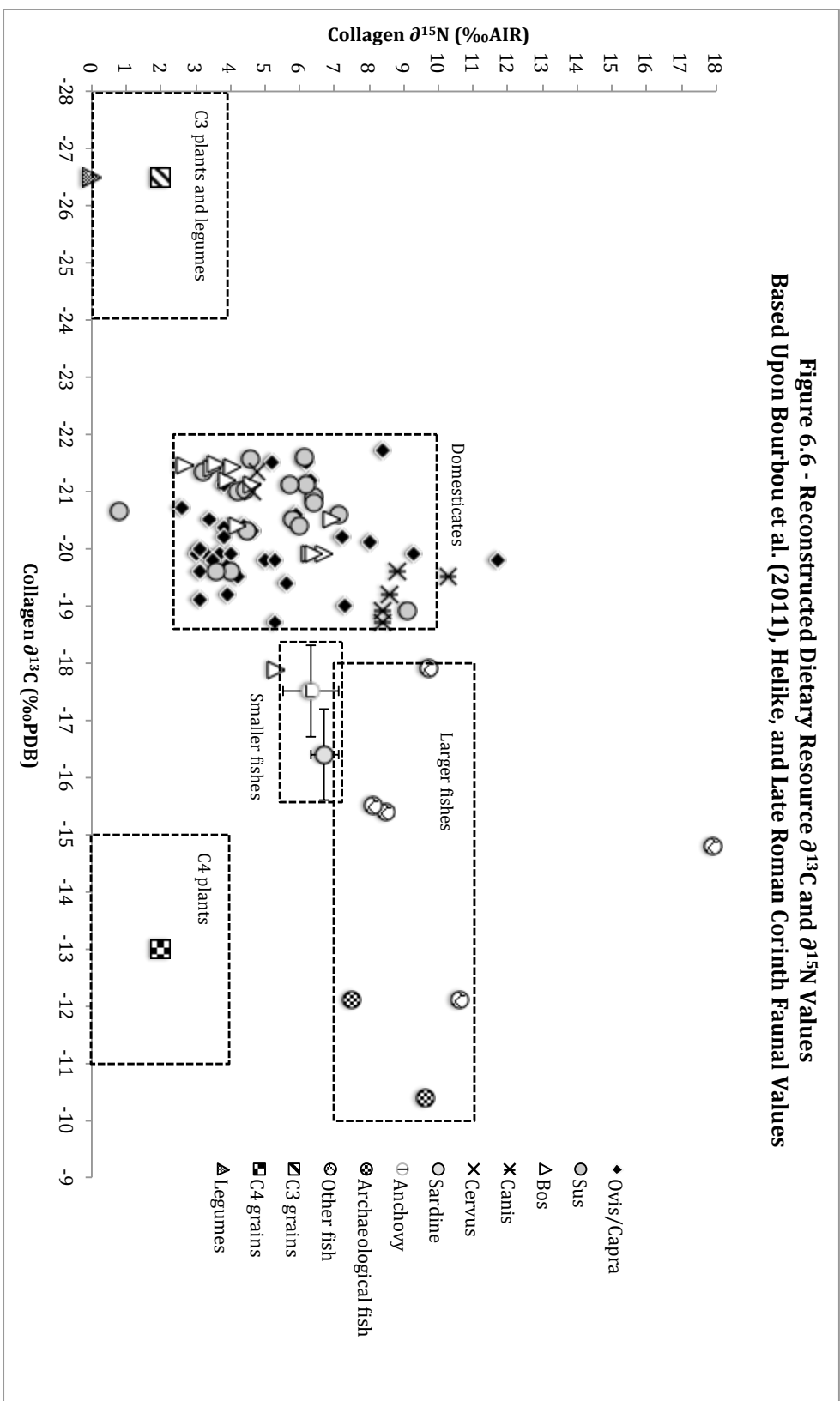
The Hellenistic Helike and Late Roman Corinth faunal samples were then combined with the data from Bourbou et al. (2011) to create generalized reconstructed collagen values for the domesticated animals ($\delta^{13}\text{C}$ = -21.7‰ to -18.7‰). This range does not include the *Bos* value,

discussed in the previous section, which has a higher stable carbon isotope value. It also does not include the outlying ovicaprine value from Bourbou et al. (2011) visible in Figure 6.6. Stable nitrogen isotope values range from 2.7‰ to 9.3‰, with the *Sus* sample from Late Roman Corinth with suspected legume foddering omitted.

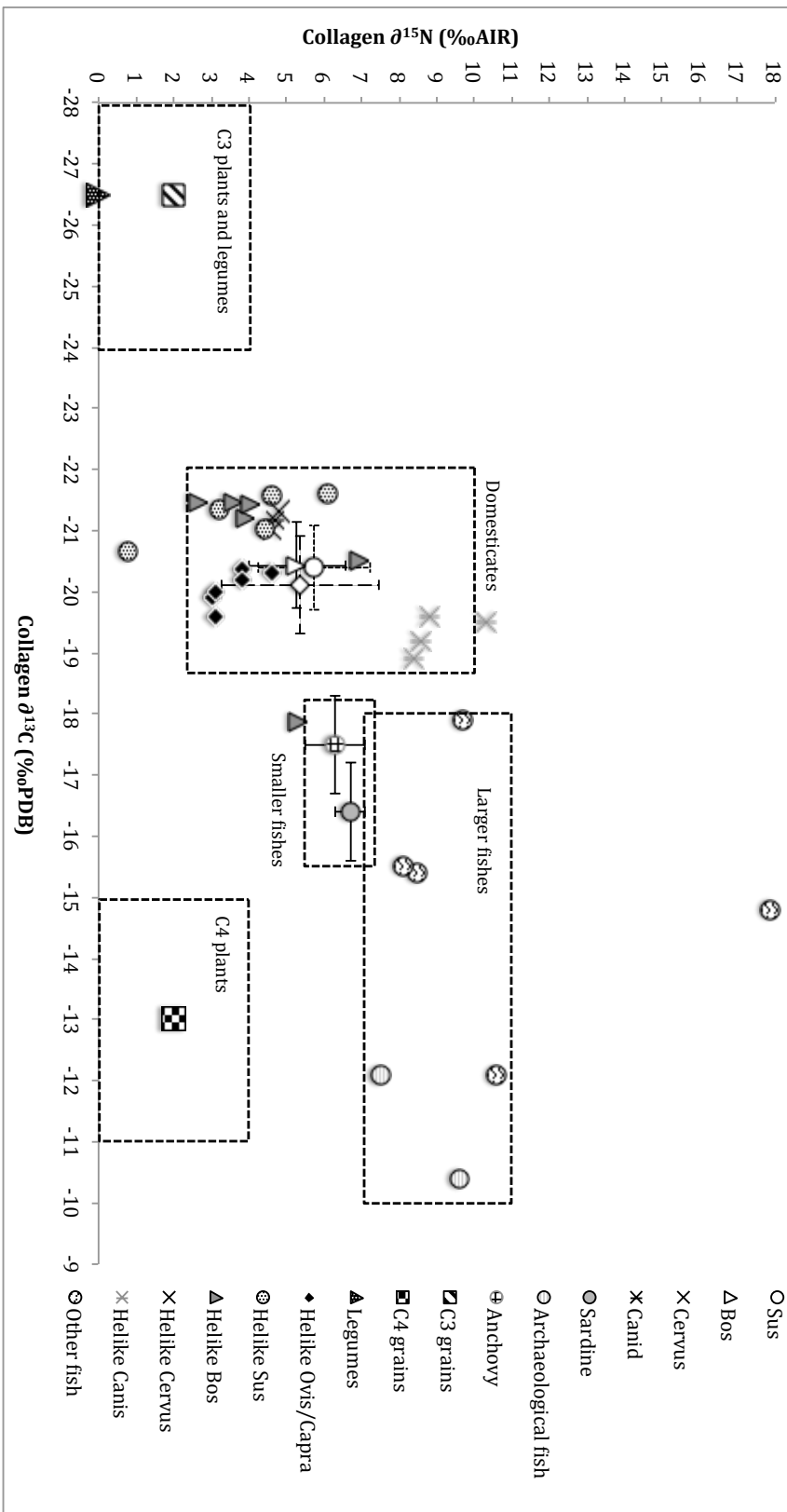
Bourbou et al. (2011) provide an estimated range for smaller fishes based upon modern sardine and anchovy bone collagen values ($\delta^{13}\text{C} = -18.3\text{‰}$ to -15.6‰ , $\delta^{15}\text{N} = 5.5\text{‰}$ to 7.3‰). Larger fishes are also represented as a dietary resource, and include species such as sea bream, mullet, and mackerel ($\delta^{13}\text{C} = -17.9\text{‰}$ to -10.4‰ , $\delta^{15}\text{N} = 7.5\text{‰}$ to 10.6‰). While these larger fish species appear to be fairly distinct from the other food resource values based upon their higher $\delta^{13}\text{C}$, the smaller fishes are much closer in stable carbon isotope values to the values found with the domesticated animals. In addition, the combination of modern and archaeological fish values in the reconstructed marine resource categories may not accurately reflect the values of the fishes found in the Gulf of Corinth during the Hellenistic, Roman, or Byzantine periods. Despite the potential for some inaccuracy in the detection of marine resource consumption, the overall ranges do provide a starting point for the interpretation of human bone collagen values and will likely be refined further as more data becomes available.

