What's For Dinner?

An Isotopic Analysis of Pleistocene Mammals of the American Southeast

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GEOL 394

Abstract

During the Rancholabrean North American Land Mammal Age of the late Pleistocene, three megaherbivores are believed to have co-existed in the southeastern region of North America. These include *Eremotherium laurillardi* (the giant ground sloth), *Mammut americanum* (the American mastodon), and *Mammuthus columbi* (the Columbian mammoth). Morphological and comparative studies suggest that these giant herbivorous animals all appear to have occupied the same feeding niche, which would have put extreme stress on food resources. An alternative interpretation suggests that *E. laurillardi* was an omnivore that occasionally consumed meat. To test these hypotheses, bones from animals that occupied a range of trophic levels (including sloths, mastodons, and mammoths) in three separate Rancholabrean-aged successions were obtained from the Smithsonian Institution. Collagen was extracted from bone samples for subsequent carbon and nitrogen isotopic analysis. Based on C/N values it was determined that bone collagen from two of the study sites were diagenetically altered. Samples from the third (the Upland Bog Formation in Smyth County, Virginia) including sloth, mastodon, and mammoth remains, however, appeared to be well preserved. The nitrogen isotope compositions of collagen from these animals suggest that the giant sloths may have occupied a higher trophic level, and should thus be tentatively classified as omnivores. To confirm this result, further analyses of bones from the Upland Bog Formation along with a detailed study of the sedimentology and diagenetic environment of all three studied deposits are warranted.

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Introduction

The study of paleoecology is important to the paleontological community because it provides information about how ancient animals lived and interacted with their environment. Examining diet can be a useful tool to help us learn more about paleoecology because, diet analysis provides insight into the food chain. Previous paleodietary studies have examined the concept of the niche (Clementz et al., 2003; Feranec and MacFadden, 2000; Zazzo et al., 2000), which is a particular function and position of an organism within a habitat. Determination of the proportions of herbivore browsers, grazers, and intermediate feeders (mixed feeders) within a community informs us about niche splitting. The partitioning of resources allows for an increase in animal diversity because the competition for food is decreased (Feranec 2003). The optimum scenario is one species per niche.

In the North American Southeast during the Rancholabrean North American Land Mammal Age (ca. 0.3 to 0.01 Ma) of the Pleistocene Epoch (1.9 to 0.01 Ma) there were three colossal animals that appeared to occupy the same megaherbivore niche: *Eremotherium laurillardi* (giant ground sloth), *Mammut americanum* (American mastodon), and *Mammuthus columbi* (Columbian mammoth). Figure 1 illustrates general body size of these animals. Considering there are presently no megaherbivores in North America, the question arises, how did these giant herbivores co-exist? One hypothesis suggests that *Eremotherium laurillardi* was not a strict herbivore, but rather an omnivore, an opportunistic scavenger, an insectivore (Farina, 1996), or even a carnivore (Farina and Blanco, 1996). This would relieve some of the stress on the community food source. Nonetheless, *Mammut americanum* and *Mammuthus columbi* still appear to occupy the same niche. They are both of similar size and would have utilized the same feeding range. If *Eremotherium laurillardi* were a true herbivore, it too would have to utilize the same ecospace.

Figure 1: Relative size compared to average sized human. From left to right, *Eremotherium laurillardi, Mammut americanum*, and *Mammuthus columbi*

It is believed the environment of the Southeastern United States following the last glacial maximum (ca. 18,000 years ago), was similar to today's environment (Pittillo et al., 1998). The vegetation diversity and plant biomass should not have radically changed since that time, and should be able to support an ecosystem as it did in the late Pleistocene. Currently, Southeastern North

America does not support an ecosystem with three megaherbivores; indeed with the loss of the bison there is none. If *E. laurillardi, M. americanum*, and *M. columbi* all occupied the same niche, then this would indicate an ecosystem that is unlike any of today.

Performing nitrogen and carbon stable isotopic fractionation studies on these giant animals as well as known carnivores and herbivores from the same deposits will produce quantitative data that can be used to examine their diet. If the data show *E. laurillardi* to be an herbivore then this species' data will be compared to the isotopic data from *M. americanum* and *M. columbi*. Evaluating these data will show if these three animals split up the megaherbivore niche. If the data show *E. laurillardi to* be an omnivore or carnivore, then the data of *M. americanum* and *M. columbi* will still be compared to see if there was a niche splitting between the two. Therefore my hypothesis is *E. laurillardi* was an herbivore that occupied the same megaherbivore niche as *M. americanum* and *M. columbi.*

Background

Previous Morphological Studies

In depth morphological studies have been preformed on *E. laurillardi* showing a wide range of results (Figure 2).

(A)  **(B)** 

Figure 2: (A) Fossil skeleton of *Eremotherium laurillardi*,
(B) Reconstruction of giant ground sloth.

Ground sloths have long been thought to be herbivores (Farina, 1996, see Burmeister, 1879). However, Farina (1996) pointed out several characteristics of megatheriids (the family that contains *E. laurillardi*) that could indicate another type of diet. Megatheriids, as well as megalonychids and nothrotheriids (other families of giant ground sloth), have great control and precision over their hands and claws (Figure 3) because of their developed brains (Farina, 1996, see Dozo, 1989) and the biomechanics of their forearms (Farina, 1996, see Aramayo, 1988). This control could indicate that sloths used their claws to slash carcasses

open and to cut their meat into smaller pieces in order to make it easier to chew (Farina, 1996). Using their claws this way would help compensate for not having sharp cutting surfaces and sectorial dentitions on their teeth, which mammalian carnivores possess (Farina, 1996). Even so, these giant sloths had transverse crests on their teeth, which presumably could have been used to help chew flesh more effectively than a true herbivore (Farina, 1996). Also, their bigger and more developed brains further are consistent with carnivory, insofar as predators and scavengers tend to have larger brains than herbivores (Farina, 1996). This adaptation would not be needed for consuming only vegetation (Jerison, 1973), as herbivores do not usually have to outwit or sneak up on a plant. Even with all of these signs of possible meat consumption, *E. laurillardi* is still generally thought to be an herbivore (Farina, 1996). Therefore Farina (1996) concludes that a more diverse diet may not have evolved until later in the sloth's evolution, which could explain why morphologic evidence for carnivory is lacking.

