ABSTRACT

Title of dissertation: A CARBON AND NITROGEN ISOTOPIC ANALYSIS OF PLEISTOCENE FOOD WEBS IN NORTH AMERICA: IMPLICATIONS FOR PALEOECOLOGY AND EXTINCTION

Christine Ann Missell France, Doctor of Philosophy, 2008

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Carbon and nitrogen isotopic reconstructions of North American Pleistocene trophic relationships were used to examine the extinction within terrestrial mammals ~10,000 years ago and distinguish between two potential causal mechanisms - human over-hunting and climate change. Additionally, individual animals were examined for unique isotopic signatures associated with feeding specializations, digestive strategies, and juveniles.

Bones representing a comprehensive set of Pleistocene mammalian genera were obtained from three fossil localities: McKittrick Brea, California; Saltville, Virginia; and several sites in Florida. Collagen, a durable bone protein whose carbon and nitrogen isotopic composition reflects dietary input, was extracted from specimens and analyzed for $\delta^{13}C$, $\delta^{15}N$, % collagen, %C, %N, and C:N. Radiocarbon dating and amino acid analyses were performed on select sample sets.

Results indicated that several specimens contained well preserved collagen, the isotopic values of which indicated both trophic position and vegetation preference. Those samples that contained residual diagenetic proteinaceous material exhibited increased hydrolysis of collagen with time and leaching of disassociated amino acids. Trophic relationships were reconstructed from well preserved specimens for Aucilla River, Florida and the herbivores of Saltville, Virginia, with a less complete reconstruction established for McKittrick Brea, California. The following notable trends emerged:

1) absence of nitrogen isotopic distinction between ruminants and non-ruminants,
2) enriched juvenile nitrogen isotopic signature,
3) distinction of giant ground sloths as omnivores,
4) C4 grass grazers and open C4 grasslands restricted to southern North American latitudes,
5) generalized and opportunistic feeding habits of herbivores,
6) potential prey specializations of carnivores.

A noticeable lack of competition and feeding specialists among herbivores suggested a stable base to these late Pleistocene ecosystems, which argues against climatically induced stress on plants. While carnivore specimens were fewer, the apex trophic levels appeared to exhibit a similar lack of competition, which would be expected in a human-driven extinction where carnivores were stressed due to rapidly over-hunted herbivores. The ultimate cause of the late Pleistocene mammalian extinction in North America can not be exclusively attributed to either of these two

mechanisms at this point in time; rather, a combination of factors should be considered.

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by

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DEDICATION

I would like to dedicate this work to all those family members and friends who always seemed to know when to encourage, when to push, and when not to ask. Your genuine interest and pride in my success always reminded me why I started on this path. And to Todd, who always knew I could do it.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

CHAPTER 1 – INTRODUCTION AND BACKGROUND THEORY

1.1 Introduction

The study of ancient ecosystems requires a combination of data to produce a well-supported understanding of the system of interest. Determination of dietary habits is a single, but important step in reconstructing prehistoric ecology. Specifically, mapping of a food web provides invaluable insight into faunal interactions and habits. The distinction of different trophic levels in a system allows for the examination of predator-prey relationships and thus general ecosystem stability.

This project utilized a geochemical approach to reconstruct ancient food chains. Trophic positions were examined through combined CN isotopic analyses in an effort to quantitatively distinguish predator-prey relationships in fauna that have traditionally been analyzed qualitatively and morphologically for such information. Geochemical distinctions between digestive strategies and between juveniles and adults of similar species were an additional focus of this study. Finally, these reconstructed ecosystems were examined for the presence of exceptionally competitive levels of the food chain and overlapping dietary preference of different species, which has direct implications for the ability of an ecosystem to sustain a stable food web. Evidence was sought for areas where a potential collapse of the food chain was imminent and subsequent extinction a high probability.

These methods were applied to terrestrial fauna from the late Cenozoic of North America. This was a dynamic time period in Earth's history that saw dramatic

climate changes, profound shifts in faunal assemblages, and the arrival of humans. The end of the Pleistocene Epoch at ~10,000 years before present (i.e. yrs BP) was a time of mass extinction when over one hundred species were lost, making way for the modern fauna of North America. Well preserved specimens from this time period and region provided an adequate fauna on which one could apply a geochemical approach to ecosystem reconstruction.

1.2 The terminal Pleistocene mass extinction

The mass extinction in the late Cenozoic of North America occurred at the end of the Rancholabrean Land Mammal Age about 12,000 -10,000 radiocarbon yrs BP (Kurten and Anderson 1980, Martin and Steadman 1999, Bell et al. 2004, Woodburne 2004, Guthrie 2006).

Prior to the extinction, North American terrestrial mammals included species from most of the major late Cenozoic families and genera (Table 1.1). Throughout the Pleistocene Epoch, various species experienced extinction, but no single event is as notable as that at the end of the Rancholabrean Land Mammal Age (~200,000 – 10,000 yrs BP) which selectively affected larger species. Figure 1.1 demonstrates the trend in the disappearance of species grouped according to size and land mammal age. This early work estimated that over 50 species above 50 kg suffered extinction at the termination of the Pleistocene. Recent work by Barnosky et al. (2004) estimated the loss at 33 genera all over 44 kg. Temporal ranges of the most representative large species in the late Pliocene and Pleistocene Epochs suggest that

Table 1.1 – Faunal list of common late Cenozoic mammals in North America

	Family	**Genus**	**Common name**
Metatheria	Didelphidae	*Didelphis*	opossum
Insectivora	Soricidae	*Sorex*	shrew
		Microsorex	shrew
		Planisorex	shrew
		Paracryptotis	shrew
		Cryptosis	shrew
		Blarina	shrew
		Notiosorex	shrew
	Talpidae	*Condylura*	mole
		Parascalops	mole
		Scapanus	mole
		Scalopus	mole
Chiroptera	Mormoopidae	*Mormoops*	bat
	Phyllostomatidae	*Leptonycteris*	bat
		Desmodus	bat
	Vespertilionidae	*Myotis*	bat
		Lasionycteris	bat
		Pipistrellus	bat
		Eptesicus	bat
		Histiosus	bat
		Lasiurus	bat
		Nycticeius	bat
		Plecotus	bat
		Antrozous	bat
		Anzanycteris	bat
	Molossidae	*Tadarida*	bat
		Eumops	bat
Edentata	Dasypodidae	*Kraglievichia*	armadillo
		Dasypus	armadillo
	Pampatheriidae	*Pampatherium*	pampathere, southern
		Holmesina	pampathere, northern
	Glyptodontidae	*Glyptotherium*	glyptodon
	Megalonychidae	*Megalonyx*	sloth, Jefferson's ground
	Megatheriidae	*Eremotherium*	sloth, ground
		Nothrotheriops	sloth, Shasta ground
	Mylodontidae	*Glossotherium*	sloth, ground
Carnivora	Mustelidae	*Martes*	marten/fisher
		Tisisthenes	weasel
		Gulo	wolverine
		Ferinestrix	
		Trigonictis	weasel
		Sminthosinus	weasel
		Canimartes	waesel

		Taxidea	badger
		Satherium	otter
		Lutra	otter
		Enhydriodon	otter
		Enhydra	otter
		Buisnictis	skunk
		Spilogale	skunk
		Brachyprotoma	skunk
		Osmotherium	skunk
		Mephitis	skunk
		Conepatus	skunk
		Mustela	mink
	Canidae	*Borophagus*	dog/wolf
		Canis	dog/wolf
		Cuon	dhole
		Protocyon	fox
		Urocyon	fox
		Vulpes	fox
		Alopex	
	Procyonidae	*Procyon*	raccoon
		Bassariscus	ring-tailed "cat"
		Parailurus	panda
	Ursidae	*Tremarctos*	bear, spectacled
		Arctodus	bear, short-faced
		Ursus	bear
	Felidae	*Megantereon*	cat
		Smilodon	cat, sabertooth
		Ischyrosmilus	
		Homotherium	cat, scimitar
		Dinofelis	cat, false sabertooth
		Panthera	lion
		Acinonyx	cheetah
		Miracinonyx	cheetah
		Felis	cat
		Lynx	lynx/bobcat
	Hyaenidae	*Chasmaporthetes*	hyaena
Rodentia	Aplodontidae	*Aplodintia*	beaver, mountain
	Sciuridae	*Paenemarmota*	marmot,giant
		Marmota	marmot
		Spermophilus	squirrel, ground arctic
		Ammospermophilus	squirrel, antelope ground
		Cynomys	praririe dog
		Tamias	chipmunk
		Eutamias	chipmunk
		Sciurus	squirrel, tree
		Tamiasciurus	squirrel

	Cryotopterus	
	Glaucomys	squirrel, flying
Geomyidae	*Thomomys*	gopher, pocket
	Pliogeomys	gopher
	Merterogeomys	gopher
	Geomys	gopher
	Cratogeomys	gopher
	Heterogeomys	gopher
Heteromyidae	*Prodipodomys*	
	Etadonomys	
	Dipodomys	rat, kangaroo
	Perognathus	mouse, pocket
	Liomys	
Castoridae	*Dipoides*	beaver
	Paradipoides	beaver
	Procastoroides	beaver
	Castoroides	beaver, giant
	Castor	beaver
Cricetidae	*Atopomys*	vole
	Baiomys	mouse, pygmy
	Bensonomys	mouse
	Clethrionomys	vole, Gapper's red-backed
	Cosomys	
	Dicrostonyx	lemming
	Lagurus	vole
	Lemmus	lemming, brown
	Microtus	vole
	Mimomys	vole
	Nebraskomys	vole
	Neofiber	vole
	Neotoma	rat, wood
	Ochrotomys	mouse, golden
	Ogmodontomys	
	Ondatra	muskrat
	Onychomys	mouse, grasshopper
	Ophiomys	rat
	Oryzomys	rat, rice
	Peromyscus	mouse, deer
	Phenacomys	vole, heather
	Pliolemmus	vole
	Pliomys	vole
	Pliophenacomys	vole
	Pliopotamys	muskrat, pygmy
	Predicrostonyx	lemming
	Proneofiber	vole
	Reithrodontomys	mouse, western harvest

		Sigmodon	rat, cotton
		Symmetrodontomys	mouse
		Synaptomys	lemming, bog
	Zapodidae	*Zapus*	mouse, jumping
		Napaeozapus	mouse, woodland jumping
	Erethizontidae	*Coendou*	porcupine
		Erethizon	porcupine
	Hydrochoeridae	*Hydrochoerus*	capybara
		Neochoerus	capybara
Lagomorpha	Ochotonidae	*Ochotona*	pika
	Leporidae	*Hyoplagus*	hare/rabbit
		Notolagus	hare/rabbit
		Pratilepus	hare/rabbit
		Aluralagus	hare/rabbit
		Brachylagus	hare/rabbit
		Nekrolagus	hare/rabbit
		Sylvilagus	rabbit
		Lepus	hare/jackrabbit
Perissodactyla	Equidae	*Nannippus*	horse
		Equus	horse
	Tapiridae	*Tapirus*	tapir
Artiodactyla	Tayassuidae	*Mylohyus*	peccary
		Platygonus	peccary
	Camelidae	*Titanotylopus*	camel
		Blancocamelus	llama
		Megatylopus	camel
		Camelops	camel
		Hemiauchenia	llama
		Palaeolama	llama
	Cervidae	*Bretzia*	deer
		Odocoileus	deer, mule and white-tail
		Blastocerus	deer, marsh
		Navahoceros	deer, mountain
		Sangamona	deer
		Rangifer	caribou/reindeer
		Alces	moose
		Cervalces	moose, stag
		Cervus	elk
	Antilocapridae	*Ceratomeryx*	pronghorn
		Tetrameryx	pronghorn
		Hayoceros	pronghorn
		Stockoceros	pronghorn
		Antilocapra	pronghorn
		Capromeryx	pronghorn
	Bovidae	*Saiga*	antelope, saiga
		Oreamnos	goat, Rocky Mountain

		Capra	goat
		Ovis	sheep, bighorn
		Euceratherium	shrub-ox
		Soergelia	muskox
		Bootherium	muskox, woodland
		Praeovibos	muckox
		Ovibos	muskox, tundra
		Bison	bison
		Platycerabos	
		Bos	cow
Proboscidea	Mammutidae	*Mammut*	mastodon
	Gomphotheriidae	*Phynchotherium*	gomphothere
		Stegomastodon	gomphothere
		Cuvieronius	gomphothere
	Elephantidae	*Mammuthus*	mammoth
Primates	Hominidae	*Homo*	human

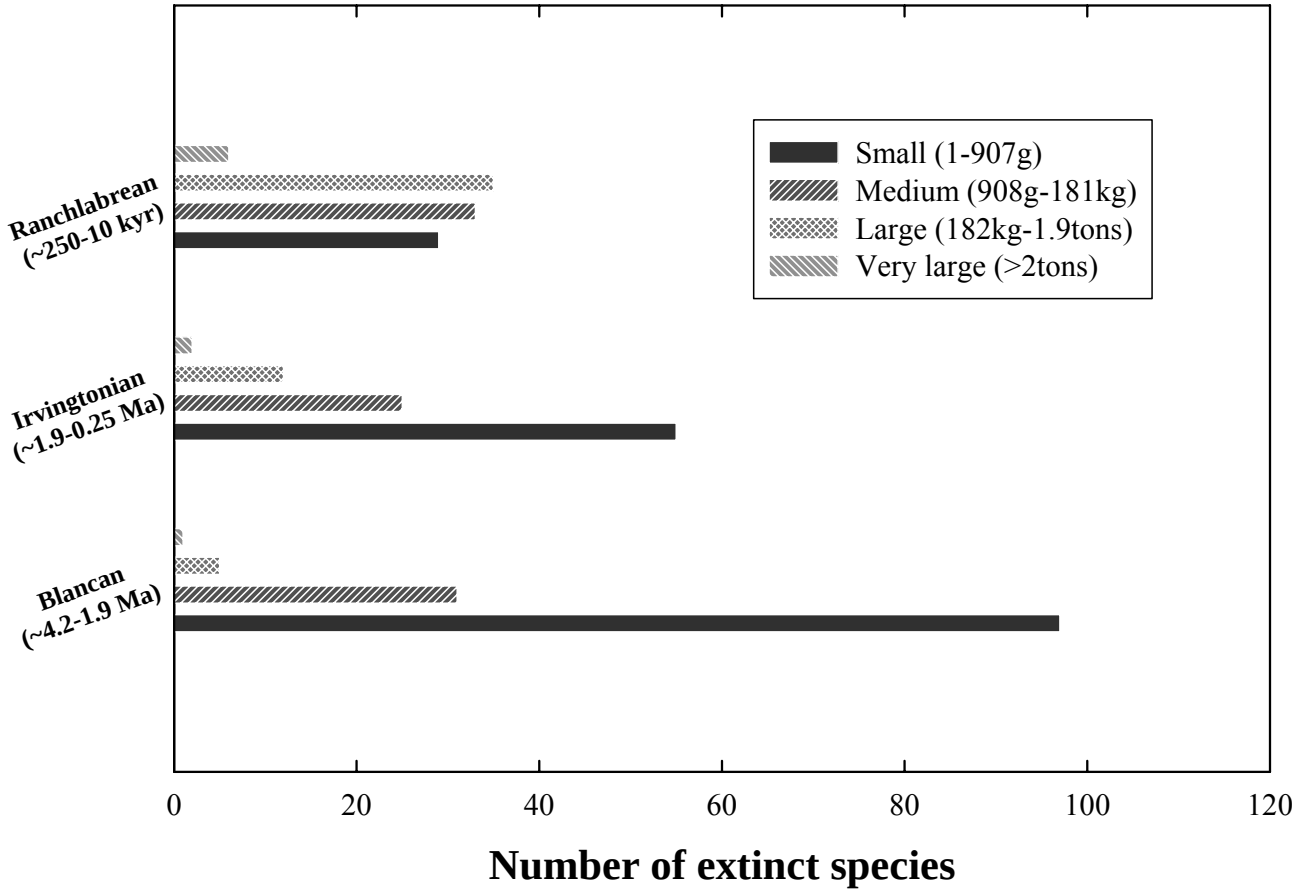


Figure 1.1 – Number of extinct Pleistocene species according to size and time period. Data compiled from Kurten and Anderson (1980), and Lyons et al. (2004).

only a relatively small number experienced extinction prior to the Rancholabrean (Figure 1.2). Examination of the Pleistocene-Holocene boundary in detail reveals extinctions in all major families and genera containing large mammals (Figure 1.3).

1.2.1 Causes

The end Pleistocene extinction event is most generally attributed to two potential causes. Major climate change at the end of the Pleistocene is often considered a contributing factor to the reorganization of ecosystems and the demise of the North American mammalian fauna. Concurrent to climatic changes, these animals were facing the arrival of humans on the continent and subsequent hunting pressures. Overkill of this fauna is often suggested as a second hypothesis for the cause of extinction. A third potential, albeit far-fetched, extinction mechanism is the presence of a "hyper-disease" that could kill multiple large species. This possibility, however, has little scientific merit and will not be considered further in this study (Lyons et al. 2004).

1.2.1.1 Climate change

Climate change as a valid hypothesis for the end Pleistocene extinction is supported by the record of extreme climatic fluctuation at this time. The late Cenozoic was a time of extreme glacial activity with repeated glacial-interglacial cycles spanning the last ~8 million years (Zachos et al. 2001). Figure 1.4 outlines these fluctuations for the last 200,000 years. Although different climate proxies vary slightly in the timing and magnitude of climatic changes, notably rapid transitions

Figure 1.2 (following page) – Temporal ranges of a representative set of species from the North American Pleistocene. Reproduced after Kurten and Anderson (1980) and Bell et al. (2004).

Figure 1.3 (following pages) – Temporal ranges of a representative set of species from the North American Rancholabrean land mammal age (Late Pleistocene). Reproduced after Kurten and Anderson (1980) and Bell et al. (2004).

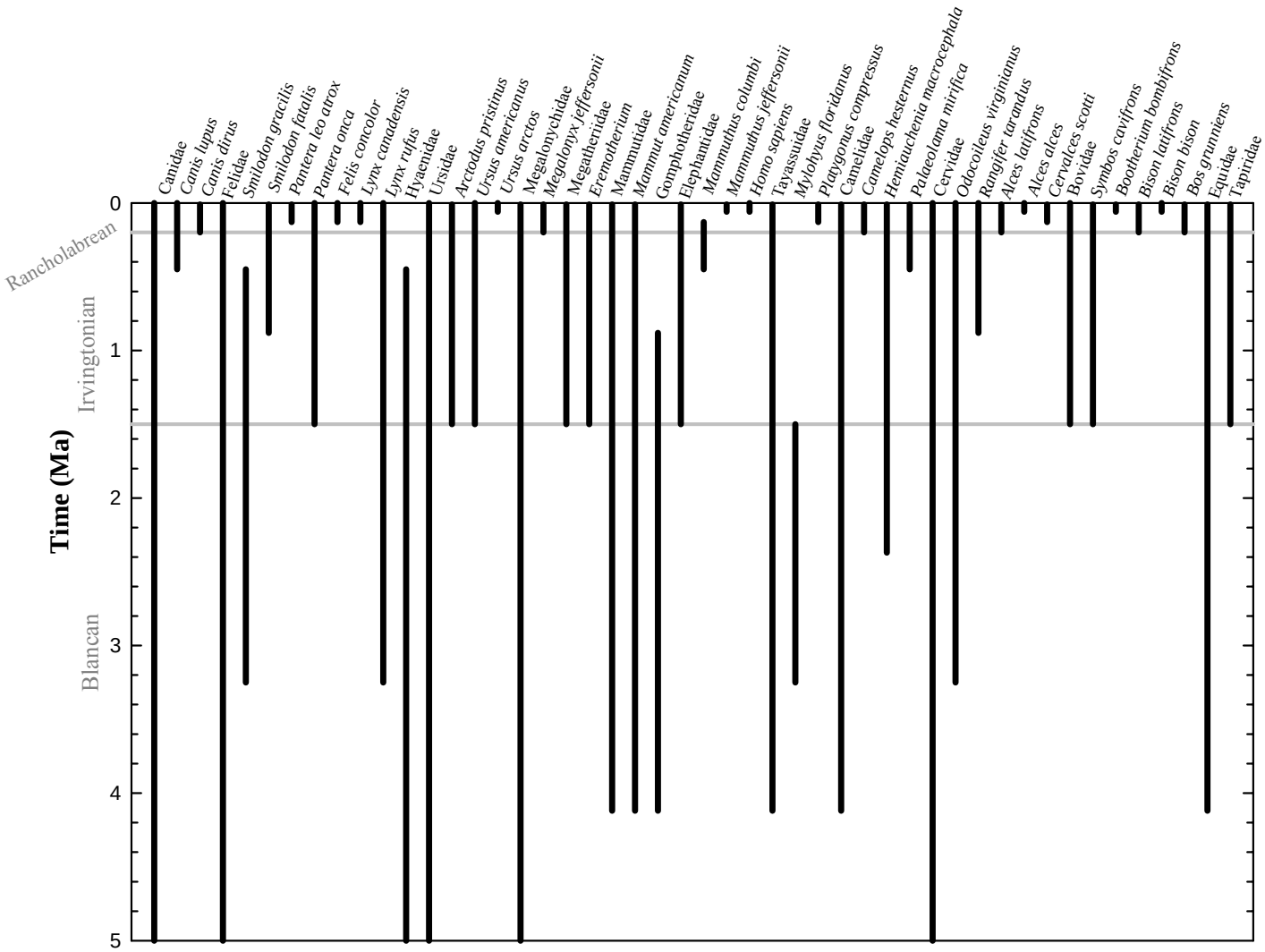
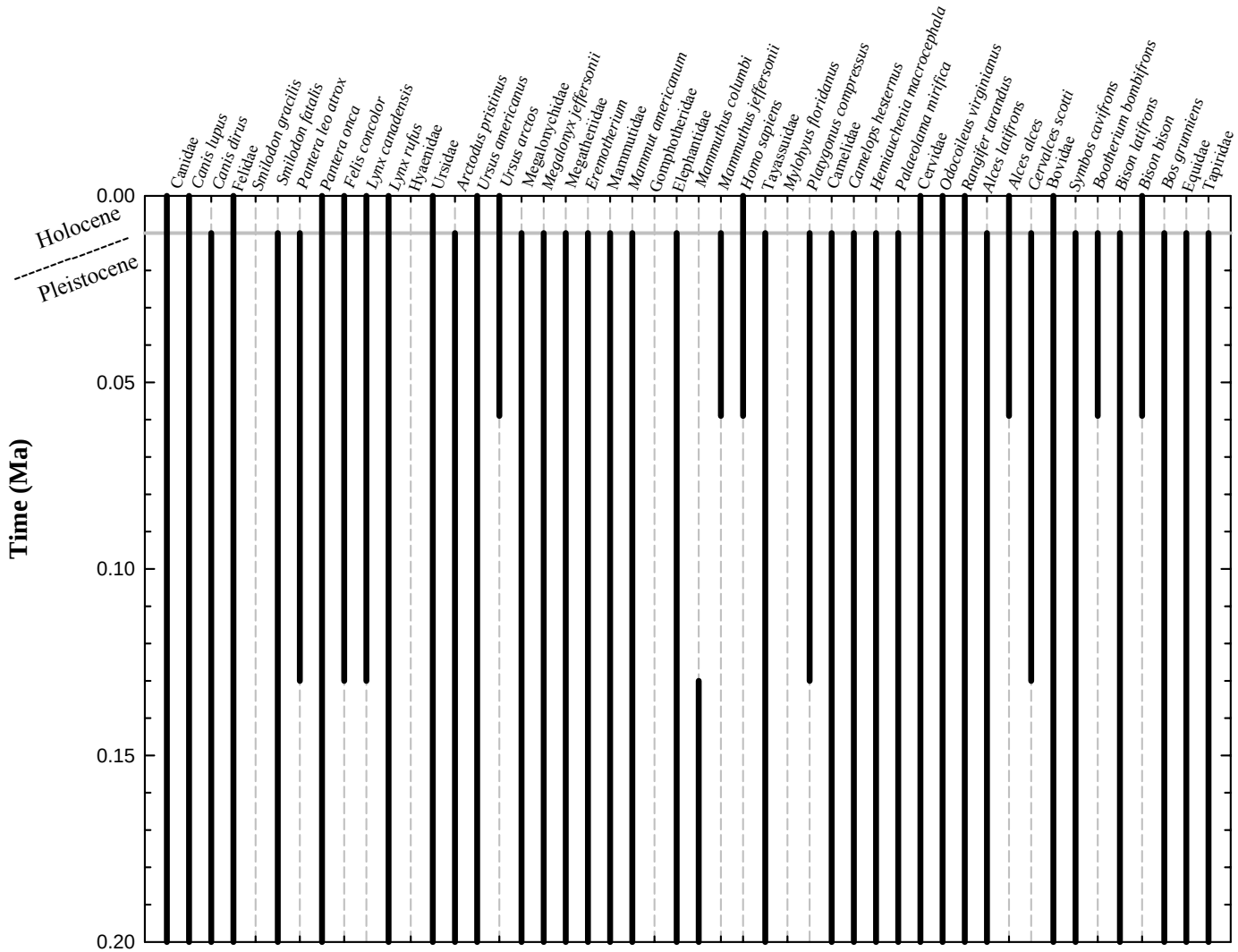

Canidae
Canis lupus
Canis dirus
Felidae
Smilodon gracilis
Smilodon fatalis
Pantera leo atrox
Pantera onca
Felis concolor
Lynx canadensis
Lynx rufus
Hyaenidae
Ursidae
Arctodus pristinus
Ursus americanus
Ursus arctos
Megalonychidae
Megalonyx jeffersonii
Megatheriidae
Eremotherium
Mammutidae
Mammut americanum
Gomphotheridae
Elephantidae
Mammuthus columbi
Mammuthus jeffersonii
Homo sapiens
Tayassuidae
Mylohyus floridanus
Platygonus compressus
Camelidae
Camelops hesternus
Hemiauchenia macrocephala
Palaeolama mirifica
Cervidae
Odocoileus virginianus
Rangifer tarandus
Alces latifrons
Alces alces
Cervalces scotti
Bovidae
Symbos cavifrons
Bootherium bombifrons
Bison latifrons
Bison bison
Bos grunniens
Equidae
Tapiridae
Rancholabrean
Irvingtonian
Blancan
Time (Ma)
0
1
2
3
4
5

Figure 2


Canidae
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Pantera onca
Felis concolor
Lynx canadensis
Lynx rufus
Hyaenidae
Ursidae
Arctodus pristinus
Ursus americanus
Ursus arctos
Megalonychidae
Megalonyx jeffersonii
Megatheriidae
Eremotherium
Mammutidae
Mammut americanum
Gomphotheridae
Elephantidae
Mammuthus columbi
Homo sapiens
Tayassuidae
Mylohyus floridanus
Platygonus compressus
Camelidae
Camelops hesternus
Hemiauchenia macrocephala
Palaeolama mirifica
Cervidae
Odocoileus virginianus
Rangifer tarandus
Alces latifrons
Alces alces
Cervalces scotti
Bovidae
Symbos cavifrons
Bootherium bombifrons
Bison latifrons
Bison bison
Bos grunniens
Equidae
Tapiridae
Holocene
Pleistocene
Time (Ma)
0.00
0.05
0.10
0.15
0.20

Figure 3

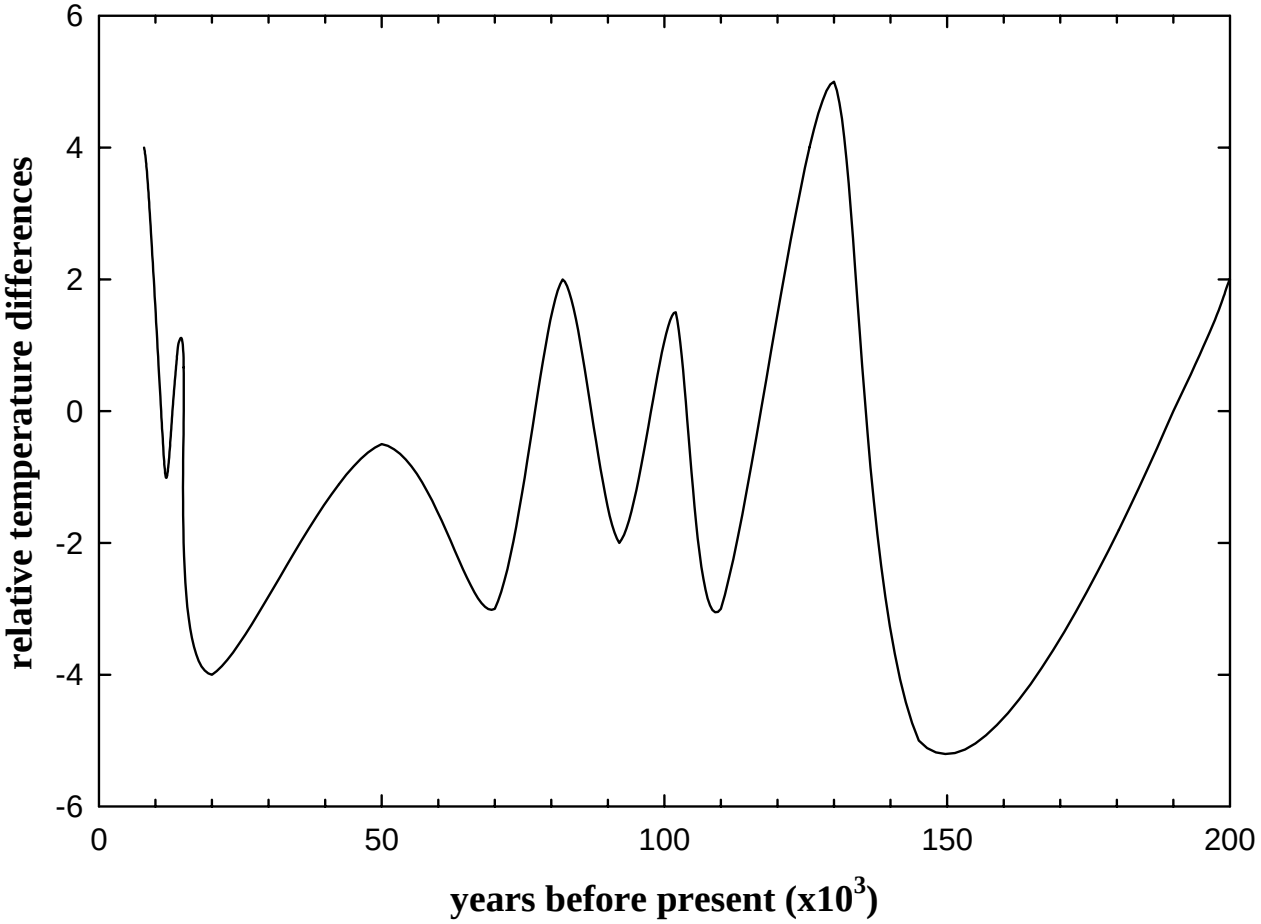

Figure 1.4 – Relative temperature fluctuations in the late Pleistocene. Data compiled from Heusser et al. (1980), Lorius et al. (1985), Dansgaard et al. (1993), Petit et al. (1999), Nordt et al. (2007).

between glacial and interglacial cycles leading up to 10,000 yrs BP are ubiquitous. The Last Glacial Maximum at ~18,000 yrs BP preceded the Bolling-Allerod, a brief period of relative warmth from ~15,000-13,000 yrs BP; this climatic optimum was followed by the Younger Dryas cooling event which lasted until ~11,000 yrs BP (Paterson and Hammer 1987, Dansgaard et al. 1993, Severinghaus and Brook 1999, Monnin et al. 2001, Nordt et al. 2007). The Younger Dryas then rapidly transitioned into the warmer interglacial that North America experiences today. The temperature shift of ~5-9ºC associated with this recent interglacial is suspected to be a comparable shift to previous thermal cycles, including a shift ~150,000 yrs BP from an interglacial to glacial climate (Lorius et al. 1985, Dansgaard et al. 1993, Petit et al. 1999, Severinghaus and Brook 1999, Nordt et al. 2007). A concurrent shift in pressure, winds, and precipitation thus resulted in wetter conditions in the west and east coast, with drier conditions in the central continent (Heusser et al. 1985, Kutzbach 1987, Webb 1987, Anderson 1988, Webb 1992, Jennings 1996, Shuman et al. 2002).

As a result of the climatic shifts, major changes in vegetation, and therefore in the food base of these animals, is also apparent in the late Pleistocene. The combination of warming temperatures and increased precipitation in the southeast of North America resulted in a shift from dry-tolerant pines, hemlock, and beech mixed woodland to moist-tolerant pines and oak of a warm mixed forest (Webb et al. 1987, Shuman et al. 2002, Webb at al. 2004). The northeast regions transitioned from dry-tolerant pine, sedge, and spruce taiga/cool mixed forests to beech, hemlock, birch, hickory, and oak temperate mixed and deciduous forests (Jacobson et al. 1987, Webb

et al. 1987, Shuman et al. 2002, Webb et al. 2004). Uncertainty exists as to the range of extensive open grasslands on the northeast coast of North America in the late Pleistocene, but evidence suggests such regions were absent north of the Carolinas and at least some tree cover was present along the entire east coast during the Pleistocene-Holocene transition (Williams et al. 2000, Webb et al. 2004, Dreise et al. 2005). The southwest coast experienced a shift from a diverse flora including pines, firs, cypresses, oak, junipers, laurels, and shrub understory to a system with a higher predominance of pine (Chaney and Mason 1934, Mason 1944, Jennings 1999). This area essentially remained a temperate deciduous and conifer forest, but now transitions into desert regions at the border of California, Nevada, and Arizona. The northwest coast shifted from drier steppe-like conditions with sagebrush, saltbrush, and some oak and mahogany trees to conifer forests (Barnosky et al. 1987, Thompson and Anderson 2000, Thompson et al. 2004). The drier conditions in the central continent resulted in replacement of forested areas with more open grasslands and prairie forbs with patchy areas of trees and small forests (Axelrod 1985, Webb et al. 1987).

Links between climate change and extinction have been made for both modern and Pleistocene ecosystems. The subsequent changes in habitat and food base that accompany climate change could have occurred at a rate which outpaced evolutionary adaptation. A mismatch between an organism and its environment could develop to the point where the animal suffers death and its community faces extinction. Evidence of shifting biomes was recognized at the end of the Pleistocene (Graham and Mead 1987, Graham 1992, Guthrie 2006). Changes in species densities,

ranges, and outright extinctions are noticeable in the Pleistocene paleontologic record within 100-1000 years of initial global warming (Barnosky et al. 2003). This pattern mimics modern systems that are facing similar ecologic variations as a result of climate change (Petchey et al. 1999, Walther et al. 2002, Post and Forchhammer 2004). The correlation between temperature variation and faunal diversity and range implicates the glacial-interglacial transitional event as the driving force of extinction for many species.

The argument against the climate change hypothesis focuses on the timing of extinctions in other global locations, as well as the lack of extinction during previous glacial-interglacial transitions. The European record suggests that the end Pleistocene extinction of large mammals was not as severe as that observed in North America with slightly fewer species lost (Stuart 1999). This region as well as the Middle East also began to experience marked extinctions at about 50,000 yrs BP which were then staggered as the ecosystem approached 10,000 yrs BP; a start time which is significantly earlier than the North American pattern (Tchernov 1984, Stuart 1999). In Australia, the majority of Pleistocene megafaunal extinctions occurred before 15,000 yrs BP with many last occurrence dates of 45,000 yrs BP or earlier (Martin and Steadman 1999, Miller et al. 2005, Prideaux et al. 2007). In China and southeast Asia, the larger mammalian fauna disappeared around 20,000 yrs BP (Liu and Li 1984). In Africa marked extinctions appeared as early as 2 Ma with various groups disappearing at different times leading up to the Pleistocene-Holocene transition and some groups persisting beyond that boundary (Klein 1984, Martin and Steadman 1999). The South American timing pattern seems to mimic that of North America

with the major pulse of extinction occurring at the very end of the Pleistocene (Martin and Steadman 1999). Alternately, certain islands such as Wrangel Island in arctic Siberia, the West Indian Islands, and Madagascar maintained some of the typical Pleistocene megafauna after 10,000 yrs BP (Dewar 1984, Martin and Steadman 1999, Steadman et al. 2005). Essentially the argument against the climate-driven extinction is that these patterns mimic the arrival of humans, or the transition of human culture to a more advanced state, in each major global geographic region rather than consistently correlating with climate change. Few explanations have been offered to explain this other than the human over-hunting scenario, which is discussed in the following section.

1.2.1.2 Human predation and overkill

Support for the hypothesis that humans over-hunted mammals to the point of extinction lies in the timing of human arrival on North America and the evidence of large animal kills. The first appearance of humans in North America has been dated at ~18,000-14,000 yrs BP with some more tenuous suggestions of a presence as early as ~40,000 yrs BP (West 1983, Bonnichsen et al. 1987, Adovasio and Pedler 2004, Fedje et al. 2004, Madsen 2004, Meltzer 2004, Sarnthein et al. 2006). These sources also suggest that, once present, humans had spread across the entire continent by ~12,500 yrs BP and possibly even earlier. Kill sites, waste burial sites, and single mammal fossils with weapon wounds suggest that large herbivores, such as proboscideans, equids, bison, and cervids were in fact the most commonly hunted species with some dietary supplementation by smaller game (Speiss et al. 1985,

Bonnichsen et al. 1987, Dunbar 1991, Frison 1991, Johnson 1991, Willig 1991, Hofman and Todd 2001, Meltzer 2004). An increase in large game hunting towards the Pleistocene-Holocene boundary is also suggested by the noticeable proliferation of early hunting weapons with a triangular fluted blade that is well suited for spears and larger kills (Bonnichsen et al. 1987, Willig 1991).