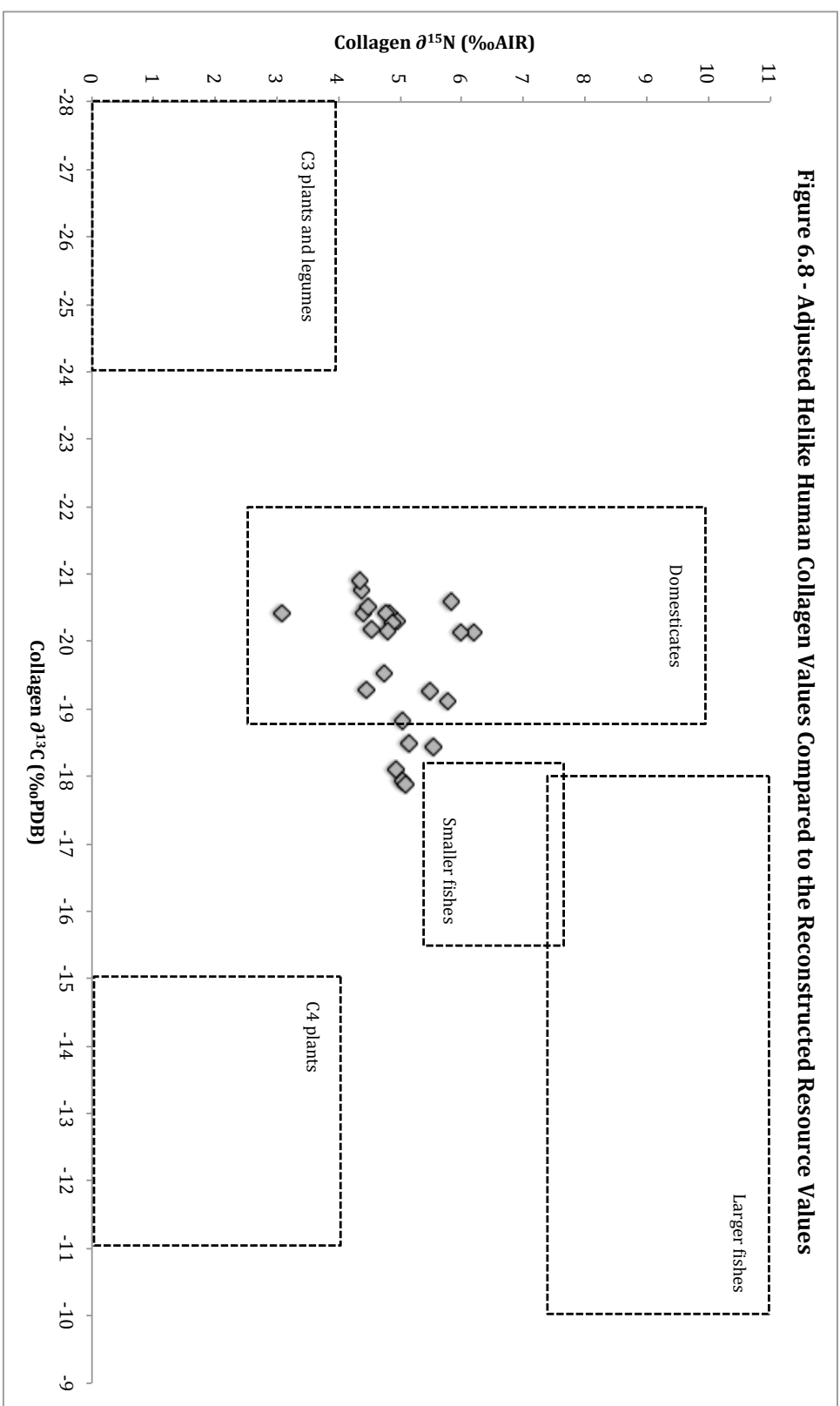
Figure 6.7 compares the Helike and Corinth faunal values with the faunal values from Bourbou et al. (2011). The domesticate animals have been plotted showing the mean values and ranges within one standard deviation. Interestingly, the majority of the Helike and Late Roman Corinth *Bos*, *Sus*, and *Ovis/Capra* $\delta^{13}\text{C}$ values are lower than those reported for similar animals in Bourbou et al. (2011). This may reflect a greater reliance on C_3 plant foddering for the Helike domesticates, as well as area-specific values that are lower than at the other sites. It is also possible that these faunal values will affect the human values by pulling them towards the lower end of the reconstructed domesticate food resource box.

Figure 6.6 - Reconstructed Dietary Resource $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Values
Based Upon Bourbon et al. (2011), Heilke, and Late Roman Corinth Faunal Values