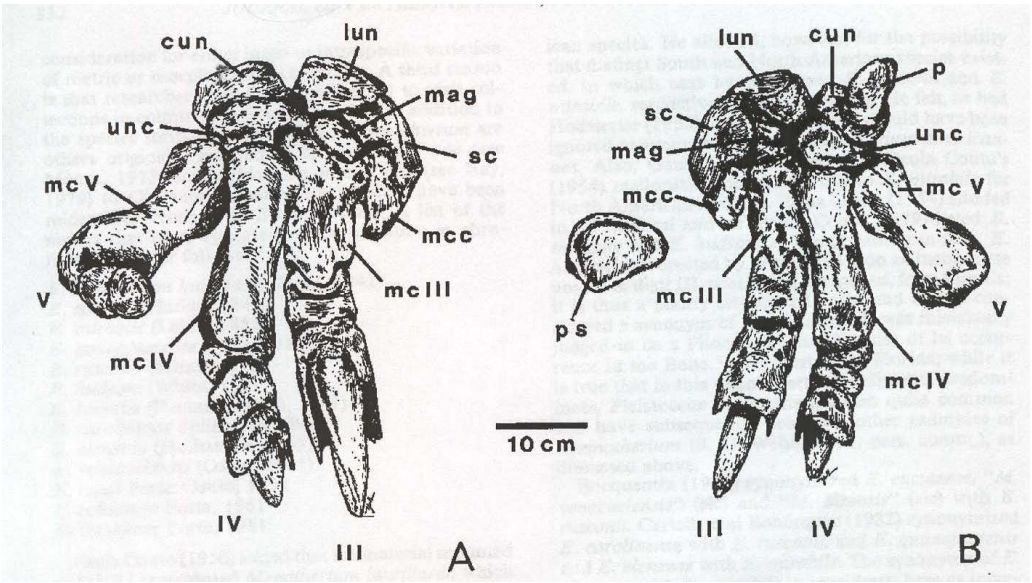
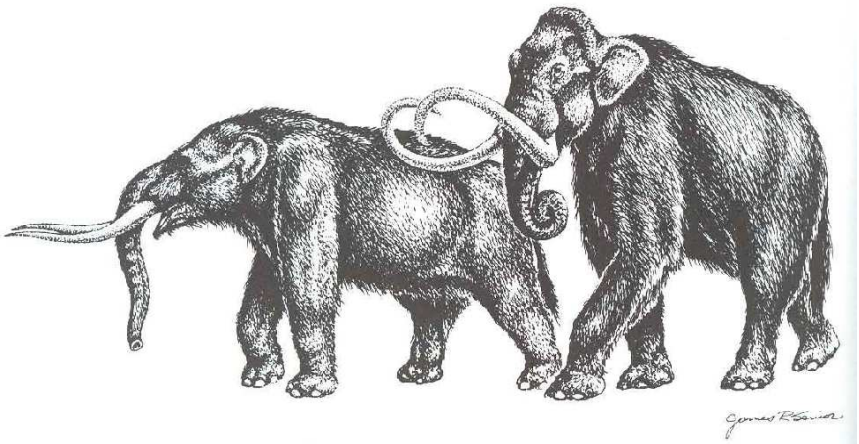


Figure 3: Dorsal (A) and palmar (B) views of *E. laurillardi*'s right manus (from Cartell 1995).

Morphological studies have similarly been preformed on *M. americanum* and *M. columbi* (Feranec, 2004; Sanchez et al., 2003; Martin, 1975) (Figure 4). One type of study involves examining the degree of hypsodonty, the condition of having high crowned teeth. It is measured by dividing the tooth crown height by the anteroposterior length of the tooth. Examining the hypsodonty index (HI) of an herbivore can indicate its food source. Using the HI, Feranec (2004) showed *M. columbi* to be a grazer. By examining the low-crowned molars of *M. columbi* Sanchez et al. (2003) concluded it also to be a grazer. In addition, Sanchez et al. (2003) examined *M. americanum*, and from its high crowned teeth concluded it to be browser. A tooth morphology study of *Megatherium* (another giant ground sloth from the Pleistocene of North America) examined its shearing molar cusps, and concluded it was a very well adapted browser (Martin 1975).



17.1 Mammutidae and Elephantidae. The dominant proboscideans during the Pleistocene were the mastodonts and mammoths. Shown are *Mammut americanum* and *Mammuthus columbi*.

Figure 4: Reconstruction of *Mammut americanum* (left) and *Mammuthus columbi* (right) (Kurten, 1992).

Another type of morphological study related to diet involves examining the muzzle shape of animals. This involves the observation of the shape of the premaxilla and the corresponding mandibular region, as well as the relative proportion of the incisor teeth. In these studies, a browser will show a narrow muzzle (i.e. short premaxillary width) and rounded incisor with the first one longer than the third. Grazers have a broader muzzle and transversely straight incisors that are of equal length (Palmqvist et al., 2003).