Although humans were hunting mammals just prior to the end Pleistocene extinction event, there is some question as to their ability to completely eradicate an entire fauna. Some models suggest that the population sizes, spreading rate, hunting rate, and nutritive demands of early humans were great enough to drive the extinctions (Martin 1982, Whittington and Dyke 1984, Choquenot and Bowman 1998, Alroy 2001). Rather than completely killing all the animals themselves, it has also been suggested that humans over-hunted one or two keystone species and/or contributed to significant habitat destruction through agricultural practices, thus instigating a collapse of the entire ecosystem (Owen-Smith 1987). The keystone species concept focuses specifically on the mammoths and mastodons. Elephants, the extant counter parts of *Mammut* and *Mammuthus*, are known to impact the types of vegetation present in an area due to their grazing habits and locomotion (Dublin et al. 1990, Plumptre 1993, Ben-Shahar 1998, Augustine and McNaughton 2004, Sheil and Salin 2004, Birkett and Stevens-Wood 2005). The habitat and food source of smaller animals is then heavily affected by the presence or absence of proboscids, thereby making the latter group the "key" controlling factor in the ecosystem of other animals. Additionally, modeling suggests that basal herbivores with a high frequency of connectedness to other species in the food web are likely to serve as keystone

species and instigate multiple secondary extinctions upon their removal from the web (Quince et al. 2005). Mammoths and mastodons may fit this description, thus their extinction could trigger an ecosystem collapse. Proboscids are thought to be especially vulnerable to over-hunting due to their high nutritive value, long gestation periods, and frequent single offspring births resulting in slow recovery from any type of population decline (Haynes 1991). Alternately, an ecosystem collapse may have been triggered by habitat destruction such as human-induced fires or farming. However, the archaeologic evidence does not currently support the widespread occurrence of either of these two practices until after the Pleistocene-Holocene transition (Schule 1990, Woodcock and Wells 1994, Lyons et al. 2004, Erickson et al. 2005, Smith 2005).

The argument against the human overkill hypothesis focuses on the inherent assumptions that must be made in order to construct simulation models. Values for late Pleistocene human population size and density are based on incomplete archaeologic evidence. The number of potential prey killed is based on estimates only of the nutritive value of a given prey item and the hunting efficiency necessary to kill it. The prey populations are estimated by a paleontologic record which is also incomplete. Some modern analogs can be used, such as the extant fauna of Africa, but the usefulness of these examples is hindered by the different climates and surrounding ecosystems of the two regions. Ultimately, over-hunting simulation models will always be plagued by an inability to verify assumptions inherent in the model.

1.3 Ecosystem collapse theory

In this scenario, extinction is fundamentally a result of ecosystem collapse; the patterns of which can vary greatly. An ecosystem can collapse starting from the top carnivores and filtering downwards to the basal herbivores (i.e. "top-down"). Likewise a collapse can start with the basal herbivores and filter upwards to the apex carnivores (i.e. "bottom-up). Other mitigating factors may play a role as well, such as stabilization of an ecosystem by the presence of omnivores or high biodiversity. Ultimately, identification of the pattern of ecosystem collapse may provide clues to the root cause.

A bottom-up collapse is usually instigated by factors affecting the top of the food chain (i.e. "top-down effects"). Specifically, the top carnivores can exert considerable influence over the basal herbivores. This predator regulation can simply maintain a baseline herbivore biomass without significant increases or decreases, or result in the extreme depletion of herbivore populations if predators are in high abundance and hunting is rampant (Erlinge et al. 1984, Schmitz et al. 1997, Crooks and Soule 1999, Terborgh et al. 1999, Schmitz et al. 2000, Finke and Denno 2004). Similarly, the behavioral response of herbivores to the mere presence of carnivores may alter prey feeding time or range, thus also limiting their population numbers (Schmitz et al. 1997, Schmitz 1998). Alternately, herbivore populations may explode and severely deplete the flora in the partial or complete absence of predators (Bock at al. 1992, Marquis and Whelan 1994, Gutierrez et al. 1997, Terborgh et al. 1999, Halaj and Wise 2001, Terborgh et al. 2001). In either scenario, predators are critical to maintaining a stable herbivore biomass. If predator populations either exceed or are

reduced from their normal population abundances, the ecosystem will lose stability in the lower parts of the food chain and collapse from the bottom-up (Pimm 1980, Quince et al. 2005). Mitigating factors in this theory include variations in predator diversity and prey susceptibility. A high diversity of predator species is thought to dampen the top-down effects as predators are now competing with and serving as prey for one another, thus eliminating some of the pressure on herbivores (Sih et al. 1998, Pace et al. 1999, Finke and Denno 2004). Also, predation does not affect all herbivores to the same degree as some have avoidance mechanisms that limit their contact with predators.

A top-down collapse is usually instigated by factors affecting the bottom of the food chain (i.e. "bottom-up effects"). Disturbances in the ecosystem can alter floral composition and reduce floral biomass such that herbivores are negatively affected. The prey base for carnivores is thereby reduced or completely deleted, thus resulting in instability at the top of the food chain and ultimate top-down collapse (Petchey 2000, Duffy 2002, Quince et al. 2005). Disturbances of this nature include drastic climate change, fire, drought, natural disasters, disease, floral invasions, over-harvesting or other human mediated habitat destruction. Herbivores with a higher competitive ability have a greater chance to withstand and recover from a disturbance (Melian and Bascompte 2002). The ability to disperse widely and seek new habitats and food sources also promotes survival (Paine et al. 1998). Finally, survivorship appears to be higher for species that have short gestation periods, short generation times, and do not limit their breeding sites (Paine et al. 1998, Vermeij 2004).

It should be noted that ecosystem disturbance is not always a negative factor. Some disturbances result in increased floral biomass and a flourishing of herbivore populations. Carnivores will benefit from the increased prey numbers. However, if the disturbance instigates a floral biomass increase that is only temporary, the system may eventually crash and faunal distributions return to pre-disturbance levels. Disturbances may also affect carnivores directly thereby resulting in a top-down effect and a subsequent bottom-up collapse (Wootton 1998). Some disturbances occur on a regular basis in certain ecosystems and do not result in long term effects on the fauna, while others actually increase faunal diversity by reducing competitive exclusion (Paine et al. 1998, Wootton 1998, Kondoh 2001, Scheffer et al. 2001).

Ecosystem traits that offer resistance to trophic collapse include high biodiversity, few linkages between species, and presence of omnivory. Both computer modeling of food webs and field studies suggest that high biodiversity with multiple species sharing a trophic level buffers the effects of a species deletion on any other single species (Tilman 1996, Naeem and Li 1997, Ives et al. 1999, Yachi and Loreau 1999, Borrvall et al. 2000, Ives et al. 2000, Chesson et al. 2002, Ebenman et al. 2004, Roopnarine 2006). This is often referred to as the 'insurance hypothesis' because high biodiversity insures that the loss of any one predator or prey will be replaced by another species that can occupy the same niche, and there is usually at least one species at each trophic position that can survive disturbances and environmental fluctuations. The ecosystem will never have any vacant roles unless multiple species experience extinction at the same time. Likewise, omnivores tend to stabilize ecosystems because they can serve multiple roles as herbivore, carnivore,

predator, and/or prey (Fagan 1997, Pace et al. 1999, Borrvall et al. 2000, Melian and Bascompte 2002). Finally, a species with few strong links to other members of the community is unlikely to cause secondary extinctions if it should disappear (Dunne et al. 2002).

While neither the top-down collapse nor bottom-up collapse scenarios seem to be favored in theoretical or field studies, they can be related to the late Pleistocene extinction event by matching the potential causes of extinction with their expected pattern of collapse. The climate-driven hypothesis of extinction dictates that climate change would reduce floral biomass and/or diversity. This is a bottom-up effect that would cause the most stress in the next highest level of the food chain – the herbivores. Destabilization would then start at the level of herbivores and work its way up the food chain. The human-driven hypothesis dictates that over-hunting of herbivores would severely reduce the prey biomass of carnivores, thus creating intense competition at the top of the food web. Destabilization and subsequent collapse would then begin at the top and filter down the food web. Therefore, locating the trophic positions in the late Pleistocene food webs that display a relatively high degree of competition will allow recognition of unstable areas of the food chain and subsequent determination of a causal mechanism.

1.4 Biogeochemical Theory

Examination of chemical clues in the well-preserved bones of late Pleistocene mammals provided the evidence necessary to address several questions surrounding

this fauna. Carbon and nitrogen stable isotopes from bone collagen were used to reconstruct trophic positions. This provided evidence for areas of competition within the ecosystem and served as a means to address the hypotheses for the end Pleistocene extinction. Additionally, evidence for unique juvenile and ruminant isotopic signatures was sought in an effort to confirm previous suggestions regarding the relative isotopic patterns of these sub-groups. Finally, diagenesis within individually reconstructed food chains and throughout time was examined.

1.4.1 Bone collagen

Bone collagen is an ideal substance from which to extract isotopic data due to its strong and stable nature. It is a triple-helix protein wherein the helices are cross-linked with hydrogen bonds. It is the primary structural protein of bone comprising about 90% of the organic material in bone. When combined with the inorganic apatite, collagen gives bone its strong solid nature. The cross-links and tight helices make collagen extremely resistant to weathering and breakdown, thus making it an ideal target for extraction from fossil bones. Collagen that is intact and not diagenetically altered can provide an original signature of nitrogen and carbon isotopes. Collagen itself is recycled approximately every 6-12 months within the bone, therefore providing an integrated look at an animal's isotopic signature just before its death (Katzenberg 1993, Fogel et al. 1997).

1.4.2 Nitrogen isotopes

Nitrogen isotopes from bone collagen provide the means to reconstruct trophic positions within a faunal assemblage. Of the two nitrogen isotopes, ^{14}N and ^{15}N, animals preferentially retain ^{15}N and excrete ^{14}N during the formation of urea (Sutoh et al. 1987). An animal's isotopic signature is therefore determined by what it eats minus what it excretes. Thus the heavier ^{15}N concentrates towards the top of the food chain. Isotopic values are measured using the standard delta notation:

$$\delta^{15}N = [(R_{sample}-R_{standard})/(R_{standard})]*1000$$

where $R=^{15}N/^{14}N$. Resulting units are parts per thousand or permil (‰), and the standard is atmospheric N_2. The $\delta^{15}N$ values from bone collagen exhibit a 3 to 4.2‰ enrichment between trophic levels (DeNiro and Epstein 1981, Minagawa and Wada 1984, Schoeninger and DeNiro 1984, Post 2002, Bocherens and Drucker 2003). This clear jump in values between steps of the food chain allows for the assignment of trophic positions. It is especially useful for determining which predators consumed both herbivores and other carnivores, as well as which animals may have been omnivores.

This technique was here applied to the end Pleistocene extinction by examining trophic levels showing extensive competition between mammals at three discrete sites in the United States by identifying regions of nitrogen isotopic overlap within their food chains. Figure 1.5 outlines the hypothetical changes expected in nitrogen isotopic patterns during two ecosystem collapse scenarios. In a top-down collapse, maximum overlap would be expected in the top carnivores. As their prey base is diminished by over-hunting, the carnivores begin to compete for food and

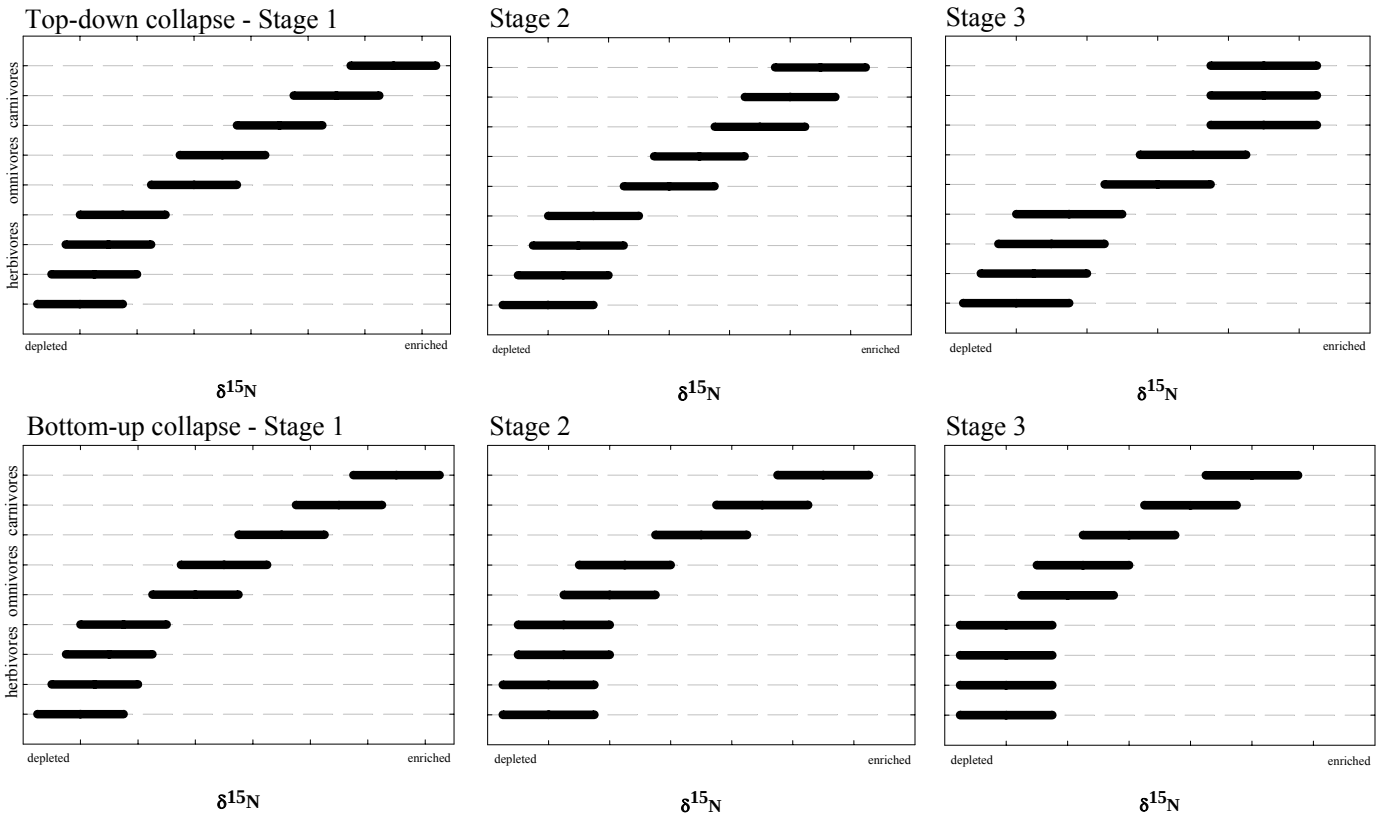

Figure 1.5 – Hypothetical scenario of top-down (top row) and bottom-up (bottom row) trophic collapse as reflected by nitrogen isotopes. Stage 1 represents a beginning stage with no competition, Stage 2 represents a mid-point in a time series with some competition in upper or lower levels of the trophic structure, Stage 3 represents the end point in a time series with intense competition at the upper or lower levels of the trophic structure.

their nitrogen isotopic signatures will overlap. In a bottom-up collapse, maximum competition and subsequent isotopic overlap would be expected at the base of the food chain (i.e. the herbivores and omnivores). It should be noted that the nitrogen isotopic signature of herbivores will overlap to some degree irregardless of competition due to the fact that are all feeding at the same trophic level. However, the carbon isotopic signatures can be used to further determine the presence or absence of competition within the herbivore trophic level (as discussed in section 1.4.3). Determination of the causal mechanism for the end Pleistocene extinction will depend on identifying one of these two patterns in the examined Pleistocene fauna.

Nitrogen isotopes from bone collagen are also useful for the identification of specific digestive strategies in mammals and the identification of juveniles. During rumination, a common digestive strategy in mammals, an animal relies heavily on microbial digestion of food in the stomach. Ruminants will often seek protein rich diets and digest both their food and their microbial symbiotes, whereas non-ruminants have a lower protein diet and rely less on microbial symbiosis (Stevens and Hume 1995). As such, rumination presents an additional step in the digestion process where nitrogen isotopic fractionation can occur. Studies of ancient ecosystems suggest that ruminants and non-ruminants tend to group together in terms of their nitrogen isotopes, but neither group currently appears to consistently show enrichment or depletion relative to the other (Bocherens et al. 1996, Coltrain et al. 2004). The production of mammalian milk presents another body process wherein nitrogen isotopes can fractionate. Modern and fossil systems indicate that juveniles often plot one trophic level higher than adults of the same species due to the nitrogen isotopic

enrichment in mammalian milk (Fogel et al. 1989, Katzenberg 1993, Bocherens et al. 1994a, Balasse et al. 1999).

1.4.3 Carbon isotopes

Carbon isotopes have the added benefit of providing information regarding different types of plant consumption. The C3 and C4 photosynthetic pathways of plants each produce characteristic isotopic signatures. The C3 plants, which consist of dicots, most trees, shrubs, herbaceous plants, and some grasses yield $\delta^{13}C$ values of ~-26.5 ± 3‰ (Smith and Epstein 1971, O'Leary 1988, Heaton 1999). The C4 plants, which consist of a specific subset of grasses and sedges, exhibit a $\delta^{13}C$ of ~-12.5 ± 3‰ (Smith and Epstein 1971, O'Leary 1988). The carbon isotopic difference between plants and herbivore bone collagen is ~2-5‰ (van der Merwe 1982, Balasse et al. 1999, Roth and Hobson 2000). This results in a $\delta^{13}C$ of ~-21.5‰ for browsers (herbivores that primarily consume trees, shrubs, and herbs that are virtually all C3). Grazers (herbivores that primarily consume grasses) will exhibit a $\delta^{13}C$ value that is indicative of the dominant type of grass in the local area. The presence of C4 variety grasses will result in a $\delta^{13}C$ of herbivore bone collagen of ~-7.5‰. An extremely low abundance or complete absence of C4 grasses will result in similar $\delta^{13}C$ signatures between browsers and grazers. While there is a certain amount of natural variation in these values, the C3 and C4 signatures are nonetheless distinct from one another. Therefore, carbon isotopic data are valuable for distinguishing both the presence of C4 grasses and the different herbivore niches within a food web that contains this specific subset of plants.

1.4.4 Examination of diagenesis

Although bone collagen has a high potential to preserve unaltered isotopic values, all ancient specimens have been exposed to potential diagenesis and must be examined for alteration during data analysis. Research on modern and fossil samples has noted that unaltered bone collagen has an atomic C:N ratio of 2.8-3.6 (DeNiro 1985, Ambrose 1990, Bocherens et al. 1996, Bocherens et al. 1997, Drucker et al. 2001, Drucker et al. 2003). The percent total carbon yields from collagen range from ~30-45%, and percent total nitrogen yields range from ~11-16% (Ambrose 1990, Bocherens et al. 1996, Bocherens et al. 1997, Drucker et al. 2003, Jorkov et al. 2007). These values are unique to bone collagen due to the unique pattern of amino acids in this protein. The relatively high amounts of proline and hydroxyproline amino acids in collagen contribute to its characteristic C:N ratio. Humic and fulvic acids, the most common products of organic degradation, are chemically and structurally variable with a much higher C:N ratio than collagen (Anderson et al. 1989, MacCarthy et al. 1990). Therefore, organic yield that has an atomic C:N ratio outside the range of 2.8-3.6 likely contains some humic components. Additionally, previous fossil studies have achieved a collagen yield of ~1-21% of the whole bone weight (Ambrose 1990, Bocherens et al. 1991, Bocherens et al. 1994a, Coltrain et al. 2004). Fossil specimens must be monitored for their C:N ratio, %C and %N in collagen extract, as well as overall yield of collagen and compared to modern bone data. Diagenesis may also be related to time elapsed and should be considered in respect to the age of individual specimens.

A secondary check of preservation quality can be accomplished through an amino acid analysis. The high levels of proline and hydroxyproline that are characteristic of collagen can be identified in a measurement of the relative abundances of the different amino acids in an organic extract. Theoretically, collagen should consist of about 30% glycine, 10% proline, 10% hydroxyproline, with the remaining fraction distributed among the other amino acids. Studies comparing fossil with modern specimens exhibit a range of glycine values from ~31-37 mol%, a range of proline values from ~8-14 mol%, and hydroxyproline values in the range ~7-11% (Ho 1965, DeNiro and Weiner 1988, Ostrom et al. 1994, Liden et al. 1995). A similar comparison of amino acid compositions between modern and fossil specimens in this study provided the final evidence to conclude that specimens with a C:N ratio in the appropriate range of ~2.8-3.6 were in fact well preserved collagen.

1.5 Concluding remarks

The primary goal of this study was to examine the late Pleistocene extinction of mammalian fauna in North America by reconstructing the trophic relationships of these animals. In so doing, the general food chain and distinct trophic position of each animal were determined with feeding specializations and dietary strategies becoming apparent in the nitrogen and carbon isotopic signatures from well preserved bone collagen. This detailed reconstruction provided a useful view of the overall ecosystem structure. It was then applied to late Pleistocene extinction in an attempt to determine if the trophic collapse followed a top-down or a bottom-up pattern. As

part of the chemical analyses, the diagenesis of organic material in the bone specimens was also examined and related to time of exposure to diagenetic mechanisms.

CHAPTER 2 – SAMPLES, METHODS, AND MATERIALS

2.1 Samples

Fossil specimens from three widely-separated locations were used to reconstruct food chains, and examine trophic level issues and the end Pleistocene extinction event. Samples from Saltville, Virginia, several sites in Florida, and one site in McKittrick, California cover a range of time periods, geologic environments, and genera from the Pleistocene Epoch. Figure 2.1 outlines the estimated age of each site and its placement in the North American Land Mammal Ages. Several modern specimens were used to monitor reproducibility of data and serve as a comparison to fossil samples.

2.1.1 Saltville, Virginia

The fossil bearing strata of the Saltville Valley are located in the southwest tip of Virginia (Figure 2.2). Sediments consist of loosely packed Quaternary alluvium composing eight different stratigraphic units ranging in age from upper Holocene to ~27,000 yrs BP. (McDonald 1984). The units are primarily fluvial and lacustrine in origin and suggest periodic transitions between moist swampy areas and drier fluvial systems as evidenced by cycles of peaty muds and silty-sandy clays. Palynology suggests the vegetation varied from sedge meadows and alder swamps to pine and spruce forests (Ray et al. 1967, McDonald 1984).

The majority of mammal remains are herbivores found in strata ranging in age from ~12 to 20 yrs BP. Animal remains include mostly large herbivores with some

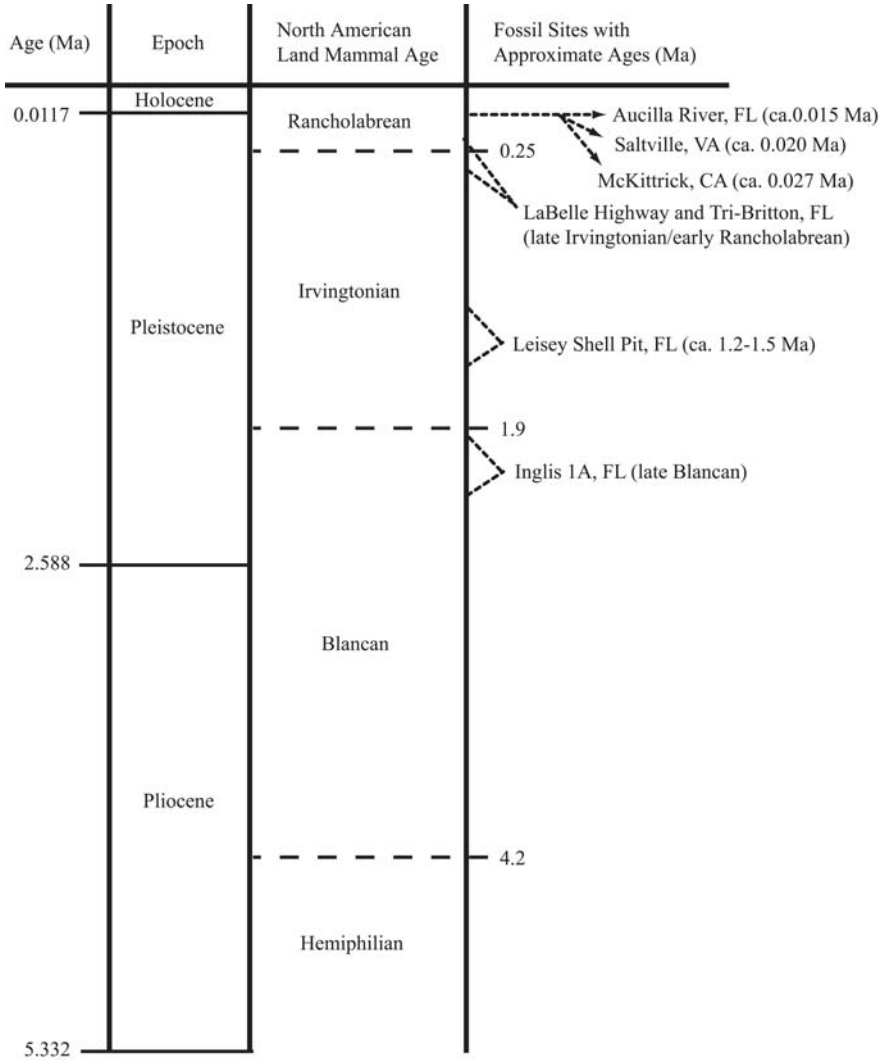

Figure 2.1 – Pliocene and Pleistocene timeline with North American Land Mammal Ages and fossil sites.

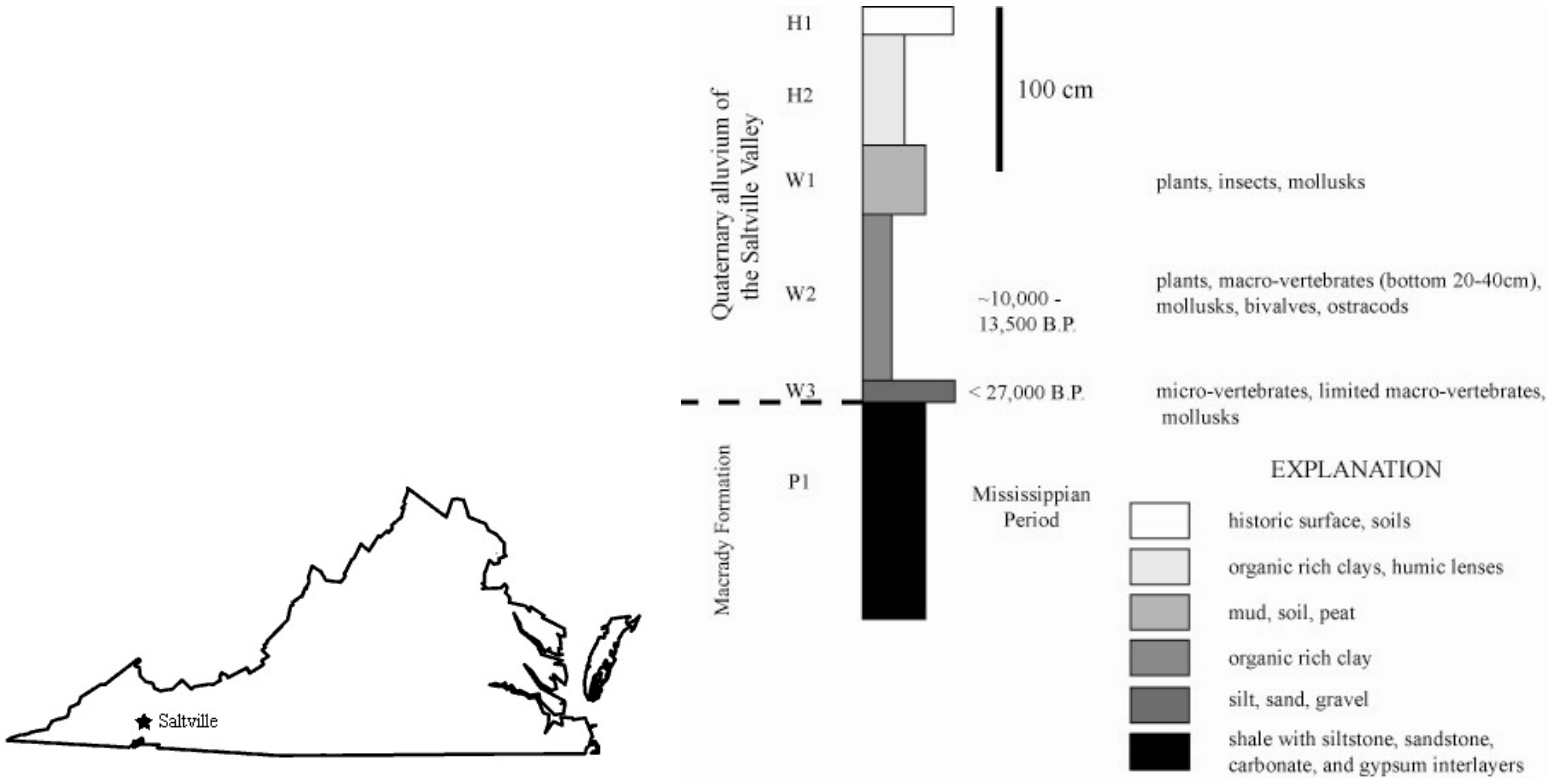

Figure 2.2 – Map of Virginia, USA showing the location of Saltville; simplified stratigraphic column of the Saltville Quarry sedimentology after McDonald (1984).

unidentified large carnivores (McDonald 1984). Samples for this study were obtained from the Smithsonian Museum of Natural History (NMNH) and the Virginia Museum of Natural History (VMNH) and are listed in Table 2.1. This fauna presents the opportunity to use carbon isotope distributions as indicators of the presence or absence of C4 grasslands as far north as Virginia. The presence of a mammoth juvenile can be used to look for a unique juvenile signature in nitrogen isotopes. Both ruminants and non-ruminants are present which will facilitate their placement in nitrogen isotopic space as well as the placement of a giant ground sloth, a mammal with an unknown digestive strategy.

In addition to mammal specimens, two sediment samples were collected. Sediment sample VMNH-A was collected directly from one of the primary sampling sites in the quarry. Sample VMNH-B was obtained from sediment removed from a mammoth tooth upon preparation of the sample. These samples provide an opportunity to examine the carbon isotopic composition of the sediment organic fraction in an effort to estimate the dominant flora available for consumption.

2.1.2 Florida

Numerous sites in Florida spanning a range of Pleistocene ages have yielded a plethora of fossil specimens (Figure 2.3). This study will focus on the following sites: Inglis 1A, Leisey Shell Pit 1A, LaBelle Highway, Tri-Britton, and Aucilla River. These sites lie in formations consisting of siliciclastic, mixed siliciclastic-carbonate, and carbonate lithologies indicative of alternating coastal terrestrial and nearshore marine environments (DuBar 1974, Zullo and Harris 1992). The Inglis 1A

Table 2.1 – Saltville, Virginia sample list

Genus/species	Common name	[a]Collection	Museum designation	Body element
Bison	bison	NMNH	8070	tooth with root
Bootherium	musk ox	NMNH	23264	cranium
Bootherium	musk ox	NMNH	364320	metacarpal
Bootherium	musk ox	NMNH	none listed	scapula
Bootherium	musk ox	VMNH	2349	mandible
Bootherium	musk ox	VMNH	10N5E	scapula (?)
Bootherium	musk ox	VMNH	59W-25S-SV2	metacarpal
Bootherium	musk ox	VMNH	92-58W-21S-1	rib
Bootherium	musk ox	VMNH	92-61N-20S-1	rib
Bootherium	musk ox	VMNH	92-61N-20S-2	rib
Bootherium	musk ox	VMNH	92-62W-25S-1	astragalus
Bootherium	musk ox	VMNH	92-63W-23S-8	ball joint
Bootherium	musk ox	VMNH	93-26S-55W-7	cranial element
Bootherium	musk ox	VMNH	95-51W-19S	rib
Bootherium	musk ox	VMNH	95-54W-25S-1	rib
Bootherium	musk ox	VMNH	95-54W-25S-2	rib
Bootherium ("Symbos cavifrons")	musk ox	NMNH	23705	cervical
Cervalces	moose	NMNH	23704	calcaneum
Cervalces	moose	NMNH	23750	antler
Equus	horse	NMNH	23703	femur
Equus	horse	NMNH	66-R-S-1-45	ankle
Equus	horse	VMNH	2356	longbone
Mammut	mastodon	VMNH	55W-27S (#038)	ankle element
Mammut	mastodon	VMNH	none listed	atlas
Mammut americanum	mastodon	NMNH	8071	patella
Mammut americanum	mastodon	NMNH	215076	longbone

Genus/species	Common name	[a]Collection	Museum designation	Body element
Mammuthus ("Elaphus primigenius")	mammoth	NMNH	none listed	thoracic centrum
Mammuthus (juvenile)	mammoth	VMNH	92-28S-63W-1	mandible
Mammuthus columbi	mammoth	NMNH	none listed	longbone
Megalonyx jeffersonii	giant ground sloth	NMNH	23737	femur
Platygonus compressus	peccary	NMNH	299961	canine with root
Rangifer tarandus	caribou	NMNH	23700	antler
Unidentified	ovibovine	NMNH	none listed	pelvis
Ursus americanus	black bear	NMNH	244222	vertebra

[a] NMNH (National Museum of Natural History); VMNH (Virginia Museum of Natural History)

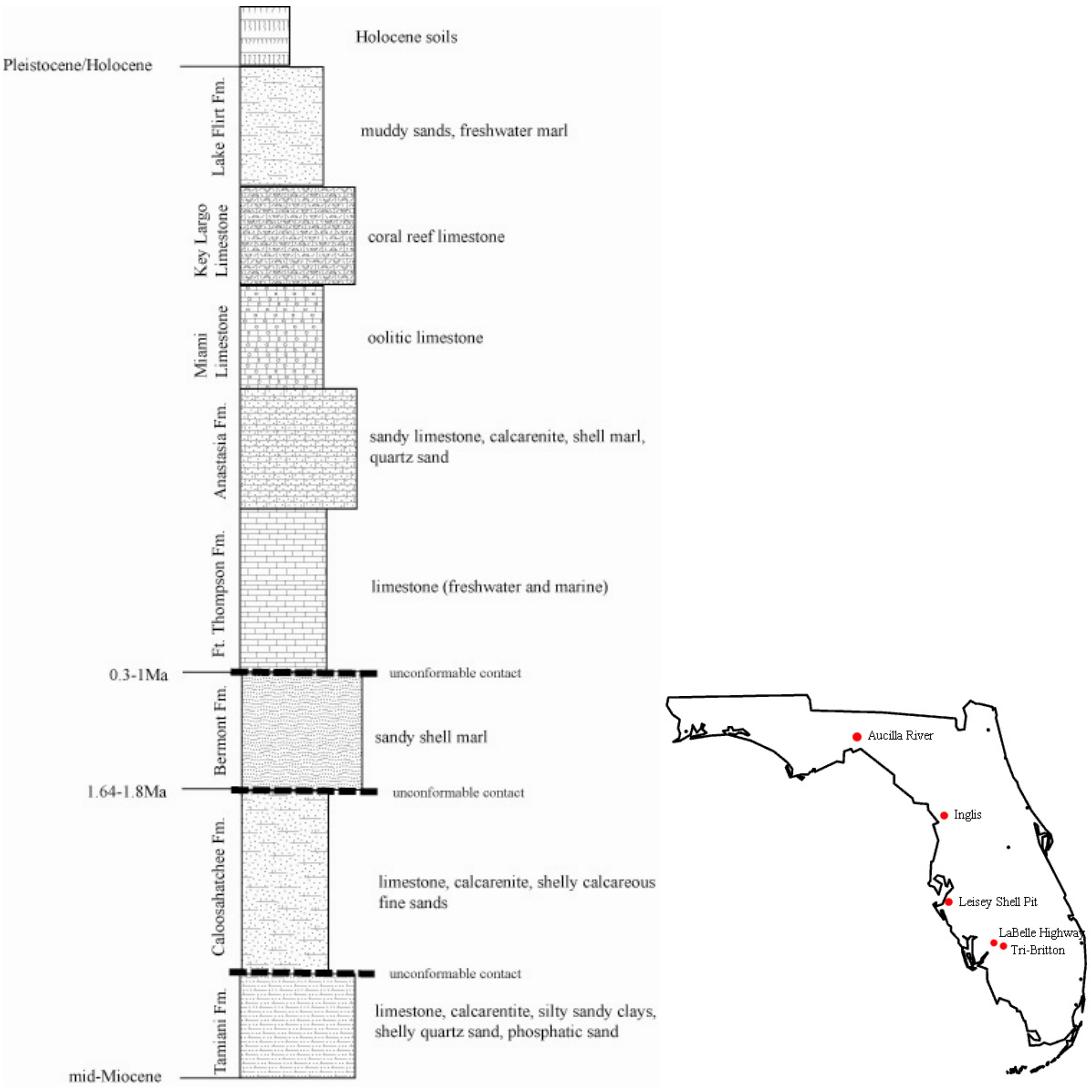

Figure 2.3 – Map of Florida, USA showing location of sample sites; simplified stratigraphic column of Florida sedimentology compiled from DuBar (1974), Jones (1992), Muhs et al. (1992), and Zullo and Harris (1992). Note relative thicknesses and presence/absence of formations varies from site to site.

and Leisey Shell Pit 1A sites are inferred to be early Pleistocene in age with the former being late Blancan (~1.9-2 Ma) and the latter dated at 1.2-1.5 Ma, which places it in the earliest Irvingtonian (Jones 1992, Woodburne and Swisher 1995). The LaBelle Highway and Tri-Britton sites are located geographically very close to one another and both date to the late Irvingtonian/early Rancholabrean (mid-late Pleistocene). The Aucilla River sites are from the terminal Pleistocene and constitute the most recent fauna of the set.

Vertebrate remains include a wide variety of species that span all the major groups present during the Pleistocene Epoch. Samples were obtained from the Florida Museum of Natural History (FLMNH) and are listed in Table 2.2. This group of samples spans a considerable length of time from the earliest to the most terminal Pleistocene and provides a means to examine diagenesis through time. Well preserved specimens include herbivores and carnivores which allows for a complete reconstruction of trophic relationships and examination of areas of competition. The presence of a unique grazing niche will be examined through carbon isotopic analyses. Unique nitrogen isotopic signatures will be sought for the juveniles and ruminants in the fauna as well.