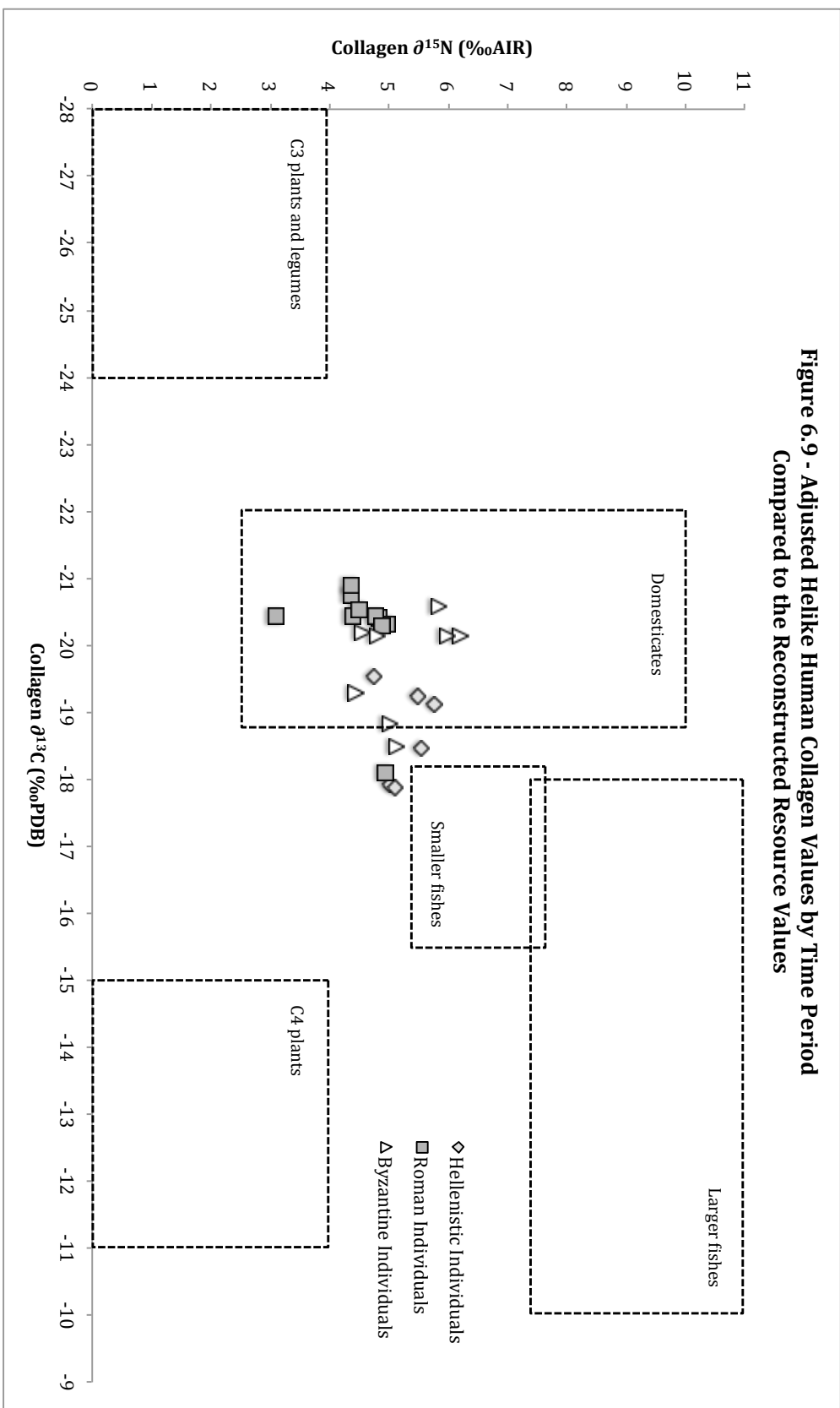


**Figure 6.7 - Reconstructed Dietary Resource $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ Values:
Comparison of Helike and Late Roman Corinth Fauna to Bourbou et al. (2011)**





**Figure 6.9 - Adjusted Helike Human Collagen Values by Time Period
Compared to the Reconstructed Resource Values**



In order to interpret the human values within the context of the reconstructed dietary resource boxes, they need to be adjusted to account for being a trophic level above the food resource values. The adjustment used was 1‰ for $\delta^{13}\text{C}$ and 4‰ for $\delta^{15}\text{N}$, based on evidence for a general trophic level effect of roughly 1‰ for $\delta^{13}\text{C}$ and between 3‰ and 5‰ for $\delta^{15}\text{N}$ (Bourbou et al., 2011; also see Chapter Three). Note that the correction used for $\delta^{13}\text{C}$ is the 1‰ overall trophic level shift and not the 5‰ shift observed between dietary protein and bone collagen. This is necessary because the faunal ranges are based upon bone collagen values and not the meat values. Since people were consuming meat and dairy products and not animal bones, it might seem reasonable to reconstruct values for these particular food items. However, doing so would be difficult because of uncertainties regarding the $\delta^{13}\text{C}$ fractionations between the milk, muscle meat, and bone collagen of individual animals and the effects of dairy product processing. For ease of interpretation and to provide a more consistent reference, this study also adjusted the Helike human $\delta^{13}\text{C}$ values downwards by 1‰ and reduced the $\delta^{15}\text{N}$ values by 4‰ in order to compare them to the reconstructed food resource values. These estimated trophic level adjustments are meant to provide a general idea of where the human values would fall within the ranges for the food values and are comparable to methods used in the ecological literature (Bourbou et al., 2011).

When the reconstructed food ranges from Figures 6.6 and 6.7 are applied to the Helike human values, the majority of the individuals appear to fall within the domesticate resource box. However, a few individuals show $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values falling very close to the reconstructed ranges for smaller fishes (Figure 6.8), suggesting the addition of marine resources to the diet.

As described in sections 6.1 and 6.3, the Helike human data points are spread horizontally along the $\delta^{13}\text{C}$ axis and the highest values belong to individuals from the Hellenistic period. Figure 6.9 shows the patterning of the adjusted human bone collagen values in relation to the reconstructed

food resource values. Notable is the extension of three of the Hellenistic data points, as well as a Byzantine and a Roman period data point, towards the smaller fishes box. This strongly suggests that these individuals were consuming some proportion of smaller fishes in addition to an overall C_3 terrestrial diet, resulting in their adjusted bone collagen values falling in between the two reconstructed food resource ranges. While all three time periods represented at Helike contain at least one individual that shows values consistent with the consumption of aquatic resources, as discussed in section 6.3, the Hellenistic individuals as a group have a statistically different mean $\delta^{13}C$ value from both the Roman and Byzantine period individuals. This patterning of stable isotope values according to time period suggests that although all the people living at Helike had access to marine resources via the coast, many of the Hellenistic individuals were using a resource with slightly different values from what the majority of the Roman and Byzantine individuals were using.

These results could possibly reflect a wider difference in fishing practices during the Hellenistic period as compared to the subsequent periods; the addition of other Hellenistic sites and data, ideally from the Peloponnese, could clarify whether or not this pattern is found elsewhere.

It is also possible that the isotopic signatures found in the Hellenistic individuals are reflective of the contributions of fish sauce to the diet. As presented in Chapter Two, *garum* recipes frequently included smaller-sized fishes as the main ingredient, although parts of larger fishes were also used. Prowse et al. (2004) analyzed *garum* residue from ten African and Italian amphorae dating from the 4th century BC to the 2nd century AD. The mean $\delta^{15}N$ and $\delta^{13}C$ values of these sauces were 6.5‰ and -14.7‰, respectively, which led the authors to suggest that the *garum* was derived from smaller fishes and possibly some shellfish (Prowse et al., 2004).

Studies of modern Asian fish sauces, which are produced in similar ways to those manufactured in antiquity, have found that these sauces contain an average of about 9% protein by weight (Leung et al., 1972). It

would only take a few tablespoons per day to contribute a minor, yet significant amount of protein to the diet (Garvie-Lok, 2001; Leung et al., 1972). Although fish sauce may have contributed to the shift seen in the human isotopic values towards the marine resource box in Figure 6.9, this contribution does not explain the full extent of these values. *Garum* was used as a condiment or flavouring for food and likely would not have been consumed in large enough amounts to be the sole cause of the Hellenistic values.

While fish sauce is an interesting potential explanation of the Hellenistic values, it is puzzling that the Roman period individuals do not show a similar range of values. Fish sauce was extremely popular during the Roman period (Garnsey, 1999; Curtis, 1991) and we would expect to see these individuals also reflecting its widespread consumption at Helike. The Roman period values are almost all well within the reconstructed domesticated resource box (Figure 6.9), suggesting that *garum* use did not affect the values of the protein component of the Roman period diet at Helike and that the Hellenistic values cannot be explained solely by fish sauce.