Dung analysis can be another tool used to determine diet of extinct animals. Studies of *M. americanum* coprolites suggest it was an opportunist (Coltrain et al., 2004) that fed on mixed foliage from spruce pinecones (Laub et al., 1994) to mosses (Gobetz and Bozarth, 2001). A study that examined *Nothrotheriops shastensis* (the smallest of the giant ground sloths; this pony-sized sloth is also known from the Pleistocene of North America) dung concluded that it was a browser (Martin, 1975). Another study of *Nothrotheriops shastensis* dung, however, showed that the diet of these sloths differ greatly from browser to grazer, even within animals that lived in relatively close environments (Hansen, 1978). *Paramylodon harlani*, another giant ground sloth native to North America, has been considered to be a grazer by Cork, (1994) based on the digestive physiology of modern sloths. Naples (1989) suggested *Paramylodon harlani* should be classified as a mixed feeder because it was probably not as efficient at digesting grasses as true grazers.

However, morphological and dung analyses both have their limitations. Hypsodonty Index, for example, can yield false results. A hypsodont animal (having high-crowned teeth) would be presumed to be a grazer, when in fact it could be a browser. Browsers are usually brachyodont, having low-crowned teeth. The animal may not eat as much as other animals of its size and therefore not show as much wear on its teeth as other brachyodonts (Palmqvist et al. 2003). One of the problems associated with fossil dung analyses is its extreme

rarity. It is also highly susceptible to post-depositional alteration (Martin 1975). Another disadvantage is that both of these methods are time consuming and are subject to considerable observer error (DeNiro and Epstein 1981).

Even so, these types of studies are useful, but do not provide the quantitative data that isotopic analysis of animal bone can yield. Many biogeochemical techniques, including the use of stable isotopes, have been shown to be effective tools in determining diet and niche placement in extinct animals (Palmqvist et al., 2003; Ostrom et al., 1993; Sanchez et al., 2003; Ostrom et al., 1994). Isotopic analyses of diet use the fractional behavior of carbon, nitrogen, and oxygen isotopes (Palmqvist et al. 2003, Ostrom et al. 1994, MacFadden et al. 2004).

This study will involve the measurement of elemental C/N as well as carbon and nitrogen isotopic compositions of bone collagen in order to make isotopic comparisons between these large animals. Bone collagen is continuously reworked throughout life, so the sample that is extracted and measured reflects an average isotopic composition of an animal's diet for a large portion of its life (Bocherens and Drucker, 2003). The chemistry of bone collagen is very well established and its amino acids vary only slightly among species (Schoeninger and DeNiro, 1983). Based on these studies, the C/N of well-preserved modern and ancient bone collagen should range between 2.9 and 3.6 DeNiro (1985), Palmqvist et al. (2003), and Coltrain et al. (2004). If the collagen is well preserved, its carbon and nitrogen isotopic compositions should reflect differences in isotopic composition of their diets, not differences in chemical composition of the bone (Schoeninger and DeNiro, 1983). In contrast, other sources of isotopes can be extracted from tooth enamel and dentin (Koch et al. 1994). These sources however, do not reflect an average isotopic composition of the animal's diet (Koch et al. 1994), because they are not reworked during the animal's lifetime and therefore are only indicative of the time when the tooth was formed (Koch et al. 1994, Palmqvist et al. 2003).

Stable Isotopes

The bones and tissue of mammalian animals are preferentially enriched in the heavy ^{15}N isotope due to their metabolic activities. Proportionally more ^{14}N is excreted as liquid and solid wastes, leaving the metabolic pool enriched in ^{15}N. Recognition of this isotopic behavior can be used in many ways, including paleodietary analysis. Isotope composition can be used in such analyses because the ^{15}N abundances recorded in animal bone are an indicator of its place in the food chain (Ostrom et al., 1993; Minagawa and Wada, 1984). Each step higher in the food chain shows approximately a 3‰ increase in $\delta^{15}N$ values (Minagawa and Wada, 1984; Ostrom et al., 1994; Schoeninger and DeNiro 1983), so by determining where an animal falls within the continuum may indicate whether it was an herbivore, omnivore, or primary or secondary carnivore.

In contrast, there is approximately a 1‰ increase in $\delta^{13}C$ values with each step up in the food chain (Ostrom et al., 1994; Schoeninger and DeNiro, 1983; DeNiro and Epstein, 1978). Because the differences would be necessarily

smaller, the use of carbon isotopes in paleodietary analyses is more difficult. Nonetheless, these values will be used to verify the increase in $\delta^{15}N$ values up the food chain.

The preferential retention of one isotope over another is also observed among plant communities. In particular, grasses, which utilize a C_4 pathway of biosynthesis, typically retain more of the heavy ^{13}C isotope than their leafy C_3 counterparts. This can be very useful in palaeodietary analysis because the different photosynthetic pathways that plants use reflect different $^{13}C/^{12}C$ ratios and these values are imprinted into the animals that consume them (DeNiro and Epstein, 1978; Zazzo et al., 2000; Clementz et al., 2003; Feranec 2003, Palmqvist et al., 2003). Therefore, ^{13}C values may indicate the type of vegetation the animal consumed. C_3 plants utilize the Calvin cycle of photosynthesis and have values for $\delta^{13}C$ in the range of –30‰ to –22‰ (Clementz et al., 2003; MacFadden et al., 2004; Sanchez et al., 2003). Examples of C_3 plants include most trees, bushes, shrubs, and high latitude grasses. C_4 plants use the Hatch-Slack photosynthetic pathway and have a $\delta^{13}C$ range of –15‰ to –10‰ (Clementz et al., 2003; MacFadden et al., 2004; Sanchez et al., 2003). Some examples of C_4 plants are warm seasoned and tropical grasses and sedges. The third type of photosynthetic pathway is the Crassulacean Acid Metabolism (CAM) process, which has $\delta^{13}C$ values ranging between C_3 and C_4 plants (Clementz et al. 2003, Sanchez et al. 2003). CAM plants make up only 5% of plant diversity, and are found in arid environments (MacFadden et al. 2004). For this study, a result within this middle range will most likely indicate an animal that feeds on both C_3 and C_4 plants, not the presence of CAM consuming animals, since the environment was not believed to be arid.