2.1.3 McKittrick Brea, California

The fossil bearing strata of the McKittrick Brea tar pits are located in the San Joaquin Valley in California near the town of McKittrick (Figure 2.4). These deposits are found in the McKittrick Formation which spans the upper Miocene to the Pleistocene (Arnold and Johnson 1910). The sedimentology includes several

Table 2.2 – Florida sample list

Genus/species	Common name	[a]Collection	Museum designation	Body element	Site
Paramylodon harlani	sloth	FLMNH	UF 65854	r. humerus	Leisey Shell Pit 1A
Nothrotheriops texanus	ground sloth	FLMNH	UF 86988	r. humerus	Leisey Shell Pit 1A
Canis edwardii	dog/wolf	FLMNH	UF 81679	l. humerus	Leisey Shell Pit 1A
Arctodus pristinus	short-faced bear	FLMNH	UF 95882	l. humerus	Leisey Shell Pit 1B
Canis armbrusteri	dog/wolf	FLMNH	UF 85124	l. metacarpal IV	Leisey Shell Pit 1A
Arctodus pristinus	short-faced bear	FLMNH	UF 129078	l. tibia	Leisey Shell Pit 1A
Procyon sp.	raccoon	FLMNH	UF 115738	l. femur	Leisey Shell Pit 1A
Smilodon gracilis	sabertooth cat	FLMNH	UF 81732	r. tibia	Leisey Shell Pit 1A
Smilodon gracilis	sabertooth cat	FLMNH	UF 67087	r. humerus	Leisey Shell Pit 1A
Equus (juvenile)	horse	FLMNH	UF 67297	l. humerus	Leisey Shell Pit 1A
Tapirus haysii	tapir	FLMNH	UF 89534	l. metacarpal III	Leisey Shell Pit 1A
Equus	horse	FLMNH	UF 88531	l. radius	Leisey Shell Pit 1A
Hemiauchenia macrocephala	llama	FLMNH	UF 206055	metacarpal	Leisey Shell Pit 1A
Hemiauchenia (juvenile)	llama	FLMNH	UF115745	metapodial	Leisey Shell Pit 1A
Palaeolama mirifica	llama	FLMNH	UF 66420	r. metacarpal III and IV	Leisey Shell Pit 1A
Mylohyus fossilis	peccary	FLMNH	UF 80241	l.scapula	Leisey Shell Pit 1A
Tayassuidae	peccary	FLMNH	UF 65263	r. humerus	Leisey Shell Pit 1A
Odocoileus cf. virginianus	deer	FLMNH	UF 87890	r. humerus	Leisey Shell Pit 1A
Platygonus vetus	peccary	FLMNH	UF 65948	metacarpal III and IV	Leisey Shell Pit 1A
Mammut americanum (juvenile)	mastodon	FLMNH	UF 115739	r. mandible	Leisey Shell Pit 1A
Lynx rufus	lynx	FLMNH	UF 87300	r. ulna	Leisey Shell Pit 1A
Mammuthus imperator	mammoth	FLMNH	UF 86979	lower mandible	Leisey Shell Pit 1A
Mammut americanum	mastodon	FLMNH	UF 81453	r. mandible	Leisey Shell Pit 1A

Genus/species	Common name	[a]Collection	Museum designation	Body element	Site
Paramylodon harlani	sloth	FLMNH	UF 208521	r. humerus	Tri-Britton
Playgonus cumberlandensis	peccary	FLMNH	UF 211011	scapula	Tri-Britton
Casteroides	beaver	FLMNH	UF 209380	astragulus	Tri-Britton
Tayassuidae	peccary	FLMNH	UF 209235	l. calcaneum	Tri-Britton
Megalonyx wheatleyi	ground sloth	FLMNH	UF 211068	l. ulna	Tri-Britton
Equus sp.	horse	FLMNH	UF 210924	r. humerus	Tri-Britton
Tapirus veroensis	tapir	FLMNH	UF 210900	r. femur	Tri-Britton
Palaeolama mirifica (juvenile)	llama	FLMNH	UF 209193	l. femur	Tri-Britton
Palaeolama mirifica	llama	FLMNH	UF 209192	r. metatarsals III and IV	Tri-Britton
Hemiauchenia macrocephala	llama	FLMNH	UF 210969	metatarsal III and IV	Tri-Britton
Mammut americanum	mastodon	FLMNH	UF 210415	l. humerus	Tri-Britton
Hemiauchenia macrocephala	llama	FLMNH	UF 219453	radioulna	LaBelle Highway
Equus	horse	FLMNH	UF 218081	r. humerus	LaBelle Highway
Palaeolama mirifica	llama	FLMNH	UF 219458	l. metatarsal III and IV	LaBelle Highway
Mammut americanum	mastodon	FLMNH	UF 219690	l. scapula	LaBelle Highway
Odocoileus virginianus	deer	FLMNH	UF 214143	r. scapula	LaBelle Highway
Hydrochoerus holmesi	capybara	FLMNH	UF 219701	r. radius	LaBelle Highway
Pantera onca	lion	FLMNH	UF 217672	l. femur	LaBelle Highway
Lynx rufus	lynx	FLMNH	UF 214129	r. tibia	LaBelle Highway
Canis ambrusteri	dog/wolf	FLMNH	UF 219575	l. calcaneum	LaBelle Highway
Tayassuidae	peccary	FLMNH	UF 214139	l. tibia	LaBelle Highway
Platygonus cumberlandensis	peccary	FLMNH	UF 217662	radioulna	LaBelle Highway
Mammuthus columbi	mammoth	FLMNH	UF 220429	humerus	LaBelle Highway

Genus/species	Common name	[a]Collection	Museum designation	Body element	Site
Eremotherium eomigrans	ground sloth	FLMNH	UF 95876	ungual phalanges	Inglis 1A
Paramylodon harlani	sloth	FLMNH	UF95821	l. humerus	Inglis 1A
Megalonyx leptostomus	ground sloth	FLMNH	UF23346	femur	Inglis 1A
Xenosmilus hodsonae	sabertooth cat	FLMNH	UF 45340	ulna	Inglis 1A
Arctodus pristinus	short-faced bear	FLMNH	UF 18069	femur	Inglis 1A
Canis edwardii	dog/wolf	FLMNH	UF 22567	r. radius	Inglis 1A
Smilodon gracilis	sabertooth cat	FLMNH	UF 18112	femur	Inglis 1A
Miracinonyx inexpectatus	cheetah	FLMNH	UF 45353	l. femur	Inglis 1A
Smilodon gracilis	sabertooth cat	FLMNH	UF 18109	ulna	Inglis 1A
Felis lacustris	cat	FLMNH	UF 18115	humerus	Inglis 1A
Miracinonyx inexpectatus	cheetah	FLMNH	UF 45350	r. tibia	Inglis 1A
Lynx rufus	lynx	FLMNH	UF 18118	r. humerus	Inglis 1A
Procyon	raccoon	FLMNH	UF 18077	humerus	Inglis 1A
Erethizon kleini	porcupine	FLMNH	UF 24157	l. femur	Inglis 1A
Leporinae	hare/rabbit	FLMNH	none listed	r. tibia	Inglis 1A
Lepus	hare/jackrabbit	FLMNH	UF 178465	r. femur	Inglis 1A
Tapirus sp.	tapir	FLMNH	UF 45310	humerus	Inglis 1A
Sylvilagus webbi	rabbit	FLMNH	UF 178325	l. femur	Inglis 1A
Geomys propinetis	gopher	FLMNH	UF 46190	l. mandible	Inglis 1A
Hydrochoerus holmesi	capybara	FLMNH	UF 223993	humerus	Inglis 1A
Sigmodon curtisi	rat	FLMNH	UF 223994	mandible	Inglis 1A
Chasmaporthetes ossifragus	hyaena	FLMNH	UF 27367	r. radius	Inglis 1A
Platygonus bicalcaratus	peccary	FLMNH	UF 176774	r. tibia	Inglis 1A
Equus	horse	FLMNH	UF 97238	metapodial	Inglis 1A
Hemiauchenia macrocephala	llama	FLMNH	UF 179742	l. humerus	Inglis 1A
Hemiauchenia macrocephala	llama	FLMNH	UF 45655	tibia	Inglis 1A
Capromeryx arizonensis	pronghorn	FLMNH	UF 177178	l. femur	Inglis 1A
Odocoileus virginianus	deer	FLMNH	UF 45014	radius	Inglis 1A
Mammut (americanum ?)	mastodon	FLMNH	UF 18121	vertebra	Inglis 1A
Mammut	mastodon	FLMNH	UF 223995	femoral head	Inglis 1A

Genus/species	Common name	Collection	Museum designation	Body element	Site
Equus	horse	FLMNH	UF 14871	calcaneum	Aucilla River 1A
Tapirus veroensis	tapir	FLMNH	UF 47931	l. metatarsal II	Aucilla River 1A
Neochoerus pinckneyi	capybara	FLMNH	UF 47938	tibia	Aucilla River 1A
Smilodon fatalis	sabertooth cat	FLMNH	UF 14894	mandible	Aucilla River 1A
Tapirus	tapir	FLMNH	UF 47935	calcaneum	Aucilla River 1A
Lamini	llama	FLMNH	UF 47942	metapodial	Aucilla River 1A
Paramylodon	giant ground sloth	FLMNH	UF 14832	limb element	Aucilla River 1A
Equus	horse	FLMNH	UF 47960	pelvis	Aucilla River 1A
Lynx rufus	lynx	FLMNH	UF 24731	tibia	Aucilla River 1A
Tapirus veroensis	tapir	FLMNH	UF 213894	r. femur	Aucilla River 1B
Camelidae (Lamini?)	llama	FLMNH	UF 47990	metacarpal	Aucilla River 1B
Megalonyx jeffersonii	giant ground sloth	FLMNH	UF 21336	l. ulna	Aucilla River 1B
Holmesina septentrionalis	northern pampathere	FLMNH	UF 217484	l. ulna	Aucilla River 1E
Odocoileus virginianus	deer	FLMNH	UF 92630	metacarpal	Aucilla River 2
Felis sp.	cat	FLMNH	UF 92617	mandible	Aucilla River 2
Equus	horse	FLMNH	UF 223997	limb	Aucilla River 2C
Odocoileus virginianus	deer	FLMNH	UF 132740	r. metatarsal	Aucilla River 2C
Urocyon cinereoargenteus	fox	FLMNH	UF 133190	mandible	Aucilla River 2C
Lynx rufus	lynx	FLMNH	UF 133191	r. humerus	Aucilla River 2C
Tapirus veroensis	tapir	FLMNH	UF 223996	mandible	Aucilla River 2C
Equus	horse	FLMNH	UF 131283	medial phalanx	Aucilla River 2C
Ondatra zibethicus	rat	FLMNH	UF 202011	r. tibia	Aucilla River 2C
Castor canadensis	beaver	FLMNH	UF 133212	r. tibia	Aucilla River 2C
Felis concolor	mountain lion	FLMNH	UF 133192	medial phalanx	Aucilla River 2C
Lutra canadensis	otter	FLMNH	UF 132633	r. metatarsal V	Aucilla River 2C

Genus/species	Common name	[a]Collection	Museum designation	Body element	Site
Vulpes vulpes	fox	FLMNH	UF 226408	metatarsal	Aucilla River 2D
Odocoileus virginianus	deer	FLMNH	UF 226402	r. humerus	Aucilla River 2D
Odocoileus virginianus	deer	FLMNH	UF 226403	r. tibia	Aucilla River 2D
Canis latrans	coyote	FLMNH	UF 226401	r. metatarsal III	Aucilla River 2E
Equus	horse	FLMNH	UF 226407	phalanx	Aucilla River 2E
Odocoileus virginianus	deer	FLMNH	UF 226406	r. metacarpal	Aucilla River 2E
Odocoileus virginianus	deer	FLMNH	UF 226405	r. radius	Aucilla River 2E
Bison	bison	FLMNH	UF 48035	metatarsal	Aucilla River 3
Mammut americanum	mastodon	FLMNH	UF 60815	radius	Aucilla River 3
Equus	horse	FLMNH	UF 14959	vertebra	Aucilla River 3A
Mammuthus columbi	mammoth	FLMNH	UF 14778	ulna	Aucilla River 3A
Odocoileus virginianus	deer	FLMNH	UF 14956	limb	Aucilla River 3A
Equus	horse	FLMNH	UF 14958	scapula	Aucilla River 3A
Bison	bison	FLMNH	UF 14982	phalange	Aucilla River 3E
Ondatra zibethica	rat	FLMNH	UF 226410	tibia	Aucilla River 3E
Mammut americanum	mastodon	FLMNH	UF 137891	humerus	Aucilla River 3E
Megalonyx jeffersonii	ground sloth	FLMNH	UF 48025	metapodial	Aucilla River 3E
Odocoileus virginianus	deer	FLMNH	UF 14979	femur	Aucilla River 3E
Equus	horse	FLMNH	UF 14981	limb	Aucilla River 3E
Bison antiquus	bison	FLMNH	UF 14982	limb	Aucilla River 3E
Platygonus compressus	peccary	FLMNH	none listed	mandible	Aucilla River 3E

Genus/species	Common name	[a]Collection	Museum designation	Body element	Site
Megalonyx jeffersonii	giant ground sloth	FLMNH	UF 103508	femur	Aucilla River 3J
Sylvilagus	rabbit	FLMNH	UF 175226	l. femur	Aucilla River 3J
Ondatra zibethica	rat	FLMNH	UF 92394	femur	Aucilla River 3J
Didelphis marsupialis	opossum	FLMNH	UF 153042	humerus	Aucilla River 3J
Canis sp.	wolf	FLMNH	UF 149517	r. tibia	Aucilla River 3J
Ursus americanus	black bear	FLMNH	UF 149521	l. mandible	Aucilla River 3J
Ursus americanus	black bear	FLMNH	UF 150475	l. mandible	Aucilla River 3J
Procyon lotor	northern raccoon	FLMNH	UF 69537	humerus	Aucilla River 3J
Procyon lotor	northern raccoon	FLMNH	UF 103567	humerus	Aucilla River 3J
Equus	horse	FLMNH	UF 153747	scapula	Aucilla River 3J
Odocoileus virginianus	deer	FLMNH	UF 153050	humerus	Aucilla River 3J
Bison antiquus	bison	FLMNH	UF 146074	humerus	Aucilla River 3J
Hemiauchenia	llama	FLMNH	UF 148598	phalanx	Aucilla River 3J
Tapirus sp.	tapir	FLMNH	UF 146089	r. astragalus	Aucilla River 3J
Paramylodon	giant ground sloth	FLMNH	UF 226409	rib	Aucilla River 3J
Equus	horse	FLMNH	UF 24972	rib	Aucilla River 3J
Paramylodon harlani	sloth	FLMNH	UF 153058	rib	Aucilla River 3J
Megalonyx jeffersonii	ground sloth	FLMNH	UF 103604	r. metacarpal III	Aucilla River 3J
Pilosa	sloth	FLMNH	UF 151910	metapodial	Aucilla River 3J
Procyon lotor	northern raccoon	FLMNH	UF 153040	r. femur	Aucilla River 3J
Palaeolama mirifica	llama	FLMNH	UF 92519	jaw	Aucilla River 3J
Tapirus veroensis	tapir	FLMNH	UF 154821	calcaneum	Aucilla River 3J
Tapirus veroensis	tapir	FLMNH	UF 146081	humerus	Aucilla River 3J
Equus sp.	horse	FLMNH	UF 176248	rib	Aucilla River 3J
Odocoileus virginianus	deer	FLMNH	UF 154482	r. tibia	Aucilla River 3J
Mammuthus columbi	mammoth	FLMNH	UF 146663	axis	Aucilla River 3J

Genus/species	Common name	[a]Collection	Museum designation	Body element	Site
Mammut americanum	mastodon	FLMNH	UF 27894	thoracic spine	Aucilla River 3J
Palaeolama mirifica	llama	FLMNH	UF 103650	r. mandible	Aucilla River 3J
Bison antiquus	bison	FLMNH	UF 175237	l. humerus	Aucilla River 3J
Ursus americanus	bear	FLMNH	UF 149521	l. mandible	Aucilla River 3J
Canis dirus	dog/wolf	FLMNH	UF 69576	mandible	Aucilla River 3J
Castor canadensis	beaver	FLMNH	UF 150474	r. mandible	Aucilla River 3J
Mephitis mephitis	skunk	FLMNH	UF 182751	l. tibia	Aucilla River 3J
Lynx rufus	lynx	FLMNH	UF 147397	r. mandible	Aucilla River 3J
Procyon lotor	northern raccoon	FLMNH	UF 153040	r. femur	Aucilla River 3J
Procyon lotor	northern raccoon	FLMNH	UF 92395	mandible	Aucilla River 3J
Lutra canadensis	otter	FLMNH	UF 153074	r. mandible	Aucilla River 3J
Sciurus carolinensis	squirrel	FLMNH	UF 92581	l. femur	Aucilla River 3J
Hydrochoeridae	capybara	FLMNH	UF 175232	r. radius	Aucilla River 3J
Didelphis virginianus	opossum	FLMNH	UF 69626	femur	Aucilla River 3J
Sylvilagus	rabbit	FLMNH	UF 175226	l. femur	Aucilla River 3J
Ondatra zibethica(?)	rat	FLMNH	UF 92394	l. femur	Aucilla River 3J

[a]FLMNH (Florida Museum of Natural History)

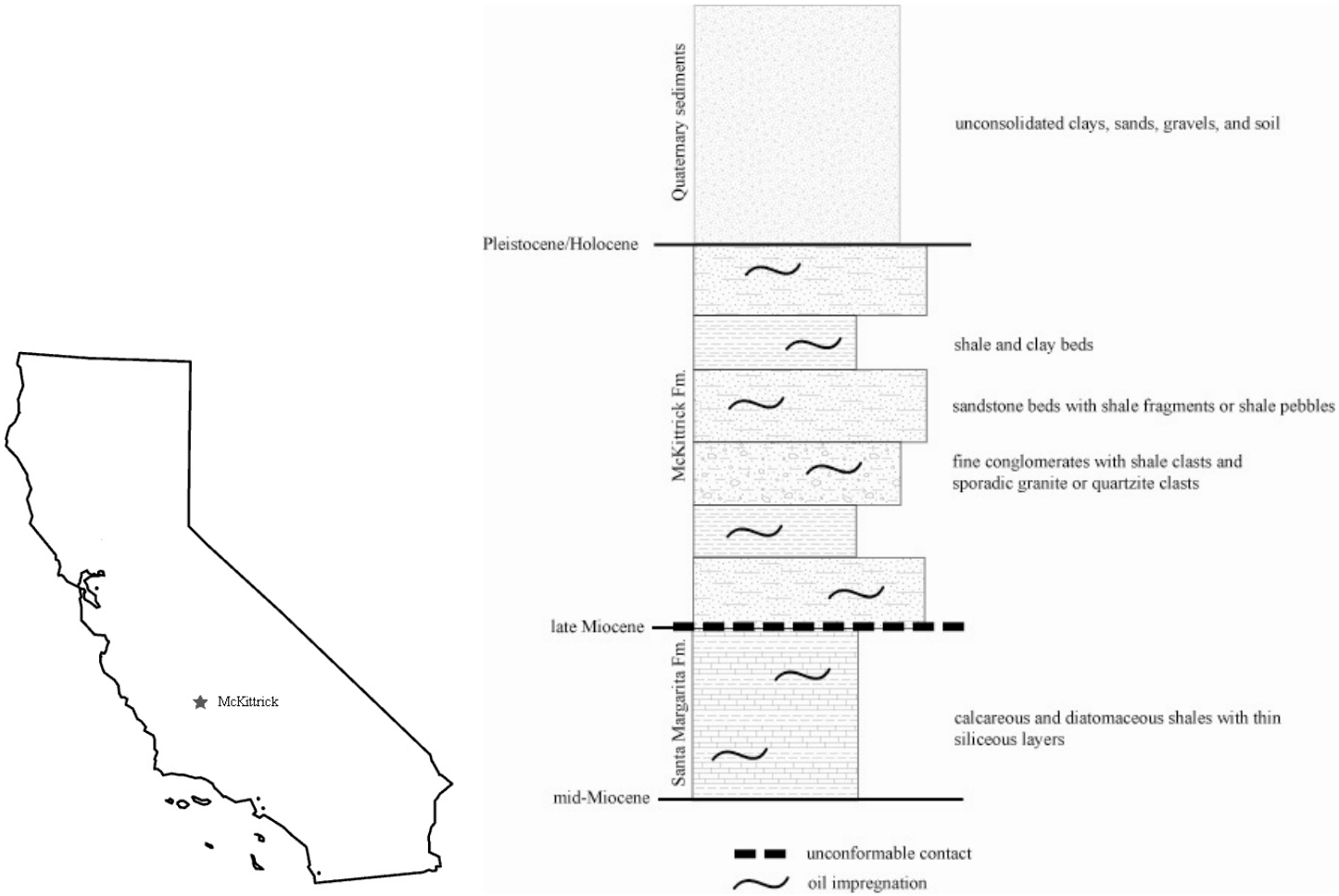

Figure 2.4 – Map of California, USA showing the location of McKittrick Brea; simplified sedimentology of the McKittrick Formation compiled from Arnold and Johnson (1910), and Merriam and Stock (1921). Note relative thickness of formations varies between outcrops. The relative thickness and sequence of beds within the McKittrick Formation also varies between outcrops.

sandstone beds with prolific shale fragments or pebbles, shale and clay beds, and fine conglomerates composed largely of shale clasts and rarer granite or quartzite clasts (Arnold and Johnson 1910). Petroleum impregnates these layers to a varying degree, reaching up to 1000 feet in thickness at some locations (Arnold and Johnson 1910). Fossiliferous layers of Pleistocene age are located near the top of the succession, often in areas where oil has reached the surface thereby impregnating both sediments and fossils (Arnold and Johnson 1910, Merriam and Stock 1921). Palynology suggests that vegetation consisted of forests dominated by pines, cypresses, and oaks, with an understory of highly diversified shrubs (Mason 1944).

Vertebrate fossils indicate a wide range of species from all major terrestrial groups present during this time period. Samples were obtained from the Los Angeles County Museum of Natural History (LACM) and are listed in Table 2.3. Stratigraphic estimates place this site as late Pleistocene. The diversity of available specimens will allow for a complete reconstruction of trophic relationships and determination of areas of maximum competition. The tar pit faunas in California have provided an exceptional array of well preserved carnivores, thus providing a good view of the upper levels of the food chain through the use of nitrogen isotopes. Unique ruminant signatures will also be examined using nitrogen isotopes, and herbivore niche splitting will be examined using carbon isotopes.

Table 2.3 – McKittrick Brea sample list

Genus/species	Common name	[a]Collection	Museum designation	Body element
Hemiauchenia	llama	LACM	151883	l. metatarsal proximal
Canis lupus	dog/wolf	LACM	107672	l. humerus proximal
Tremarctotherium	bear	LACM	107968	r. tibia distal
Ursus	bear	LACM	108008	metapodial
Canis dirus	dog/wolf	LACM	105591	l. tibia distal
Vulpes	fox	LACM	107786	r. tibia distal
Canis latrans	coyote	LACM	101505	r. radius distal
Canis dirus	dog/wolf	LACM	106425	l. metatarsal
Taxidea taxus	badger	LACM	108180	l. femur proximal
Canis latrans	coyote	LACM	101373	r. humerus distal
Felis atrox	cat/lion	LACM	151872	tibia proximal
Mammuthus	mammoth	LACM	151875	fibula proximal
Equus	horse	LACM	151879	r. metacarpal III proximal
Glossotherium harlani	ground sloth	LACM	151877	rib proximal fragment
Arctodus	short-faced bear	LACM	151878	r. metacarpal III
Bison	bison	LACM	151880	l. lunar
Lynx rufus	lynx	LACM	151874	radius proximal
Smilodon	sabertooth cat	LACM	151876	phalanx I proximal fragment
Equus occidentalis	horse	LACM	151881	l. tibia distal
Cervus	elk	LACM	151884	phalanx I
Antilocapra	pronghorn	LACM	151885	r. radius proximal
Camelops	camel	LACM	151882	metapodial distal
Bison	bison	LACM	151886	ulna proximal
Leporidae	hare/rabbit	LACM	151887	humerus distal
Rodentia	rodent	LACM	151889	humerus proximal
Rodentia	rodent	LACM	151890	humerus proximal
Rodentia	rodent	LACM	151891	humerus proximal
Spermophilus beecheyi	squirrel	LACM	151888	humerus distal

[a]LACM (Los Angeles County Museum of Natural History)

2.1.4 Modern specimens

Two modern bones from extant species were used to monitor intrabone variability and serve as a comparison to fossil specimens during diagenetic analysis. The first specimen was a *Bos taurus* (domestic cow) phalange that was obtained as road kill. The second was an *Odocoileus virginianus* (white-tailed deer) skull found in a pasture in Aldie, Virginia. Both specimens were naturally desiccated and flesh was completely decomposed by bacterial, fungal, insectivorous, and other natural decomposition processes.

2.2 Methods and Materials

Mechanical sample preparation consists of drilling a bone plug using a common rotary tool and diamond tip powdering bit or a plug bit. Approximately 150-300mg of bone was obtained. No effort was made to separate the compact and cancellous portions of the bone.

Modern bone is decalcified using a soak of 0.6M HCl (~1mL acid:20mg bone) at 4ºC. The acid solution is changed every day until bubbles disappear. The bone material is then washed copiously with ultra-pure 18.2 MΩ water by either gravity filtration or centrifuging. After drying under moderate heat, the resulting collagen is analyzed by EA mass spectrometry. Some of the modern bone is also processed using the procedure outlined below for fossil specimens for comparison purposes.

Fossil specimens were processed according to Stafford et al. (1988). Specimens are first sonicated in ultra-pure water to remove any clinging sediment material or any labile salts. They are then dried overnight. This is followed by a demineralization soak in 0.6M HCl (~1mL acid:20mg bone) at 4ºC with fresh acid added every 24 hours until bubbling stops. The solid bone residue is then rinsed copiously with ultra-pure water and dried overnight. The dried bone material is soaked in 5mL of 0.03M HCl at 90ºC for 24 hours. The acidic solution is separated from the solid bone residue and saved. This solution is freeze-dried producing a crude collagen extract.

This extract is soaked in 10mL of 6M HCl at 100ºC for 24 hours. It is centrifuged and the supernatant containing the denatured protein as well as any humic contaminants is poured off and saved. A 5cc plastic syringe is loaded with 2-3cc of Serdolit® PAD-I resin (0.1-0.2mm particle size). The resin is conditioned using 3 bed volumes of 6M HCl. The supernatant is then allowed to pass through the resin at ~200µL/min and the eluant is saved. The column is then rinsed with 2 bed volumes of 6M HCL; both rinses are saved with the eluant. The resulting solution is diluted up to 50mL with ultra-pure water and freeze-dried.

The resin for this process is thoroughly washed before use. It is first rinsed by gravity filtration with 10 bed volumes of 50% acetone followed by 10 bed volumes of ultra-pure water. It is then soaked in alternating baths of 3M NaOH and 3M HCl at 80ºC for 30 minutes per soak. The resin is soaked three times in each bath and then rinsed copiously with ultra-pure water. It is transferred into a Soxhlet apparatus and extracted for 24 hours in 100% acetone. It is rinsed excessively with water and then

extracted again using 100% methanol. The resin is given a final water rinse and stored in 1M HCl until use.

The McKittrick samples present an additional contaminant that requires removal prior to isotopic analysis as per Coltrain et al. (2004). These specimens were impregnated with tar and other hydrocarbon contaminants during burial. Prior to the procedure outlined above, they are soaked in a 2:1 toluene:methanol solution for 48 hours (changing the solution after 24 hours). The solid bone material is then extracted by Soxhlet method in a 2:1 toluene:methanol solution for 24 hours. Samples are dried in a vacuum oven at 110ºC overnight to remove the organic solvents. At this point, these samples continue with the previously outlined procedure starting with the demineralization in 0.6M HCl.

Select samples were carbon dated by accelerator mass spectrometry. The isolated collagen from these samples was reduced to filamentous graphite by catalytic deposition onto iron-group metals according to the procedures of Vogel et al. (1987). This graphite was then used as sputtering targets for the mass spectrometer.

Select samples were analyzed for their amino acid composition. Isolated collagen from these samples was hydrolyzed in acid and diluted. The diluted hydrolysate was then introduced to the amino acid analyzer.

2.3 Mass spectrometry and amino acid analyzers

For isotopic analyses, all isolated bone collagen is weighed and packed into tin boats. The samples are introduced into a Eurovector Elemental Analyzer where

CO_2 and N_2 gas are produced by combustion of the collagen at 1040ºC. Helium carrier gas moves the CO_2 and N_2 through a GC column wherein they are separated. The isolated gases are then introduced into a GV continuous flow gas source mass spectrometer to obtain the $\delta^{15}N$ and $\delta^{13}C$ values, C:N, and percent yields of carbon and nitrogen for each sample.

Reproducibility and machine stability for the stable isotope analyses were monitored using NIST-912a urea standard. Standard operating procedure included a minimum of two urea standards for every ten samples. The standard exhibited approximately ±0.1‰ (1σ) for both $\delta^{15}N$ and $\delta^{13}C$, and approximately ±0.25% for the percent yields of carbon and nitrogen during individual runs of samples. The NIST-912a urea standard as well as an in-house glycine standard (Acros Chemical Company) was used to monitor the quality and reproducibility of $\delta^{13}C$, $\delta^{15}N$, and C:N values. The modern bovine bone was also used to determine intra sample variability.

Radiometric ^{14}C dates were obtained from the accelerator mass spectrometry apparatus at the Multiuser Tandem Laboratory at the Lawrence Livermore National Laboratory, University of California, USA as described in Davis et al. (1990). Radiocarbon concentrations were obtained as fraction modern, $\Delta^{14}C$, and conventional radiocarbon age according to the conventions of Stuiver and Polach (1977). Errors ranged from ±50 to ±590 years. Two in-house standards were used to monitor the quality of sample preparations and quality of data – Thistle Creek IUIS6 Bear bone and ACTIII Holocene whale vertebra.

Amino acid abundance analyses were performed on a Beckman 6300 spectrophotometer and a Hitachi L8900 amino acid analyzer at AAA Service Laboratory in Damascus, Oregon, USA.

CHAPTER 3 – RESULTS

3.1 Preservation

Before discussion of any isotopic data or carbon dates, it is first necessary to determine the quality of preservation of the samples. A total of 64 specimens exhibited good quality preservation and the presence of intact collagen. The identification of these specimens was based on the % yield of organic extract, % nitrogen and carbon within that extract, C:N ratio, and amino acid analysis.

3.1.1 Organic yields and C:N ratios

Approximately one-quarter of sampled specimens yielded an appropriate C:N ratio, % collagen, % nitrogen in collagen, and % carbon in collagen to be considered well-preserved. Each site was examined individually and then combined with other sites to determine prevailing trends in organic yields and ultimately delineate diagenetically altered specimens from well preserved ones. Figure 3.1 summarizes the % collagen, % nitrogen in collagen, and % carbon in collagen for all samples, both well preserved and diagenetically altered.

3.1.1.1 Organic yields and C:N ratios in McKittrick Brea, California specimens

Samples from McKittrick Brea, California produced the fewest number of well preserved specimens. The overall yield of organic material from McKittrick samples ranged from 0-17.2% (Table 3.1). Six samples showed a good quality of preservation indicating the organic extract was in fact collagen. For these six

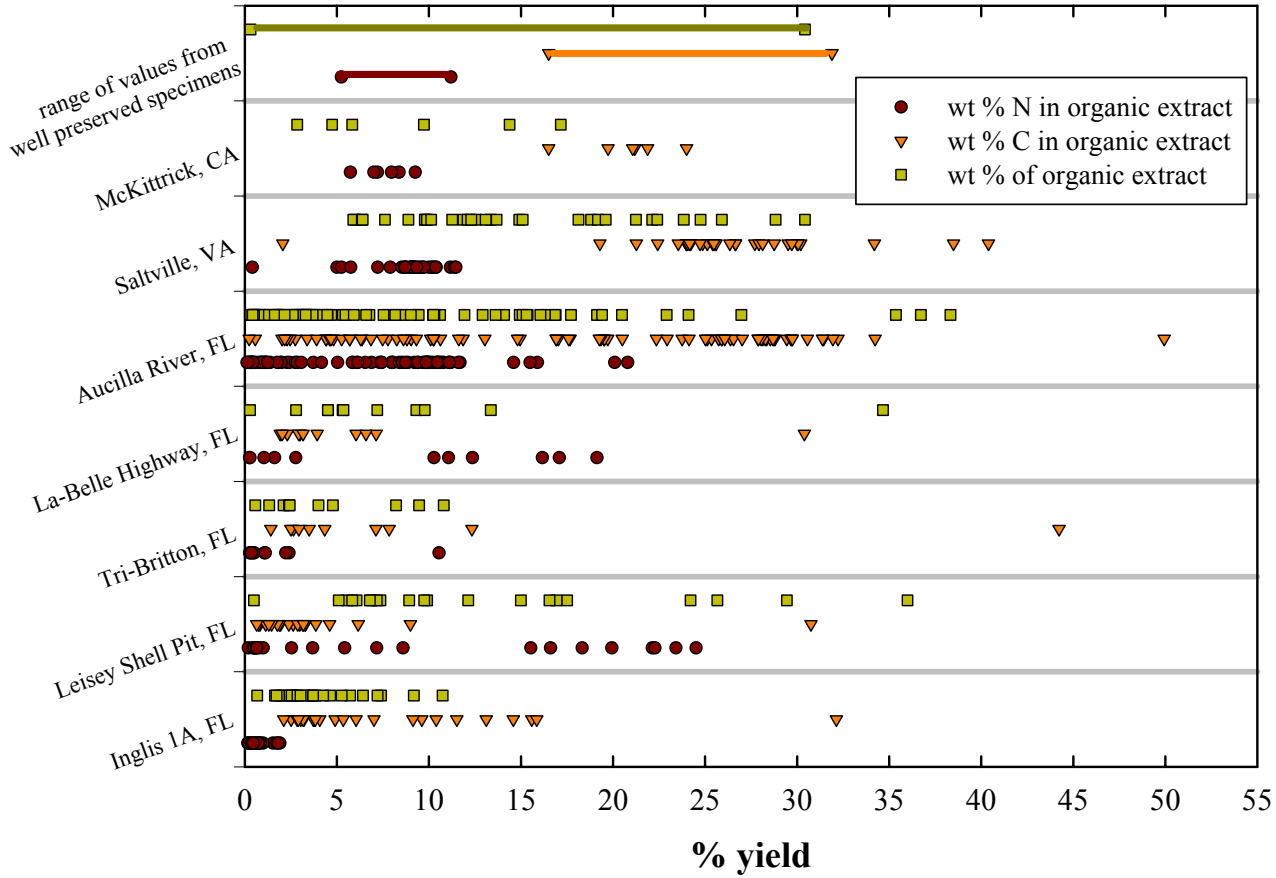

Figure 3.1 – Summary of the yield of organic extract (represented by the weight percent of the whole bone), weight percent nitrogen in the organic extract, and weight percent carbon in the organic extract for all samples that produced an organic extract upon completion of chemical processing. One outlier (LSP-7) with an anomalously high % organic extract yield of 72.9% was excluded from the figure.

Table 3.1 – Data table for McKittrick Brea, California samples

Genus/species	Common name	[a]% Yield	[b]"Collagen" %N	[b]"Collagen" %C	[c]$\delta^{15}N$	[c]$\delta^{13}C$	C:N
Antilocapra	pronghorn	no yield					
Arctodus	short-faced bear	no yield					
Bison	bison	no yield					
Bison	bison	no yield					
Camelops	camel	no yield					
Canis dirus	dog/wolf	no yield					
Canis dirus	dog/wolf	no yield					
Canis latrans	coyote	no yield					
Canis latrans	coyote	no yield					
Canis lupus	dog/wolf	no yield					
*Cervus**	elk	17.2	9.26	24.00	8.99	-19.25	3.02
Equus	horse	no yield					
Equus occidentalis	horse	no yield					
Felis atrox	cat/lion	no yield					
Glossotherium harlani	ground sloth	no yield					
*Hemiauchenia**	llama	5.8	5.74	16.51	13.61	-18.46	3.35
Leporidae*	hare/rabbit	14.4	8.38	21.88	7.36	-19.58	3.05
*Lynx rufus**	lynx	4.8	7.21	21.19	9.59	-20.62	3.43
Mammuthus	mammoth	no yield					
Rodentia	rodent	no yield					
Rodentia*	rodent	2.8	7.01	19.73	7.24	-21.00	3.28
Rodentia*	rodent	9.7	7.97	21.08	9.85	-19.62	3.09
Smilodon	sabertooth cat	no yield					
Spermophilus beecheyi	squirrel	no yield					
Taxidea taxus	badger	no yield					
Tremarctotherium	bear	no yield					
Ursus	bear	no yield					
Vulpes	fox	no yield					

[a] % yield represents the % organic extract obtained from the whole bone sample

[b] "collagen"%N and "collagen"%C represent the % of N and C, respectively, obtained from the organic extract (±0.5%, 2σ). In samples noted with an *, the organic extract has been determined to be well preserved collagen.

[c] $\delta^{15}N$ and $\delta^{13}C$ values are in ‰ units with respect to atmospheric air and V-PDB, respectively. Errors (2σ): $\delta^{15}N < \pm 0.27‰$, $\delta^{13}C < \pm 0.18‰$

samples, the %N in the collagen ranged from 5.7-9.3%, the %C in the collagen ranged from 16.5-24.0%, and the C:N ranged from 3.02-3.43.

3.1.1.2 Organic yields and C:N ratios in Saltville, Virginia specimens

Samples from Saltville, Virginia exhibited a larger number of well preserved specimens. Organic yields from this site ranged from 0-30.4% (Table 3.2). A total of 26 samples showed good quality preservation with %N in collagen ranging 5.0-11.5%, %C in collagen ranged from 19.3-30.2%, and C:N ranged from 3.06-3.65.

3.1.1.3 Organic yields and C:N ratios in Florida specimens

Specimens from Florida exhibited a decreasing quality of preservation with age. Inglis 1A, the oldest site, failed to produce any specimens with well preserved collagen. The organic yield ranged from 0-10.7% (Table 3.3). Those samples that produced an organic extract exhibited extremely low %N values ranging from 0.2-1.9%, thus indicating a lack of well preserved protein. The %C in the organic extract varied widely from 2.1-32.1%. This wide range in %N and %C yields and the large disparity between the two values produced C:N ratios ranging from 5.00 to as high as 58.58; these values are indisputably outside the range of C:N values for well preserved collagen.