Given the historical and geological evidence for the formation of a lagoon over the *chora* of ancient Helike after 373 BC, another possible explanation is that the exploitation of this particular food resource is the cause of the increased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the Hellenistic individuals. As the lagoon is thought to have started silting up after the Hellenistic (Soter and Katsonopoulou, 2011), its resources may not have been as extensive in the Roman period and may not have been available at all by the Byzantine era, when the area was occupied by a small, freshwater marsh. The specific carbon and nitrogen signatures created in this lagoon environment would have resulted in an isotopically distinct range of values in the organisms living within it, including higher carbon values. The higher stable nitrogen isotope values typically found in marine environments would also explain the elevation of the Hellenistic values above the Roman values. As described in Chapter Three, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are both generally higher in marine and

lagoon environments, but these values are not always tied together; an elevation in one may not be reflected in an elevation in the other, especially with multiple carbon or nitrogen inputs into a system or with mixed resource consumption.

When considering the Hellenistic individuals, it is important to note that one of the data points which fall close to the reconstructed range for smaller fish resources is from H25.6. This is the one juvenile individual in the Helike sample, who was estimated to be 3-4 years old at death. This individual shows signs consistent with rampant caries, with the majority of teeth recovered affected by large carious lesions. The effect of this condition and of the individual's age on its diet is unclear, but both factors should be kept in mind when considering this value.

The patterning of the other data points from the Roman and Byzantine time periods suggests that a few individuals were also consuming more marine resources than others. Unfortunately, the remains associated with these individuals were either in too poor condition or too fragmented to assign a sex category; increased presence of marine resources in the diet has been tentatively associated with males at other sites and in the literature (Craig et al., 2009; Keenleyside et al., 2006; Dalby, 1996). Overall, there is no strong evidence for status or sex-based differences in marine resource use at Helike, but there is evidence that dietary resource use differed between the various time periods. Hellenistic individuals show values consistent with the exploitation of smaller fishes, while the Roman individuals cluster more closely within the domesticate meat or dairy resource ranges. The Byzantine individuals show values mostly similar to the Roman individuals, but there are also a few individuals with values consistent with marine resource use.

This patterning of predominantly C₃ terrestrial resource use with the occasional addition of aquatic dietary resources has been found at other sites. The next section compares the results from Helike to the other studies discussed in Chapter Two. Particularly pertinent are those sites that had access to local marine or freshwater resources.

6.5. Comparison to Other Stable Isotope Studies Cited in Background

In this section, the Classical and Hellenistic stable isotope evidence from Classical and Hellenistic Thebes (Vika, 2011; Vika et al., 2009), Classical Apollonia (Keenleyside et al., 2006), Classical Metaponto (Henneberg and Henneberg, 2003), and Classical and Hellenistic Sagalassos (Fuller et al., 2012) are compared to the values from Hellenistic period Helike. Roman period sites such as Leptiminus (Keenleyside et al., 2009), Isola Sacra (Prowse et al., 2004; 2005), Velia (Craig et al., 2009), Rome (Killgrove and Tykot, 2013), and Roman Sagalassos (Fuller et al., 2012) provide further comparisons for the Roman individuals at Helike. Finally, the Byzantine stable isotopic data, including the sites cited in Bourbou et al. (2011) as well as Late Roman/Early Byzantine Stymphalos (Pennycook, 2008) and Byzantine Sagalassos (Fuller et al., 2012), are examined.

Classical and Hellenistic Sites

Table 6.5 shows the mean and standard deviations for the human stable carbon and nitrogen isotope values from Classical and Hellenistic sites, including those located both within Greece and for Greek colonies. With the exception of Sagalassos, all of these sites are located either in coastal areas or had access to freshwater resources. Hellenistic Thebes (Vika, 2011) and Sagalassos (Fuller et al., 2012) are argued to not show any signs of marine resource use, while the others are suggested to show significant amounts of marine protein in the diet. The stable nitrogen isotope values for the sites with significant marine resource or freshwater resource use range from 10.1‰ to 10.7‰, while the terrestrial-only sites fall range from 9.6‰ to 9.7‰. The stable carbon isotope values of all these sites are roughly similar at around -19.2‰ to -19.4‰, with the exception of Apollonia at -18.5‰ (Keenleyside et al., 2006). These values roughly demonstrate the generally higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values found with marine and freshwater resource use, as well as some of the difficulties in applying general isotopic cut-off points

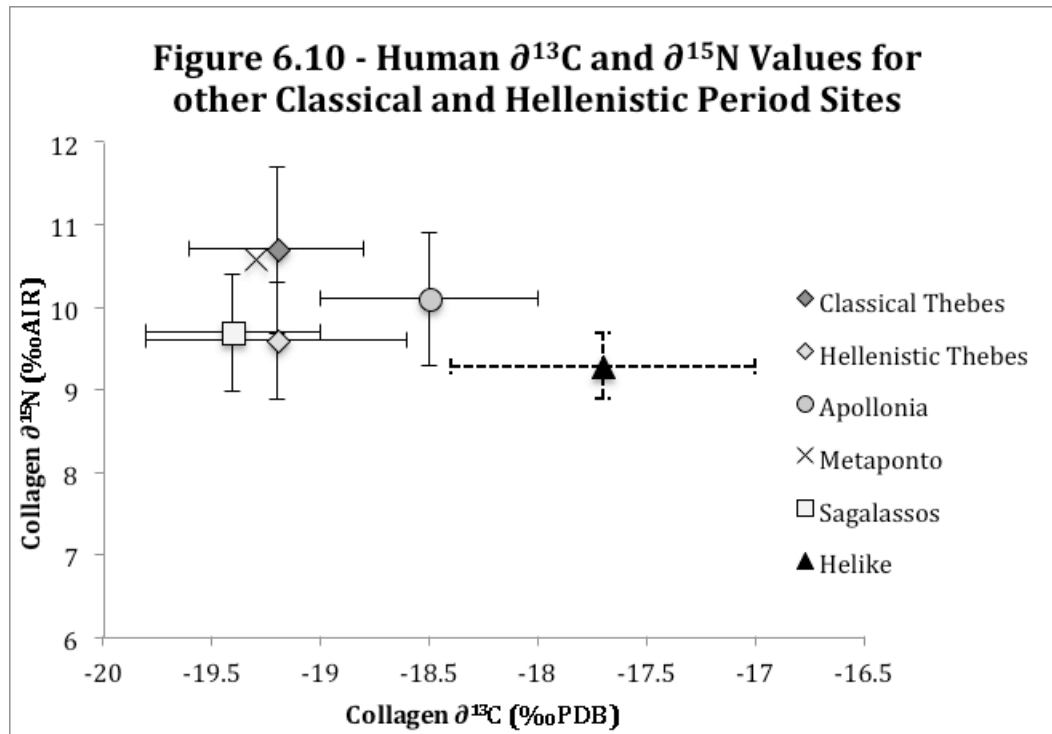
for terrestrial-only and marine-only consumption (Schoeninger and DeNiro, 1984).

Table 6.5 – Adult Human Collagen Values from Classical and Hellenistic Greek Sites (mean \pm 1 s.d.)