Therefore, herbivores may be split into categories based on what they eat. Generally browsers consume C_3 plants and grazers consume C_4 plants, while mixed feeders eat both. When a mammal eats a plant and its constituents are metabolized, a fractionation of approximately 5‰ $\delta^{13}C$ (Lee-Thorpe et al., 1989) is imparted to bone collagen. Roughly speaking, herbivores that consume C_3 plants will have $\delta^{13}C$ values of between –30 ‰ to –17 ‰, and herbivores that consume C_4 plants will have $\delta^{13}C$ values between –11‰ and –3‰ (Plamqvist, 2003). Knowledge of the $\delta^{13}C$ compositions of animal bone collagen can thus allow us to infer what types of plants they consumed.

Trophic Levels

A trophic level is a metabolic hierarchy that certain groups of organisms will occupy in the food chain. For example, a leopard, a lion, and a tiger are all on the same trophic level: carnivore. As stated above, there is an approximate 3‰ increase in $\delta^{15}N$ with each step up in the food chain. Figure 5 illustrates a schematic of this concept.

Examining trophic levels requires comparing unknown animals to ones that are unquestionably herbivores, omnivores and carnivores. The animals in this study that will be compared to *E. laurillardi, M. columbi, and M. americanum* are still living or have very close living relatives. Therefore their diets can be

directly observed. These known animals and their isotope values are required as a baseline to which the unknowns can be compared.

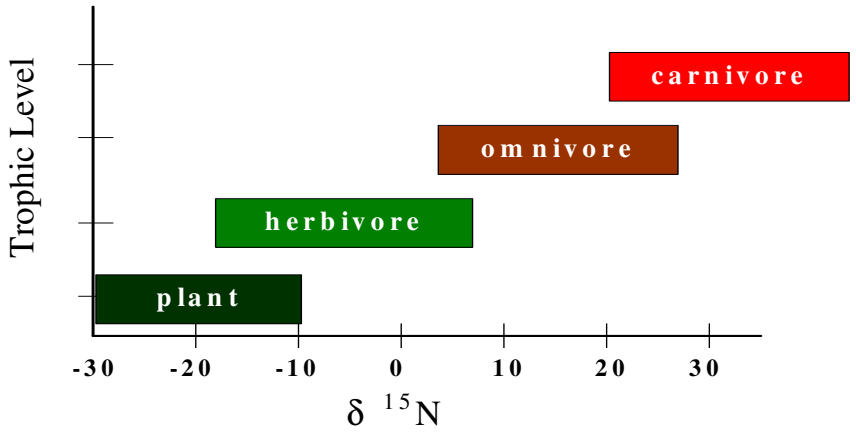


Figure 5: The stepwise increase in $\delta^{15}N$ ‰ compositions of organisms from different trophic levels.

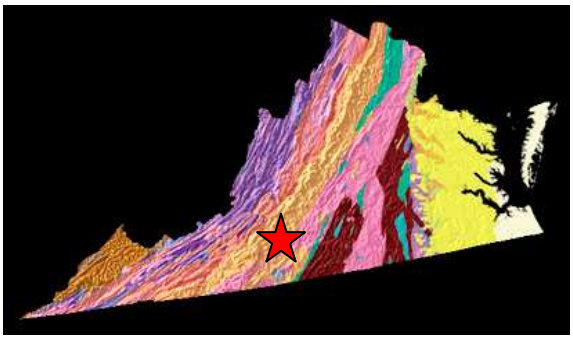
Previous Isotopic Studies

Isotopic analyses of *M. americanum* (Koch et al., 1998; Coltrain et al., 2004; Hoppe, 2004) and *M. columbi* (Koch et al., 1998; Feranec, 2004; Feranec and MacFadden, 2000; Hoppe, 2004) have previously been completed. Koch et al. (1998) suggested *M. americanum* was a browser, while Coltrain et al. (2004) indicated it was a grazer. Similarly, Koch et al. (1998) and Feranec and MacFadden (2000) suggested *M. columbi* was a grazer, but Feranec (2004) indicated that *M. columbi* was an intermediate feeder with an intense fondness for grasses and sedges. Although no isotopic determinations have been reported on *E. laurillardi,* there has been some completed on other ground sloths. *Paramylodon harlani,* for example, was determined to be a browser by Coltrain et al. (2004).

Samples

The *E. laurillardi,* samples that are the main focus of the study are from the Watkins Quarry of coastal Georgia in Glynn County (Hulbert and Pratt 1998). It is an estuarine deposit from the Rancholabrean North American Land Mammal Age. Additional samples come from two other Rancholabrean deposits, including the Saltville Quarry in Smyth County, Virginia and the Ladds Quarry in Bartow County, Georgia (see Figure 6 for relative location of the quarries in the southeastern USA). Samples from Saltville Quarry are from the Upland Bog Formation, which is carbon-14 dated to 13,460 ± 420 yrs (Smithsonian Museum of Natural History Archives). All samples have been donated by the Smithsonian Museum of Natural History. A description of the specimens and a list of the samples are found in Appendix 1.

(A)



(B)

Figure 6: Localities of the studied quarries in the southeastern USA. (A) Red star is Saltville Quarry, VA. (B) Blue star is Ladd's Quarry and green star is Watkins Quarry, GA.

Methods

To acquire the bone samples, a Multipro 3961-02 Dremel drill with a diamond tipped drill bit was used to first remove about 1 mm of surficial material, which was discarded. A hole was then drilled into the fresh bone surface until approximately 200 mg of powder was collected.