The Leisey Shell Pit site exhibited higher yields of organic extract, but an equal lack of well preserved collagen (Table 3.3). Organic yields ranged from 0% to one anomalously high yield of 72.9%. The %N in the organic extracts ranged from 0.2-24.5%, and the %C ranged from 0.7%-30.8%. While the C:N ratios ranged from

Table 3.2 – Data table for Saltville, Virginia samples

Genus/species	Common name	[a]% Yield	[b]"Collagen" %N	[b]"Collagen" %C	[c]$\delta^{15}N$	[c]$\delta^{13}C$	C:N
Bison	bison	19.6	9.34	29.71	4.51	-24.93	3.71
Bootherium	musk ox	no yield					
Bootherium	musk ox	15.1	9.11	34.2	2.25	-22.54	4.12
*Bootherium ("Symbos cavifrons")**	musk ox	30.4	11.47	30.06	1.99	-20.90	3.06
*Bootherium**	musk ox	18.8	8.73	24.22	5.81	-19.99	3.24
*Bootherium**	musk ox	18.1	7.90	21.27	2.29	-20.48	3.14
*Bootherium**	musk ox	9.8	7.22	22.43	2.20	-20.95	3.62
*Bootherium**	musk ox	9.8	9.19	24.87	2.35	-20.40	3.16
*Bootherium**	musk ox	24.8	10.23	27.71	2.66	-20.61	3.16
*Bootherium**	musk ox	11.9	8.75	23.98	2.36	-20.94	3.20
*Bootherium**	musk ox	7.6	9.17	26.66	5.58	-20.36	3.39
*Bootherium**	musk ox	8.9	8.53	24.06	5.55	-20.55	3.29
*Bootherium**	musk ox	22.4	10.40	27.93	5.68	-19.91	3.13
*Bootherium**	musk ox	28.8	10.00	26.35	2.49	-20.67	3.07
*Bootherium**	musk ox	13.3	8.68	23.55	2.33	-20.64	3.17
*Bootherium**	musk ox	13.7	9.02	24.17	2.50	-20.71	3.13
*Bootherium**	musk ox	12.6	9.22	24.73	2.19	-20.81	3.13
*Cervalces**	moose	14.9	10.37	28.76	1.53	-21.11	3.24
*Cervalces**	moose	12.1	9.58	25.60	1.39	-20.28	3.12
Equus	horse	6.0	0.41	2.07	N/A	-24.23	5.89
Equus	horse	6.4	5.76	38.5	2.32	-24.34	6.684
*Equus**	horse	23.8	11.39	30.02	3.87	-22.71	3.08
Mammut	mastodon	no yield					
*Mammut americanum**	mastodon	9.9	9.65	30.20	3.60	-22.40	3.65
*Mammut americanum**	mastodon	11.3	8.71	25.48	2.52	-22.08	3.41
*Mammut**	mastodon	25.9	8.99	24.00	3.04	-21.15	3.11

Genus/species	Common name	[a]% Yield	[b]"Collagen" %N	[b]"Collagen" %C	[c]$\delta^{15}N$	[c]$\delta^{13}C$	C:N
*Mammuthus ("E. primigenius")**	mammoth	6.4	5.25	19.29	3.96	-22.00	3.53
Mammuthus (juvenile)*	mammoth	21.2	10.16	26.64	5.60	-20.59	3.06
*Mammuthus columbi**	mammoth	12.3	9.43	25.42	5.60	-21.35	3.15
*Megalonyx jeffersonii**	giant ground sloth	19.2	11.17	29.52	4.65	-20.66	3.08
Platygonus compressus	peccary	no yield					
*Rangifer tarandus**	caribou	13.1	9.06	25.54	0.79	-20.28	3.29
Unidentified*	ovibovine	10.1	9.73	28.13	2.37	-21.79	3.37
Ursus americanus	black bear	5.9	5.01	40.4	3.12	N/A	N/A

[a] % yield represents the % organic extract obtained from the whole bone sample

[b] "collagen"%N and "collagen"%C represent the % of N and C, respectively, obtained from the organic extract (±0.5%, 2σ). In samples noted with an *, the organic extract has been determined to be well preserved collagen.

[c] $\delta^{15}N$ and $\delta^{13}C$ values are in ‰ units with respect to atmospheric air and V-PDB, respectively. Errors (2σ): $\delta^{15}N < \pm 0.15‰$, $\delta^{13}C < \pm 0.20‰$

Table 3.3 – Data table for Florida samples

Site	Genus/species	Common name	[a]% Yield	[b]"Collagen" %N	[b]"Collagen" %C	[c]$\delta^{15}N$	[c]$\delta^{13}C$	C:N
Inglis 1A	*Arctodus pristinus*	short-faced bear	no yield					
Inglis 1A	*Canis edwardii*	dog/wolf	2.1	0.50	3.80	N/A	-24.44	8.87
Inglis 1A	*Capromeryx arizonensis*	pronghorn	5.3	0.45	2.12	N/A	-24.39	5.50
Inglis 1A	*Chasmaporthetes ossifragus*	hyaena	4.3	0.55	7.02	N/A	-25.27	14.89
Inglis 1A	*Equus*	horse	5.7	0.41	2.52	N/A	-24.35	7.17
Inglis 1A	*Eremotherium eomigrans*	ground sloth	1.9	1.91	15.59	N/A	-26.08	9.52
Inglis 1A	*Erethizon kleini*	porcupine	3.9	0.65	4.91	N/A	-24.13	8.81
Inglis 1A	*Felis lacustris*	cat	no yield					
Inglis 1A	*Geomys propinetis*	gopher	2.5	0.17	3.04	N/A	-24.03	20.86
Inglis 1A	*Hemiauchenia macrocephala*	llama	7.2	0.21	3.21	N/A	-25.49	17.83
Inglis 1A	*Hemiauchenia macrocephala*	llama	9.2	0.35	2.85	N/A	-24.99	9.50
Inglis 1A	*Hydrochoerus holmesi*	capybara	3.7	0.20	3.73	N/A	-24.37	21.76
Inglis 1A	Leporinae	hare/rabbit	2.4	0.45	5.35	N/A	-23.89	13.87
Inglis 1A	*Lepus*	hare/jackrabbit	3.3	0.24	4.08	N/A	-24.4	19.83
Inglis 1A	*Lynx rufus*	lynx	2.6	0.64	32.14	N/A	-28.19	58.58
Inglis 1A	*Mammut*	mastodon	3.0	0.46	2.91	N/A	-24.11	7.38
Inglis 1A	*Mammut (americanum ?)*	mastodon	10.7	0.74	3.17	N/A	-23.42	5.00
Inglis 1A	*Megalonyx leptostomus*	ground sloth	2.7	0.81	15.86	N/A	-27.27	22.84
Inglis 1A	*Miracinonyx inexpectatus*	cheetah	no yield					
Inglis 1A	*Miracinonyx inexpectatus*	cheetah	3.6	0.66	13.12	N/A	-27.04	23.19
Inglis 1A	*Odocoileus virginianus*	deer	6.4	0.85	10.40	N/A	-26.01	14.27
Inglis 1A	*Paramylodon harlani*	sloth	1.6	1.54				
Inglis 1A	*Platygonus bicalcaratus*	peccary	3.7	0.52	3.83	N/A	-25.09	8.59
Inglis 1A	*Procyon*	raccoon	4.6	0.34	9.62	N/A	-25.65	33.01
Inglis 1A	*Sigmodon curtisi*	rat	2.9	1.81	11.52	N/A	-26.26	7.42
Inglis 1A	*Smilodon gracilis*	sabertooth cat	1.7	1.74	14.59	N/A	-24.54	9.78
Inglis 1A	*Smilodon gracilis*	sabertooth cat	no yield					
Inglis 1A	*Sylvilagus webbi*	rabbit	no yield					
Inglis 1A	*Tapirus sp.*	tapir	0.7	0.74	9.14	N/A	-25.86	14.41
Inglis 1A	*Xenosmilus hodsonae*	sabertooth cat	7.4	0.99	6.05	N/A	-26.3	7.13

Site	Genus/species	Common name	[a]% Yield	[b]"Collagen" %N	[b]"Collagen" %C	[c]$\delta^{15}N$	[c]$\delta^{13}C$	C:N
Leisey Shell Pit	*Arctodus pristinus*	short-faced bear	7.4	0.58	2.63	N/A	N/A	5.29
Leisey Shell Pit	*Arctodus pristinus*	short-faced bear	36.0	0.61	0.82	N/A	N/A	1.57
Leisey Shell Pit	*Canis armbrusteri*	dog/wolf	5.8	0.79	6.16	N/A	-24.33	9.10
Leisey Shell Pit	*Canis edwardii*	dog/wolf	6.9	0.44	9.00	N/A	-26.48	23.86
Leisey Shell Pit	*Equus*	horse	7.2	0.52	2.40	N/A	N/A	5.38
Leisey Shell Pit	*Equus* (juvenile)	horse	12.1	18.33	1.94	-15.35	-25.72	0.12
Leisey Shell Pit	*Hemiauchenia* (juvenile)	llama	17.5	1.00	2.88	N/A	-24.92	3.36
Leisey Shell Pit	*Hemiauchenia macrocephala*	llama	15.0	22.14	1.14	-15.7	N/A	0.06
Leisey Shell Pit	*Lynx rufus*	lynx	8.9	5.43	1.46	-13.01	-24.83	0.31
Leisey Shell Pit	*Mammut americanum*	mastodon	5.1	2.55	3.19	-9.48	-24.72	1.46
Leisey Shell Pit	*Mammut americanum* (juvenile)	mastodon	25.7	23.42	1.77	-14.14	-25.63	0.09
Leisey Shell Pit	*Mammuthus imperator*	mammoth	16.6	15.54	1.31	-15.99	-25.45	0.10
Leisey Shell Pit	*Mylohyus fossilis*	peccary	6.8	3.69	4.61	-16.22	-24.87	1.46
Leisey Shell Pit	*Nothrotheriops texanus*	ground sloth	6.1	0.20	3.15	N/A	-24.18	18.37
Leisey Shell Pit	*Odocoileus cf. virginianus*	deer	0.5	0.65	30.75	N/A	-26.51	55.19
Leisey Shell Pit	*Palaeolama mirifica*	llama	16.9	16.61	3.28	-15.7	-24.12	0.23
Leisey Shell Pit	*Paramylodon harlani*	sloth	5.6	0.56	3.29	N/A	-24.12	6.85
Leisey Shell Pit	*Platygonus vetus*	peccary	24.2	19.94	1.88	-18.24	-24.65	0.11
Leisey Shell Pit	*Procyon sp.*	raccoon	72.9	24.52	0.75	-14.06	N/A	0.04
Leisey Shell Pit	*Smilodon gracilis*	sabertooth cat	no yield					
Leisey Shell Pit	*Smilodon gracilis*	sabertooth cat	9.9	8.60	2.99	-13.31	-24.43	0.41
Leisey Shell Pit	*Tapirus haysii*	tapir	9.8	7.17	3.86	-14.6	-25.38	0.63
Leisey Shell Pit	Tayassuidae	peccary	29.4	22.28	0.65	-16.53	N/A	0.03

Site	Genus/species	Common name	[a]% Yield	[b]"Collagen" %N	[b]"Collagen" %C	[c]$\delta^{15}N$	[c]$\delta^{13}C$	C:N
LaBelle Highway	*Canis ambrusteri*	dog/wolf	9.8	19.13	2.04	-20.35	-24.68	0.12
LaBelle Highway	*Equus*	horse	34.7	16.17	3.94	-24.45	-23.92	0.28
LaBelle Highway	*Hemiauchenia macrocephala*	llama	no yield					
LaBelle Highway	*Hydrochoerus holmesi*	capybara	0.3	0.28	30.40	N/A	-28.21	126.65
LaBelle Highway	*Lynx rufus*	lynx	9.3	11.07	1.93	-20.42	-24.14	0.20
LaBelle Highway	*Mammut americanum*	mastodon	5.3	12.37	6.05	-23.34	-25.12	0.57
LaBelle Highway	*Mammuthus columbi*	mammoth	5.4	1.05	3.17	N/A	-23.45	3.52
LaBelle Highway	*Odocoileus virginianus*	deer	4.5	10.28	2.93	-23.52	-24.17	0.33
LaBelle Highway	*Palaeolama mirifica*	llama	4.5	0.26	3.00	N/A	-23.34	13.46
LaBelle Highway	*Pantera onca*	lion	7.2	17.09	2.31	-25.22	-24.26	0.16
LaBelle Highway	*Platygonus cumberlandensis*	peccary	13.4	1.62	6.59	N/A	N/A	4.75
LaBelle Highway	Tayassuidae	peccary	2.8	2.77	7.14	N/A	N/A	3.01
Tri-Britton	*Casteroides*	beaver	2.1	0.52	3.50	N/A	-23.61	7.85
Tri-Britton	*Equus sp.*	horse	no yield					
Tri-Britton	*Hemiauchenia macrocephala*	llama	0.6	2.23	44.23	N/A	-27.5	23.14
Tri-Britton	*Mammut americanum*	mastodon	2.4	0.37	2.94	N/A	-23.54	9.27
Tri-Britton	*Megalonyx wheatleyi*	ground sloth	4.8	10.55	2.51	-23.99	-25.38	0.28
Tri-Britton	*Palaeolama mirifica*	llama	8.2	2.41	7.13	N/A	N/A	3.45
Tri-Britton	*Palaeolama mirifica* (juvenile)	llama	10.8	1.12	12.34	N/A	-26.63	12.85
Tri-Britton	*Paramylodon harlani*	sloth	1.3	1.07	7.85	N/A	-24.21	8.56
Tri-Britton	*Playgonus cumberlandensis*	peccary	4.0	0.47	2.65	N/A	-23.25	6.58
Tri-Britton	*Tapirus veroensis*	tapir	9.5	0.33	1.42	N/A	-25.62	5.02
Tri-Britton	Tayassuidae	peccary	2.4	0.26	4.35	N/A	-23.46	19.52

Site	Genus/species	Common name	[a]% Yield	[b]"Collagen" %N	[b]"Collagen" %C	[c]$\delta^{15}N$	[c]$\delta^{13}C$	C:N
Aucilla River	*Bison antiquus**	bison	7.7	10.61	29.44	4.26	-10.56	3.24
Aucilla River	*Bison antiquus**	bison	19.4	10.70	28.14	3.98	-19.69	3.07
Aucilla River	*Bison antiquus**	bison	13.6	10.49	31.44	4.21	-20.64	3.50
Aucilla River	*Bison**	bison	6.6	9.39	27.90	5.46	-12.42	3.47
Aucilla River	*Bison**	bison	9.0	10.47	31.39	4.23	-18.31	3.50
Aucilla River	*Camelidae (Lamini?)*	llama	no yield					
Aucilla River	*Canis dirus*	dog/wolf	2.6	2.43	13.04	N/A	-22.89	6.26
Aucilla River	*Canis latrans*	coyote	1.3	0.92	2.17	N/A	N/A	2.75
Aucilla River	*Canis sp.**	wolf	12.9	8.87	27.02	8.90	-20.52	3.55
Aucilla River	*Castor canadensis*	beaver	1.8	1.07	5.70	4.66	-23.03	6.21
Aucilla River	*Castor canadensis**	beaver	3.8	6.55	17.03	-0.08	-19.69	3.03
Aucilla River	*Didelphis marsupialis*	opossum	0.4	5.04	17.60	N/A	-21.17	4.07
Aucilla River	*Didelphis virginianus*	opossum	5.3	1.98	6.86	6.15	-22.76	4.04
Aucilla River	*Equus*	horse	2.0	0.74	4.43	N/A	-25.09	6.98
Aucilla River	*Equus*	horse	1.0	0.88	8.26	N/A	-28.05	10.95
Aucilla River	*Equus*	horse	no yield					
Aucilla River	*Equus*	horse	0.6	3.07	19.35	N/A	-21.04	7.35
Aucilla River	*Equus*	horse	0.5	1.25	10.25	N/A	-23.95	9.57
Aucilla River	*Equus*	horse	5.9	0.15	0.24	N/A	N/A	1.87
Aucilla River	*Equus*	horse	10.3	7.33	32.22	4.31	-32.97	5.13
Aucilla River	*Equus sp.**	horse	20.5	11.10	28.35	4.62	-22.20	2.99
Aucilla River	*Equus**	horse	16.9	10.10	25.90	2.48	-8.64	3.04
Aucilla River	*Equus**	horse	16.1	9.94	29.67	5.48	-23.05	3.48
Aucilla River	*Equus**	horse	7.5	9.86	29.75	2.08	-12.74	3.52
Aucilla River	*Felis concolor (Puma concolor)*	mountain lion	3.7	0.13	0.57	N/A	N/A	5.11
Aucilla River	*Felis sp.*	cat	1.5	0.34	3.90	N/A	N/A	13.38
Aucilla River	*Hemiauchenia**	llama	6.8	8.56	25.03	4.8	-22.93	3.41

Site	Genus/species	Common name	[a]% Yield	[b]"Collagen" %N	[b]"Collagen" %C	[c]$\delta^{15}N$	[c]$\delta^{13}C$	C:N
Aucilla River	*Holmesina septentrionalis*	northern pampathere	3.2	1.17	5.71	N/A	-23.40	5.69
Aucilla River	Hydrochoeridae	capybara	2.0	1.39	7.78	N/A	-24.40	6.66
Aucilla River	*Lamini*	llama	1.1	0.19	3.39	N/A	-24.35	20.81
Aucilla River	*Lutra canadensis*	otter	3.3	2.75	11.63	1.23	-24.91	4.93
Aucilla River	*Lutra canadensis*	otter	1.8	0.35	8.62	N/A	-24.47	28.73
Aucilla River	*Lynx rufus*	lynx	0.6	0.44	4.42	N/A	-27.96	11.72
Aucilla River	*Lynx rufus*	lynx	15.3	8.02	26.07	9.18	-21.74	3.79
Aucilla River	*Lynx rufus**	lynx	10.2	8.40	24.13	6.38	-20.19	3.35
Aucilla River	*Mammut americanum*	mastodon	35.4	14.60	20.51	-4.16	-20.71	1.64
Aucilla River	*Mammut americanum**	mastodon	10.6	11.20	26.39	1.32	-20.83	2.75
Aucilla River	*Mammut americanum**	mastodon	19.1	10.80	28.80	3.75	-21.85	3.10
Aucilla River	*Mammuthus columbi*	mammoth	36.7	20.10	10.10	-12.02	-9.33	0.59
Aucilla River	*Mammuthus columbi**	mammoth	22.9	9.41	25.13	4.39	-21.76	3.12
Aucilla River	*Megalonyx jeffersonii*	ground sloth	38.3	20.80	9.04	-13.56	-21.06	0.51
Aucilla River	*Megalonyx jeffersonii**	ground sloth	15.1	9.22	25.35	6.01	-21.03	3.21
Aucilla River	*Megalonyx jeffersonii**	ground sloth	8.2	9.78	28.55	7.69	-20.86	3.41
Aucilla River	*Megalonyx jeffersonii**	ground sloth	10.2	9.94	28.70	7.63	-20.84	3.37
Aucilla River	*Mephitis mephitis*	skunk	3.4	0.28	2.75	N/A	-25.71	11.46
Aucilla River	*Neochoerus pinckneyi*	capybara	3.0	0.38	2.47	N/A	-25.93	7.58
Aucilla River	*Odocoileus virginianus*	deer	15.4	11.60	23.76	1.51	-21.84	2.38
Aucilla River	*Odocoileus virginianus*	deer	0.3	1.97	49.94	N/A	-26.96	29.57
Aucilla River	*Odocoileus virginianus*	deer	0.3	6.10	17.67	-8.35	-22.80	3.38
Aucilla River	*Odocoileus virginianus*	deer	no yield					
Aucilla River	*Odocoileus virginianus**	deer	14.1	9.93	25.74	4.18	-22.30	3.02
Aucilla River	*Odocoileus virginianus**	deer	8.6	10.02	29.72	4.70	-22.59	3.46
Aucilla River	*Odocoileus virginianus**	deer	9.4	9.89	28.28	2.11	-23.48	3.34
Aucilla River	*Odocoileus virginianus**	deer	3.7	7.43	22.36	3.89	-23.19	3.51
Aucilla River	*Odocoileus virginianus**	deer	4.5	9.83	28.74	3.07	-22.28	3.41
Aucilla River	*Odocoileus virginianus**	deer	17.7	10.52	30.56	4.40	-23.44	3.39

Site	Genus/species	Common name	[a]% Yield	[b]"Collagen" %N	[b]"Collagen" %C	[c]$\delta^{15}N$	[c]$\delta^{13}C$	C:N
Aucilla River	*Ondatra*	rat	2.8	4.17	14.82	2.61	-21.68	4.15
Aucilla River	*Ondatra zibethica(?)*	rat	5.5	2.85	10.63	3.69	-22.33	4.39
Aucilla River	*Ondatra zibethica**	rat	2.2	7.43	22.94	6.73	-22.72	3.60
Aucilla River	*Ondatra Zibethicus*	rat	3.2	1.17	4.69	3.04	-23.04	4.68
Aucilla River	*Palaeolama mirifica*	llama	16.9	11.70	19.72	-1.77	-21.81	1.97
Aucilla River	*Palaeolama mirifica*	llama	4.7	8.05	27.89	4.85	-23.61	4.04
Aucilla River	*Paramylodon*	giant ground sloth	1.9	0.11	3.36	N/A	-24.59	35.63
Aucilla River	*Paramylodon*	giant ground sloth	3.3	8.74	34.24	7.51	-24.81	4.57
Aucilla River	*Paramylodon harlani*	giant ground sloth	24.1	15.90	6.37	-18.33	-21.83	0.47
Aucilla River	Pilosa*	sloth	14.9	10.40	26.55	5.28	-20.57	2.98
Aucilla River	*Platygonus compressus*	peccary	3.6	6.90	14.96	0.15	-16.04	2.53
Aucilla River	*Procyon lotor*	northern raccoon	3.0	10.20	19.51	-1.74	-22.43	2.23
Aucilla River	*Procyon lotor*	northern raccoon	2.6	0.88	4.82	N/A	-25.90	6.39
Aucilla River	*Procyon lotor*	northern raccoon	1.6	0.46	6.30	N/A	-23.78	15.98
Aucilla River	*Procyon lotor**	northern raccoon	4.2	5.88	16.92	2.58	-21.07	3.36
Aucilla River	*Procyon lotor**	northern raccoon	2.6	6.14	19.26	6.73	-22.53	3.66
Aucilla River	*Sciurus carolinensis*	squirrel	no yield					
Aucilla River	*Smilodon fatalis*	sabertooth cat	0.4	3.72	5.27	N/A	N/A	1.65
Aucilla River	*Sylvilagus*	rabbit	4.5	1.01	8.81	N/A	-25.69	10.18
Aucilla River	*Sylvilagus*	rabbit	0.5	0.37	7.49	N/A	-24.45	23.61
Aucilla River	*Tapirus*	tapir	3.4	0.52	2.30	N/A	N/A	5.16
Aucilla River	*Tapirus sp.**	tapir	16.3	11.11	31.94	3.78	-23.32	3.35
Aucilla River	*Tapirus veroensis*	tapir	27.0	15.50	19.53	-7.00	-22.90	1.47
Aucilla River	*Tapirus veroensis*	tapir	no yield					
Aucilla River	*Tapirus veroensis*	tapir	3.0	0.56	4.62	N/A	-24.99	9.62
Aucilla River	*Tapirus veroensis*	tapir	0.9	1.12	10.27	N/A	-22.93	10.70
Aucilla River	*Tapirus veroensis*	tapir	11.9	7.96	25.92	4.07	-23.20	3.80

Site	Genus/species	Common name	[a]% Yield	[b]"Collagen" %N	[b]"Collagen" %C	[c]$\delta^{15}N$	[c]$\delta^{13}C$	C:N
Aucilla River	*Urocyon cinereoargenteus*	fox	4.0	0.32	2.03	N/A	-26.14	7.40
Aucilla River	*Ursus americanus**	black bear	8.2	5.84	16.92	5.18	-21.94	3.38
Aucilla River	*Ursus americanus**	black bear	9.4	9.66	28.06	3.07	-22.05	3.39
Aucilla River	*Ursus americanus*	black bear	1.9	2.30	11.86	0.85	-24.58	6.02
Aucilla River	*Vulpes vulpes*	fox	0.9	1.77	9.32	1.42	-22.15	6.14

[a] % yield represents the % organic extract obtained from the whole bone sample

[b] "collagen"%N and "collagen"%C represent the % of N and C, respectively, obtained from the organic extract (±0.5%, 2σ). In samples noted with an *, the organic extract has been determined to be well preserved collagen.

[c] $\delta^{15}N$ and $\delta^{13}C$ values are in ‰ units with respect to atmospheric air and V-PDB, respectively. Errors (2σ): $\delta^{15}N < \pm 0.37‰$, $\delta^{13}C < \pm 0.39‰$

0.04-55.19, none fell within the range of ~2.8-3.6 which would indicate well preserved collagen.

The Tri-Britton and LaBelle Highway sites, while both slightly younger than Leisey Shell Pit, exhibit similar trends to the older sites (Table 3.3). Organic yields ranged from 0%-34.7%. Samples that yielded an organic extract showed %N in that extract of 0.3%-19.1%, and %C ranged from 1.4%-44.2%. The C:N ratios ranged from 0.12-126.65. However, no specimens showed a C:N ratio in the range of ~2.8-3.6, thus indicating a lack of well preserved specimens in the Tri-Britton and LaBelle Highway sites.

Aucilla River, the latest Pleistocene site in Florida, exhibited a much higher quality of preservation than the older Florida sites. Organic yields ranged from 0%-38.3% (Table 3.3). The organic extracts showed a range of %N content from 0.1%-20.8%, and a %C content of 0.2%-49.9%. The C:N ratios ranged from 0.47-35.63 with a total of 32 specimens showing a C:N ratio in the range of ~2.8-3.6. These 32 specimens were considered to be well preserved collagen.

3.1.1.4 Selection of well preserved specimens

The range of C:N ratios used to classify samples as well preserved collagen from these sites was determined by first observing broad trends in collagen yield, and % N and % C yields within the collagen of all specimens that produced organic material upon completion of the chemical extraction process. Figure 3.1 shows these yields for all sample sites and compares them to the range of values ultimately

determined to indicate acceptable collagen preservation. The following discussion summaries the details and trends used to make this determination.

Examination of all specimens that produced an obtainable yield of organic extract shows a noticeable increase in the variability of C:N ratios with decreasing yield (Figure 3.2). As the amount of organic material extracted from bone decreased, it is likely that some or all of the original collagen was destroyed during diagenesis. That which is left may have suffered deterioration to the point where amino acids and proteinaceous substances remain, but intact collagen no longer exists. As such, the C:N ratio will differ from that of well preserved collagen.

This idea is also reflected in an examination of the organic yield versus the percent of nitrogen in that organic substance (Figure 3.3). As the amount of organic extract decreases, the amount of nitrogen in that substance also decreases. Of all the organic molecules in a vertebrate bone, nitrogen is most prevalent in protein. The trend of decreasing nitrogen content therefore suggests that degradation of organic material is happening at the protein level. Several samples also exhibited variable carbon contents beyond that expected in collagen as the organic yield decreased (Figure 3.4). Again this suggests that diagenesis of organic material results in the degradation of some of the protein and produces a by-product which still contains organic molecules, but not necessarily intact collagen.

An examination of the percent nitrogen yield and percent carbon yield versus the C:N ratios (Figures 3.5 and 3.6, respectively) reiterates the conclusion that diagenetic processes have deteriorated collagen protein in several of the samples. Once the C:N ratio ranges outside the values of ~2.8-3.6 (i.e. the accepted range of

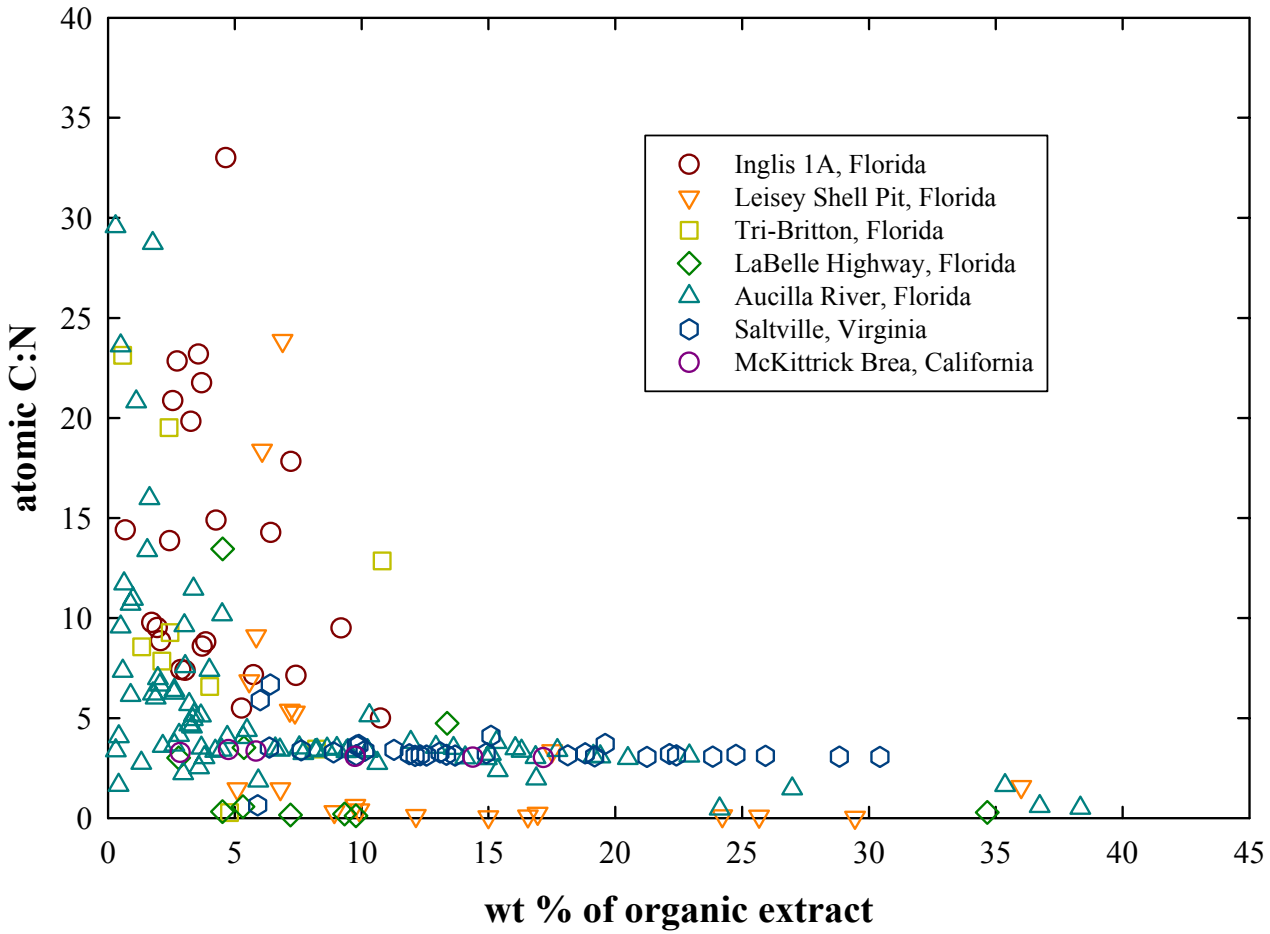

Figure 3.2 – Atomic C:N ratio versus the yield of organic extract (represented by the weight percent of the whole bone) for all samples that produced an organic extract upon completion of chemical processing. One outlier (LSP-7) with an anomalously high % organic extract yield of 72.9% was excluded from the figure.

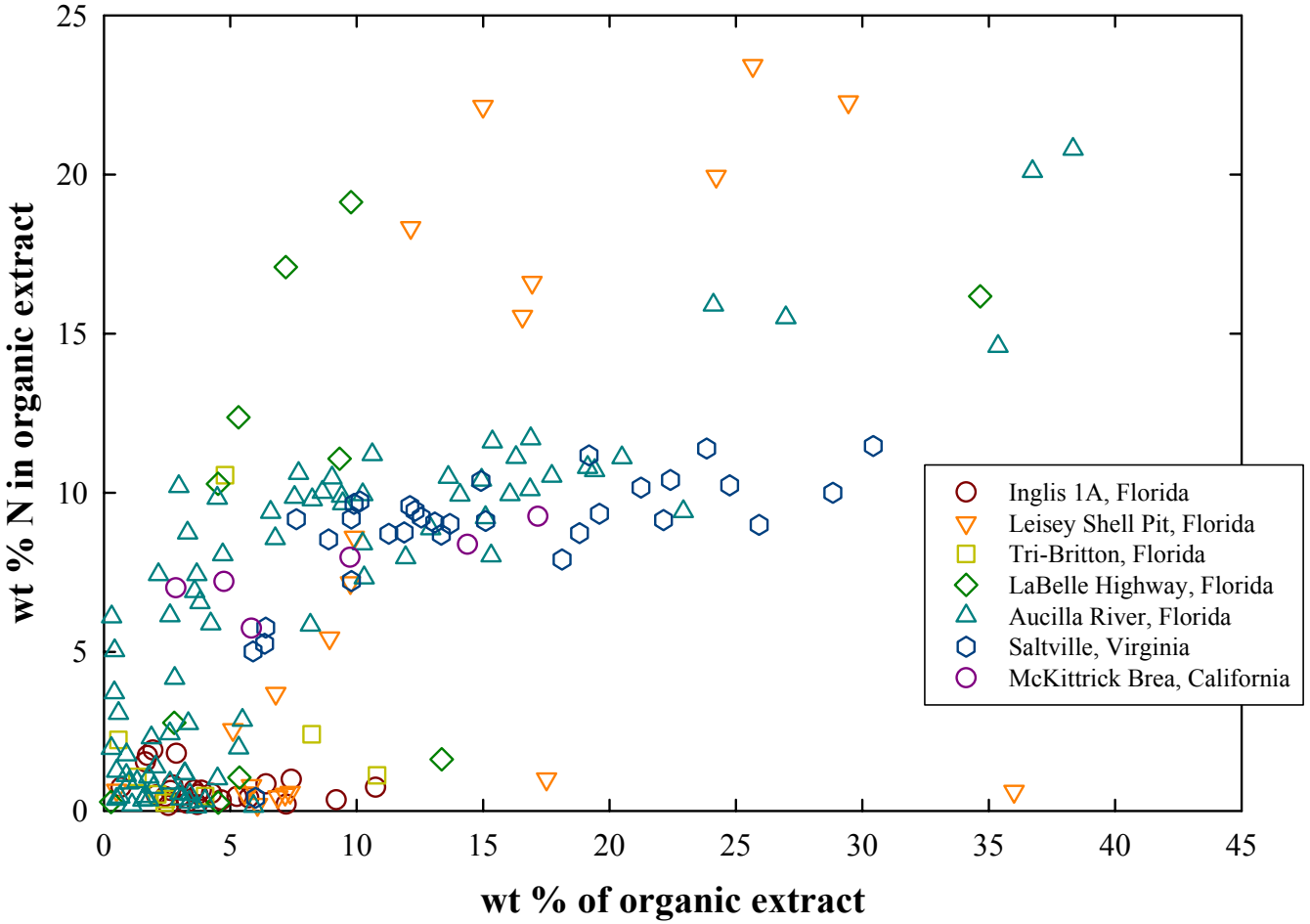

Figure 3.3 - Yield of organic extract (represented by the weight percent of the whole bone) versus the weight percent of nitrogen in that extract for all samples that produced an organic extract upon completion of chemical processing. One outlier (LSP-7) with an anomalously high % organic extract yield of 72.9% was excluded from the figure.

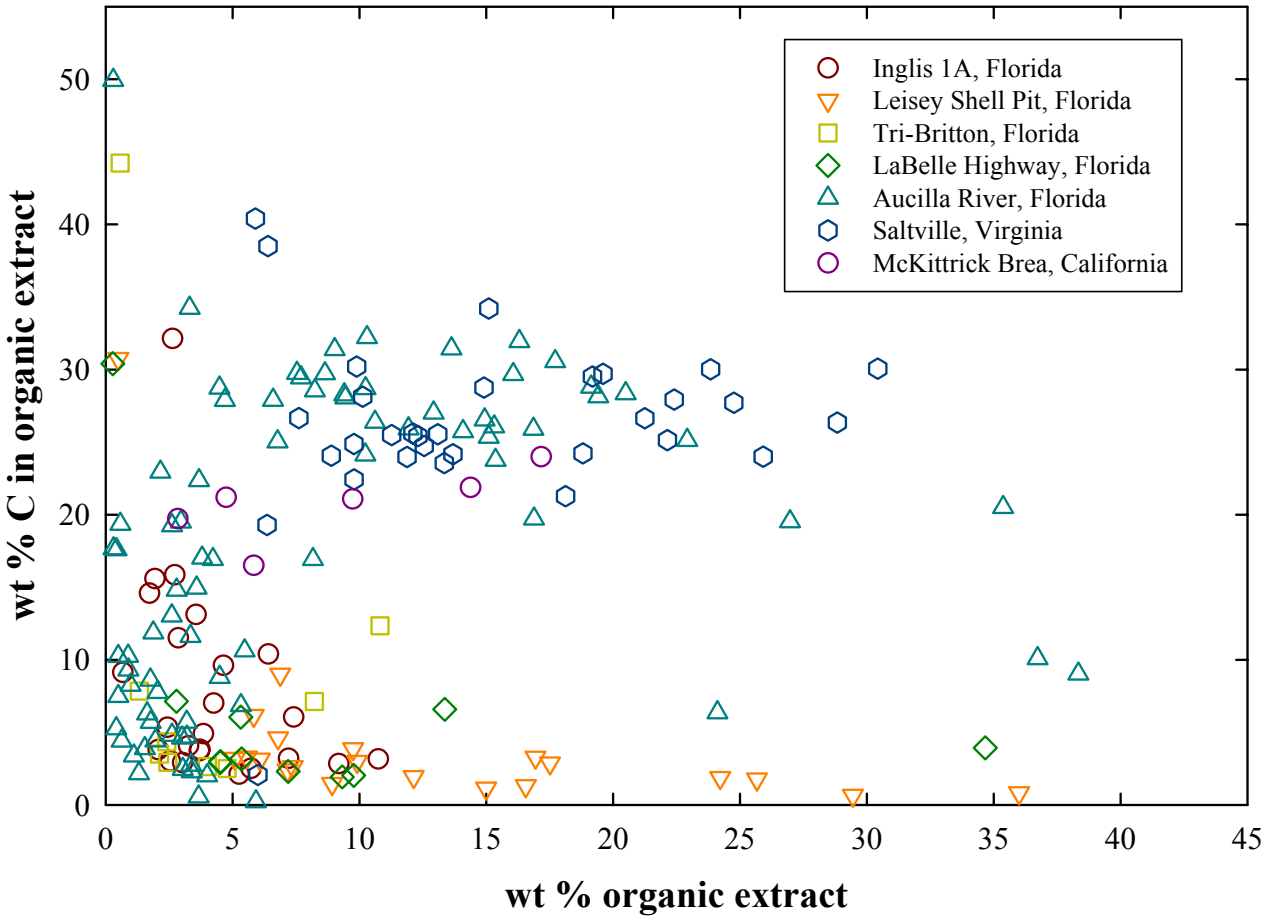

Figure 3.4 - Yield of organic extract (represented by the weight percent of the whole bone) versus the weight percent of carbon in that extract for all samples that produced an organic extract upon completion of chemical processing. One outlier (LSP-7) with an anomalously high % organic extract yield of 72.9% was excluded from the figure.