Study	Site	Date	Location	Mean $\delta^{13}\text{C}$ (‰)	Mean $\delta^{15}\text{N}$ (‰)	Dietary resource use
Vika et al. 2009	Thebes (Greece)	5 th to 3 rd cent. BC	Inland, with access to sea and freshwater lakes	-19.2 \pm 0.4	10.7 \pm 1.0	C ₃ terrestrial resources with addition of freshwater protein
Vika 2011	Thebes (Greece)	3 rd to 1 st cent. BC	Inland, with access to sea and freshwater lakes	-19.2 \pm 0.6	9.6 \pm 0.7	C ₃ terrestrial resources
Keenleyside et al. 2006	Apollonia (Bulgaria)	5 th to 2 nd cent. BC	Coastal	-18.5 \pm 0.5	10.1 \pm 0.8	C ₃ terrestrial resources with significant marine protein
Henneberg and Henneberg 2003	Metaponto (Italy)	7 th to 2 nd cent. BC	Coastal	-19.3 (no s.d. given)	10.6	C ₃ terrestrial resources with significant marine protein
Fuller et al. 2012	Sagalassos (Turkey)	400–200 BC	Inland	-19.4 \pm 0.4	9.7 \pm 0.7	C ₃ terrestrial resources

Figure 6.10 shows the means for each of the sites and the spread within one standard deviation in addition to the human values from Hellenistic period Helike. The mean $\delta^{13}\text{C}$ values for Helike (-17.7‰) are noticeably higher than those at the other contemporary sites while the mean $\delta^{15}\text{N}$ values (9.3‰) are the lowest. This is not entirely unexpected, as all of these sites are subject to geographical and geological variations in their carbon and nitrogen sources. The available dietary resources for the studies cited in Table 6.5 include freshwater lakes, marine environments, and terrestrial resources, all of which can differ dramatically from each other in isotopic patterning, as discussed in Chapter Three. The Hellenistic period lagoon at Helike would have had its own specific isotopic values different

from these other resources, potentially causing the relatively higher $\delta^{13}\text{C}$ and relatively lower $\delta^{15}\text{N}$ values of the individuals at Helike.



Roman Period Sites

Table 6.6 lists the Roman period sites discussed in Chapter Two. The variability in location and cultural affiliations is reflected in the $\delta^{13}\text{C}$ values, which range from -19.5‰ at coastal Velia to -17.7‰ at Leptiminus, Tunisia. The $\delta^{15}\text{N}$ values range from 8.2‰ at Velia to 13.4‰ at Leptiminus, again demonstrating the site-specific variables affecting diet and stable isotope values. Even within-site variation is apparent, with isotopic differences found between urban and suburban Romans (Killgrove and Tykot, 2013) and between two groups of individuals at Velia (Craig et al., 2009). Group I at Velia is argued to have less of a marine component to the diet than Group II, a difference that is reflected in the stable nitrogen isotope values (Table 6.6). This does not appear to be related to sex, age, burial type, or status of the individuals (Craig et al., 2009). The majority of these sites had either coastal,

marine, or riverine resource access, and show varying amounts of aquatic resources in the diet. The overall terrestrial C₃ plant and animal tissue consumption pattern remains predominant throughout and is best illustrated at Roman period Sagalassos, where the human $\delta^{15}\text{N}$ values are suggestive of frequent pork consumption when compared to the site's faunal values (Fuller et al., 2012).

Table 6.6 – Adult Human Collagen Values from Roman Sites
(mean \pm 1 s.d.)

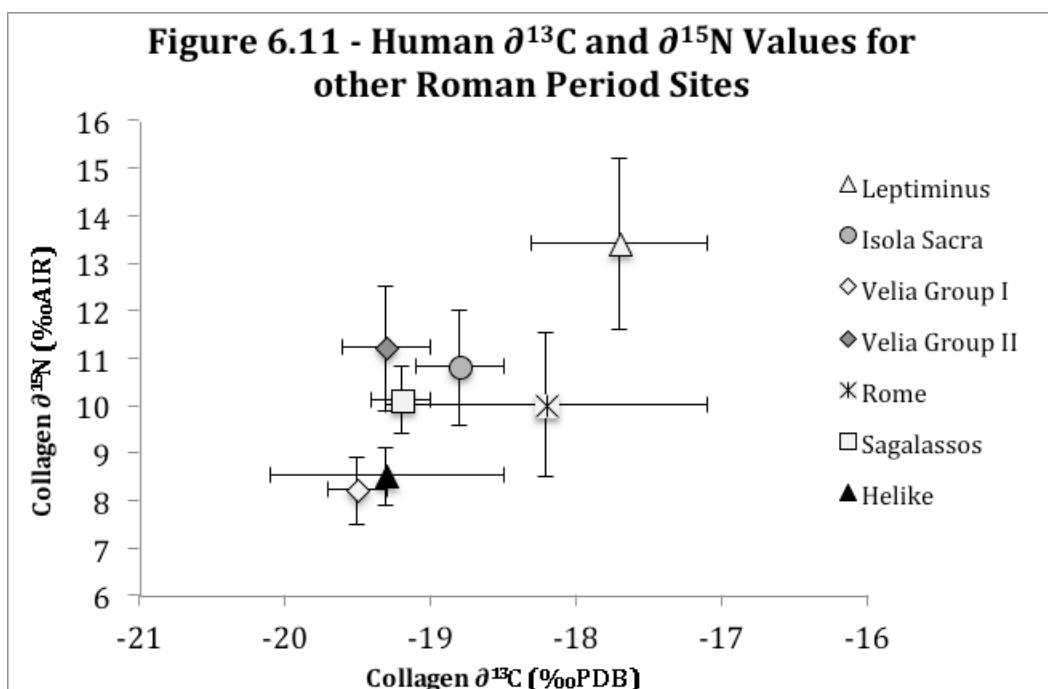
Study	Site	Date	Location	Mean $\delta^{13}\text{C}$ (‰)	Mean $\delta^{15}\text{N}$ (‰)	Dietary resource use
Keenleyside et al. 2009	Leptiminus (Tunisia)	2 nd to 5 th cent. AD	Coastal	-17.7 \pm 0.6	13.4 \pm 1.8	C ₃ terrestrial resources with significant marine protein
Prowse et al. 2004; 2005	Isola Sacra (Rome, Italy)	1 st to 3 rd cent. AD	Coastal	-18.8 \pm 0.3	10.8 \pm 1.2	C ₃ terrestrial resources with significant marine protein
Craig et al. 2009	Velia (southern Italy)	1 st to 2 nd cent. AD	Coastal	-19.5 \pm 0.2 (Group I) -19.3 \pm 0.3 (Group II)	8.2 \pm 0.7 (Group I) 11.2 \pm 1.3 (Group II)	C ₃ terrestrial resources, minor marine contribution
Killgrove and Tykot 2013	Urban and suburban Rome (Italy)	1 st to 3 rd cent. AD	Inland, some river access	-18.2 \pm 1.1	10.0 \pm 1.5	C ₃ terrestrial resources, with some C ₄ millet use in <i>suburbium</i> and more aquatic resource consumption in urban individuals
Fuller et al. 2012	Sagalassos (Turkey)	300-450 AD	Inland	-19.2 \pm 0.2	10.1 \pm 0.7	C ₃ terrestrial resources

Figure 6.11 shows a visual representation of the mean values and standard deviations for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from these sites. Roman period Helike (mean $\delta^{13}\text{C}$ = -19.3‰ and mean $\delta^{15}\text{N}$ = 8.5‰) falls closest to the values from Group I at Velia followed by the values at Sagalassos. Even though it is difficult to compare sites in different locations with different populations,

these similarities in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ further support the interpretation that the Roman period individuals at Helike were following a largely C_3 terrestrial plant and animal protein diet. This corresponds well with the historical literature and zooarchaeological evidence, both of which indicate that the Roman diet was more focused on meat than the typical Mediterranean diet.