An initial test for secondary contaminates was preformed on a few of the samples before the resin procedure (see below) was adopted. For samples LQ-11 and WQ-22 approximately 100 mg of the bone powder was weighed out and 50 ml of 1M HCl was added to remove residual carbonates. The solution was agitated and soaked over night in a refrigerator at approximately 4°C. The following day the samples were centrifuged for 10 minutes on medium power. There was no bone powder remaining in sample LQ-11 so it was disregarded. The preparation of sample WQ-22 continued and the supernatant was decanted. Then 50 ml of ultra-pure milli-Q water was added. The solution was agitated and

centrifuged for 10 minutes; the supernatant was decanted and discarded. This rinsing was repeated with ultra-pure milli-Q water four times. The sample was then freeze-dried and then run on a Micromass Isoprime continuous flow gas source mass spectrometer.

Approximately 500 μg of purified collagen is weighed and placed in tin cups. The Micromass Isoprime continuous flow gas source mass spectrometer is connected to an Elemental Analyzer (EA) (all EA settings are found in Appendix 2). The sample is placed into the auto sampler and injected into the combustion column with O_2 where it flash combusts at 1020°C, releasing carbon dioxide and nitrous oxide gases. These gases then flow into the reduction column (650°C) via a stream of helium carrier gas where the nitrous oxides are reduced to N_2. These gases then flow through a magnesium perchlorate water trap where residual water vapor is removed and is then led into the gas chromatography (GC) column. The GC separates the gases into discrete N_2 and CO_2 pulses, which are quantified by the elemental analyzer before flowing into the source of the continuous flow mass spectrometer in a stream of ultra-high purity helium.

The N_2 and CO_2 pass the ion source, which is a thorium filament that produces electrons upon exposure to an electrical current. The carrier gas then pulls the sample past the filament as the electrons ionize the gas molecules, thus giving the N_2 and CO_2 molecules a negative charge. The ions are then passed through two negatively charged accelerating plates that repel and propel them down the flight tube. The accelerating voltage for N_2 is 3620-3625 volts and 3600-3605 volts for CO_2.The mass spectrometer is tuned daily, so the precise accelerating voltages will change slightly from day to day. The ionized molecules are accelerated down the flight tube where a magnet bends the paths of each mass fraction into separate collecting cups. When the ions hit the collector cups they are recorded as electrical pulses. The integrated computer compiles the data in an Excel file, applies appropriate corrections, and reports the elemental and isotope abundances relative to their standard materials. Carbon and nitrogen isotope compositions of the samples are reported in the standard delta notation relative to Vienna Peedee Belemnite (VPDB) and atmospheric N_2, respectively, where

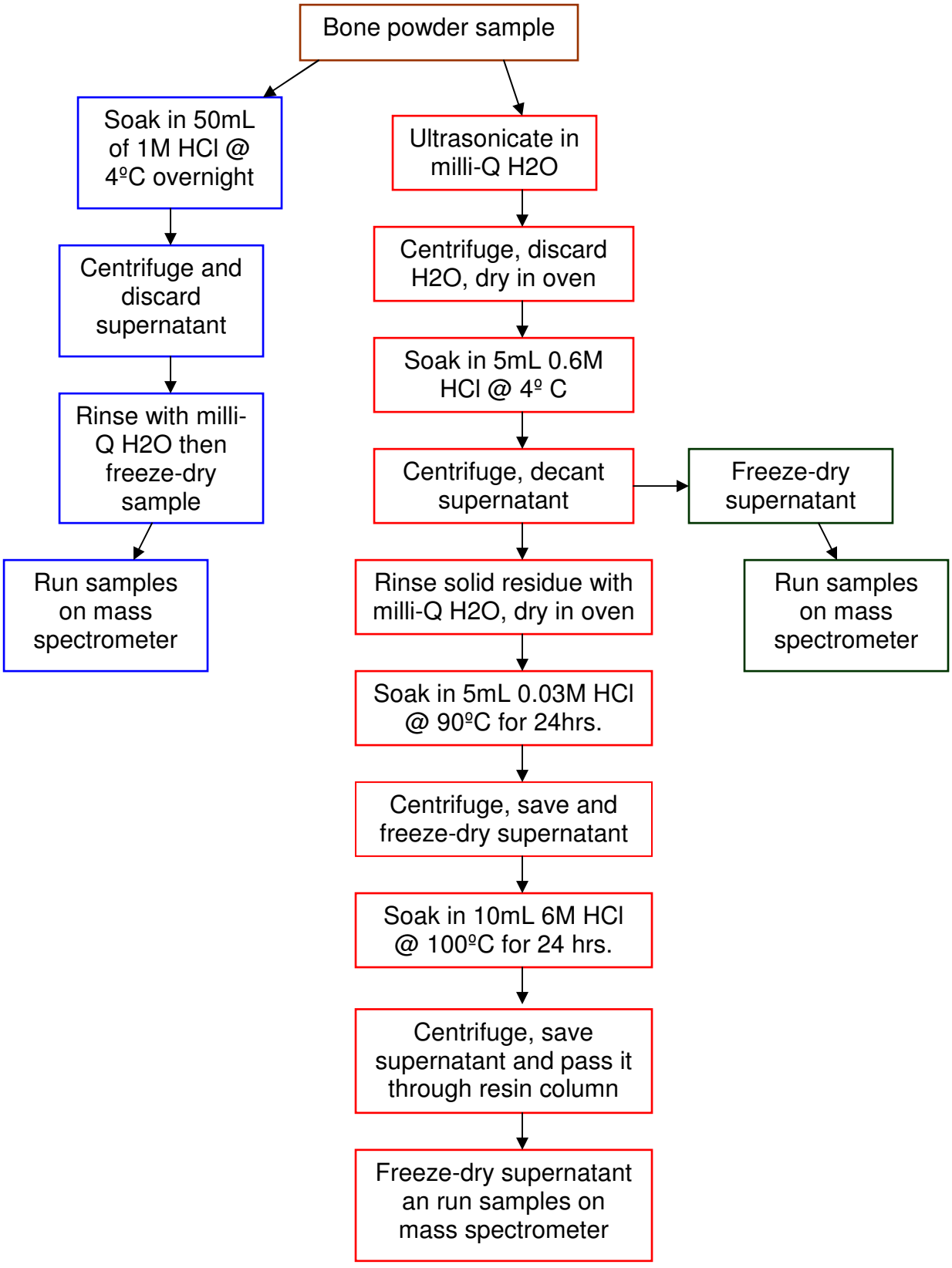
$$\delta^{13}C = \{[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}] - 1\} \times 1000\ ‰$$