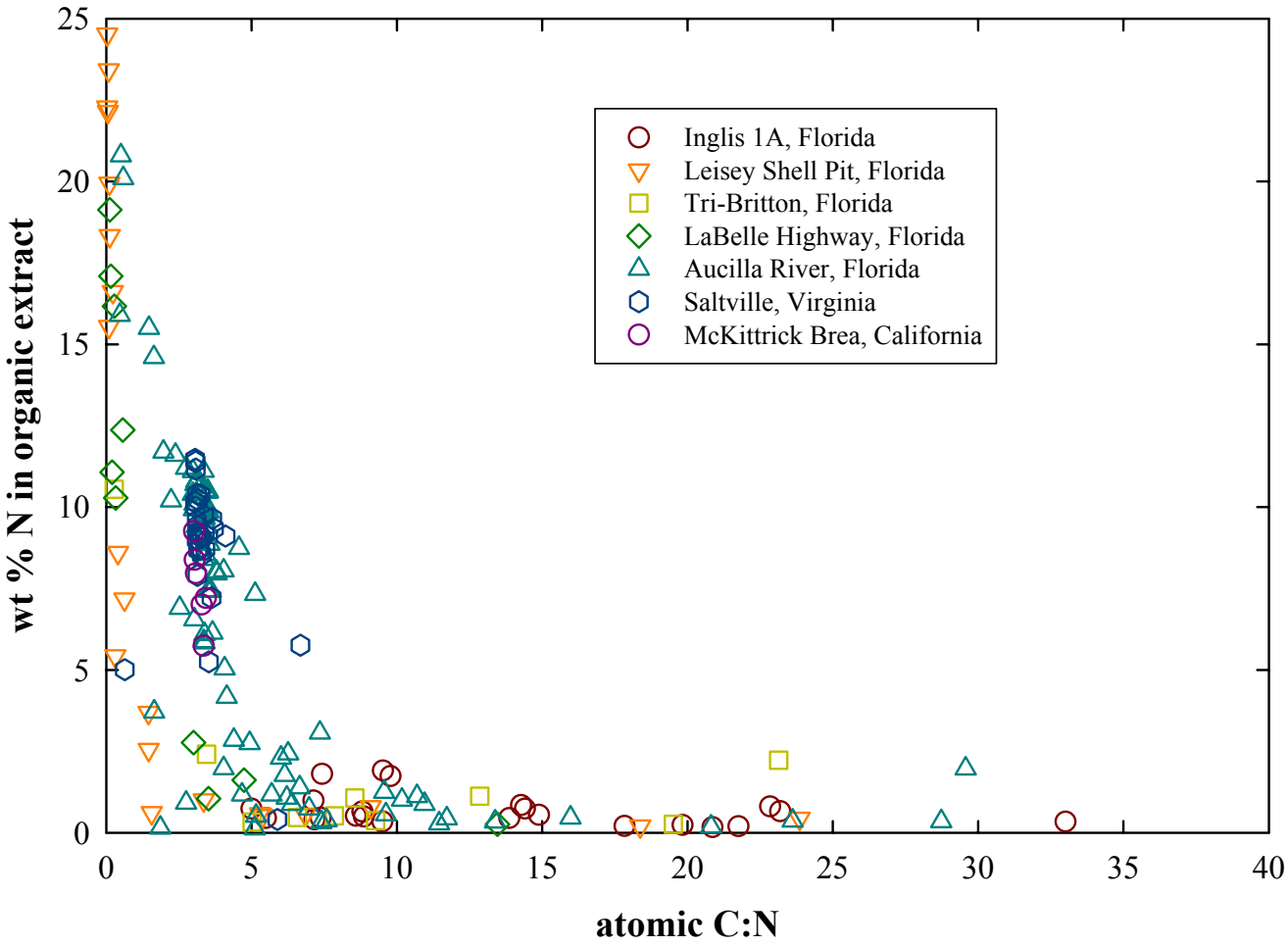

Figure 3.5 – Atomic C:N ratios versus the weight percent of nitrogen in the organic extract for all samples that produced an organic extract upon completion of chemical processing.

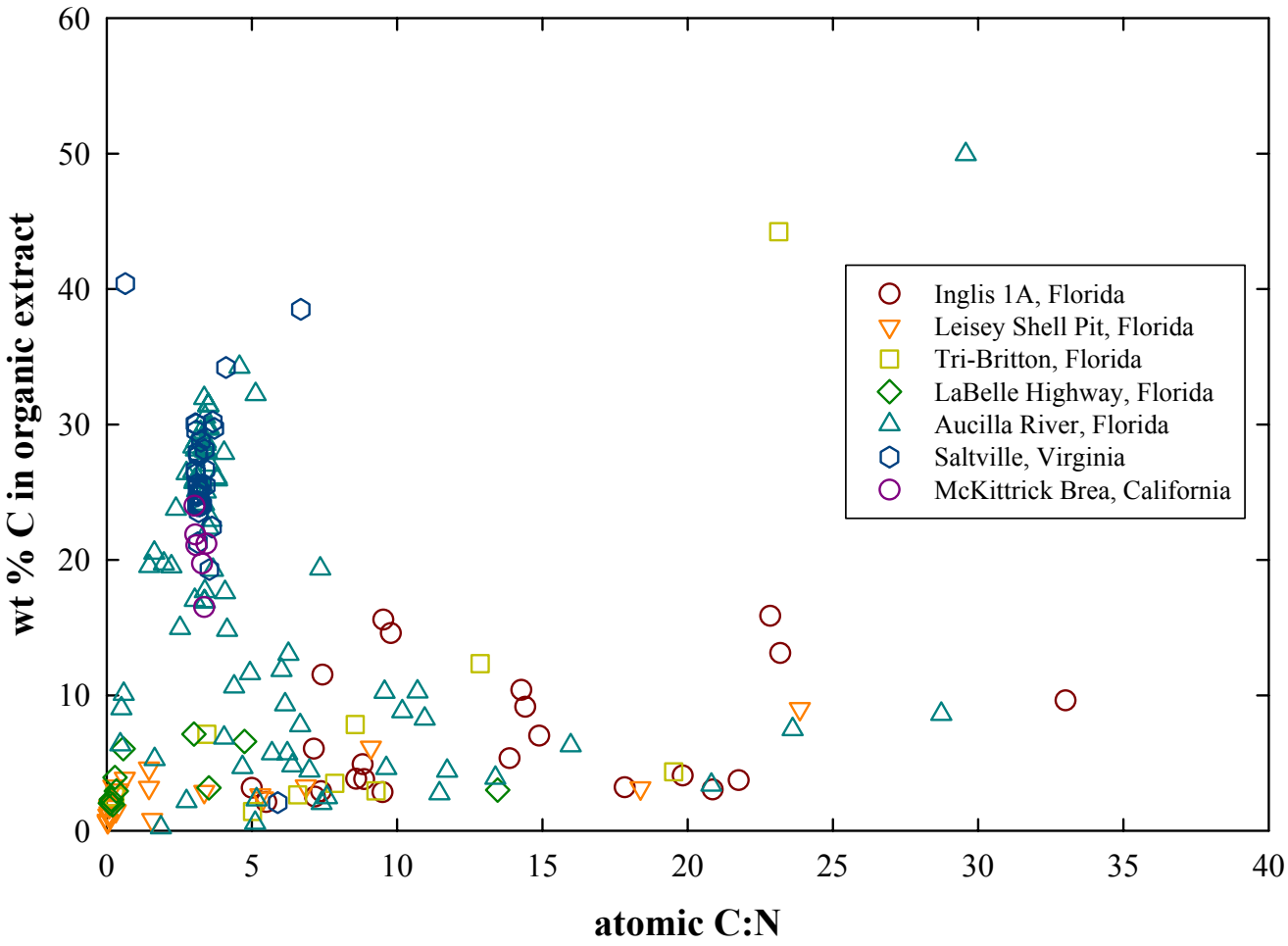

Figure 3.6 - Atomic C:N ratios versus the weight percent of carbon in the organic extract for all samples that produced an organic extract upon completion of chemical processing.

C:N ratios for well preserved collagen), the nitrogen and carbon yields within the organic extract vary widely. Both carbon and nitrogen show an inverse relationship with C:N whereby they decrease as the ratio increases. This suggests that the by-products of collagen degradation in these particular samples are unidentified organic molecules having higher C:N ratios than collagen and were not removed by the chemical process. However, it should be noted that within the range of ~2.8-3.6 for C:N ratios, the nitrogen and carbon yields exhibit an apparent independence from the aforementioned inverse relationship. Examination of this independent relationship was the next step in determining which samples contained well preserved collagen.

The range of C:N ratios for well preserved collagen samples in this study was determined to be 2.75-3.66; the limits of this range are based on the observed independent relationships between collagen yield, percent nitrogen and carbon yields within the collagen, and C:N ratios. Three sites produced specimens with yields and C:N ratios close to the accepted literature values for well preserved collagen (DeNiro 1985, Ambrose 1990, Bocherens et al. 1996, Bocherens et al. 1997, Drucker et al. 2001, Drucker et al. 2003): Aucilla River, Florida; Saltville, Virginia; and McKittrick Brea, California. Examination of the collagen yield versus the C:N ratio for well preserved specimens from these sites (Figure 3.7) shows a lack of correlation between these variables ($R^2 = 0.255$). This suggests that specimens with a low collagen yield may have lost some of their protein to diagenesis, but that which remains is intact. Any diagenetic products, such as humic and fulvic acids, were removed during chemical processing. Likewise, correlation of the collagen yield with the percent yield of carbon in the collagen ($R^2 = 0.188$) and percent yield of nitrogen ($R^2 = 0.362$)

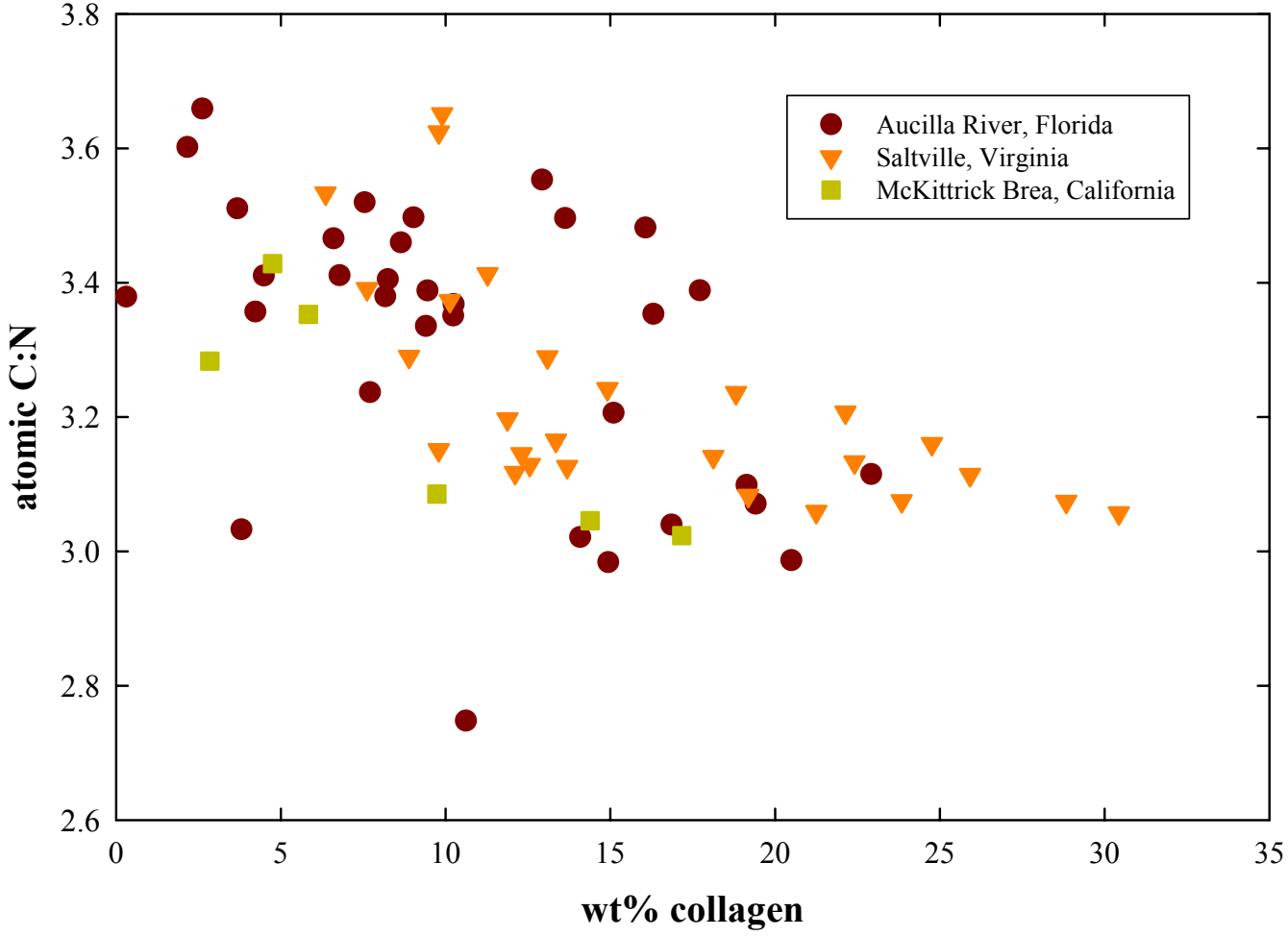

Figure 3.7 – Atomic C:N ratios versus the collagen yield (represented by the weight percent of the whole bone) for samples containing well preserved collagen.

within these same samples suggests an independent relationship (Figure 3.8). As the collagen yield decreases, the amount of both carbon and nitrogen begins to decrease slightly. As was previously discussed, this suggests that there is a threshold of organic degradation beyond which the protein itself begins to disintegrate and the nitrogen content drops. The lower limit of nitrogen yields that is hereby considered acceptable was determined by the maintenance of the independent relationship between nitrogen yield and overall collagen yield. Inclusion of samples that increase the R^2 value, thereby indicating a correlation, were excluded from the group of specimens classified as well preserved. A similar approach was used to examine the relationship between the C:N ratios themselves and the percent yields of carbon and nitrogen (Figure 3.9). Samples within the C:N ratio range of 2.75-3.66 fail to correlate with the percent yield of nitrogen in the collagen ($R^2 = 0.165$) and the percent yield of carbon ($R^2 = 0.123E-4$). Again, this suggests that while some protein may have degraded, that which remains is intact collagen. Therefore, the range of C:N ratios of 2.75-3.66 was used to determine which samples would be included in any isotopic analyses.

The ranges of collagen yields from well-preserved specimens and nitrogen and carbon yields from that collagen agree closely with previously published literature values. Collagen yields ranged from 0.3%-30.4%. Previous fossil studies extracted a collagen yield of ~1-21% of the whole bone weight (Ambrose 1990, Bocherens et al. 1991, Bocherens et al. 1994a, Coltrain et al. 2004). The slightly higher yields in this study are likely due to the different chemical processing methods. The majority of the cited values were accomplished by methods that soaked samples

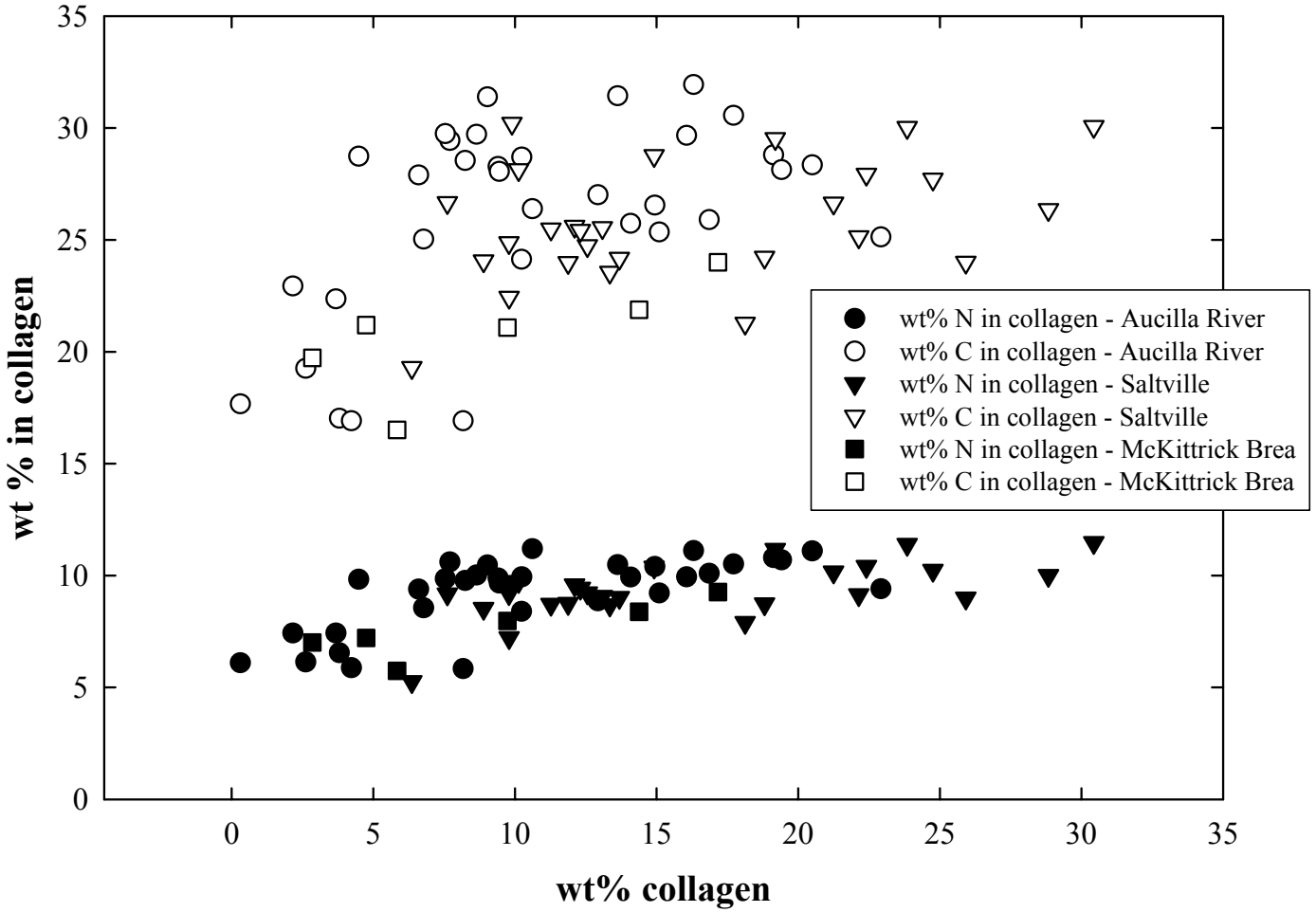

Figure 3.8 - Collagen yield (represented by the weight percent of the whole bone) versus the weight percent of carbon and nitrogen in the collagen for samples containing well preserved collagen.

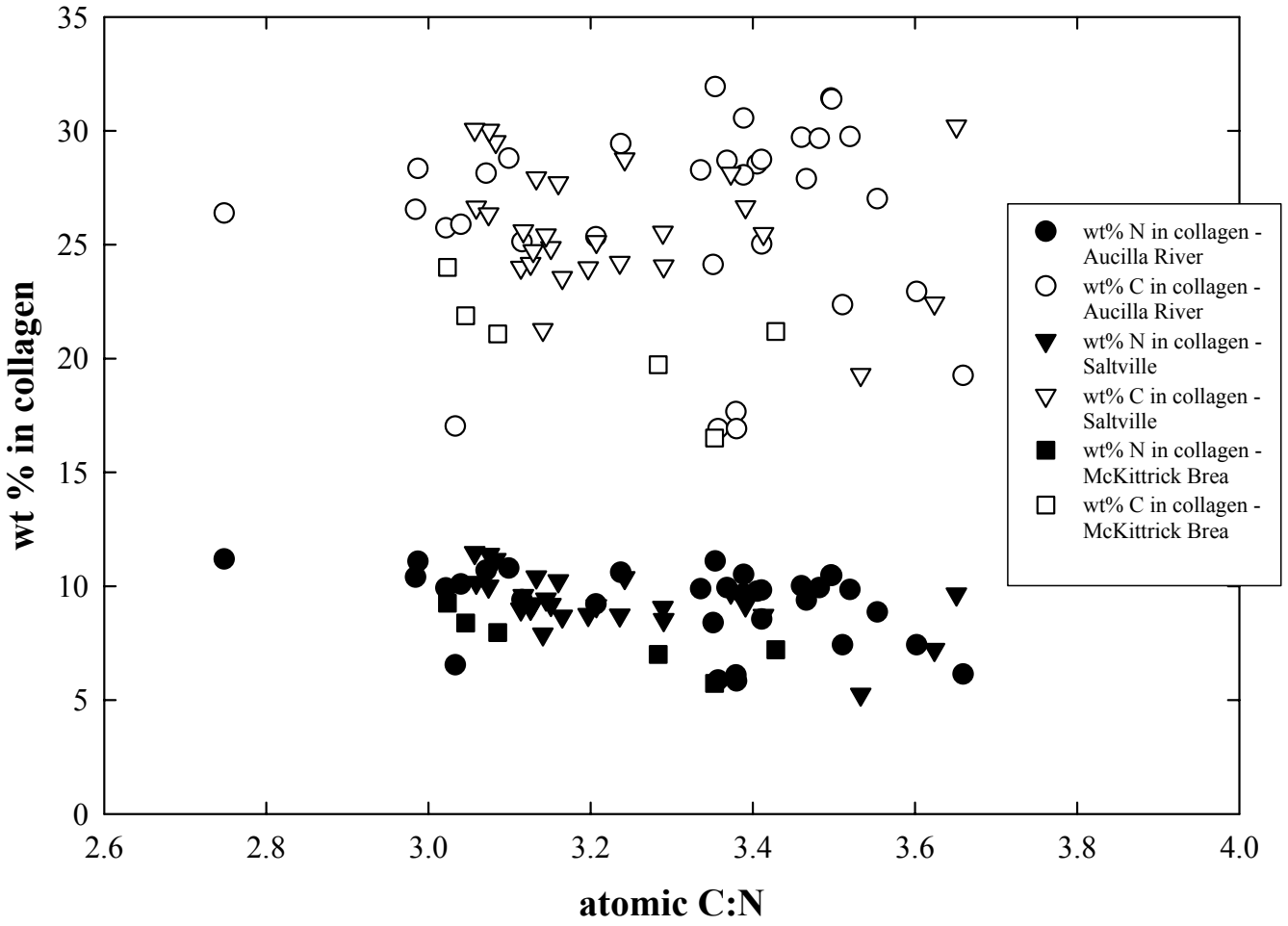

Figure 3.9 – Atomic C:N ratios versus weight percent of carbon and nitrogen in the collagen for samples containing well preserved collagen.

in NaOH to remove humic and fulvic acids. This process runs the risk of hydrolyzing collagen and removing it too early in the extraction process, thus resulting in lower overall yields (Liden et al. 1995). The resin method used in this study avoids this risk and achieves slightly higher yields from smaller specimens. In contrast, the percent yield of nitrogen and carbon was slightly lower than the published literature values. This study produced nitrogen yields of 5.25%-11.47% and carbon yields of 16.51%-31.94%, compared to the literature values of ~11-16% and ~30-45%, respectively (Ambrose 1990, Bocherens et al. 1996, Bocherens et al. 1997, Drucker et al. 2003). The nitrogen and carbon yields from this study are likely lower due to the hydroscopic nature of the final collagen product. It had a tendency to hydrate quickly after the final freeze drying stage. As a result, the weight of the sample that was analyzed by mass spectrometry contained an unknown quantity of water. This would affect the calculated yields of nitrogen and carbon, but not the C:N ratio or isotopic values.

3.1.2 Amino acid analysis

Additional evidence that specimens with a C:N ratio of 2.75-3.66 are in fact well preserved collagen was obtained through an amino acid analysis of both modern and fossil bone. Two specimens from Aucilla River that showed well preserved collagen based on their organic yields and C:N ratios were compared to the two modern bones (domestic bovine and wild deer). Table 3.4 and Figure 3.10 show that the pattern of amino acid abundances between the modern and fossil collagen is nearly identical. The abundance of glycine, proline, and hydroxyproline ranges from

Table 3.4 – Amino acid abundances (mol %)[b]

[a]Sample	CYS	HYP	ASP	THR	SER	GLU	PRO	GLY	ALA	VAL	MET	ILE	LEU	NLE	TYR	PHE	HIS	HLYS	LYS	ARG
BB-26	0.0	9.8	5.4	1.8	2.9	8.2	12.9	37.4	12.7	2.5	0.3	1.1	2.4	0.0	0.2	0.8	0.4	0.4	2.7	5.4
DBIII-2	0.0	9.9	6.1	2.3	3.3	9.1	13.7	39.9	13.4	2.9	0.5	1.1	2.5	0.0	0.5	0.6	0.7	1.2	3.0	6.1
AR-11	0.0	9.1	4.6	1.9	2.6	7.6	12.8	35.5	11.7	2.6	0.3	0.8	1.7	0.0	0.0	0.4	0.4	0.5	2.6	5.1
AR-14	0.0	10.5	5.3	2.0	3.3	6.5	14.5	40.1	14.1	2.8	0.2	1.0	2.1	0.0	0.0	0.5	0.4	0.3	3.3	5.8
AR-39	0.0	0.0	16.5	0.4	1.9	10.2	12.7	40.6	14.3	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TB-2	0.0	8.6	4.4	1.3	3.1	6.5	12.9	39.6	12.4	2.2	0.0	0.0	1.9	0.0	0.0	0.0	0.0	0.0	2.1	5.1
TB-5	0.0	0.0	17.9	0.0	5.3	10.7	0.0	66.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSP-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LSP-22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ING-26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ING-29	0.0	0.0	6.3	3.9	29.4	0.5	7.0	25.9	10.4	2.7	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	11.4	0.0

[a] Sample key: BB-26=modern bovine bone, DBIII-2=modern deer bone, AR-11=Aucilla River (*Equus*), AR-14=Aucilla River (*Mammut americanum*), AR-39=Aucilla River (*Urocyon cinereoargenteus*), TB-2=Tri-Britton (*Platygonus cumberlandensis*), TB-5=Tri-Britton (*Megalonyx wheatleyi*), LSP-6=Leisey Shell Pit (*Arctodus pristinus*), LSP-22=Leisey Shell Pit (*Mammuthus imperator*), ING-26=Inglis 1A (*Hemiauchenia macrocephala*), ING-29=Inglis 1A (*Mammut*)

[b] Amino acid abbreviations: CYS=cysteine, HYP=hydroxyproline, ASP=aspartate, THR=threonine, SER=serine, GLU=glutamate, PRO=proline, GLY=glycine, ALA=alanine, VAL=valine, MET=methionine, ILE=isoleucine, LEU=leucine, NLE=norleucine, TYP=tyrosine, PHE=phenylalanine, HIS-histidine, HLYS=hydroxylysine, LYS=lysine, ARG=arginine

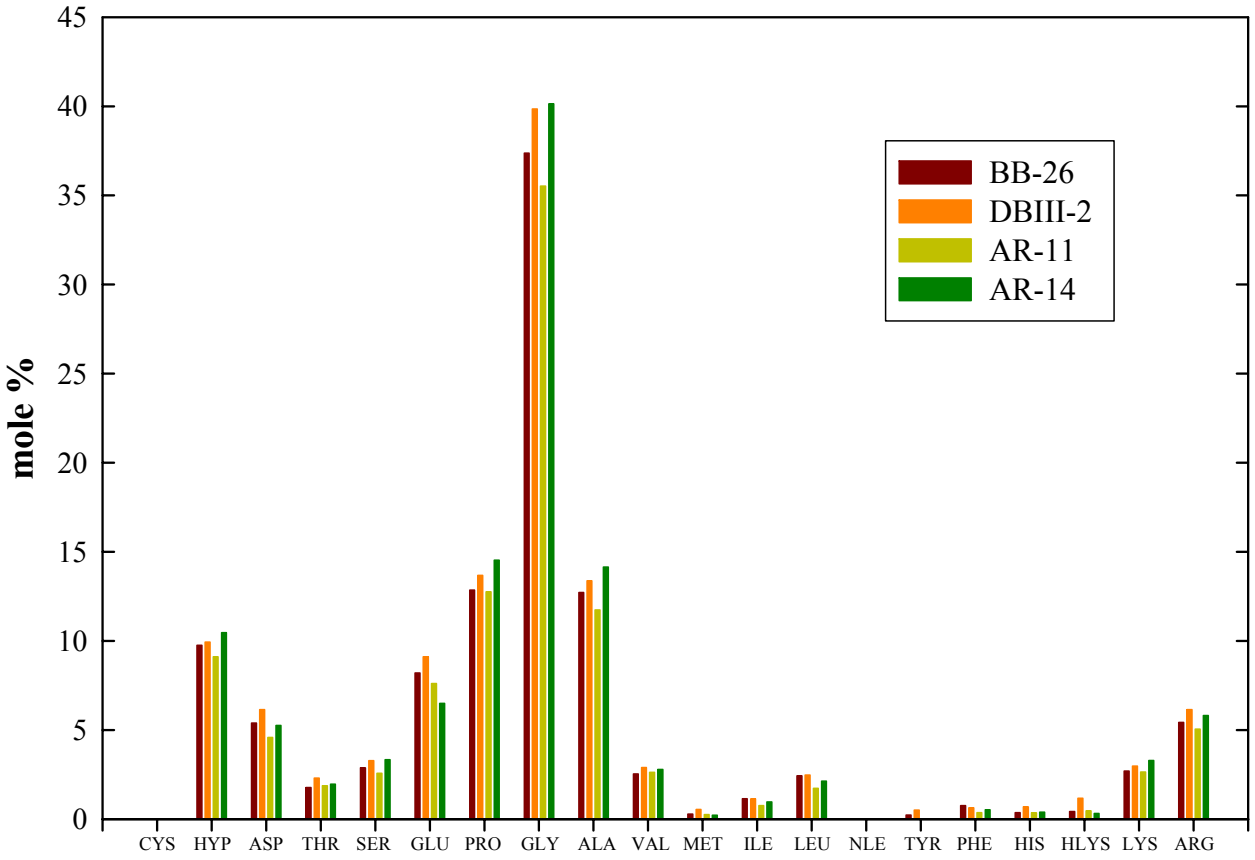

Figure 3.10 – Amino acid abundances for modern bones and well preserved fossil specimens. Sample key: BB-26=modern bovine bone, DBIII-2=modern deer bone, AR-11=Aucilla River (*Equus*), AR-14=Aucilla River (*Mammut americanum*)
Amino acid abbreviations: CYS=cysteine, HYP=hydroxyproline, ASP=aspartate, THR=threonine, SER=serine, GLU=glutamate, PRO=proline, GLY=glycine, ALA=alanine, VAL=valine, MET=methionine, ILE=isoleucine, LEU=leucine, NLE=norleucine, TYP=tyrosine, PHE=phenylalanine, HIS-histidine, HLYS=hydroxylysine, LYS=lysine, ARG=arginine

35.5-40.1 mol%, 12.8-14.5 mol%, and 9.1-10.5 mol%, respectively. This agrees with the previously published values for glycine, proline, and hydroxyproline in collagen of ~31-37 mol%, ~8-14 mol%, and ~7-11 mol%, respectively (Ho 1965, DeNiro and Weiner 1988, Ostrom et al. 1994, Liden et al. 1995). This identical pattern between the fossil and modern collagen suggests that specimens with a C:N ratio in the determined range of 2.75-3.66 are composed of well preserved collagen.

Amino acid analyses were performed on additional fossil specimens from Florida that did not exhibit C:N ratios in the range 2.75-3.66 (Table 3.4). These particular specimens were selected because they yielded an unidentified organic extract which, based on the C:N ratios and yield percent, was clearly not well preserved collagen. Table 3.5 shows that the overall yield of amino acids in the organic extract decreases with increased age of the samples. One sample from Aucilla River (AR-39), one sample from Tri-Britton (TB-2), one from Leisey Shell Pit (LSP-6), and two from Inglis 1A (ING-26, ING-29) were selected because they showed exceptionally low yields of carbon and nitrogen in their organic extracts (Table 3.5). Compared to the modern bovine bone, AR-39, TB-2, and ING-29 appear to have at least some amino acid residues present, while LSP-6 and ING-29 do not (Figure 3.11). The two younger sites (i.e. Aucilla River and Tri-Britton) exhibit the most similar amino acid distributions to the modern bovine bone with relatively high levels of glycine (39.6-40.1 mol%), proline (12.7-12.9 mol%), alanine (12.4-14.3 mol%), and glutamate (6.5-10.2%). However, the Aucilla River fossil specimen (AR-39) is noticeably lacking hydroxyproline. The presence of a relatively high amount of hydroxyproline generally indicates the presence of collagen, therefore it is expected

Table 3.5 – Amino acid yield from organic extracts

[a]Sample	ug of amino acid per mg raw material	%N in organic extract	%C in organic extract
BB-26	561.77	11.15	28.77
DBIII-2	618.83	11.53	29.17
AR-11	519.68	11.1	28.35
AR-14	581.68	10.8	28.8
AR-39	13.42	0.32	2.03
TB-2	40.98	0.47	2.65
TB-5	2.02	10.55	2.51
LSP-6	0.00	0.61	0.82
LSP-22	0.00	15.54	1.31
ING-26	0.00	0.35	2.85
ING-29	16.80	0.74	3.17

[a] Sample key: BB-26=modern bovine bone, DBIII-2=modern deer bone, AR-11=Aucilla River (*Equus*), AR-14=Aucilla River (*Mammut americanum*), AR-39=Aucilla River (*Urocyon cinereoargenteus*), TB-2=Tri-Britton (*Platygonus cumberlandensis*), TB-5=Tri-Britton (*Megalonyx wheatleyi*), LSP-6=Leisey Shell Pit (*Arctodus pristinus*), LSP-22=Leisey Shell Pit (*Mammuthus imperator*), ING-26=Inglis 1A (*Hemiauchenia macrocephala*), ING-29=Inglis 1A (*Mammut*)

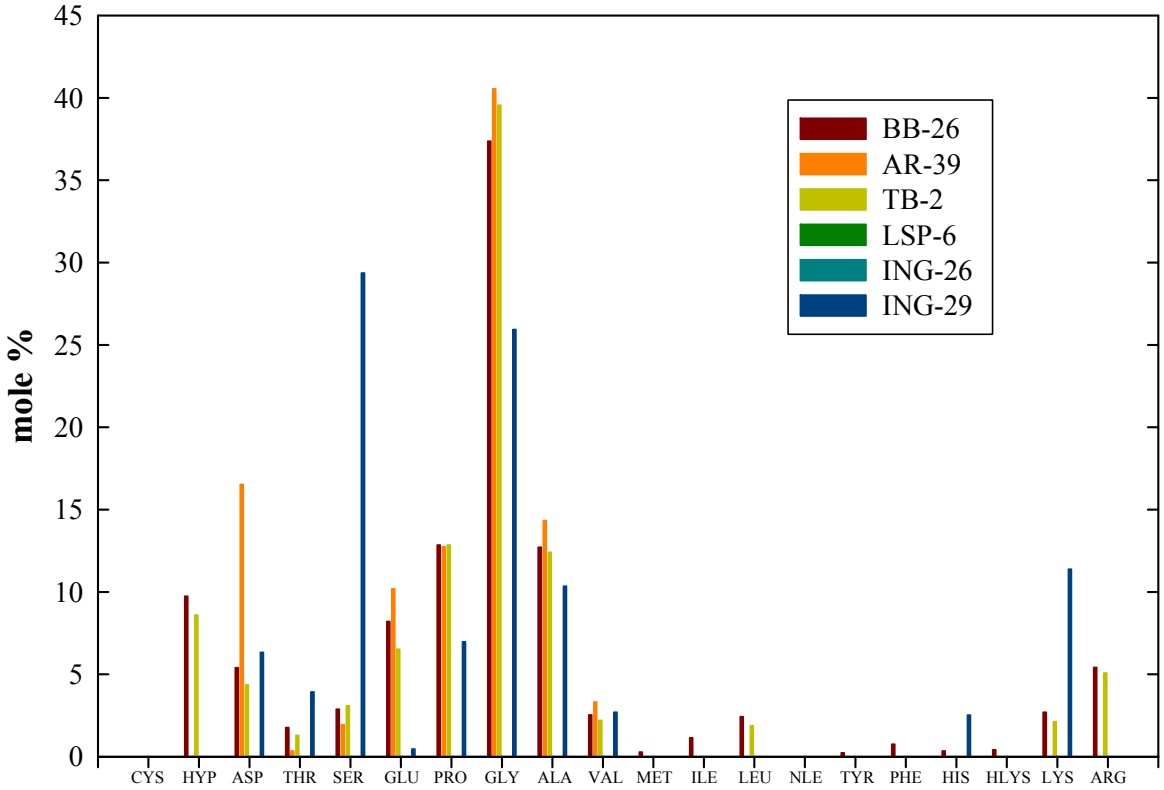

Figure 3.11 - Amino acid abundances for a modern bone and fossil specimens exhibiting a low yield of nitrogen and carbon in their organic extracts. Sample key: BB-26=modern bovine bone, AR-39=Aucilla River (*Urocyon cinereoargenteus*), TB-2=Tri-Britton (*Platygonus cumberlandensis*), LSP-6=Leisey Shell Pit (*Arctodus pristinus*), ING-26=Inglis 1A (*Hemiauchenia macrocephala*), ING-29=Inglis 1A (*Mammut*)
Amino acid abbreviations: CYS=cysteine, HYP=hydroxyproline, ASP=aspartate, THR=threonine, SER=serine, GLU=glutamate, PRO=proline, GLY=glycine, ALA=alanine, VAL=valine, MET=methionine, ILE=isoleucine, LEU=leucine, NLE=norleucine, TYP=tyrosine, PHE=phenylalanine, HIS-histidine, HLYS=hydroxylysine, LYS=lysine, ARG=arginine

that specimens missing hydroxyproline would not contain well preserved collagen. The Tri-Britton specimen (TB-2) contains some hydroxyproline, but the overall distribution of amino acids does not conform to that of collagen and does not produce the expected C:N ratio. One Inglis 1A specimen (ING-29) shows an unusually high abundance of serine and lysine. It is also missing hydroxyproline, which supports the previous determination that this specimen does not contain well preserved collagen. Finally, the second specimen from Inglis 1A (ING-26) and a specimen from Leisey Shell Pit (LSP-6) exhibited not only a low carbon and nitrogen yield in their organic extract, but also a lack of amino acids. The post-diagenetic content of these two specimens is organic in nature, but not proteinaceous.