The Roman period diet at Helike was possibly supplemented by small amounts of marine resources, but not to the same extent as the individuals from the Hellenistic and Byzantine time periods. As discussed previously, *garum* was likely frequently consumed, although this is not evident isotopically. The focus on terrestrial resources could simply reflect the general dietary preferences of the Roman era. It could also be that the delta was particularly attractive for pastoralism at this point because of the water supplied by the rivers and freshwater lagoons, allowing for grazing of Roman period livestock within the area. The borehole evidence from Helike shows that channels, flood plains, transient lakes and lagoons, and high-energy seasonal floods from the rivers and streams all occurred at various times, although the complex stratigraphy and environment of the delta make it difficult to accurately correlate these to any specific time period (Soter and Katsonopoulou, 2011; Alvarez-Zarikian et al., 2008). The effect of these aquatic sources on animal husbandry practices at Helike remains unknown.

The values of the Roman period individuals may also relate to the status of many of these individuals at Helike; several were buried with grave goods suggesting they were quite prosperous and one of the excavated Roman residences contained luxury materials such as marble. These may have been individuals who had more security in their food choices, allowing them to follow a terrestrial diet more reliably than individuals who needed a more diverse range of subsistence strategies.



Byzantine Period Sites

As mentioned previously, the Byzantine period in Greece is somewhat better documented in terms of dietary stable isotope values because of the studies found in Bourbou et al. (2011). Table 6.7 lists the sites and results from Bourbou et al. (2011), as well as from Pennycook (2008) and Fuller et al. (2012). The table also shows the range of values and resource use occurring during this time period in the various parts of Greece and Turkey.

Mean $\delta^{13}\text{C}$ values range from -19.3‰ at Stymphalos to -18.7‰ at Servia, the latter showing evidence for C_4 resource use (Bourbou et al., 2011). Mean $\delta^{15}\text{N}$ values range from 8.2‰ at Eleutherna to 9.5‰ at Sourtara and Petras. The Byzantine period is generally characterized by the increased use of millet, a C_4 cereal, which was detected at Sagalassos, Sourtara, and Servia. None of these three sites had access to local marine resources, although trade may have brought preserved foods into the area. The lack of dietary marine input, coupled with detection of C_4 foddering in some of the sites'

domesticates, helped to prevent the C₄ isotopic contributions from being confused with marine values (Bourbou et al., 2011).

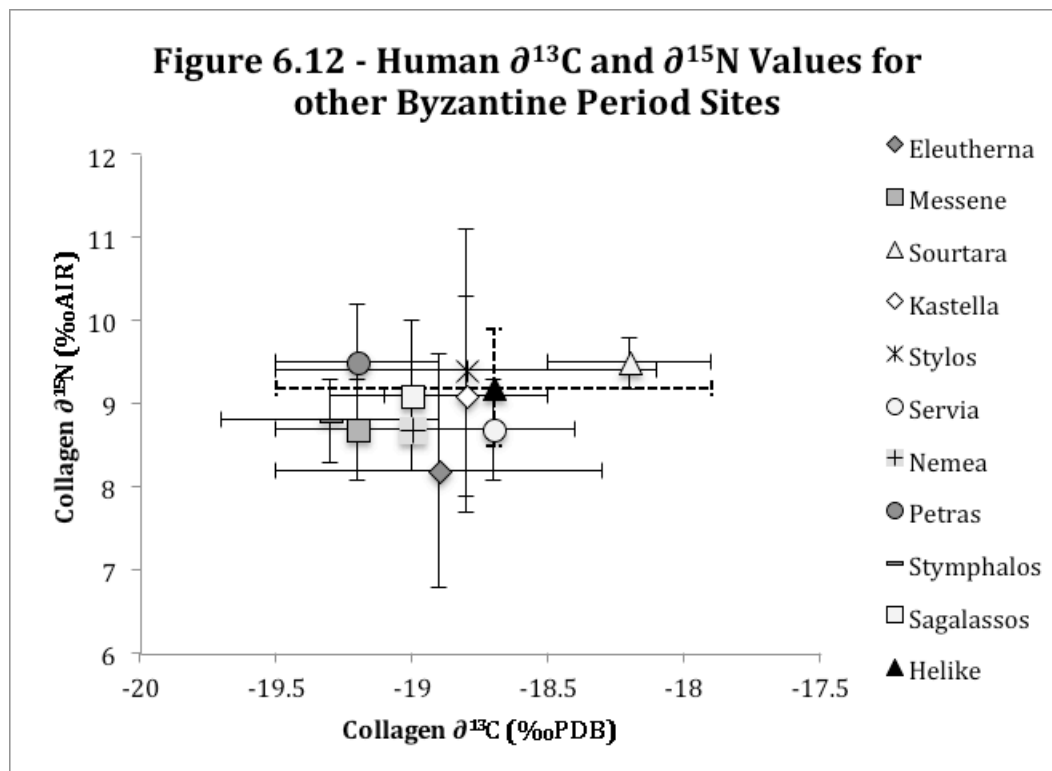
As found in the earlier time periods, the sites where marine resources were available tend to show at least a small contribution of these to the diet. The inland sites with no marine access show dietary stable isotopic values consistent with the typical C₃ terrestrial and animal protein diet seen throughout the time periods. Figure 6.12 shows the means and one standard deviation of the sites listed in Table 6.7 with the addition of the Byzantine Helike individuals for comparison.

Table 6.7 – Adult Human Collagen Values from Byzantine Greek Sites
(mean \pm 1 s.d.)

Study	Site	Date	Location	Mean $\delta^{13}\text{C}$ (‰)	Mean $\delta^{15}\text{N}$ (‰)	Dietary resource use
Bourbou et al. 2011	Eleutherna (Crete)	6 th to 7 th cent. AD	Inland	-18.9 \pm 0.6	8.2 \pm 1.4	C ₃ terrestrial resources
Bourbou et al. 2011	Messene (Peloponnese)	6 th to 7 th cent. AD	Inland	-19.2 \pm 0.3	8.7 \pm 0.6	C ₃ terrestrial resources
Bourbou et al. 2011	Sourtara (Northern Greece)	6 th to 7 th cent. AD	Inland	-18.2 \pm 0.3	9.5 \pm 0.3	C ₃ terrestrial resources, with some C ₄
Bourbou and Richards 2007; Bourbou et al. 2011	Kastella (Crete)	11 th cent. AD	Coastal	-18.8 \pm 0.3	9.1 \pm 1.2	C ₃ terrestrial resources, with some marine input
Bourbou et al. 2011	Stylos (Crete)	11 th to 12 th cent. AD	Inland	-18.8 \pm 0.7	9.4 \pm 1.7	C ₃ terrestrial resources
Garvie-Lok 2001; Bourbou et al. 2011	Servia (Northern Greece)	11 th to 15 th cent. AD	Inland	-18.7 \pm 0.3	8.7 \pm 0.6	C ₃ terrestrial resources, with some C ₄
Garvie-Lok 2001; Bourbou et al. 2011	Nemea (Peloponnese)	12 th to 13 th cent. AD	Inland	-19.0 \pm 0.3	8.7 \pm 0.5	C ₃ terrestrial resources
Garvie-Lok 2001; Bourbou et al. 2011	Petras (Crete)	12 th to 13 th cent. AD	Coastal	-19.2 \pm 0.3	9.5 \pm 0.7	C ₃ terrestrial resources, with some marine input
Pennycook 2008	Stymphalos (Peloponnese)	4 th to 6 th cent. AD	Inland, with lake access	-19.3 \pm 0.4	8.8 \pm 0.5	C ₃ terrestrial resources
Fuller et al. 2012	Sagalassos (Turkey)	800 –1200 AD	Inland	-19.0 \pm 0.3	9.1 \pm 0.9	C ₃ terrestrial resources with some C ₄

The mean human values for Byzantine period Helike ($\delta^{13}\text{C}$ = -18.7‰, $\delta^{15}\text{N}$ = 9.2‰) fall very close to those from Kastella and also to Stylos, both sites that are located on the island of Crete, some distance away from Helike. The Kastella individuals show some marine contribution to their diets, while the Stylos individuals do not. Overall, the Byzantine diet in Greece remained heavily reliant on C₃ terrestrial resources and is more uniform in this regard than the earlier time periods. The addition of C₄ cereals is minimal and is

consistent with the occasional use of millet for foddering animals. The addition of marine protein to the diet, even in small amounts, appears to be site-specific. Although trade foods and items may have contributed to the diet, especially in urban centres, the consumption of marine foods in rural areas appear dependent on direct access to that particular resource. Overall, the Byzantine individuals at Helike appear to follow a similar dietary resource use pattern as at other Byzantine Greek sites, including a lack of evidence for legume consumption.