$$\delta^{15}N = \{[(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard}] - 1\} \times 1000\ ‰.$$

The house standards used to quantify uncertainties of the continuous flow mass spectrometric technique included NIST 912a (urea) for $\delta^{13}C$ and $\delta^{15}N$ compositions, and glycine for C/N ratios, from the Acros Chemical Company.

Experiments indicated the presence of contaminates after the initial treatments, so our procedures were modified according the more rigorous methods of Stafford et al. (1998). In this procedure (Figure 7), bone powder is first decalcified with 4°C 0.6 M HCl.

Figure 7: Flow chart of procedures preformed on bone samples.



The acid-insoluble residue is then concentrated through centrifugation, and then rinsed three to four times with ultra-pure milli-Q water. Concern about the possibility of releasing degraded collagen in the initial acidification step led to a series of tests using different volumes and lengths of reaction. Practice samples were treated with the 0.6 M HCl for 5, 15, and 30 minutes, with volumes from 5 to 40mL. Results of these tests suggest that sample powders should be decalcified with 5mL of 0.6 M HCl for 5 min., and all subsequent samples were treated in this manner.

The residue is put into solution by heating the decalcified bone powder with dilute 0.03 M HCl (pH of 2-3) for 24 hours at 90°C. The solution is then centrifuged and the solid portion containing sands, minerals, and other contaminates is discarded. The supernatant is then freeze-dried. The resulting freeze-dried solid is then hydrolyzed with 10 ml of 6 M distilled HCl for 24 hours at 100°C. Remaining solids are removed by centrifugation before the filtered hydrolysate is purified by XAD chromatography. In this method, the analytical grade resin PAD-1 (0.1-0.2mm) is cleaned before use by repeatedly washing in acetone and water. The aqueous suspension is alternately extracted three times for 30 minutes each with 80°C 3 M HCl and 3 M NaOH and then washed with ultra pure milli-Q water before Soxhlet extraction for 24 hours each with methanol and acetone. After a final distilled water wash, the resin is stored in 1 M HCl.

The filtered hydrolysate is then treated with the pre-cleaned resin. A 0.45 μm VWR brand filter (PDVF membrane) is added to the base of a 5ml syringe and approximately 3 ml of resin is packed into the syringe. The resin is then washed with three bed volumes of 6 M HCl. The protein hydrolysate is passed through the resin at 200μl/min and collected. Two bed volumes of 6M HCl are then passed through the resin and added to the sample. The resin procedure removes any humic and fulvic acids in the sample. The resulting liquid is then freeze-dried and the residual purified collagen analyzed with the Eurovector elemental analyzer and Micromass Isoprime continuous flow gas source mass spectrometer as described above. As a precautionary measure, instead of discarding the supernatant from the first decalcification step, which might contain some percentage of degraded collagen, this fraction was also saved, freeze-dried and then analyzed.

Results

Standards

Standard materials were analyzed repeatedly throughout this study to provide quantitative precisions on the elemental ratios and isotope compositions of the samples. The standards included glycine for C/N and urea for carbon and nitrogen isotope compositions. The glycine standards show an average of 1.74 (± 0.07 2σ) for C/N, and the urea standards reveal average values of 2.01‰ (± 0.16 2σ) for $\delta^{15}N$ and 19.04‰ (± 0.24 2σ) for $\delta^{13}C$. Figure 8 illustrates the range of $\delta^{15}N$ and $\delta^{13}C$ compositions for the urea standards. Their distribution is extremely tight indicating very good reproducibility. Similarly, Figure 9 shows the theoretical

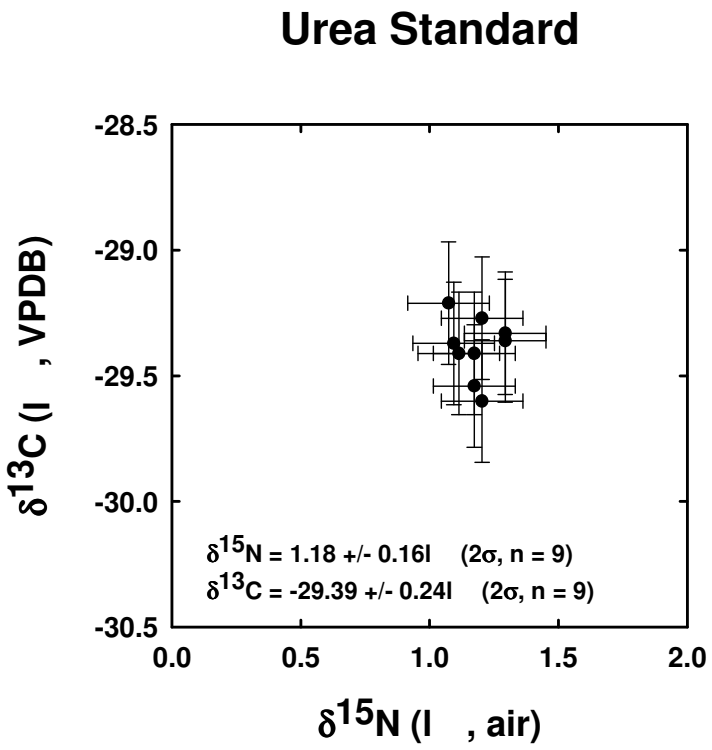


Figure 8: $\delta^{15}N$ plotted against $\delta^{13}C$ from multiple measurements of the urea standard to show precision of the mass spectrometer.