Two additional specimens, one from Tri-Britton (TB-5) and one from Leisey Shell Pit (LSP-22) were examined because they exhibited unusually high yields of nitrogen in their organic extracts, but low yields of carbon and a C:N ratio that was outside the determined range for well preserved collagen (Figure 3.12). Compared to the modern bovine bone, TB-5 shows an exceptionally high abundance of glycine (66.1 mol%) and a relatively high abundance of aspartate (17.9 mol%). It is noticeably lacking proline and hydroxyproline, thus confirming the lack of well preserved collagen. LSP-22 showed no measurable levels of amino acids despite the high nitrogen content of this sample. This suggests the presence of a non-proteinaceous organic compound.

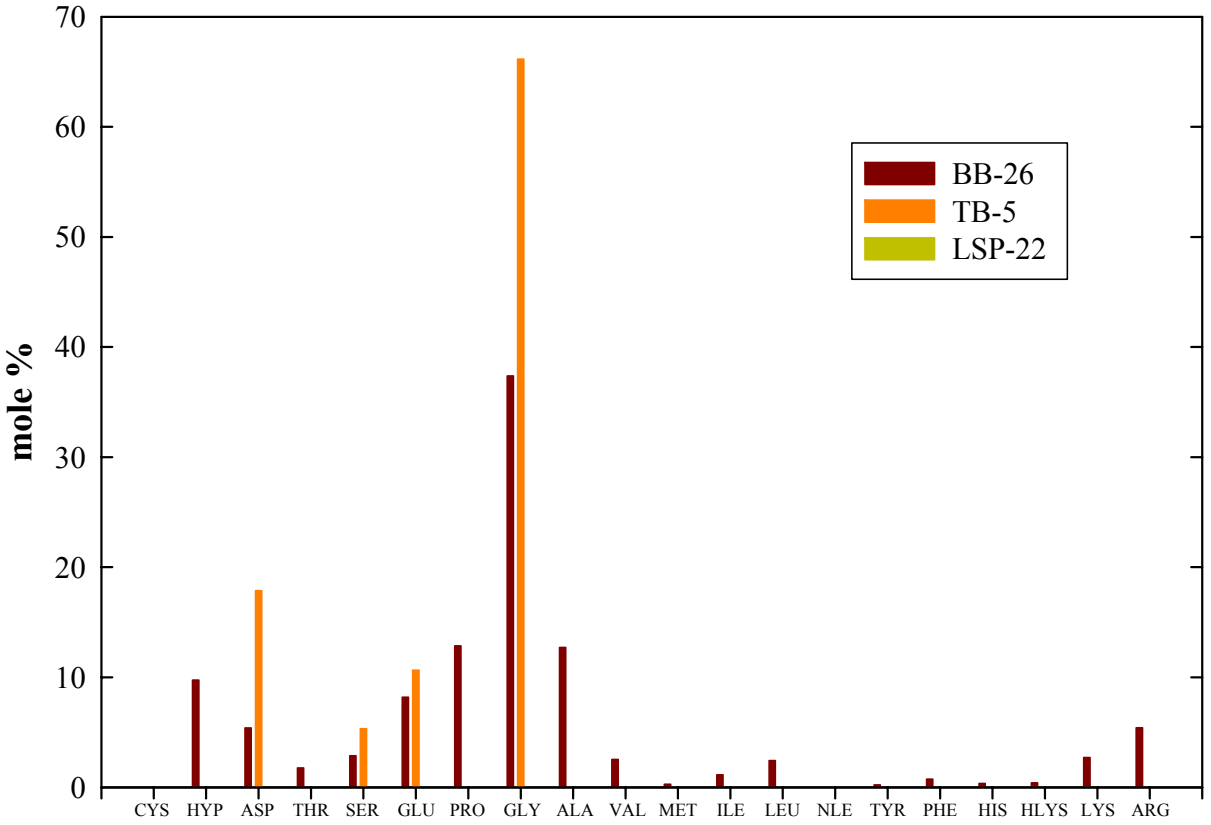

Figure 3.12 - Amino acid abundances for a modern bone and fossil specimens exhibiting a high yield of nitrogen and low carbon yield in their organic extracts. Sample key: BB-26=modern bovine bone, TB-5=Tri-Britton (*Megalonyx wheatleyi*), LSP-22=Leisey Shell Pit (*Mammuthus imperator*)
Amino acid abbreviations: CYS=cysteine, HYP=hydroxyproline, ASP=aspartate, THR=threonine, SER=serine, GLU=glutamate, PRO=proline, GLY=glycine, ALA=alanine, VAL=valine, MET=methionine, ILE=isoleucine, LEU=leucine, NLE=norleucine, TYP=tyrosine, PHE=phenylalanine, HIS-histidine, HLYS=hydroxylysine, LYS=lysine, ARG=arginine

3.2 Carbon dating results

Five specimens that yielded well preserved collagen were carbon-dated – all showed ages in the latest Pleistocene (Table 3.6). Aucilla River, Florida was the youngest site with two specimens yielding ages of 12,259±90 yrs BP and 15,700±140 yrs BP. Saltville, Virginia was close in age with two samples yielding ages of 15,680±140 yrs BP and 20,030±250 yrs BP. McKittrick Brea was the oldest site with an age of 26,850±590 yrs BP.

3.3 Nitrogen and carbon isotopes

Samples that contained well preserved collagen showed a wide range of nitrogen and carbon isotopes (Figure 3.13). Specimens from McKittrick Brea, California exhibited some of the highest $\delta^{15}N$ values with an overall range of ~6‰ (Table 3.1 and Figure 3.14). These values ranged from +7.24‰ for a rodent up to +13.61‰ for *Hemiauchenia*. It should be noted that the *Hemiauchenia*, an herbivore, shows a higher $\delta^{15}N$ value than *Smilodon*, a carnivore. This may be due to the presence of a ruminant digestive strategy or an adaptation to internal water conservation (see Chapter 4).

Specimens from Saltville, Virginia yielded a nitrogen isotopic range of ~5‰ with various trends exhibited amongst the different animals (Table 3.2 and Figure 3.15). The *Rangifer* specimen exhibited the lowest value with a $\delta^{15}N$ of +0.79‰. The highest values were included in the *Bootherium* specimens which showed a range in $\delta^{15}N$ values of +1.99‰ to +5.81‰. Observations to note include the apparent

Table 3.6 – ^{14}C dates

Site	Genus/species	Common name	^{14}C date (yrs BP)	1σ error (yrs)
Aucilla River, Florida	*Megalonyx jeffersonii*	ground sloth	15700	140
Aucilla River, Florida	*Bison antiquus*	bison	12250	90
Saltville, Virginia	*Bootherium*	musk ox	15680	140
Saltville, Virginia	*unknown*	proboscidian	20030	250
McKittrick Brea, California	*Hemiauchenia*	llama	26850	590

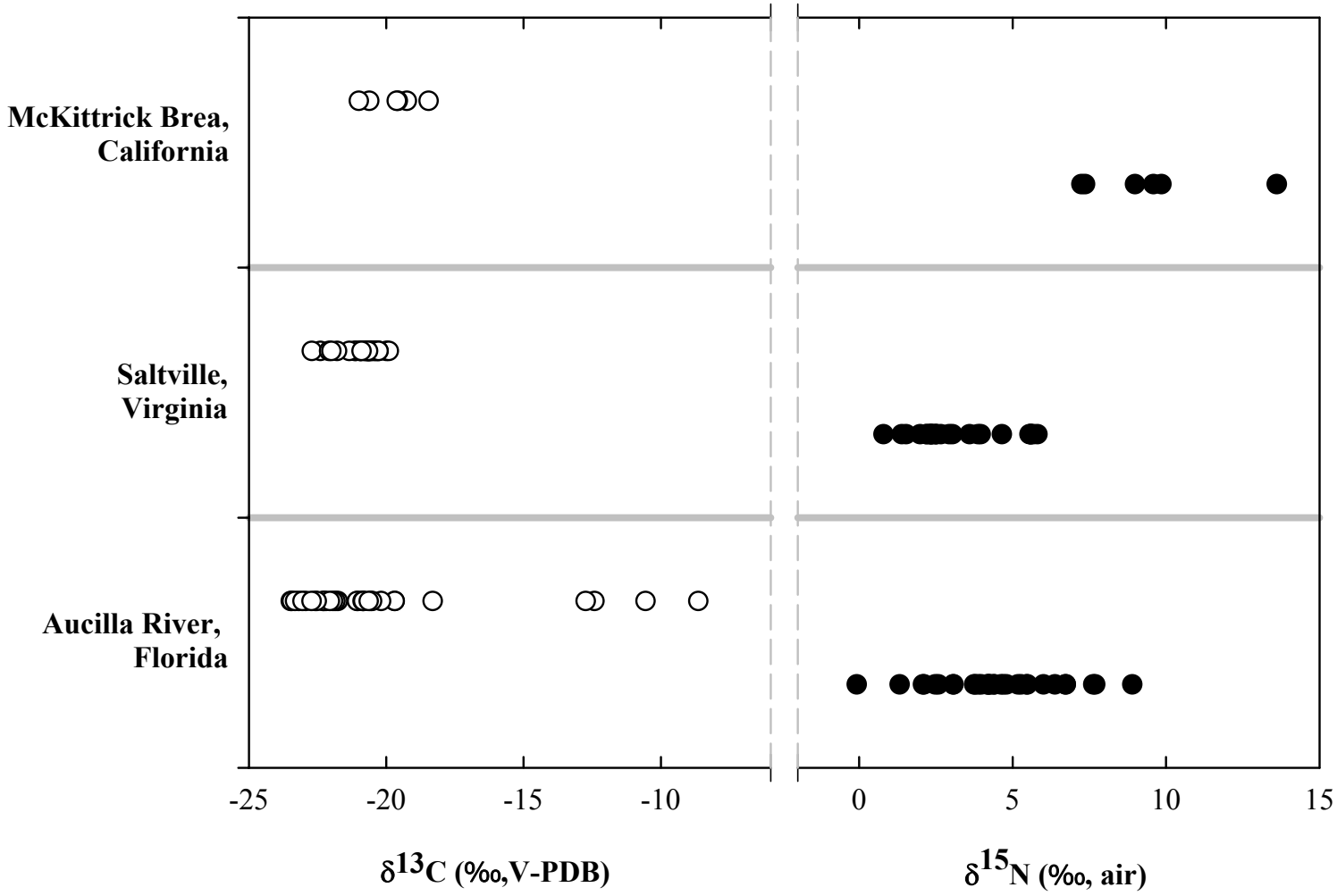

Figure 3.13 – Range of carbon and nitrogen isotopic values for all three sites that yielded well preserved collagen.

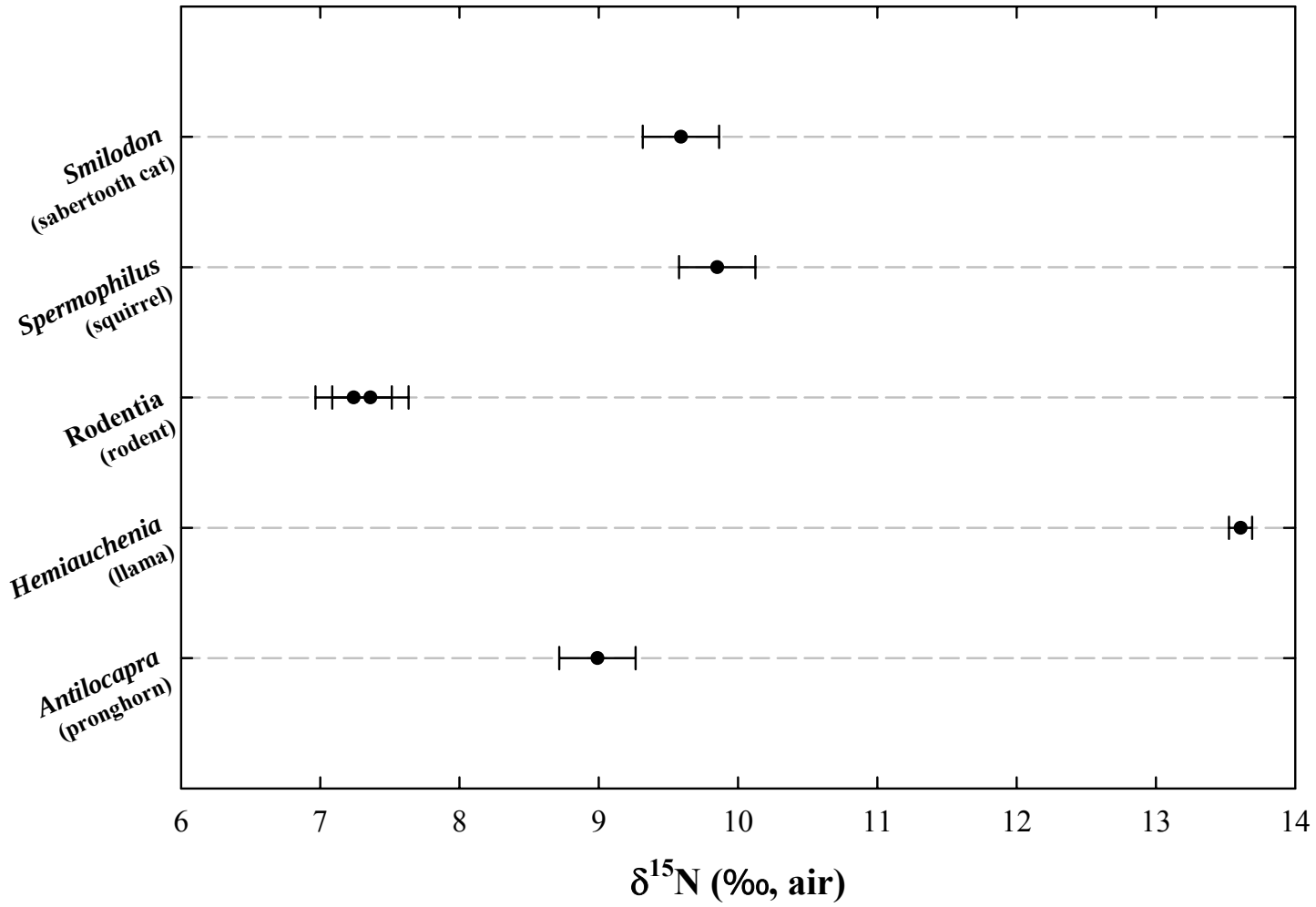

Figure 3.14 – $\delta^{15}N$ values for individual animals from McKittrick Brea, California

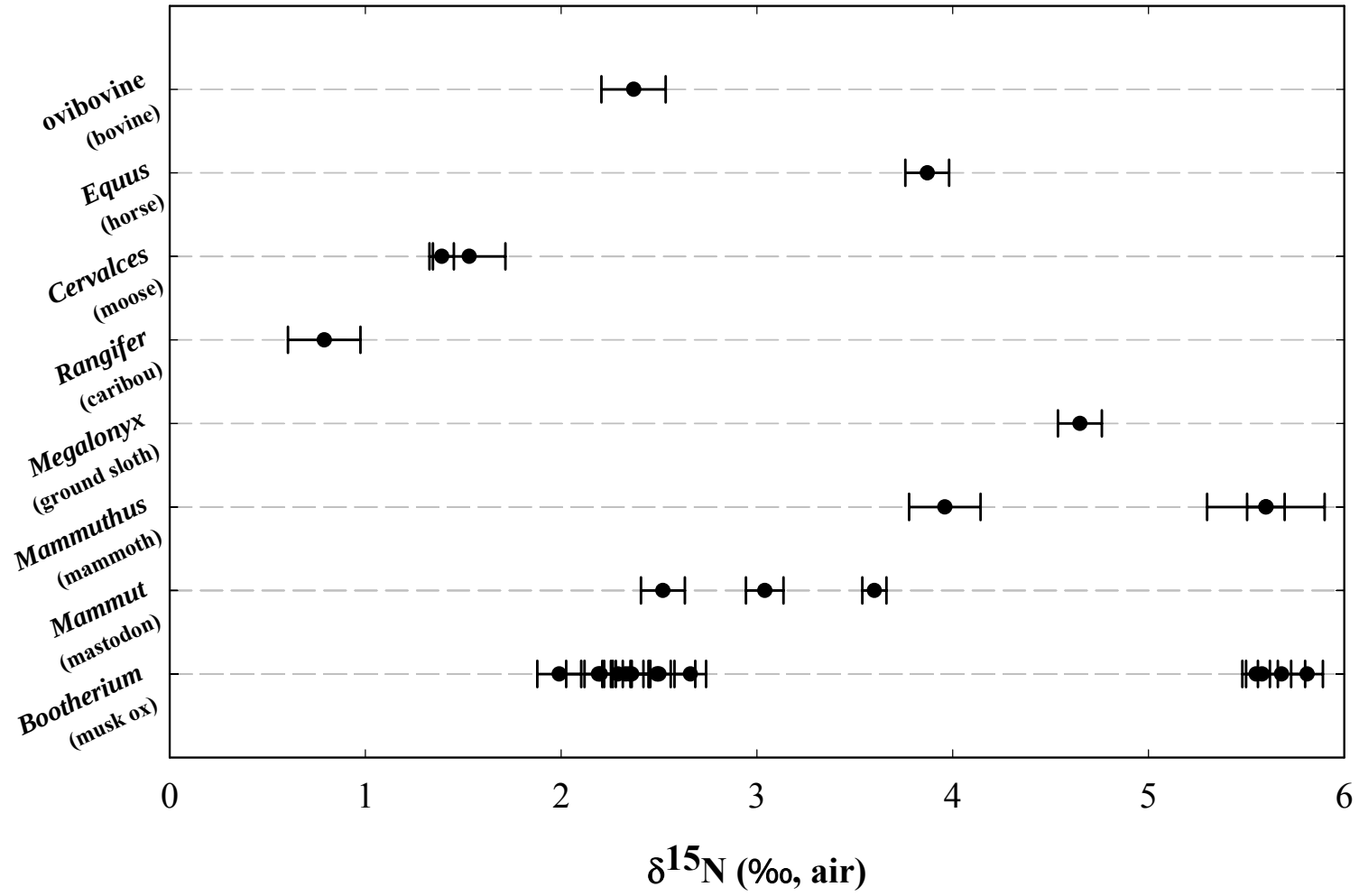

Figure 3.15 – $\delta^{15}N$ values for individual animals from Saltville, Virginia

separation into two distinct groups of the *Bootherium* as well as the consistency of the *Megalonyx* data with that of the other herbivores in the sample group. The mammoths show a range of values (+3.96‰ to +5.60‰) that rival the highest $\delta^{15}N$ exhibited by the *Bootherium*. However, this apparent similarity in ranges may be due to the presence of a mammoth juvenile (see Chapter 4).

Well preserved specimens from Aucilla River, Florida produced a wide range of nitrogen isotopic values (~9‰) and a variety of trends among and between animals (Table 3.3 and Figure 3.16). The highest $\delta^{15}N$ value was observed in the carnivore *Canis* (+8.90‰). There is a noticeable trend of decreasing $\delta^{15}N$ values towards the herbivores. A *Castor* specimen exhibits the lowest $\delta^{15}N$ of -0.08‰. The suspected omnivores, *Ursus* and *Procyon*, plot between the herbivores and carnivores. The *Mammuthus* and *Mammut* nitrogen isotopic values fall well within the range of values for all herbivores as opposed to a slightly more enriched value as was observed in Saltville. The sloth specimens, however, behave in a manner similar to the Saltville specimens. The *Megalonyx* and Pilosa show $\delta^{15}N$ values in the high end of the herbivore range and even overlap with the suspected omnivores and some of the carnivores.

The carbon isotopic data from all three sites shows the majority of specimens have a tight range of values around -20‰ with considerable overlap between animals. At McKittrick Brea, a rodent has the lowest $\delta^{13}C$ of -21.00‰ and the *Hemiauchenia* has the highest value of -18.46‰ (Figure 3.17). At Saltville, *Equus* exhibits the lowest $\delta^{13}C$ value of -22.71‰ and *Bootherium* shows the most enriched value of

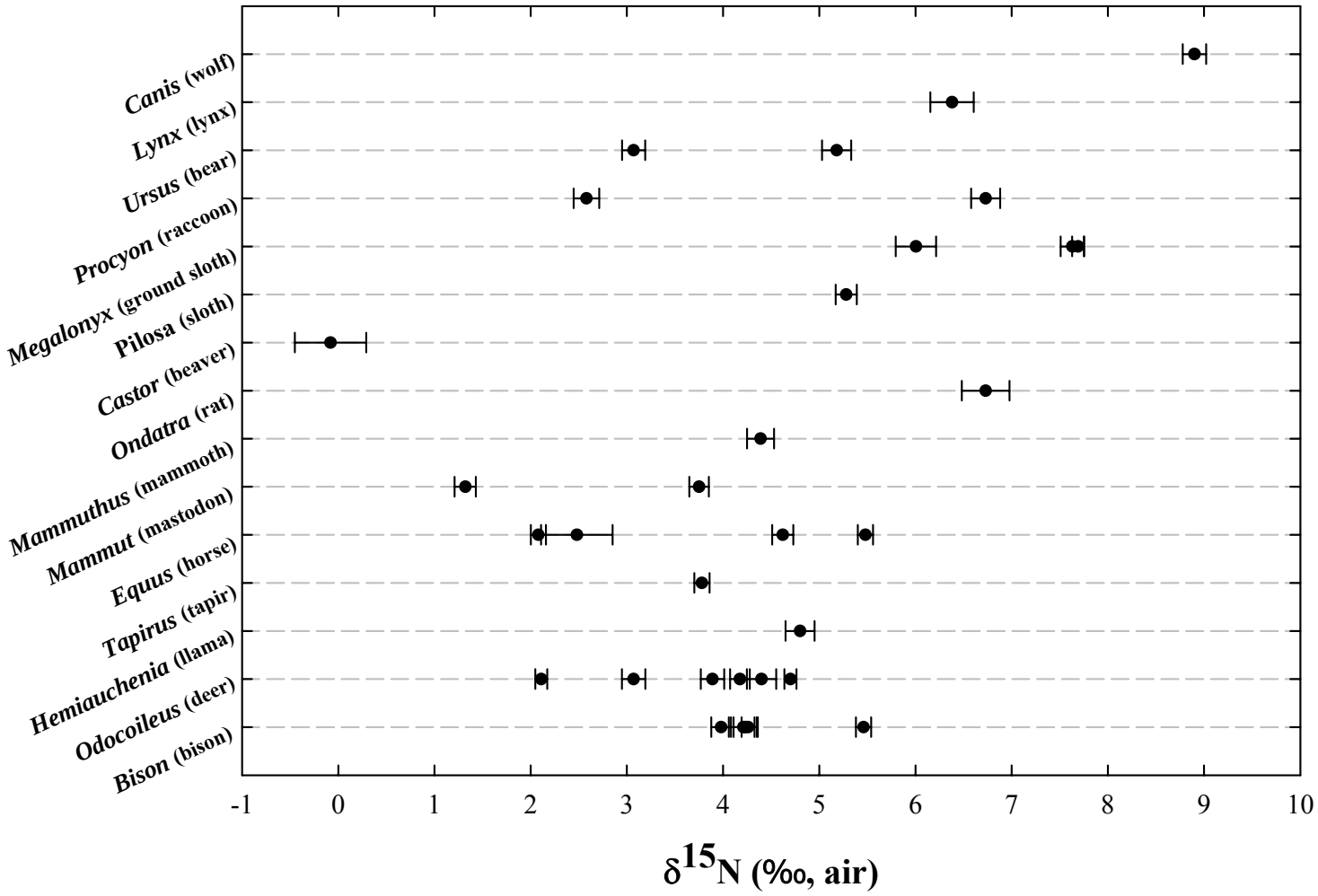

Figure 3.16 – $\delta^{15}N$ values for individual animals from Aucilla River, Florida

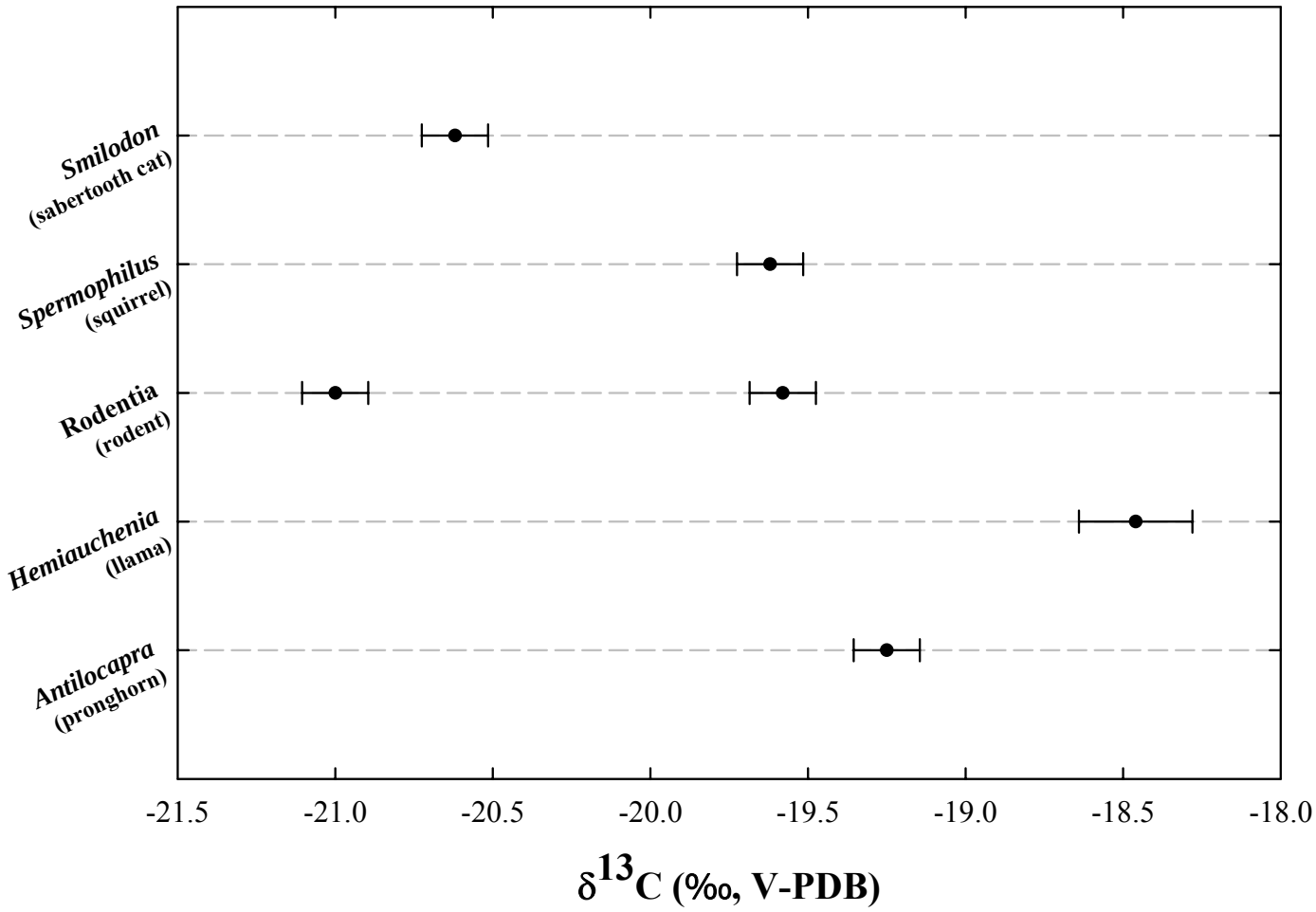

Figure 3.17 – $\delta^{13}C$ values for individual animals from McKittrick Brea, California

-19.91‰ (Figure 3.18). Most of the Aucilla River specimens plot in a similar range with four specimens showing significant enrichment (Figure 3.19). In the depleted range, an *Odocoileus* shows the lowest $\delta^{13}C$ of -23.48‰. *Equus* and *Bison* both exhibit a distinct group of enriched specimens ranging as high as -8.64‰ and -10.56‰ respectively. This distinction is likely due to a grazing dietary habit and the presence of C4 type grasses in the region (see Chapter 4).

The sediment samples from Saltville, Virginia yielded $\delta^{13}C$ values of -25.58‰ and -26.45‰. Considering the fractionation between diet and bone collagen in herbivores of ~5‰, these values mimic those observed in the animals.

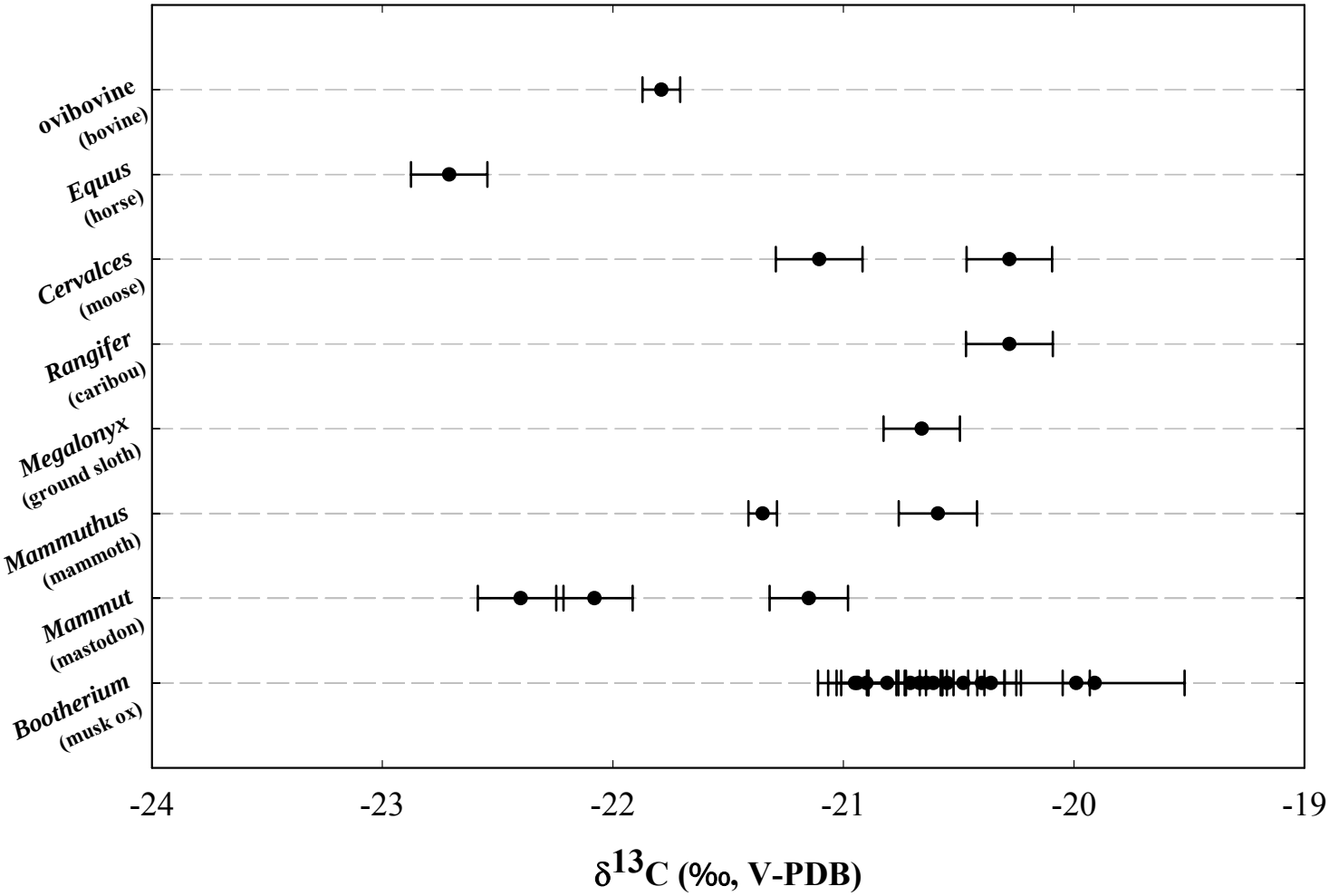

Figure 3.18 – $\delta^{13}C$ values for individual animals from Saltville, Virginia

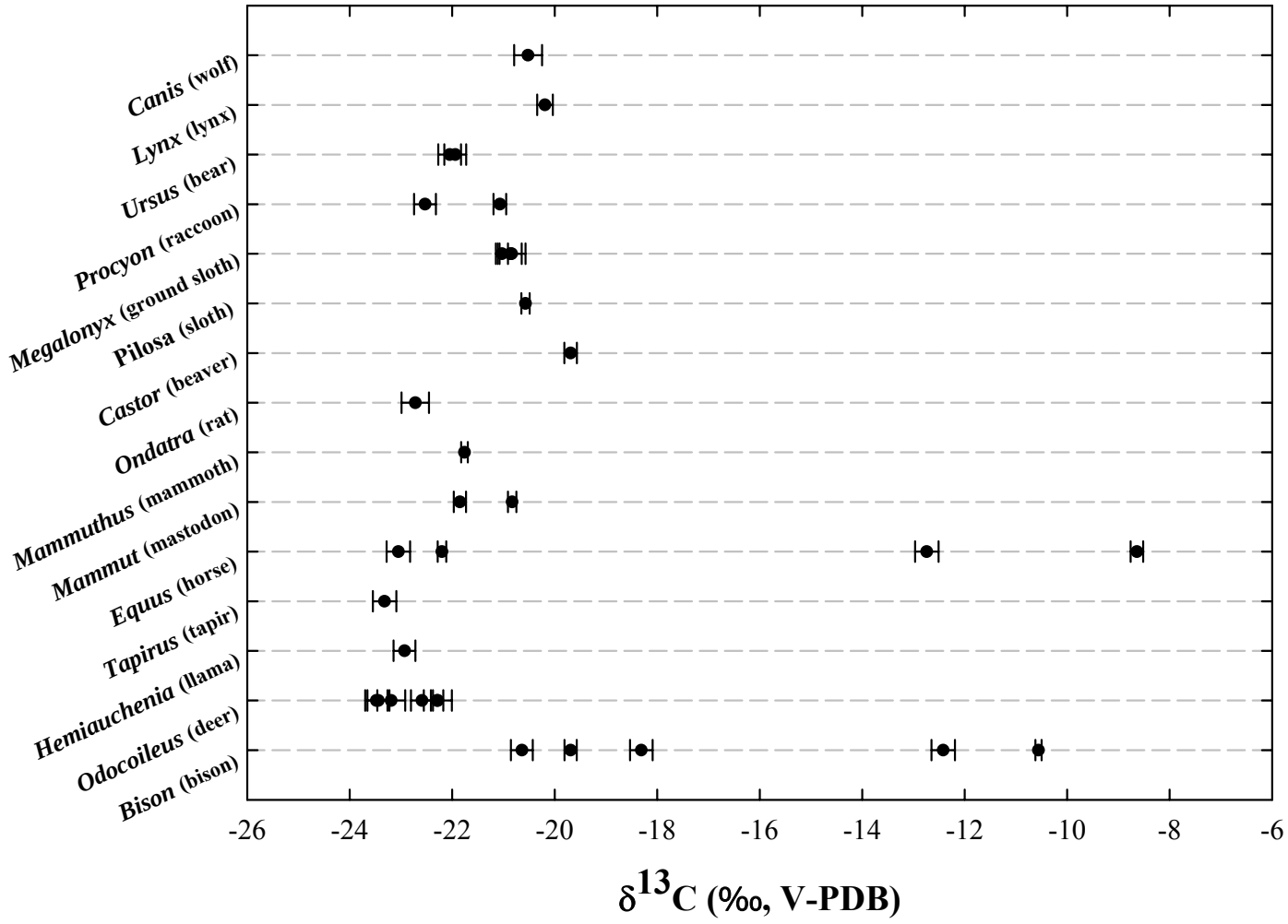

Figure 3.19 – $\delta^{13}C$ values for individual animals from Aucilla River, Florida

CHAPTER 4 – DISCUSSION

4.1 Introduction

The data obtained in this study can be applied to four main topics. First, radiocarbon dating places the sample sites chronologically within the Pleistocene and associates each sample set with climatic events occurring during its time period. Amino acid analysis facilitates the examination of bone diagenesis through time and provides insight into the duration of collagen preservation in fossil materials. Isotopic data is used to reconstruct the trophic relationships within each sample site and examine specific feeding specializations within each community. Finally, the information obtained for each ecosystem is considered in the context of the late Pleistocene extinction of primarily large mammals that occurred ~10,000 yrs BP. The reconstructed tropic patterns are applied to the two competing explanations for this extinction (i.e. human over-hunting and climate change) in an attempt to gain new insight into the cause of this event.

The examination of these various topics is made possible by the relative completeness of the data set and large number of well preserved specimens obtained from the sample sites. The sample set that yielded well preserved collagen includes a fairly comprehensive cross section of the late Pleistocene fauna, the breadth of which rivals any data set that is currently published. The sample set yielding non-collagenous organic extracts provided a unique opportunity to extensively examine diagenesis in relation to time. However, before any discussion of data interpretation ensues, it should be noted that this data set should be considered statistically small.

In most cases, interpretations are based on a repetition of data (multiple individuals from the same species, for example). However, in some cases it is necessary to base conclusions on a statistically sub-optimal number of data points due to the limited availability of samples and the need to avoid destructive analyses of sparse specimen collections. Statistical analyses take into account these smaller sample numbers and the reported uncertainties can be considered accurate despite the absence of an ideal statistical scenario.