6.6. Addressing the Original Research Questions: Diet in Helike During the Hellenistic, Roman, and Byzantine Periods

The purpose of this study was to explore two aspects of diet at Helike: general dietary resource use and any temporal changes in resource use.

Although the literary and historical information from ancient Greece addresses some aspects of food and diet, these sources tend to be skewed towards higher status individuals, focus on certain regions, or consist of artistic representations that use food symbolically rather than realistically. Previous stable isotope analyses of diet in Greece have supported the historical data indicating that most individuals subsisted on a diet based upon terrestrial plants and animals, with varying amounts of aquatic foods contributing to protein intake. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of both the human and faunal samples from Helike are consistent with the findings from these other sources. C_3 terrestrial plants, including cereal crops, formed a major part of the diet at Helike, while domesticated animals or their products provided the main dietary source of protein. Since animal meat and milk have similar stable isotopic values, it is not possible to determine exactly which was most exploited but it is likely that secondary products played a more important role in the typical diet at Helike than meat itself.

The stable nitrogen isotope values of the humans and the fauna (with the exception of the pig sample from Late Roman Corinth) do not provide evidence of significant legume consumption for either group. These findings fit well with other recent isotopic studies of diet that show little or no legume consumption in Greece. This is surprising given the emphasis placed on legume use by the historical sources discussed in Chapter Two. Some potential explanations for this finding at Helike include the possibility that these individuals truly were not consuming legumes at all or in any significant quantities. If this is the case, the stable nitrogen isotope values do not show evidence for legume consumption because it simply did not occur. Another possibility is that the practice of fertilizing fields through manuring

increased the soil nitrogen content enough that the legume plants did not need to rely on diazotrophic relationships in order to access nitrogen for growth. These legume values would then reflect soil values and be indistinguishable isotopically from other C3 plants in the diet. Another possibility is that seasonal consumption of legumes was occurring at Helike. As discussed in Chapter Three, this would result in an averaging of the dietary values in the human individuals, reflecting both legume and domesticated animal protein contributions although the dietary emphasis on these differed throughout the year. The exact reasons for why the isotopic evidence does not show significant legume consumption remain unclear and are unknown at this time.

Except for a few individuals, there is no clear indication that marine resources contributed large amounts of protein to the diet. Having said this, however, there do appear to be differences in marine resource use between the Hellenistic, Roman, and Byzantine time periods at Helike.

The hypothesis for this study was that the Byzantine individuals would have higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than the Roman or Hellenistic individuals because of an increase in marine resource consumption during this time period. If higher values were apparent in the Byzantine individuals, they could have been attributable to the Byzantine Orthodox dietary and fasting edicts that promoted abstinence from animal meats and some animal products for a large portion of the year. As shellfish, and occasionally fishes, were considered acceptable meat substitutes, their consumption would have increased. Although there is evidence in the literature that higher status urban individuals would have been able to better afford seafood, and especially those larger fishes and invertebrates that tend to have higher stable isotope values, it is not clear what the typical rural individual living on the coast would have eaten. When considering the expected effects of Byzantine Orthodox fasting edicts, the results of this study are surprising. There are differences in resource use apparent between the time periods, but it is the Hellenistic period, not the Byzantine, where the dietary signatures

are consistent with increased marine resource consumption. This finding may be related to the documented fishing activities that occurred within the lagoon that had formed after the earthquake of 373 BC and that lasted for part of the Hellenistic period at Helike. As lagoon stable isotope values tend to be intermediate between the local terrestrial and marine values, the consumption of smaller fishes from this lagoon would be reflected in human bone collagen values also intermediate to these dietary resource values.

The subsequent individuals living at Helike would have still had access to the marine resources but potentially of different isotopic values. Resources from the sea would still have been available and aquatic resources from the other lagoons, lakes, or rivers within the delta may have been accessible as well. Interestingly, the Roman period individuals do not show values consistent with significant dietary marine resource use at all, with the exception of one individual. This person may have lived mostly in a region where marine resource use was common and then moved to the Helike area, or may have shown an individual dietary preference different from the majority of the individuals. The tightly clustering values of many of the Roman individuals suggest that dietary protein during this time came more from agricultural activities and that the products of these activities were being consumed locally. If imported foods were being consumed in significant amounts, we might expect to see more variation between individual isotopic values reflective of the different origins of the food. The clustering of the Roman values is similar to what is seen at Stymphalos and Nemea, where resources were largely limited to those found in the area, suggesting that this is one possible interpretation of the values. Another reason may be due to the effects of socioeconomic status on food choices. Some of the Roman individuals at Helike appeared prosperous and may have been able to afford meat, although dairy products could have played a part in their diet as well. It is not possible to distinguish between the two isotopically, but the narrow range of values suggests that if these individuals had fewer economic limitations in regards to dietary resource use, they were

able to preferentially consume terrestrial resources over marine. This patterning corresponds well with Vika (2011), where the $\delta^{13}\text{C}$ values associated with “rich” Classical and Hellenistic burials showed a narrower range than those from the “poor” burials, although $\delta^{15}\text{N}$ values were only slightly higher in the former. It also contrasts with Roman period Isola Sacra, where the more middle-class population accessed both marine and terrestrial resources for food (Prowse et al., 2004, 2005).

As compared to the Roman period individuals, the Byzantine individuals show more variability in stable carbon isotope values, perhaps as a result of the different sources of food available at this time or of individual variation in food choices. While the majority of the Byzantine individuals at Helike appear to have consumed a terrestrial C_3 diet, their stable nitrogen isotope values are not statistically different from those of the Hellenistic period individuals. This suggests that some were consuming more marine resources while others did not or that manure fertilization was occurring on some local fields. It is not possible to conclude with any certainty that the Byzantine individuals were eating more marine resources in general or why this may have occurred, but the results of this study suggest that not everyone at Helike followed the Orthodox dietary and fasting edicts to the same degree or in the same manner.

This pattern is evident at other Byzantine Christian and western Christian sites. The studies in Bourbou et al. (2011), discussed in more detail above, found variable amounts of marine resources contributing to diet across the sites. Gregoricka and Sheridan (2013) also found a range of $\delta^{15}\text{N}$ values within the Byzantine monastic community of St. Stephen's in Jerusalem, where dietary rules would have theoretically been stringently followed. These values suggest that some individuals were consuming foods that would have not been compliant with fasting regulations, such as meats and dairy.