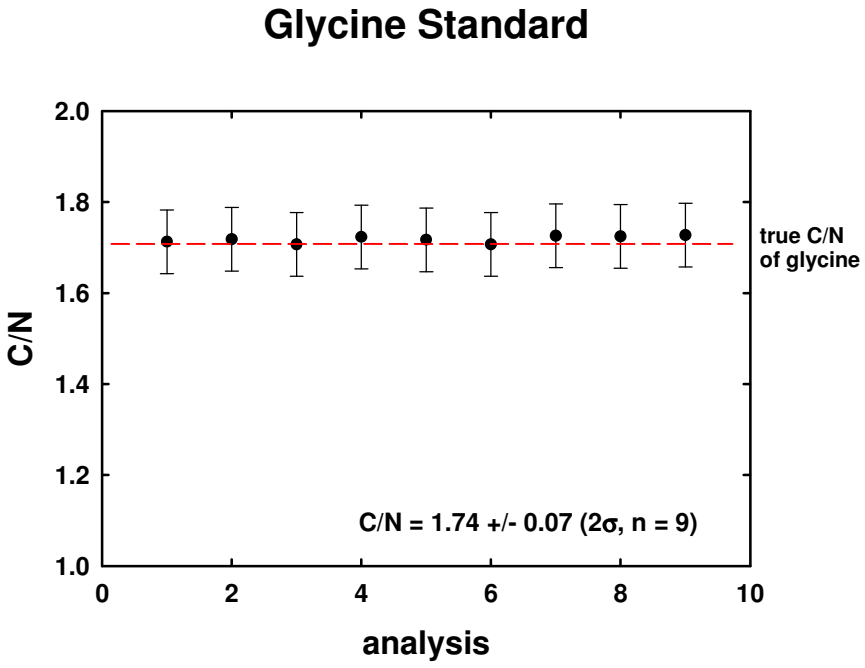


Figure 9: Actual C/N yield with 2σ uncertainties plotted relative to the expected C/N for glycine of 1.7 shown by the red stippled line.

yield of glycine C/N, which is 1.7, compared to the elemental ratios measured by the Eurovector elemental analyzer. The theoretical yield is within the error bars of the actual yield (1.74 ± 0.07 2σ), which indicates the reliability of the data.

Samples

Results of the test for secondary contaminates from the preparation of replicate bone powders from an *E. laurillardi* sample are shown in Table 1. The low abundance of both nitrogen and carbon in the residual fractions and low C/N suggest that this material was not purified collagen, so this procedure was abandoned.

Sample	N peak height (nA)	%N	δ^{15}N (‰)	C peak height (nA)	%C	δ^{13}C (‰)	C/N
WQ-22_01	0.09	0.46	-9.79	0.18	1.09	-27.55	2.3697
WQ-22_02	0.09	0.39	-13.10	0	0	-74.34	0
WQ-22_03	0.09	0.45	-8.91	0.17	0.99	-26.37	2.2

Table 1: Data for one *Eremotherium laurillardi* sample from the Watkins Quarry, treated with the initial test for secondary contaminates δ^{15}N (‰) values are corrected with respect to air, δ^{13}C (‰) values are corrected with respect to VPDB.

Analyses of the freeze-dried residues from the supernatant produced from the initial decalcification in the modified technique are shown in Table 2. Little nitrogen was detected in any of these samples, although some peaks were large enough to be measured. The range in δ^{15}N values of these supernatant samples spans from -4 to -11‰. Carbon concentrations were expectedly higher in these residues, which yielded δ^{13}C values ranging from -20 to -28‰.

Samples that were purified by the ion exchange resins showed a wide range of C/N and isotope results (Table 3). Collagen samples from all animals collected from both Watkins and Ladd quarries contained little nitrogen and high C/N, in contrast to animals analyzed from the Upland Bog Formation in the Saltville Quarry, where nitrogen concentrations ranged from 2 to 8 wt.%, C/N from 3.0 to 5.7, δ^{15}N values from 0.2 to 8.1‰, and δ^{13}C values from -19.0 to -22.5‰.

Sample	N peak height (nA)	%N	δ^{15}N (‰)	C peak height (nA)	%C	δ^{13}C (‰)	C/N
01spnt	-	1.2	-	1.43	3.1	-20.785	2.583
03spnt	-	1.33	-	1.73	3.81	-22.625	2.865
04spnt	-	0.5	-	1.18	2.23	-25.085	4.460
05spnt	-	.46	-	0.43	0.96	-23.895	2.087
06spnt	-	1.15	-	1.33	3.13	-20.025	2.722
07spnt	-	1.59	-	2.07	4.37	-20.695	2.748
08spnt	-	1.47	-	2.22	4.44	-21.535	3.020
09spnt	-	0.52	-	0.49	-	-22.385	-
10spnt	0.13	0.47	-4.598	0.06	0.2	-26.262	0.4255
11spnt	0.13	0.53	-10.288	0.14	0.42	-25.442	0.7925
12spnt	0.14	0.48	-11.908	0.19	0.48	-25.682	1
13spnt	0.14	0.51	-10.938	0.17	0.46	-25.132	0.9020
15spnt	0.14	0.44	-10.878	0.12	0.3	-25.192	0.6818
16spnt	0.13	0.46	-10.278	0.11	0.29	-25.882	0.6304
18spnt	0.13	0.47	-10.758	0.14	0.4	-27.562	0.8511
19spnt	0.12	0.39	-9.978	0.42	0.94	-24.862	2.4103
20spnt	0.09	0.27	-7.756	0.29	0.59	-24.91	2.1852
21spnt	0.09	0.34	-14.786	0.21	0.55	-26.31	1.6177
22spnt	0.09	0.34	-11.126	0.29	0.76	-25.89	2.2353