4.2 Chronologic placement of specimens

The five radiocarbon dated samples from Virginia, Florida, and California span a range of time from before the Last Glacial Maximum to the peak of the Bolling-Allerod warm period, thereby providing a view of trophic structures across the dramatic climate shift at the end of the Pleistocene Epoch (Figure 4.1). The McKittrick, California sample, dated at 26,850±590 radiocarbon years before present (i.e. ^{14}C yrs BP), provides an age that is just prior to the Last Glacial Maximum. These samples represent an ecosystem that existed during extremely harsh and cold conditions when ice coverage reached its southern-most expansion into the state of Washington on the west coast and Pennsylvania on the east coast (Booth et al. 2004, Mickelson and Colgan 2004). The Saltville, Virginia samples, dated at 20,030±250 and 15,680±140 ^{14}C yrs BP, fall in the time period when the Last Glacial Maximum reached its peak and began to wane into the Bolling-Allerod, a brief warm period that peaked ~12,000 ^{14}C yrs BP. These samples provide a view of mammals during a

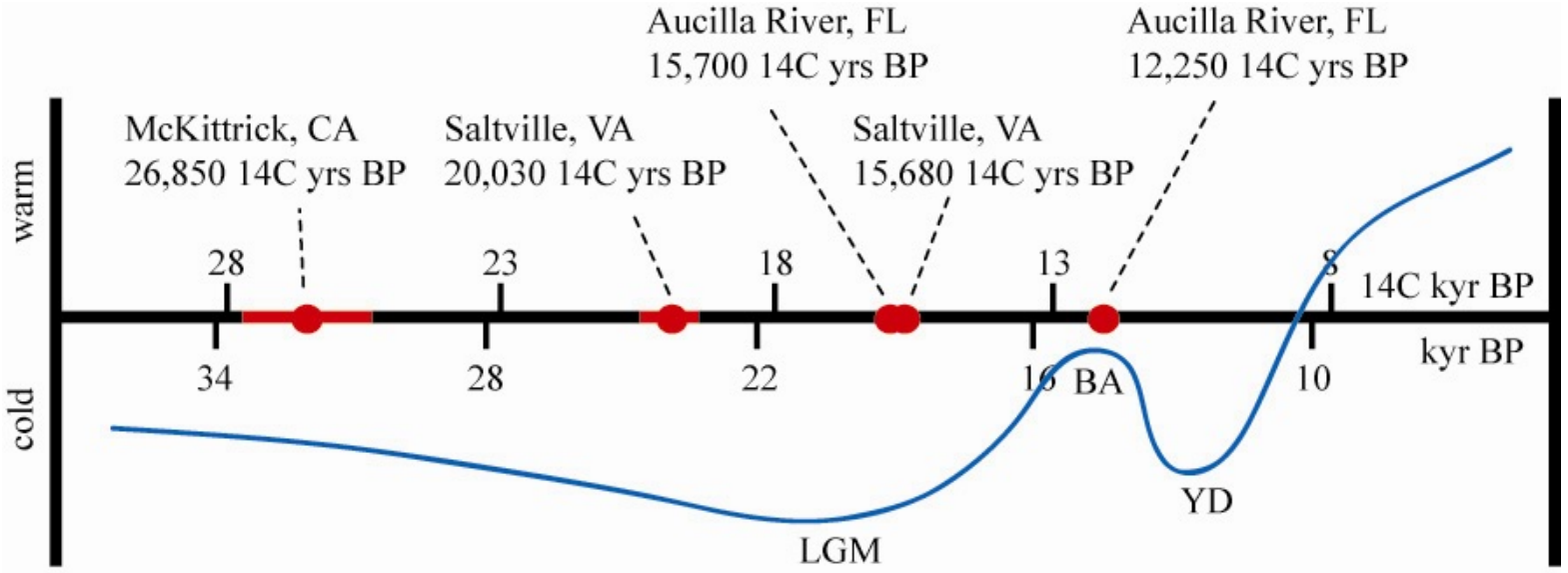

Figure 4.1 – Chronologic placement of radiocarbon dated samples. Errors are encompassed within the symbol unless otherwise noted by an additional error bar. Relative temperature trends are compiled from Heusser et al. (1980), Lorius et al. (1985), Dansgaard et al. (1993), Petit et al. (1999), and Nordt et al. (2007).
Abbreviations: LGM = Last Glacial Maximum, BA = Bolling-Allerod, YD = Younger Dryas

shift to much warmer climates when ice coverage retreated northward towards Canada. The Aucilla River, Florida samples, dated at 15,700±140 and 12,250±90 ^{14}C yrs BP, were also deposited during the rise and peak of the Bolling-Allerod event. These samples represent an ecosystem that survived the Last Glacial Maximum and continued to exist well into a more temperate period. The Aucilla River fauna slightly precedes the Younger Dryas, a brief cooling from ~11,000-10,000 ^{14}C yrs BP accompanied by a brief pulse of glacial advance, which subsequently transitioned into warmer climates and rapidly retreating glaciers.

The chronologic placement of these sample sites suggests that Late Pleistocene ecosystems in North America were thriving until the very termination of the epoch when ~100 species or more experienced extinction, the majority of which were >1kg. The mass extinction began ~12,000 -10,000 ^{14}C yrs BP (Kurten and Anderson 1980, Martin and Steadman 1999, Bell et al. 2004, Woodburne 2004, Guthrie 2006). The Aucilla River, Florida samples directly precede this event. While the studied sites are from geographically distant regions of the country, one may nonetheless consider the isotopic data from Aucilla River and the sites preceding it as a time series of ecosystem reconstructions leading up to the mass extinction that eliminated numerous mammal species from North America.

While radiocarbon dates on Pleistocene specimens are not rare, the dates obtained in this study represent new information for these three sites. The dates obtained for Saltville, Virginia and Aucilla River, Florida are especially valuable because these sample sets are extensive compared to similar studies of Pleistocene ecosystems. Not only can these sites now be placed in chronologic context, but the

isotopic data can be used to reconstruct complete ecosystems and consider them in light of prevailing climate conditions and human dispersal patterns for a particular time period (see sections 4.4 and 4.5 for a full discussion of these topics). These considerations applied across the time series of sample sites in this study represent a novel approach to the study of extinct ecosystems. Additional Pleistocene fossil localities yielding well preserved collagen should now be dated, compared, and combined with the results of this study to produce a more extensive time series of the changing trophic structures preceding the terminal Pleistocene extinction event.

4.3 Diagenesis with respect to time

While samples that are well preserved are useful for isotopic reconstructions of ecosystems, those specimens that lack intact collagen can be equally useful for discerning the diagenetic environment of a particular site or formation. The specimens from Florida, in particular, represent a unique opportunity to examine the diagenesis of collagen and amino acids through time insofar as these sites span a range of almost two million years.

Before examining these diagenetic trends, it is first necessary to consider the composition of poorly preserved organic extracts based on their C:N ratios, percent yields of carbon and nitrogen, and amino acid compositions. The primary pathway of collagen destruction is the hydrolysis of the peptide bond between amino acids, which breaks a long and complex protein chain into smaller peptides or free amino acids (Bada and Man 1980, Hare 1980, Bada 1998). Select reactions commonly occur by

other pathways as well. For example, serine, threonine, and cysteine decompose readily via β-elimination, aldol cleavage, dehydration, and/or decarboxylation (Bada and Man 1980, Bada 1998). In addition, aldol cleavage of threonine and serine produces glycine, and dehydration of serine produces alanine (Bada and Man 1980). Bonds between asparagine and proline have a tendency to spontaneously decompose (Collins et al. 1998). Valine, leucine, and isoleucine tend to be the most resistant amino acids to diagenetic break-down, while aspartic acid and serine are more rapidly cleaved from the protein structure (Bada and Man 1980, Bada 1998, Collins et al. 1998).

Previous research suggests that smaller peptides and free amino acids produced as a result of collagen degradation are eventually leached from the inorganic mineral matrix on the scale of 10^4 years, but can be retained for much longer (Bada and Man 1980, Bada 1998). The total abundance of all amino acids tends to decrease with time, but certain amino acids degrade more rapidly than others. Due to their tendency to rapidly degrade, serine, threonine, cysteine, and arginine are generally not preserved on geologic time scales (Bada and Man 1980, Bada 1998, Collins 1998). If they are present at levels outside their normal range for collagen, it likely indicates the presence of secondary contamination as opposed to lingering amino acids whose original source was bone collagen (Hare 1965, Bada and Man 1980). Some free amino acids and peptides can undergo condensation reactions with sugars, a process which produces a polymer that is much more resistant to hydrolysis than collagen and may linger in the inorganic mineral matrix of the bone for a much longer time (Bada 1998). If peptides and amino acids are completely degraded, the

most common product will be small hydrocarbons on the order of C_1-C_5 (Collins et al. 1998).

The organic residues of these various diagenetic pathways can be grouped into four broad categories: 1) non-collagenous protein composed of small peptide chains reduced from originally intact collagen, 2) complete cleavage of peptides resulting in free amino acids that remain in bone mineral matrices, 3) complete removal of nitrogen-bearing amino acids leaving behind only carbon-bearing organic compounds, or 4) complete degradation and subsequent removal of all organic material. The first case will result in a measurable amino acid signature, but the C:N ratios will depend on the sequences of the remaining peptides. Loss of short-chain amino acids, such as glycine and alanine, and the relative gain of longer chain amino acids tend to increase the C:N ratio (Masters 1987). These non-collagenous proteins tend to adhere to the inorganic mineral matrix more readily than collagen and thus remain in a diagenetically altered fossil specimen (Hare 1974, Masters 1987). If only free amino acids remain, their relative abundances will depend on the degree to which each individual amino acid has been leached from the specimen. If all amino acids have been removed, a small amount of organic material may be left (such as hydrocarbons), but it will contain little to no nitrogen. Finally, if a specimen has been continually and extensively leached with water, degradation of proteins will occur in a steep downward trend until no organic material remains (Hare 1974).

4.3.1 Collagen diagenesis in Florida samples

The analyzed samples from Florida showed a general decrease in the overall abundance of amino acids with time (Table 3.5), with noticeable patterns in the relative abundances of specific amino acids that facilitates interpretation of degradation pathways (Figures 3.10, 3.11, 3.12). The four sites included in these analyses span a range of Pleistocene time periods – Inglis 1A is early Pleistocene (~1.9 Ma), Leisey Shell Pit is mid-Pleistocene (~1.2-1.5 Ma), Tri-Britton is late Pleistocene (~250,000 yrs BP), and Aucilla River is latest Pleistocene (~12,000-15,000 yrs BP). One specimen from Aucilla River (AR-39) did not contain well preserved collagen, but exhibited relative abundances of glycine, proline, alanine, and glutamate similar to modern collagen. The remaining amino acids occurred in relative abundances outside the range for collagen, thereby suggesting that this sample contains peptides and amino acids degraded from collagen that have not yet been completely leached from the specimen. The samples from Tri-Britton (TB-2 and TB-5) showed a similar pattern of partial degradation and incomplete leaching. Sample TB-2 contained several amino acids, such as glycine, proline, and hydroxyproline, in a pattern of relative abundances that are similar to collagen. However, this sample is missing several amino acids altogether, which suggests partial leaching of collagen degradation products. Sample TB-5 is missing hydroxyproline and proline, but shows high relative abundances of glycine and aspartate. It appears to be leached more extensively than TB-2. Several of the oldest samples (LSP-6, LSP-22, and ING-26) contained no amino acids in their organic extracts. It is likely that these specimens experienced complete hydrolysis of

collagen and extensive subsequent leaching. The organic material that remains could be carbohydrates or organic molecules derived from, but not including, intact amino acids. One specimen from Inglis 1A (ING-29) showed an abnormally high relative abundance of serine and threonine, two of the more easily degraded and more mobile amino acids. The overall low abundance of amino acids and the lack of hydroxyproline suggest extensive hydrolysis of collagen and subsequent leaching of degraded materials, but the relatively high levels of serine and threonine suggest secondary contamination. In summary, these samples showed increased hydrolysis and leaching with age, with potential secondary contamination in the oldest samples.

Several additional broad conclusions can be made from this examination of protein diagenesis through time. First, studied specimens with a C:N ratio of ~2.8-3.6 show an amino acid relative abundance pattern that is indicative of well preserved collagen (Figure 3.10). Therefore, the amino acid analyses confirm that the C:N ratio is a good elemental indicator of preservation. However, it should be noted that preservation quality varies within individual sites. The Aucilla River site from Florida is a good case in point. Some samples from this site exhibited high organic yields and C:N ratios in the acceptable range for well preserved collagen, while others clearly did not. Visual inspection of Aucilla River specimens showed some bones that appeared pristine while others appeared desiccated and were exposed to extensive dry surface weathering before burial. These latter specimens might be considered poor candidates for organic preservation, but if one considers that water is a critical reagent in hydrolysis of proteins, the drier desiccated specimens are in fact more likely to contain well preserved collagen (Collins et al. 1998). Samples from

older sites such as Tri-Britton did not exhibit C:N ratios or organic yields in the acceptable range for collagen. However, the amino acid analysis indicated the presence of proteinaceous remains in these specimens. While it is no longer intact collagen, the presence of this material suggests that proteinaceous compounds can survive in the geologic record longer than previously suspected. A review of published isotopic values from well preserved collagen suggests that the oldest specimens containing intact protein in bones and teeth are ~50,000 years old (Bocherens et al. 1994b, Bocherens et al. 1995, Fizet et al. 1995, Bocherens et al. 1996, Bocherens et al. 1997, Iacumin et al. 2000, Richards and Hedges 2003, Coltrain et al. 2004) with rare exceptions beyond this age (Bocherens et al. 1988, Ostrom et al. 1993, Jones et al. 2001). Therefore it has become a common "rule of thumb" that proteinaceous material can only be extracted from bone or tooth specimens less than ~50,000 years old, thus limiting nitrogen isotopic studies of animal tissues to the late Pleistocene. The proteinaceous material extracted from Tri-Britton specimens is 150,000-400,000 years old, which suggests that exceptional preservation conditions can adequately preserve protein in bones considerably longer than 50,000 years. When searching for well preserved protein, sites older than 50,000 years should not be dismissed out of hand. This could potentially open the mid-Pleistocene to similar types of isotopic studies.

One final conclusion can be made regarding the rate of leaching of amino acids from these bones. Maintenance of consistent relative abundances of the various amino acids as a protein degrades suggests that the rate of diffusion of the different amino acids is similar (Bada and Man 1980). This trend was noted in one sample

from Tri-Britton (TB-2). Although the overall abundance of amino acids is considerably less in this sample as compared to the modern samples (~40 μg amino acids/mg raw sample in TB-2 vs. ~600 μg/mg in the modern bones), the relative abundances remained fairly constant. For this particular site, it would appear that the amino acids are being leached from the bones at rates similar to one another. However, it should be noted that this trend was only visible in one specimen and warrants further study with additional samples to confirm this hypothesis.

4.4 Isotopic indications of trophic relationships

After well preserved samples were identified, it was then possible to analyze the isotopic data from these specimens and apply it to broader paleoecological questions. It is necessary to examine the natural isotopic variability resulting from plant biodiversity as well as isotopic variations caused by trophic structure, digestive strategy, and age.

4.4.1 Nitrogen isotopic variability

The variation observed in the nitrogen isotopic values from well preserved specimens can be attributed to several coexisting factors. The overall range of values within and between sites is due in part to the natural variation in nitrogen isotopic composition of plant species, a factor which is site dependant. Several individual species are very selective in their food choice which is then reflected in their $\delta^{15}N$ values. Finally, digestive physiology and animal age may also affect the nitrogen

isotopic composition of an animal's body, thereby contributing to the overall variation observed in a food web.

4.4.1.1 Nitrogen isotopic ranges

The ranges and overlaps observed in the $\delta^{15}N$ values from all sites may be due in part to the natural range in isotopic values of the plants at the base of the food chain. This variation would then be reflected in the $\delta^{15}N$ values of the herbivores at each site. The herbivores at Saltville, Aucilla River, and McKittrick Brea exhibited $\delta^{15}N$ ranges of ~5‰ or greater (Figure 3.13). Observations of various terrestrial flora and associated herbivore fauna suggest ranges of at least 5‰ or more (Schoeninger and DeNiro 1984, DeNiro 1985, Ambrose and DeNiro 1989, Ambrose 1991, Bocherens et al. 1994a, Bocherens et al. 1996, Handley et al. 1999, Drucker et al. 2001, Coltrain et al. 2004). In modern California, for example, $\delta^{15}N$ values of woody and herbaceous terrestrial plants vary from ~0-12‰ with potential variation of >1‰ within a given family (Virginia and Delwiche 1982, Shearer and Kohl 1989, Cloern et al. 2002). The natural variation in soil $\delta^{15}N$ values and the fractionation during different methods of nitrogen incorporation into plant tissues (i.e. nitrogen fixing versus non-nitrogen fixing plants) are the primary factors contributing to the observed $\delta^{15}N$ range in plant tissues (Shearer and Kohl 1989, Virginia et al. 1989, Gebauer 1991, Nadelhoffer and Fry 1994). It is highly likely that these processes operated in the past, and that these are the primary factors influencing the nitrogen isotopic composition of the herbivores from these three sites. If this is the case, then both the observed overlap and range of $\delta^{15}N$ values within each site is expected given that the

animals would all be consuming plants from a similar soil and nutrient base while still potentially being slightly selective over plant choice.

While each of the three sites showed a $\delta^{15}N$ range consistent with natural variation in plant isotopic values, the range of values for each site varied in comparison to the others (Figure 3.13), which may suggest differences in nitrogen cycling that are site dependant. Aucilla River and Saltville showed herbivore $\delta^{15}N$ values primarily in the range of ~1-6‰. Aucilla River had two herbivores outside this range (*Castor* -0.08‰ and *Ondatra* 6.73‰), while Saltville only had one (*Rangifer* 0.79‰). Specimens from McKittrick lay within a $\delta^{15}N$ range of ~7-14‰, which is considerably more ^{15}N enriched than that of the east coast sites. This suggests the likelihood of differences between the proportion of nitrogen-fixing and non-nitrogen-fixing plants on the east and west coast of North America during the latest Pleistocene. Plants that fix nitrogen typically have depleted $\delta^{15}N$ values compared to those that do not fix nitrogen, (Virginia and Delwiche 1982, Shearer et al. 1983, Shearer and Kohl 1989, Virginia et al. 1989, Nadelhoffer and Fry 1994), which suggests that the flora of east coast North America was comprised of a higher proportion of nitrogen-fixers as compared to the west coast. The east coast did in fact show an increase in the percentage of birch trees towards the end of the Pleistocene, a family which is one of the very few non-leguminous tree families to contain nitrogen-fixing species. In contrast, the California region showed a relative increase in pines and other non-nitrogen-fixing species towards the end of the Pleistocene; information on the relative percentages of nitrogen-fixing sedges and herbaceous plants is currently not available. Additionally, nitrogen cycling in drier conditions results in

^{15}N enrichment of soil and vegetation, which subsequently leads to enrichment in the animal tissues (Virginia and Delwiche 1982, Heaton et al. 1986, Heaton 1987, Cormie and Schwarcz 1996, Handley et al. 1999). The McKittrick specimens were chronologically placed in the time just prior to the Last Glacial Maximum, a time that was significantly cooler and drier than the post-glacial time period in which the Aucilla River and Saltville fauna lived (Figure 4.1).

An alternate explanation for different $\delta^{15}N$ values observed on the east and west coast could be the contribution, or lack thereof, of oceanic sea spray and the nitrogenous compounds therein to the flora's nutrient supply. The organic nitrogen compounds in seawater are known to be enriched in ^{15}N compared to those of terrestrial environments (Wada et al. 1975). This suggests that the flora being consumed by herbivores at McKittrick Brea in California was more heavily influenced by oceanic waters than the east coast sites (Virginia and Delwiche 1982, Heaton 1987). Notably, modern terrestrial plants within ~100 km of the California coast show plant tissue $\delta^{15}N$ values that mimic nearby Pacific Ocean dissolved organic matter $\delta^{15}N$ values (Sweeney and Kaplan 1980, Virginia and Delwiche 1982, Shearer and Kohl 1989, Williams et al. 1992, Altabet et al. 1999, Cloern et al. 2002), whereas east coast terrestrial plant $\delta^{15}N$ values are significantly depleted compared to nearby Atlantic Ocean dissolved organic matter $\delta^{15}N$ values (Fry 1988, Benner et al. 1997, Guo et al. 2003, Nadelhoffer et al. 2004, Billings and Richter 2006, McLauchlan et al. 2007, Pardo et al. 2007, Templer et al. 2007). Given that a majority of weather systems in California currently originate offshore and move inland while east coast weather systems tend to move offshore, it is plausible that

modern California terrestrial plants are more heavily influenced by oceanic nitrogen sources. Climate models suggest similar weather patterns for the late Pleistocene (Kutzbach 1987, Webb et al. 1987, Anderson et al. 1988), thus supporting the idea that the oceanic contribution to terrestrial soil nitrogen was higher on the west coast of North America than the east coast during this time period.

4.4.1.2 Nitrogen isotopic indications of food preference in select species

Several herbivore specimens showed unique nitrogen isotopic values indicative of very specific dietary habits. From Saltville, the *Rangifer* (caribou) and *Bootherium* (musk ox) specimens are particularly notable. The *Rangifer* exhibited the lowest $\delta^{15}N$ for the entire site (+0.79‰), a pattern that had been previously observed in both modern and Pleistocene ecosystems (Bocherens et al. 1994b, Fizet et al. 1995, Iacumin et al. 2000, Drucker et al. 2001). This is likely due to the caribou's selective preference for lichen, a food which is commonly ^{15}N depleted (Virginia and Delwiche 1982). The *Bootherium* showed a noticeable split in their $\delta^{15}N$ values with one group exhibiting far more depleted values than the other (Figure 3.15). This may be due to the presence of a juvenile isotopic signature, which will be discussed in section 4.4.1.3.

At Aucilla River, Florida, two specimens yielded $\delta^{15}N$ data beyond the average range observed for all other herbivores at this site. The *Castor* (beaver) specimen had an extremely depleted $\delta^{15}N$ value of -0.08‰, while the *Ondatra* (muskrat) showed significant enrichment with a $\delta^{15}N$ value of +6.73‰. Based on the diet of modern beavers, it is likely that *Castor* included a significant portion of wood

in its diet. Studies have shown that woody tree bark tends to be more depleted in ^{15}N compared to other portions of the tree, although this trend can be species and habitat specific (Shearer et al. 1983, Nadelhoffer et al. 2004, Liu et al. 2006, Ometto et al. 2006). This idea is a plausible explanation for the observed depletion in the *Castor* specimen; however nitrogen isotopic analyses of preserved tree material from the Aucilla River site would be necessary to fully confirm it. The observed nitrogen isotopic enrichment in the *Ondatra* specimen is also likely to be a result of food choice. Rats are known to consume seeds and grains. In some species, the reproductive tissues of plants are slightly more enriched than leaves and stems (Shearer et al. 1983, Bergersen et al. 1985, Shearer and Kohl 1989). The muskrat specifically has a partially aquatic habitat and often consumes a significant quantity of aquatic vegetation (Lewis et al. 2000). Aquatic plants tend to be enriched in ^{15}N relative to strictly terrestrial plants, a trend which would explain the enriched values in the muskrat from Aucilla River (Virginia and Delwiche 1982, Cloern et al. 2002). The plausibility of this explanation would also benefit from a complete analysis of preserved plant material at Aucilla River.

At McKittrick Brea, California, the *Hemiauchenia* (llama) showed an anomalously high $\delta^{15}N$ of +13.61‰, a value that is higher even than the only carnivore data obtained from this site. This is difficult to explain given that the carnivore should show more enriched nitrogen isotopic values than the herbivores. It is possible that this particular *Smilodon* (sabertooth cat) relied on more ^{15}N depleted species as its primary prey, thus giving the appearance of relative depletion compared to the *Hemiauchenia*. An alternate explanation lies in the modern extant analogs for

Hemiauchenia (i.e. the camels and llamas), most of which are particularly well adapted to and tolerant of arid conditions. Internal water conservation tends to enrich an animal's tissues in ^{15}N due to the constant recycling of organic compounds in an attempt to minimize aqueous excretion via urea (Heaton et al. 1986, Ambrose 1991, Cormie and Schwarz 1996). The *Hemiauchenia* at McKittrick lived during a time that is considered to be relatively dry, which could therefore result in a nitrogen isotopic enrichment of its bone collagen. Data from sites of similar age and climate conditions suggest that camels tend to plot towards the more enriched range of nitrogen isotopic values compared to other herbivores (Coltrain et al. 2004). If *Hemiauchenia* and the modern camel, *Camelus*, are used as a phylogenetic bracket for the trait of water conservation, this then suggests that the majority of species in the subfamily Camelinae possessed the physiologic capability to conserve water (Honey et al. 1998). This subfamily originates in the middle Miocene, thereby suggesting that the trait of water conservation evolved prior to the Pleistocene (Honey et al. 1998). As this site did not yield enough well preserved specimens to completely reconstruct the food chain, this interpretation is necessarily considered tenuous at this time.

This discussion of nitrogen isotopic variability has so far focused on the herbivorous species to the exclusion of carnivores. The $\delta^{15}N$ values of known carnivores, and some omnivores, are notably more enriched than the herbivores. This is to be expected given their location at the apex of the food chain. A full discussion of the use and implications of nitrogen isotopic data for the determination of trophic levels is included in section 4.4.3.

4.4.1.3 Juvenile isotopic signatures

The previously noted nitrogen isotopic enrichment of juvenile specimens was noted in the one known juvenile bone that yielded well preserved collagen in this study. A single *Mammuthus* juvenile from Saltville yielded a relatively high $\delta^{15}N$ value compared to the other herbivores from this site (+5.60 ± 0.10‰, 2σ). This specimen plotted ~1.6‰ higher than a *Mammuthus* adult from the same site, a pattern which has been observed in previous studies (Fogel et al. 1989, Bocherens et al. 1994, Balasse et al. 1999). It should be noted that an equally high $\delta^{15}N$ value was observed in a second *Mammuthus* adult. This overlap in isotopic data could potentially be explained by taxonomic differences as the mammoth adult and juvenile were not identifiable at the species level. Alternately, this "juvenile" signature may in fact have come from a sub-adult whose collagen tissues were just beginning to record a more adult dietary signature.

The noted juvenile signature in the nitrogen isotopic values of Saltville is potentially useful for understanding the spread in $\delta^{15}N$ values for the *Bootherium* specimens at this site. The *Bootherium* data plotted into two subsets, one of which was enriched relative to the other (Figure 3.15). Those musk oxen in the enriched range of $\delta^{15}N$ values were all sampled from rib fragments which were not age indicative and may in fact be juveniles or sub-adults. Further comparisons with known musk ox juveniles are necessary to determine if the higher $\delta^{15}N$ values observed in some of the musk oxen from this study are due to the same juvenile effect observed in the mammoth.

4.4.1.4 Ruminant and non-ruminant digestive strategies

The differences in nitrogen isotopic values between ruminants and non-ruminants can be most readily tested in specimens from Saltville, Virginia and Aucilla River, Florida. McKittrick Brea, California did not yield enough well preserved specimens to perform a rigorous statistical analysis and will not be considered in the subsequent discussion of ruminant versus non-ruminant nitrogen isotopic signatures. Based on modern analogs and extant taxa, the ruminants in this study consist of the Bovidae (*Bison*, *Bootherium*), Camelidae (*Hemiauchenia*), and Cervidae (*Odocoileus*, *Rangifer*, *Cervalces*). Non-ruminants consist of Equidae (*Equus*), Tapiridae (*Tapirus*), Rodentia (*Castor, Ondatra*), Proboscidae (*Mammuthus, Mammut*), and Edentata (*Megalonyx,* Pilosa).

Statistical comparisons were performed on the means of the ruminant and non-ruminant populations from Saltville and Aucilla River, with exclusions of certain taxa in various iterations (Table 4.1). All tests were two-tailed t-tests and considered actual population variances to be unknown, but equal. Population means were considered significantly different if $p<0.1$. In all iterations from Saltville, the juvenile *Mammuthus* (mammoth) data was excluded. Its elevated $\delta^{15}N$ is believed to be a result of its juvenile age as opposed to its digestive strategy (see section 4.4.1.3). In four of the iterations, the sloth groups (*Megalonyx* and Pilosa) were excluded from the non-ruminant populations due to their uncertain status as full herbivores. An omnivorous diet would result in higher $\delta^{15}N$ values than a typical non-ruminant herbivore and increase the average $\delta^{15}N$ value for the entire population of non-ruminants. The *Castor* (beaver) specimen from Aucilla River was also excluded from

Table 4.1 – Statistical parameters for ruminant and non-ruminant herbivores from Saltville, Virginia and Aucilla River, Florida. All tests were two-tailed t-tests comparing the difference in the means of two populations assuming unknown and equal population variances.

Site	[a]Comparison	$\delta^{15}N$ average	1σ	n	df	p-value
Aucilla River	Non-ruminants (all) vs.	4.37	2.33	14	24	0.717
	Ruminants (all)	4.12	0.85	12		
Aucilla River	Non-ruminants (excluding *Megalonyx* and Pilosa) vs.	3.46	2.03	10	20	0.322
	Ruminants (all)	4.12	0.85	12		
Aucilla River	Non-ruminants (excluding *Castor*) vs.	4.71	2.03	13	23	0.350
	Ruminants (all)	4.12	0.85	12		
Aucilla River	Non-ruminants (excluding *Megalonyx*, Pilosa, and *Castor*) vs.	3.85	1.71	9	19	0.651
	Ruminants (all)	4.12	0.85	12		
Saltville	Non-ruminants (all) vs.	3.89	1.02	7	23	0.138
	Ruminants (all)	2.89	1.59	18		
Saltville	Non-ruminants (all) vs.	3.89	1.02	7	19	<0.001
	Ruminants (excluding upper range of *Bootherium* specimens)	2.10	0.52	14		
Saltville	Non-ruminants (excluding *Megalonyx*) vs.	3.77	1.05	6	22	0.225
	Ruminants (all)	2.89	1.59	18		
Saltville	Non-ruminants (excluding *Megalonyx*) vs.	3.77	1.05	6	18	<0.001
	Ruminants (excluding upper range of *Bootherium* specimens)	2.10	0.52	14		

[a] see text for full description and reasoning for exclusions of genera

two iterations due to its anomalously low $\delta^{15}N$ value (refer to section 4.4.1.2 for a full discussion of this specimen). One subset of *Bootherium* (musk ox) specimens from Saltville exhibited anomalously high $\delta^{15}N$ values compared to other *Bootherium* and ruminants from this site (Figure 3.15). As was previously discussed, this may be due to the presence of a juvenile isotopic signature (see section 4.4.1.3). Due to the unknown age classification of this subset of specimens and the subsequent effect it may have on the $\delta^{15}N$ signature, it is worth examining two iterations of t-tests that alternately include and exclude this subset.

Previous research has suggested that the average $\delta^{15}N$ between populations of ruminants and non-ruminants from the same fossil site should be distinct (Bocherens et al. 1996, Coltrain et al. 2004); a pattern which is observed in Saltville, but absent in Aucilla River. Two iterations of t-tests performed on Saltville specimens found significant differences between populations of ruminants and non-ruminants (p-values <0.001). Both of these iterations excluded the enriched group of *Bootherium* specimens. The two iterations that included this enriched group were only significant at higher p-values ($p = 0.138$ and 0.225). The inclusion/exclusion of potentially enriched non-ruminant sloths did not alter the results. This finding agrees with that of Bocherens et al. (1996), but disagrees with Coltrain et al. (2004). No significant differences were observed between the ruminant and non-ruminant populations from Aucilla River ($p = 0.322$-0.717). Inclusion or exclusion of the sloths and *Castor* specimens did not alter these results. This suggests that the lack of difference in $\delta^{15}N$ values between ruminants and non-ruminants in Florida is a real result as opposed to an apparent result that is affected by one or two outlying data points.

The discrepancies between previous studies and this study regarding the isotopic separation of ruminants and non-ruminants suggest that this method of distinguishing the two groups is tenuous at best. Where one study suggests ^{15}N enrichment in ruminants, another suggests depletion or no significant difference at all. This may mean that there is in fact no additional nitrogen isotopic fractionation occurring during the process of rumination and fore-gut fermentation. However, it is also possible that the fractionation exists; it is simply masked by the much greater natural variation observed in plant material. A difference of ~5‰ between the $\delta^{15}N$ of plant species could conceivably mask a much smaller fractionation resulting from rumination. Selectivity for certain plants, which may span the range of available $\delta^{15}N$ values, leads to the observed variation in the $\delta^{15}N$ values of herbivore bone collagen.

4.4.2 Carbon isotopic variability and indications of C3 and C4 grasses

The carbon isotopes from all three sites were fairly uniform in distribution with the exception of four specimens from Aucilla River, Florida which were considerably more enriched and indicate the presence of C4 grass grazers in the food web. Two *Equus* (horse) and two *Bison* (bison) samples from Aucilla River ranged in $\delta^{13}C$ from -12.74‰ to -8.64‰ while all other values from this site fell below -18‰ (Figure 3.19). Specimens from McKittrick Brea, California and Saltville, Virginia also plotted in the more depleted range of less than -18‰ (Figures 3.17 and 3.18). The herbivores showing the more depleted $\delta^{13}C$ values consumed plants that utilized the C3 photosynthetic pathway whereas the herbivores exhibiting more enriched values were consuming C4 vegetation.

The distribution of herbivores that consume C4 vegetation has direct implications for the presence or absence of open grasslands in a given area. The data suggests the general absence of C4 plants in the California and Virginia sites. As most grasses utilize the C4 photosynthetic pathway, this then suggests the general absence of open grasslands in these areas. This is supported by the sediment samples from Saltville which show a strong C3 signature, and previous studies which failed to find C4 signatures in the $\delta^{13}C$ values of late Pleistocene mammals (Bocherens et al. 1996, Coltrain et al. 2004). Additionally, grass pollen counts in the Saltville, Virginia region are low with relative abundances generally less than 10% (Ray et al. 1967, Williams et al. 2000). However, it should be noted that the complete absence of open grassy areas was unlikely. It is more likely that the herbivores from McKittrick and Saltville commonly considered quintessential grazers, such as *Equus*, did in fact consume grasses and herbaceous vegetation. They were simply reliant on the dominantly C3 herbaceous vegetation and less common C3 grasses. Despite a dental morphology that indicates strict grazing, the Pleistocene *Equus* is known to have consumed herbaceous C3 vegetation in the absence of C4 grasses (Koch et al. 2004). The presence of clear C4 grazers at Aucilla River suggests a geographic transition to open C4 grasslands somewhere between Florida and Virginia during the time period ~12,000-20,000 years ago. This is supported by previous studies that have found a definite presence of C4 grass consumption on the east coast of North America during this time period (MacFadden and Cerling 1996, Koch et al. 1998, Feranec and MacFadden 2000, Kohn et al. 2005). When the geographic localities from these sites are considered in conjunction with Saltville, it appears that the transition between C3

and C4 grasses was located between Virginia and South Carolina during this time period.

The transition between grass types was most likely climatically induced. Plants that utilize a C4 pathway generally do so as an adaptation to warmer and drier climates. The region including Saltville consisted of primarily cool mixed forests while regions further south exhibited slightly more open temperate forests and warmer climates. The McKittrick herbivores were living at a time when the climate was drier, but also much cooler compared to the time period of Aucilla River and Saltville (Figure 4.1). It would then be expected that McKittrick flora would be primarily C3, a trend which is in fact reflected in the $\delta^{13}C$ values of the sampled mammals.

It should be noted that the herbivores from Aucilla River that consume C4 vegetation (i.e. *Equus* and *Bison*) also showed $\delta^{13}C$ values in the range of C3 plants. These species were apparently capable of consuming a variety of plant types and did not rely solely on open grassland grazing, an observation that is supported by both this study and previous research (Feranec and MacFadden 2000, Feranec 2004, Koch et al. 2004, Kohn et al. 2005). It is possible that this site represents a brief look at a time period when this area was in transition to a more open grassland habitat and the isotopic values from the herbivores are also recording that transition. Carbon-dated specimens from this site span ~3000 years, a time interval during which floral turnover could have resulted in more widespread C4 grasses. However, previous research suggests the arrival of C4 plants in Florida as early as ~5 Ma and noticeable mammalian reliance on C4 plants prior to the time period of Aucilla River

(MacFadden and Cerling 1996, Koch et al. 1998). The idea that the Aucilla River data is capturing a floral transition is still viable, it is, however, more likely that this transition represents a shift in the relative abundances of C3 and C4 plants as opposed to the first appearance of C4 plants in Florida. *Equus* and *Bison* from Florida have shown varying relative abundances of C4 plants in their diets since the initial arrival of these plants in the region (MacFadden and Cerling 1996, Koch et al. 1998, Ferenec and MacFadden 2000).

It is also possible that the variability in $\delta^{13}C$ values of the *Equus* and *Bison* is a result of seasonal dietary shifts. Individuals exhibiting the more depleted signatures indicative of a dominantly C3 diet may have died shortly after the colder part of the year when these plants were dominant. Likewise, the individuals exhibiting a more enriched signature indicative of a C4 diet may have died shortly after the warm season when grass biomass was at its peak. Collagen in mammals can be recycled in as little as six months, suggesting that the isotopic signature recorded in herbivore bones is most indicative of the seasonal plant diversity immediately preceding death (Katzenberg 1993, Fogel et al. 1997). Previous research suggests that seasonality in $\delta^{13}C$ values is observable in micro-sampled specimens of *Equus* and *Bison* (Feranec and MacFadden 2000). If this is the dominant factor controlling the observed range in $\delta^{13}C$ values of these two species, it still supports the previous conclusion that these particular animals were well adapted for consuming a variety of vegetation types and were not solely reliant on open grassland grazing.

The absence of quintessential grazers at McKittrick Brea, California may lead to the erroneous assumption that there were no C4 grasses present in this area. The

extremely small yield of well preserved specimens from this site did not include any species that typically show a grazing habit (specifically *Equus* and *Bison*). Therefore, it should be noted that the absence of a C4 signature may be due to sampling error as opposed to actual geographic distribution of C4 grasses on the west coast of North America during this time period. However, a similar study from this region including *Equus* and *Bison* failed to show enriched $\delta^{13}C$ values and supports the idea that C3 vegetation dominated the area (Coltrain et al. 2004). Without additional well preserved specimens from McKittrick, this conclusion remains plausible, but unconfirmed.

4.4.2.1 Feeding specialization of mammoths and mastodons

The extant North American fauna does not include any species equal in size to the mammoths and mastodons of the late Pleistocene. Carbon isotopic signatures can be used to examine potential feeding specializations that would facilitate the coexistence of these large herbivores with one another and with other herbivores. It has been suggested that *Mammuthus* (mammoth) consumed a primarily grassy diet (Koch et al. 1998, Feranec and MacFadden 2000) while *Mammut* (mastodon) was a browser or more general opportunistic feeder based on hypsodonty and carbon isotopic data (Laub et al. 1994, Koch et al. 1998, Coltrain et al. 2004). Only one such study actually includes both genera and shows a distinct isotopic difference between the two (Koch et al. 1998). Alternative evidence suggests that both *Mammuthus* and *Mammut* utilized both grazing and browsing habits with the proportion of graze included in the diet depending on the dominant type of vegetation available in the

area (Davis et al. 1985, Agenbroad and Mead 1989, Gobetz and Bozarth 2001, Feranec 2004, Koch et al. 2004).