In the western Christian world, Müldner and Richards (2005) examined several English medieval sites, including an Augustinian friary, and

were able to detect the presence of freshwater fish in the diet. These results suggest that religious fasting regulations played a large part in everyday subsistence, at least in this particular region. Rutgers et al. (2009) found a similar freshwater isotopic signature in Christian individuals from 3rd to 5th century Rome, but attributed this to the socioeconomic status of the particular individuals analyzed rather than to adherence to the ascetic traditions that promoted fish consumption. Although the individuals in Müldner and Richards (2005) and Rutgers et al. (2009) would have followed western Christian practices, the similarities in fasting requirements and expected adherence make these results relevant for comparison to Byzantine Orthodox populations. There is variability apparent in the dietary practices of medieval Christian populations; other studies have not found an increase in aquatic resource consumption at all. Individuals from the Early Christian coastal site of Ridanäs, Sweden, consumed mostly terrestrial resources and actually showed a decrease in the amount of marine consumption when compared to the earlier, pre-Christian period. Kosiba et al. (2007) suggest that at this particular site, increased economic reliance on the trade of fishing products, coupled with the relocation of the main settlement, were the main influences on dietary choices. In this case, economic factors appeared to outweigh any religious dietary rules, which illustrates the difficulties in applying generalizations about diet to specific regions and cultures.

Economic factors again emerge as important variables in Salamon et al. (2008). The authors argue that it was the increased availability of fish products, and not religious dietary edicts, that is linked to increased marine resource consumption in the medieval Mediterranean world. Access to the European North Atlantic fish stocks and improvements in food preservation methods would have not only increased the supply of fish available in medieval Italy, but would have allowed these products to be kept for longer and shipped farther distances. If the Mediterranean really was as barren as some claim, the import of fish and seafood from the Atlantic would have created a more regular supply. The distribution of this particular resource

would have been highly dependent on economic status and rural or urban residence. Lacking the economic resources of wealthier, urban individuals, rural individuals may have continued to depend mainly on the local resources available to them (Koder, 2003; Kislinger, 1999).

Chapter 7: Conclusions

After considering the stable carbon and nitrogen isotope values of the individuals from Hellenistic, Roman, and Byzantine period Helike, some inferences can be made about their use of dietary marine resources. In this thesis, the general topography and climate of Greece, and the historical background of the Classical, Hellenistic, Roman, and Byzantine periods were discussed. Sources of information about diet during these times were presented, including the historical, literary, archaeobotanical, zooarchaeological, and stable isotopic evidence. These sources generally indicate that the “Mediterranean triad” of cereals, wine, and oils played a large part in the diet during these periods, while the consumption of meat and marine resources varied according to site and population. Theoretical and methodological considerations regarding stable carbon and stable nitrogen isotopes were presented along with the typical values found within terrestrial, marine, freshwater, and lagoon environments and organisms. Special considerations for interpreting dietary stable isotope values from human bone collagen were discussed.

The archaeological site of Helike and the context of the human and faunal samples analyzed in this study were presented with the final sample of 24 human individuals, five faunal samples from Helike, and 17 faunal samples from Late Roman Corinth described in further detail. A brief discussion of collagen extraction methods and the indicators of collagen sample quality provided the background for the process used in this study. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values obtained from the samples were presented, collagen quality indicators were assessed, and the results were used to reconstruct dietary resource use at Helike. These results indicate that there are no obvious differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between age categories and sex categories, although small group sizes limit the inferences that can be drawn from these. Differences are apparent between the time periods at Helike, with the Hellenistic individuals showing the highest overall values

and the Roman individuals showing the lowest; the Byzantine individuals have values intermediate to these. While overall diet at Helike appears to have been based on C₃ terrestrial resources, the differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the time periods suggests that marine or other aquatic resources contributed to the diet to varying degrees as well. The higher values of the Hellenistic individuals correspond with the presence of a large lagoon that had formed after the earthquake of 373 BC. The values of the Roman individuals are consistent with the addition of few marine resource components to the diet, while the Byzantine individuals appear to have utilized a range of resources of different isotopic values, resulting in some individuals showing isotopic signatures consistent with marine resource consumption and some showing more terrestrial resource consumption. Although the number of Byzantine individuals in this study was small, these values suggest that Byzantine Orthodox fasting and dietary rules had varying effects on the food resource choices of the individuals at Helike.

7.1. Diachronic Changes in Stable Isotope Values

This study looked for a difference in human $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between the Hellenistic, Roman, and Byzantine time periods at Helike. It was hypothesized that the Byzantine individuals would have consumed more marine foods in order to comply with the religious dietary edicts of the time, thus showing higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Instead, it was found that the Hellenistic period individuals stand apart from the others, with isotopic evidence supporting more marine resource use. In Chapter Six, some potential explanations were considered. Although a wider survey of Hellenistic groups will be needed to thoroughly test these, the simplest and most attractive explanation is that the Hellenistic individuals were consuming lagoon resources in conjunction with terrestrial resources. The Roman period shows evidence of a more homogeneous terrestrial diet based more upon land animal resources and less on marine. The Byzantine period shows a range of terrestrial and marine resource consumption values,

consistent with differences in exploitation of the various dietary resources available. The individuals from this period do not show consistent values indicating that marine resources formed a major part of the diet, despite the Orthodox Christian dietary rules that would have been around at the time. These values may reflect varying levels or ways of adherence to the dietary rules or may reflect exploitation of isotopically distinct inland lagoons or freshwater resources, of which several are documented in the area. There is evidence of more marine consumption during the Byzantine period as compared to the Roman period, but the evidence for a significant increase in marine resource use and any correlation to religious fasting edicts remains inconclusive.

7.2. The Importance of Marine Resources and of Accounting for Changes in Local Resources Over Time

As became evident with the stable isotope results, marine resources varied in importance both within and between time periods, ranging from minimal to possibly very important. Cultural, religious, and individual factors notwithstanding, one likely reason for why resource use is different between the time periods is availability. While it is tempting to think of past resources as being constantly accessible, the seismic activity that occurs in the Mediterranean means that changes can, and did, affect the landscape in a major way. The creation of a new subsistence resource, the lagoon over the *chora* of ancient Helike, could have allowed the Hellenistic period individuals to exploit smaller fishes and seafood that were isotopically intermediate to the general marine and terrestrial values of the area. While the previous stable isotope research cited in this study has shown that resource use is linked with local availability, for the individuals at Helike we have the benefit of historical documentation that provides some information about the landscape of the Helike Delta after the earthquake of 373 BC. These results act as a reminder that some assumptions about the constancy of landscape

and resource accessibility may be inaccurate, especially in seismically active regions.

The results from this study also confirm other findings that indicate that although C₃ terrestrial plants and animals were the mainstays of Greek diet, variability in marine resource consumption occurred throughout the Mediterranean world. The use of marine resources appears to be more dependent on availability than on specific cultural factors, at least in the past populations in this region. By applying this knowledge to future stable isotope reconstructions of diet, researchers are better positioned to look for and detect smaller amounts of aquatic resources in addition to the main Mediterranean foods of cereals, wine, oils, and likely dairy. The stable isotope analysis of more individuals from Helike would help to further illustrate the differences between dietary resource use during the Hellenistic, Roman, and Byzantine periods; if further burials are found and excavated, these could be added to the data from this study. Comparisons to contemporary burial groups from other nearby areas along the coast would also be useful, as it would allow testing of the hypothesis that the distinctive Hellenistic values represent a response to a new local resource rather than a broader cultural or economic shift. Analysis of marine fauna local to Helike, whether modern or archaeological in nature, would also help to refine the reconstructed food resource values of the marine resources. Potential future research could also include analysis of strontium in the individuals with outlying $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to detect the effects of mobility on the population at Helike.

This study has provided a site-specific analysis of dietary resource use at the archaeological site of Helike, Greece. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the individuals at Helike have indicated the usefulness of stable isotope analysis in not only reconstructing general diet in past populations, but also in detecting changes that occurred over time. The consideration of other sources of information in addition to the stable isotope data helps to both strengthen and refine interpretations about resource use.

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