This study suggests that the mammoths and mastodons were both more opportunistic generalized feeders. Neither genera showed the more enriched carbon isotopic values of the C4 grass grazers (Figures 3.18 and 3.19), nor did they show a significant difference from one another based on a two-tailed t-test where actual population variances were considered to be unknown, but equal ($p=0.924$, $df=6$). In the Saltville region, there were no apparent open C4 grasslands in the area, thus precluding the inclusion of these plants into the diet of mammoths and mastodons, a pattern that is seen elsewhere in ecosystems lacking C4 vegetation (Bocherens et al. 1994b). In the Aucilla River region, C4 grasses were present, but it appears that these animals preferred the C3 plants. This is not to say that mammoths and mastodons did not graze on herbaceous vegetation, it simply implies that neither of these two animals dwelled exclusively in open grassy areas. They were apparently capable of surviving on a variety of vegetation types.

4.4.2.2 Carnivore and omnivore carbon isotopic signatures

Examination of the carbon isotopic signature of the carnivores and omnivores provides some preliminary insight into their food choices. Carnivores and suspected omnivores from Aucilla River, Florida showed depleted $\delta^{13}C$ signatures with values below -20‰. This suggests that these animals were selectively consuming animals that relied on C3 vegetation, or the C3 vegetation itself in the case of the omnivores. It appears that *Equus* (horse) and *Bison* (bison), the two species that consumed C4

grasses, were not the primary prey for *Lynx* (lynx) or *Canis* (wolf) in this location. Likewise, the meat component of the omnivorous diet appears to be genera other than *Equus* and *Bison*. The likely predators of *Equus* and *Bison* at Aucilla River would then be the feline genera from which no well preserved specimens were obtained for this site. Without a full range of feline species for comparison, the degree of prey specialization among carnivores at Aucilla River is difficult to determine, but it can be stated that at least some specialization is apparent. *Canis* and *Lynx* do not appear to be open plains hunters that attacked prey in open grassland areas. It is more likely that the absent felines filled that niche, much like their counterparts on the plains of modern Africa.

The lone carnivore from McKittrick Brea also appears to have preyed upon herbivores reliant on C3 vegetation. The *Smilodon* (sabertooth cat) specimen from this site showed a relatively depleted $\delta^{13}C$ value of -20.62‰. However, this may simply be a result of the previously noted lack of C4 grazers in the area (refer to section 4.4.2). A conclusive determination of carnivore prey preference at McKittrick requires a more complete sample set.

4.4.3 Distinction of trophic levels

One of the most useful pieces of information that can be determined from an isotopic analysis of a food web is the placement of animals within broad trophic groups. To this end, animals were placed in the categories of herbivore, carnivore, and omnivore based on modern analogs and extant taxa for the purposes of statistical testing. The incomplete nature of the reconstructed food chain from McKittrick,

California renders it inadequate for rigorous statistical analysis and it is not considered in the remainder of this section. Data from Saltville, Virginia and Aucilla River, Florida was combined in the following statistical tests. Herbivores included the Bovidae (*Bison*, *Bootherium*), Cervidae (*Cervalces*, *Rangifer*, *Odocoileus*), Equidae (*Equus*), Tapiridae (*Tapirus*), Rodentia (*Castor*, *Ondatra*), Camelidae (*Hemiauchenia*), and Proboscidea (*Mammut*, *Mammuthus*). Carnivores included the Canidae (*Canis*) and Felidae (*Lynx*). Omnivores included the Ursidae (*Ursus*) and Procyonidae (*Procyon*). The sloths were considered to be unknowns in regards to their feeding strategy; this group included the Edentates (*Megalonyx*, Pilosa). The only exclusion from these analyses was the *Mammuthus* juvenile from Saltville, which appears to fall into a higher trophic level due to its elevated juvenile nitrogen isotopic signature (see section 4.4.1.3 for a full discussion).

A first approximation of trophic levels can be obtained from an examination of nitrogen isotopes alone. To this end, t-tests were performed on the various trophic groups as described above; results are listed in Table 4.2. All tests were two-tailed t-tests and considered actual population variances to be unknown, but equal. Population means were considered significantly different if $p<0.1$. Herbivores and carnivores show a clear difference in their nitrogen isotopic values ($p<0.001$), which is to be expected given the well documented enrichment in nitrogen isotopic values observed in the upper levels of food chains. The omnivores plotted between the herbivores and carnivores and failed to show a significant difference from either group at $\alpha=0.1$.

Table 4.2 – Statistical parameters for broad trophic level comparisons based on nitrogen isotopic values from Saltville, Virginia and Aucilla River, Florida combined. All tests were two-tailed t-tests comparing the difference in the means of two populations assuming unknown and equal population variances.

[a]Comparison	δ^{15}N average	1σ	n	df	p-value
Herbivores vs.	3.47	1.52	47	47	<0.001
Carnivores	7.64	1.78	2		
Herbivores vs.	3.47	1.52	47	49	0.257
Omnivores	4.39	1.93	4		
Carnivores vs.	7.64	1.78	2	4	0.118
Omnivores	4.39	1.93	4		
Herbivores vs.	3.47	1.52	47	50	<0.001
Edentates	6.25	1.37	5		
Carnivores vs.	7.64	1.78	2	5	0.308
Edentates	6.25	1.37	5		
Omnivores vs.	4.39	1.93	4	7	0.133
Edentates	6.25	1.37	5		

[a] see text for full description of the animals included in each group listed

The relationship between the omnivores and carnivores provides insight into the dietary preferences of the latter group. The failure of omnivores to exhibit a statistical difference from carnivores suggests either that omnivores had a significant meat component in their diets, or that the carnivores preferentially preyed upon herbivores as opposed to omnivores or other carnivores. While both factors likely contributed to the observed pattern, the latter is a particularly likely conclusion, especially considering that the omnivore group includes ursids (bears). While it is possible for wild dogs and felines to prey upon bears and one another, modern analogs suggest that this is not the preferred prey of these carnivores. The *Canis* (wolf) from Aucilla River has the most enriched $\delta^{15}N$ value (Figure 3.16) which plots >3‰ higher than the most enriched herbivores and omnivores. Given that each step up the food chain enriches an animal's $\delta^{15}N$ value by ~3‰, it is likely that this particular *Canis* consumed carnivorous or omnivorous prey. Additional *Canis* specimens are necessary to confirm this hypothesis. While the McKittrick Brea, California specimens were not included in these statistical analyses, it should be noted that the carnivorous *Smilodon* (sabertooth cat) from this site plotted in the same range of nitrogen isotopic values as the local herbivores – a pattern that is similar to the carnivorous *Lynx* (lynx) from Aucilla River. These specimens appear to plot only one trophic step above the herbivores. This suggests that the felids (cats) may have preferentially preyed upon herbivores while the canids (dogs) may have included omnivores and other carnivores in their diet. This pattern is supported by some similar studies (Bocherens et al. 1994a, Bocherens et al. 1994b, Fizet et al. 1995), but refuted by others (Bocherens and Drucker 2003, Coltrain et al. 2004). However, it

should be noted that the limited number of data points for carnivores necessarily renders this a preliminary conclusion only.

According to the t-tests, the Edentates (sloths) show a similarity to the upper levels of the food chain. They are significantly different from the herbivores ($p<0.001$), but similar to the omnivores and carnivores ($p=0.133$ and 0.308, respectively). The sloths were in fact most similar to the carnivores, but do not show quite as much nitrogen isotopic enrichment as this group (Figure 3.16). Based on these statistical analyses, the Edentates should be considered omnivorous. This agrees with previous research that has suggested omnivory, opportunistic scavenging, carnivory, and insectivory as sloth dietary strategies (Martin 1975, Hansen 1978, Naples 1989, Cork 1994, Farina 1996, Farina and Blanco 1996). However, previous findings for a Pleistocene *Megalonyx* from Alberta (Bocherens et al. 1994b) and the Pleistocene sloth *Paramylodon harlani* (Coltrain et al. 2004), exhibited carbon and nitrogen isotopic values similar to those of strict herbivores from the same ecosystem. This then suggests that the Edentates likely followed a dietary strategy similar to modern omnivores in that they will consume meat when and if it is available, but will subsist on vegetation when it is not. The sloths from Aucilla River appear to show a nitrogen isotopic enrichment compared to herbivores that is greater than the enrichment observed in Saltville. The dietary preferences of sloths may vary by region depending on the availability of plants and prey at different sites.

An alternate method of examining trophic groups from these sites is to combine the nitrogen and carbon isotopic data into a multivariate statistical analysis. This method describes the extent to which a combination of $\delta^{15}N$ and $\delta^{13}C$ values can

accurately define different trophic groups. Carnivore, herbivore, and omnivore groups are organized in the same manner as the t-tests with the Edentates variously included in the herbivores, omnivores, and carnivores. Results are listed in Table 4.3. All statistical models produced a p-value less than 0.1 which suggests that there is a significant difference between at least two of the trophic groupings within each model. This result is identical to the t-tests. The discriminant function analysis also suggests that placement of the Edentates within the omnivore group produces the highest percentage of correct trophic group determinations (69%) based on carbon and nitrogen isotopic signatures. This supports the conclusions from the t-tests that sloths are best considered as omnivores. Overall, the alternative statistical models produced a range of total percentages of correct group determinations of 50%-69%. There was considerable overlap between groups as indicated in Figures 4.2, 4.3, 4.4, 4.5, and 4.6. The separation between herbivores and carnivores is consistently distinct, while the omnivores and sloths represent a continuum between the two. Removal of the sloths into their own category did not resolve the observed overlap or improve the percentage of correct group determinations, thus confirming that the Edentates are most closely associated with the omnivores. In sum, the resolution for group determination using the multivariate analysis was only marginally better than a simple t-test. The multivariate analysis has the advantage of determining the amount of overlap between groups and suggesting the quality of subjective groupings by way of the percent correct statistics.

Table 4.3 – Results of discriminant function analysis for isotopic data from Saltville, Virginia and Aucilla River, Florida

	[a]Comparison	Wilk's λ	Approximate F	p-value	% correct
1	Carnivores	0.760	3.596	0.00890	100
	Herbivores				53
	Omnivores				25
	total				*53*
2	Carnivores	0.830	2.630	0.0383	100
	Herbivores (including Edentates)				50
	Omnivores				25
	total				*50*
3	Carnivores	0.697	5.338	<0.001	50
	Herbivores				72
	Omnivores (including Edentates)				56
	total				*69*
4	Carnivores (including Edentates)	0.666	6.078	<0.001	71
	Herbivores				53
	Omnivores				25
	total				*53*
5	Carnivores	0.651	4.225	<0.001	50
	Herbivores				53
	Omnivores				25
	Edentates				40
	total				*50*

[a] see text for full description of the animals included in each group listed

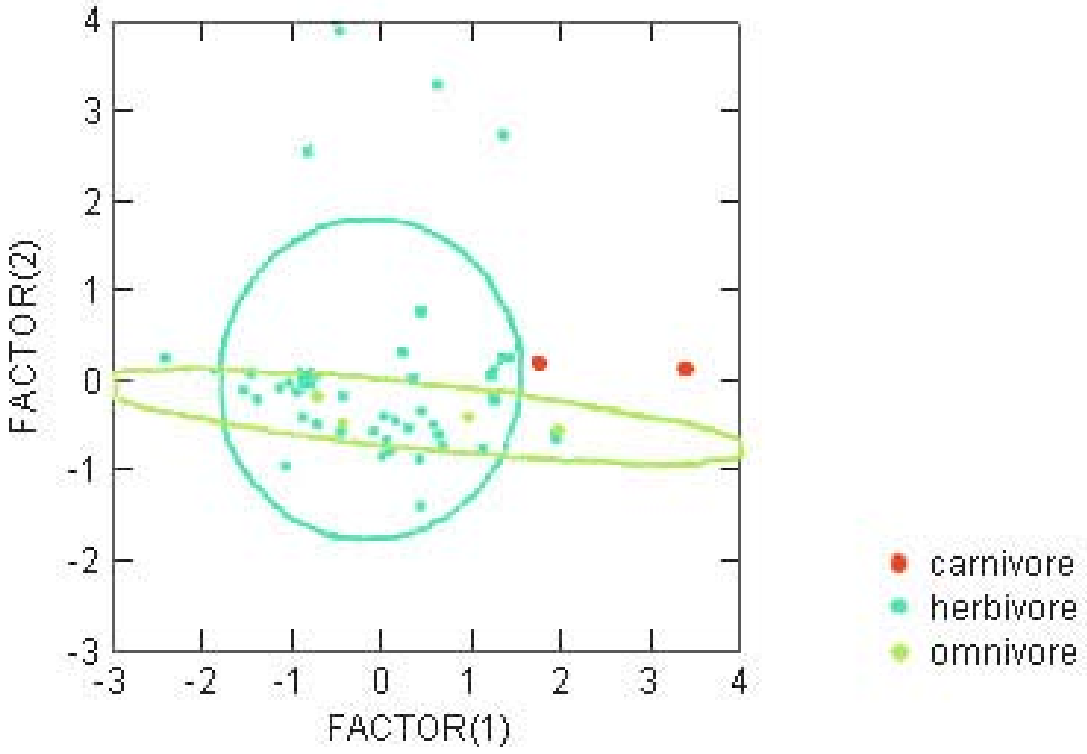

Figure 4.2 – Discriminant function analysis of isotopic data from Saltville, Virginia and Aucilla River, Florida. Factor 1 represents the synthetic variable derived from $\delta^{13}C$ values. Factor 2 represents the synthetic variable derived from $\delta^{15}N$ values. Edentates (sloths) are excluded from this analysis. See text for a full description of the animals included in each group.

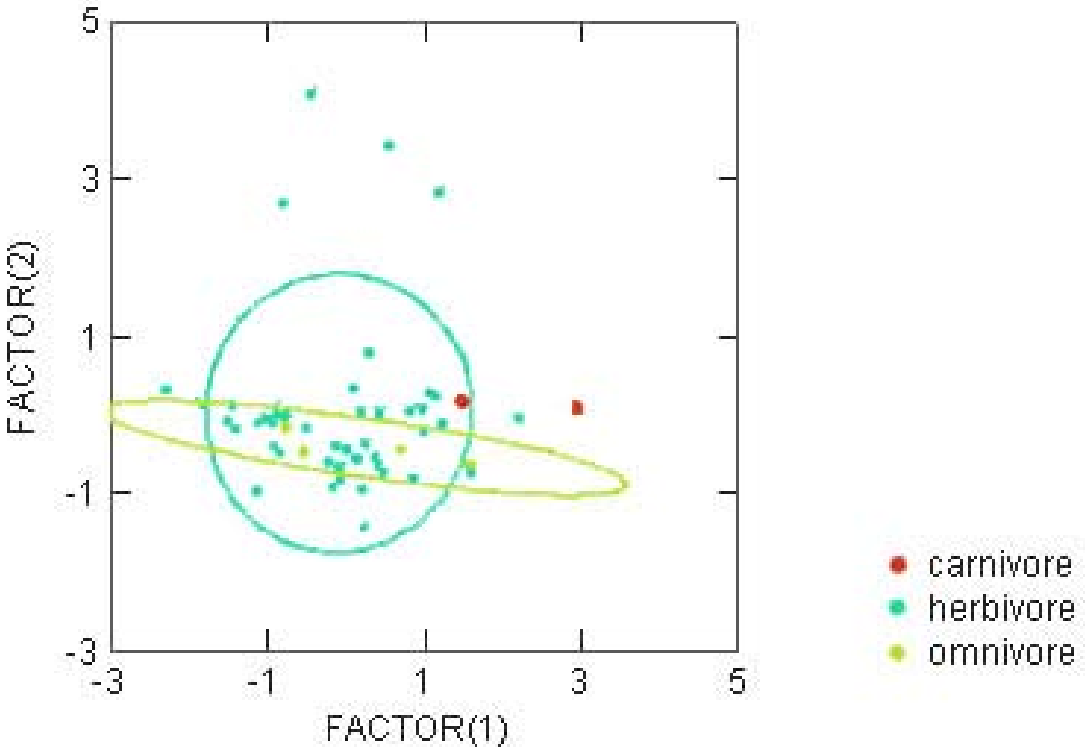

Figure 4.3 – Discriminant function analysis of isotopic data from Saltville, Virginia and Aucilla River, Florida. Factor 1 represents the synthetic variable derived from $\delta^{13}C$ values. Factor 2 represents the synthetic variable derived from $\delta^{15}N$ values. Edentates (sloths) are considered as herbivores for this analysis. See text for a full description of the animals included in each group.

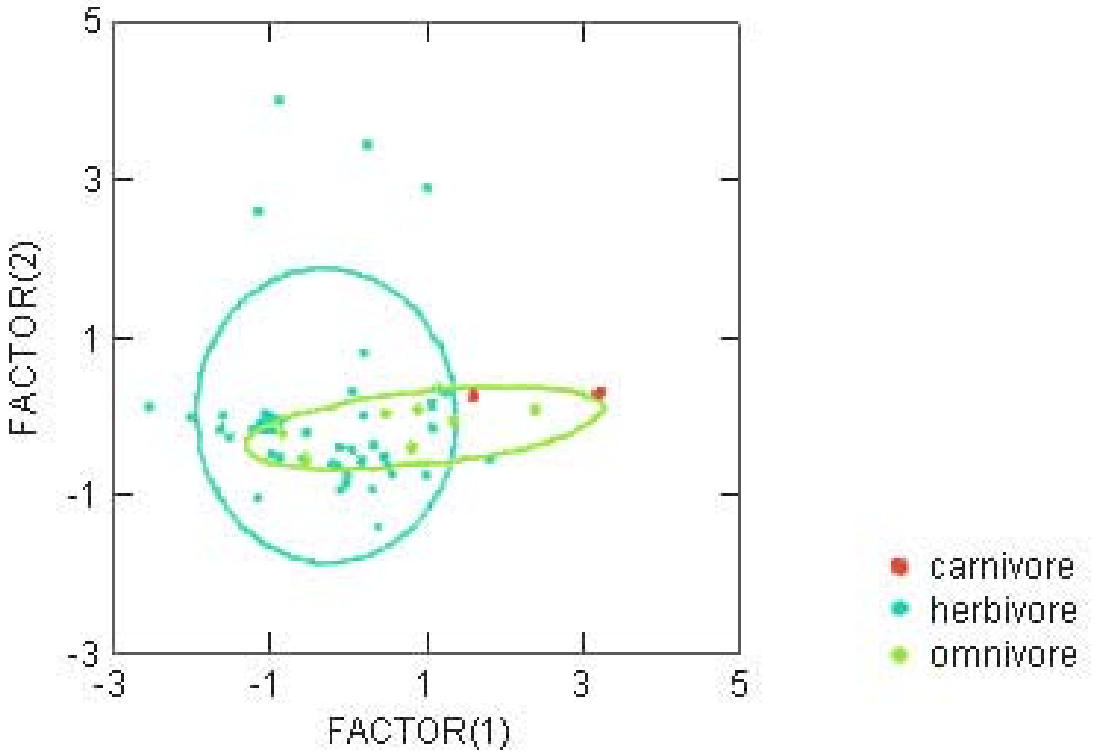

Figure 4.4 – Discriminant function analysis of isotopic data from Saltville, Virginia and Aucilla River, Florida. Factor 1 represents the synthetic variable derived from $\delta^{13}C$ values. Factor 2 represents the synthetic variable derived from $\delta^{15}N$ values. Edentates (sloths) are considered as omnivores for this analysis. See text for a full description of the animals included in each group.

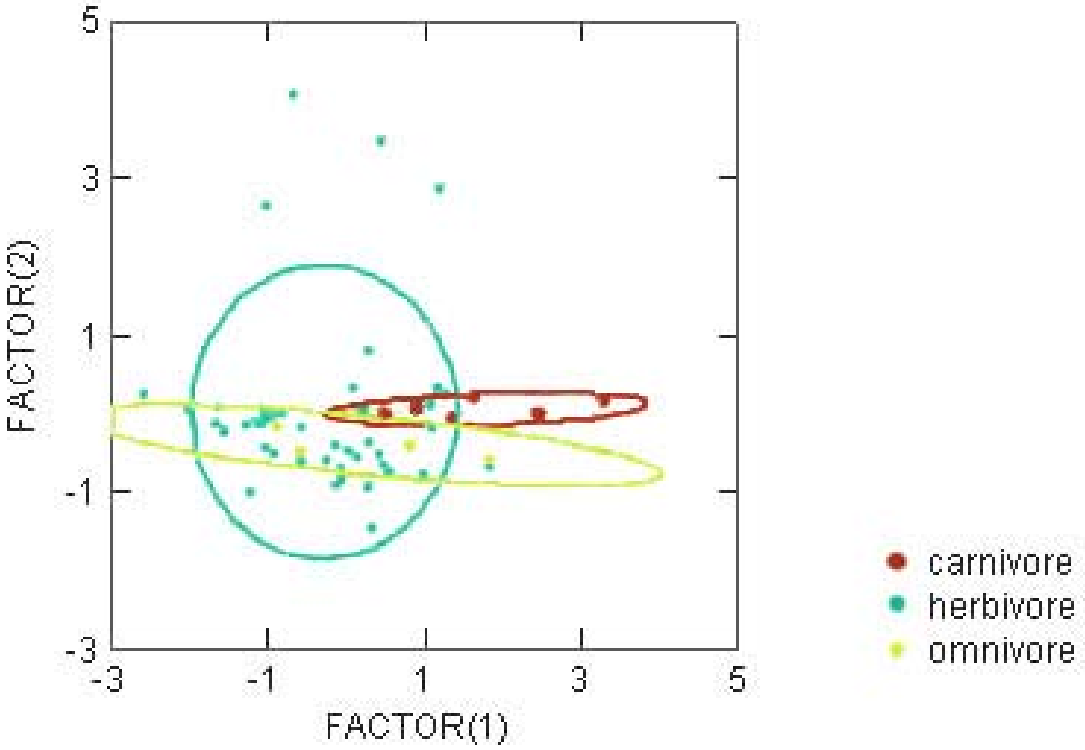

Figure 4.5 – Discriminant function analysis of isotopic data from Saltville, Virginia and Aucilla River, Florida. Factor 1 represents the synthetic variable derived from $\delta^{13}C$ values. Factor 2 represents the synthetic variable derived from $\delta^{15}N$ values. Edentates (sloths) are considered as carnivores for this analysis. See text for a full description of the animals included in each group.

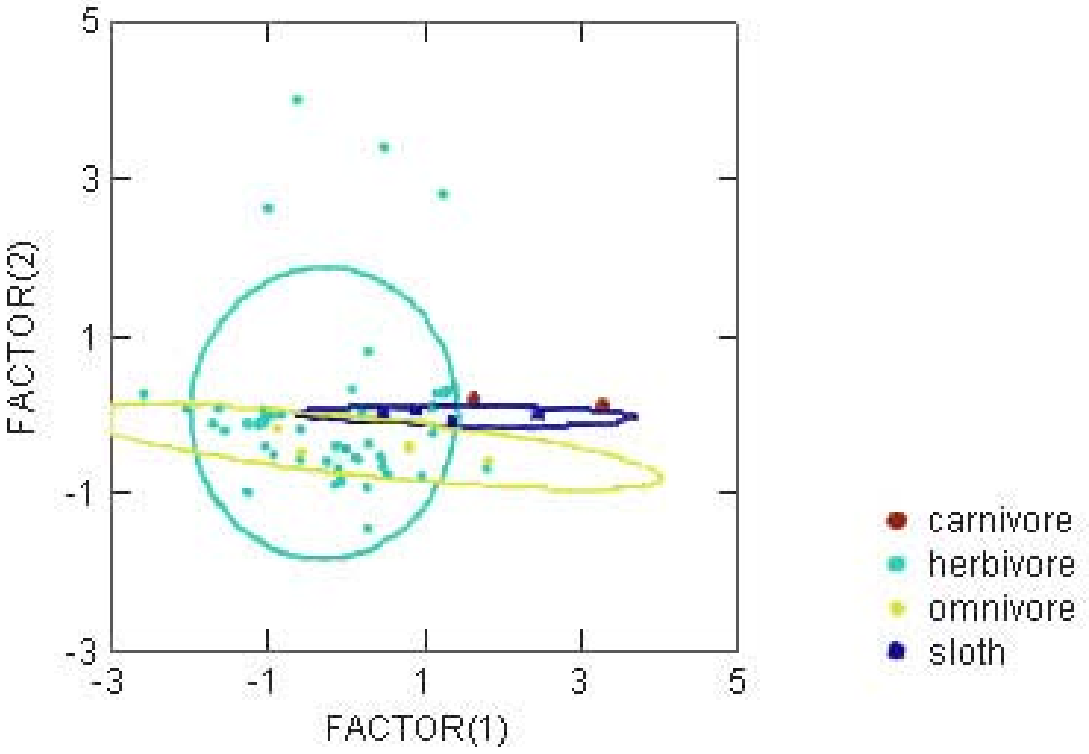

Figure 4.6 – Discriminant function analysis of isotopic data from Saltville, Virginia and Aucilla River, Florida. Factor 1 represents the synthetic variable derived from $\delta^{13}C$ values. Factor 2 represents the synthetic variable derived from $\delta^{15}N$ values. Edentates (sloths) are considered separately as their own distinct group for this analysis. See text for a full description of the animals included in each group.

4.5 Pleistocene extinctions

The initial intention of this study was to examine reconstructed trophic structures from a time series of fossil sites across the entire Pleistocene Epoch in an attempt to gain insight into the late Pleistocene faunal extinctions in North America. However, only two sites produced well preserved collagen from an adequate number of animals to reconstruct the trophic relationships (i.e. Aucilla River, Florida and Saltville, Virginia). These two sites were dated between ~12-20 yrs BP. While the broad analysis across the epoch is not possible, these sites may still be considered as final end members of the sequence of events leading up to the late Pleistocene extinction. As such, certain suppositions can be made about the presence of top-down versus bottom-up ecosystem collapses that would indicate a human-driven versus a climate-driven extinction, respectively.

Before examining this issue, it should be noted that the well preserved samples represent a fairly comprehensive list of herbivores present during this time period, but there was a paucity of well preserved carnivores. As such, conclusions concerning the lower levels of the food chain and a bottom-up ecosystem collapse are necessarily more robust than those put forth for the upper levels of the food chain and a top-down collapse.

4.5.1 Top-down versus bottom-up ecosystem collapse

A top-down ecosystem collapse would be represented by an isotopic signature showing competition at the highest levels of the food chain (Figure 1.5) as opposed to each individual carnivorous species occupying a unique ecologic space with a unique

food base (i.e. niche-splitting). While only a very few carnivores produced well preserved collagen, these appear to span a range of several permil in nitrogen isotopic space at the Aucilla River, Florida site. The felids (cats) appear to be more depleted than the canids (dogs), thereby indicating that these two groups preyed upon herbivores and other meat eaters, respectively (see section 4.3.2.2). While the carnivore sample set is limited, it does appear that there was a sufficient prey base for these animals to split the prey base and show some selectivity in their prey choice. This pattern is mimicked in a late Pleistocene faunal assemblage from California where the felids and canids show differences in prey selection with the latter group exhibiting a more generalistic and possibly scavenging behavior (Fox-Dobbs et al. 2003). The absence of large feline genera in the well preserved Florida sample set, such as *Felis* or *Smilodon*, prevents an examination of potential niche splitting between open grassland hunters and closed forest hunters. It remains possible that additional carnivores from this site would suggest such a split thus supporting the idea that competition was limited at the highest levels of the food chain and top-down collapse was not the driving force behind the extinction. However, given the small size of this data set, this conclusion must remain tentative at this time so that the possibility of a top-down collapse caused by human over-hunting remains a viable hypothesis.

The herbivore record is far more complete and provides a good look at potential bottom-up collapse of the ecosystem. At first glance, the Saltville, Virginia herbivores appear to show significant overlap in their dietary choices. However, this is most likely a result of a lack of C4 grasses in the area (see section 4.4.2) as opposed

to a lack of sufficient vegetation in general. At Aucilla River, the herbivores clearly had the choice to feed in open grassy areas containing C4 plants as evidenced by the enriched carbon isotopic signatures in the *Equus* (horse) and *Bison* (bison). However, none of the genera show a complete reliance on open grassland grazing. Even the *Equus* and *Bison* show evidence of significant C3 plant components in their diet. This could indicate one of two scenarios. The first is that the vegetation biomass was too low to support the entire herbivore biomass and the plant eaters were subsequently forced to consume any plant that was available. However, given the climate shift towards a warmer and more equable climate, this scenario seems unlikely. The second scenario focuses on food choice versus morphologic specialization. While some animals, such as horses or bison, could subsist entirely on grasses, they are not necessarily limited to this diet if the ecosystem is capable of providing a significant C3 plant component to their diets. The mammoths are a case in point (see section 4.4.2.1). Their dental morphology suggests that they could adequately process large amounts of grasses, and the flora at Aucilla River clearly contains open C4 grasslands, yet these animals preferentially consumed C3 plants. The presence of mammoths in Saltville, where open grasslands were not prevalent, further suggests that these particular animals were generalistic in their dietary habits. These multiple occurrences of generalized feeders at different sites argue against the idea of a bottom-up trophic collapse.

Further consideration of the generalized feeding habits of certain herbivores suggests that the late Pleistocene fauna were actually quite versatile and potentially resistant to ecosystem disturbances. As discussed above, the mammoths and the

mastodons apparently possessed the capability of subsisting on the far more abundant C3 vegetation as opposed to relying solely on open C4 grasslands. The Edentates (sloths) also showed a variety of dietary preferences with a higher meat component in the Aucilla River sloths as opposed to the one from Saltville (see section 4.4.3). Extant sloths will eat different plant species depending on availability (Hofreiter et al. 2000). The group as a whole appears to employ a variety of dietary strategies that are location dependant with the ability to adapt to floral and faunal changes.

It is tempting to suggest that the coexistence of numerous large herbivores necessarily requires niche separation and specialization to maintain a stable ecosystem. However, the late Pleistocene ecosystems examined in this study suggest that generalization was the dietary habit of choice during the latest Pleistocene. Omnivores and generalists tend to stabilize ecosystems and make them more resistant to dramatic climate and subsequent floral changes (Fagan 1997, Pace et al. 1999, Borrvall et al. 2000, Melian and Bascompte 2002). This may explain why the Pleistocene fauna was able to survive several dramatic glacial-interglacial cycles throughout this epoch. Open C4 grasslands alternately grew and diminished with the constantly fluctuating climate. The herbivores consumed these grasses when available, but were able to survive without them when they were in low abundance.

Thus far, this study has provided insight into the potential ecosystem collapse at the end of the Pleistocene, but fails to indicate clearly an absolute mechanism. Pure bottom-up or top-down collapse in unclear, however the former appears unlikely while the latter remains plausible. No single trophic level shows intense competition and the fauna as a whole appears fairly resistant to ecosystem disturbances. Without

a complete time series of ecosystems prior to the Aucilla River and Saltville sites, it is impossible to indicate where the areas of maximum competition were shifting as the climate changed and humans arrived in North America. However, these two end member sites provide a fairly complete view of the herbivores and suggest that this trophic level at least was a versatile one with several generalist feeders. As such, it is unlikely that the late Pleistocene climate change was responsible for a bottom-up collapse of the food chain. A top-down human-driven collapse of the food chain is also unsupported due to the apparent lack of competition at the higher trophic levels. However, given the small and incomplete set of carnivore data obtained in this study, an absolute conclusion regarding this hypothesis in not possible and it therefore remains a viable possibility. In summary, one of the two extinction hypotheses is hereby considered implausible (i.e. climate-driven extinction), while the other remains viable (i.e. human over-hunting). A more complete set of carnivorous animals would facilitate a more conclusive view regarding a top-down collapse caused by human over-hunting.

A more thorough resolution of this issue requires complete trophic relationship reconstructions of numerous well-dated late Pleistocene sites that span a range of time from well before the Last Glacial Maximum (>18,000 years ago) to the last occurrence of this fauna (~10,000 years ago). While this study provides a good initial view of the latest Pleistocene ecosystems, it does not provide the broad time series necessary to examine the *shifts* within trophic structures. It is necessary to have a view of a food web prior to major climate change and human arrival as a baseline for comparison. Once this baseline is established, the latest Pleistocene

trophic relationships from Saltville, Virginia and Aucilla River, Florida in this study can be compared and contrasted to locate where the areas of competition within these ecosystems are shifting. This will indicate the dominant stress on the ecosystem (i.e. humans or climate). It is also necessary to radiometrically date any sites that would be included in this endeavor; rough date estimates based on stratigraphy are insufficient. Precise dates are necessary to place samples within the rapidly fluctuating climate record where cold and warm periods may be separated by only ~2000 years.

4.5.2 Alternate possible extinction mechanisms

Since this study apparently indicates a lack of extreme competition in any one trophic level and subsequent directional collapse of the ecosystem, it is worth considering alternative extinction mechanisms that may have contributed to the demise of the late Pleistocene fauna. A mechanism that affects all trophic levels equally would be more consistent with the observed data in this study. One such possibility is lower latitude drought resulting from the rapid disappearance of glacial meltwater as the ice retreated northward. Within only a few thousand years of the Last Glacial Maximum (~18,000 years ago) most of the continental United States was free of ice. The lower and middle latitudes, including Florida and Virginia, would have experienced a reduction in overall water sources leading up to the extinction at 10,000 yrs BP. Drought affects all animals, regardless of trophic position. A second possibility is the hunting of all animal species as opposed to just herbivores. This would reduce competition at all levels of the food chain, but excessive hunting could

still reduce numbers to the point of extinction. However, this scenario seems unlikely. Human kill sites including carnivores in equal abundance to herbivores have yet to be located.

The potential keystone role of mammoths and mastodons remains a viable hypothesis for the demise of the late Pleistocene fauna. While their roles as generalist feeders would likely have a stabilizing effect on the ecosystem, their role in destroying or creating habitats, thereby significantly affecting other animals in the ecosystem (i.e. a "keystone" role), is not examined in this study. Additionally, the isotopic values of the proboscids and the carnivores in this study suggest that the former was part of the prey base for the latter. Species with numerous connections to other levels of the food chain are the most likely candidates to serve as keystone species. Their low reproductive rates would have exacerbated their inability to recover from environmental stress, thus supporting the possibility that they may have been keystone species.

CHAPTER 5 – CONCLUSIONS

Preservation of organic bone collagen from Saltville, Virginia; McKittrick Brea, California; and several sites from Florida varied by location and age. The youngest late Pleistocene samples yielded diagenetic products indicative of partial protein chains and amino acids. Older samples showed a complete lack of organic material or secondary contamination by mobile amino acids. Those samples that showed good quality collagen preservation were indicated by a C:N ratio between 2.8-3.6 and amino acid composition comparable to modern bones. This study indicates that bone collagen could be preserved under conditions of exceptional preservation in sites as old as ~400,000 years. Each fossil site is unique in its preservation potential and should be examined for the presence of well preserved collagen despite an age that may traditionally be considered too old for the preservation of proteins.

Carbon and nitrogen isotopic analysis of bone collagen provided the means to identify unique dietary strategies among different animal species. Isotopic analyses distinguished the distinct groups of herbivores, carnivores, and omnivores, as well as placed the previously uncharacterized group of sloths as omnivores. A few select species such as beavers, rats, and caribou showed selectivity for specific types of vegetation. Therefore it is suggested that species which typically rely on one very unique food source should be carefully considered before inclusion in broad isotopic comparisons between different groups of animals. Their unique isotopic signatures may erroneously alter results. The inclusion of juveniles in isotopic comparisons

should also be carefully considered. This study confirmed the nitrogen isotopic enrichment of juvenile collagen, which is useful for locating potential juvenile bones that are not in and of themselves age identifiable. Additionally, ruminants and non-ruminants appeared to overlap in their nitrogen isotopic signatures, thus precluding the use of isotopes to distinguish the two groups. Rather, the primary factor controlling the observed range of nitrogen isotopes in herbivores was the natural variation in plant isotopic composition. This reconstruction of a late Pleistocene ecosystem is a valuable tool that can now be applied to a variety of paleoecologic questions including, but not limited to, the correlation of dominant grass type to various North American sites and the identification of the dominant extinction mechanism for the late Pleistocene fauna.

The isotopic compositions of the fauna have various implications for the distribution of plant types in the continental United States at the end of the Pleistocene. Nitrogen fixation was potentially more prominent in the eastern United States, while the west coast may have incorporated a significant marine component into its water source and soils. Open C4 grasslands were apparently absent on the west coast and as far north as Virginia on the east coast. The transition between C4 and C3 grasses likely occurred in the region between South Carolina and Virginia in the latest Pleistocene.

The chronologic placement of the sample sites yielding well preserved collagen material provided the means to examine ecosystems at the very end of the Pleistocene just prior to the extinction event and potentially gain insight into the extinction mechanism. The limited number of carnivores appeared to have a variety

of food choices and minimal competition between carnivorous genera which does not support a top-down human-driven collapse of the ecosystem. However, given that this observation is based on such a small number of samples, the human-driven extinction scenario can not be conclusively disproved by this study and therefore remains a viable possibility. Omnivores showed a range in feeding preferences with meat and vegetation proportions in their diet that varied by region. Herbivores appeared to be opportunistic generalized feeders that could alternately graze on open grassy vegetation, or browse on herbaceous and woody vegetation. It is this pattern of generalization in the omnivores and herbivores that suggests a relatively robust ecosystem at the end of the Pleistocene that could withstand the potential changes in vegetation distribution resulting from climate change. A bottom-up climate-driven ecosystem collapse is not apparent.

The inability of this study to clearly indicate a human-driven or a climate-driven ecosystem collapse does not necessarily preclude either of these mechanisms from being the driving force behind the late Pleistocene extinctions. Severe climate shifts during this time are well supported as is the concurrent arrival of humans onto the North American continent. Rather, the extinction was likely influenced by both mechanisms to at least some extent. Prior research has attempted to separate the two mechanisms and argue strictly for one as opposed to the other. This study suggests that such an approach is far too simplistic and should be abandoned. Multiple factors facilitated the extinction of the late Pleistocene fauna and future models must consider human-driven, climate-driven, and other potential mechanisms (e.g. the keystone species hypothesis) when attempting to resolve this issue.

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