Table 2: The supernatant sample number (spnt) corresponds to the bone sample number shown in Table 3. All supernatant samples were the product of the first decalcification step. δ^{15}N (‰) values are corrected with respect to air, δ^{13}C (‰) values are corrected with respect to VPDB.

Sample number	N peak height (nA)	%N	δ^{15}N (‰)	C peak height (nA)	%C	δ^{13}C (‰)	C/N
SQ-01	3.99	7.97	3.676	14.98	24.69	-20.098	3.0979
SQ-04	1.06	2.87	1.626	6.09	12.12	-21.818	4.2223
SQ-05	0.86	1.96	8.056	6.64	11.3	-22.578	5.7653
SQ-06	3.18	6.96	0.176	12.55	22.39	-19.008	3.2170
SQ-07	1.09	3.24	0.2225	4.52	10.49	-19.805	3.2377
SQ-08	2.64	6.25	2.6725	9.45	18.71	-20.245	2.9936
SQ-09	1.28	3.2	0.4925	5.7	11.48	-20.595	3.5875
LQ-13	0.1	1.01	-4.8675	5.47	25.74	-26.462	25.485
LQ-14	0.15	0.82	-4.03	4.64	11.2	-24.64	13.658
LQ-15	0.09	0.31	-8.536	1.71	3.22	-24.77	10.387
LQ-17	0.10	0.42	-6.92	0.71	1.80	-22.37	4.29
WQ-19	0.09	0.32	-12.756	0.34	0.81	-24.69	2.5312
WQ-20	0.09	0.36	-10.296	0.34	0.83	-24.22	2.3056
WQ-21	0.09	0.24	-12.816	0.42	0.82	-24.81	3.4583
WQ-22	0.09	0.3	-15.856	0.31	0.71	-23.61	2.3667

Table 3: All samples were treated with the resin procedure. δ^{15}N (‰) values are corrected with respect to air, δ^{13}C (‰) values are corrected with respect to VPDB. SQ denotes Saltville Quarry, LQ denotes Ladds Quarry, WQ denotes Watkins Quarry.

Discussion

Precision of Instruments

The precision of instruments is important in any study, but extreme precision is very essential in this study. The range of values that are being examined are so small that any significant error can skew the results greatly. By performing numerous runs of the standards, we have shown that the reproducibility is well within the range of acceptable values. Each step up in trophic level shows an ~3‰ increase in $\delta^{15}N$ and an ~1‰ increase in $\delta^{13}C$ values. It is likely that some animals in the study will show differences from one another that are even less than these values. Consequently, any standard deviation produced by the mass spectrometer of 0.5‰ or greater would render the data useless. The standard deviation produced by the Micromass Isoprime continuous flow gas source mass spectrometer is well below that value for both $\delta^{15}N$ (± 0.16 2σ) and $\delta^{13}C$ (± 0.24 2σ). Therefore we feel exceptionally confident of our ability to discern subtle differences in trophic levels. The same is true for the C:N values. A standard deviation of ± 0.07 (2σ) is well within the range of acceptable variations.

Parameters for Evaluating Sample Data

Collagen samples were evaluated as altered or unaltered based on their nitrogen abundances and C/N ratios. Our baseline of acceptable N peak heights is >1nA for a rough approximation of the presence of organic matter. Samples with less than this are considered unreliable for the determination of organic matter present. The peak height must be above 4nA for reliable isotopic data (manufacturer's recommendation and this lab's observation). If the sample has a usable C and N peak height then its' C/N ratio will be considered. If the C/N ratio is approximately three (Bocherens et al. 1994, Bocherens et al. 1991) then this indicates the collagen samples have been cleaned properly, no longer containing secondary contamination, and are not diagenetically altered. A range of 2.9 to 3.6, for C/N values, was indicated by DeNiro (1985), Palmqvist et al. (2003), and Coltrain et al. (2004). Our precision (± 0.07 2σ) is more than adequate to detect this range. In cases where the C/N and N peak heights are not usable, it is likely due to the fact that the collagen was degraded by diagenetic reactions.

Alteration of Collagen

The results from our first run of a resin treated sample were unsuccessful. The sample used was LQ-14 (a bear), its' data showed a very low nitrogen content. We were fearful that the nitrogen along with the collagen was being lost somewhere in the process. So we began to analysis each step of the procedure. The problem may be within the chemistry of the resin procedure itself. Therefore, extractions were stopped until the procedure could be reevaluated. The first decalcification step could be pulling the collagen into solution, which is discarded.

This could be occurring because of the physical structure and the type of bonds that make up collagen (Figure 10).

Collagen is a triple helix structure that has various branching bonds that interlock the three intertwined amino acid chains (Kucharz, 1992). These interlocking chains, however, are linked by hydrogen bonds, which under acidic conditions may weaken and break apart (Figure 10). Thus we considered the possibility that the initial decalcification step may be releasing some of the more degraded collagen in the samples (see Table 2 and Methods).

Therefore the decalcification step was reexamined. Practice samples were treated with the acid on different time intervals, such as, 5 minutes, 15 minutes, and 30 minutes. A shorter amount of time should adequately decalcify the sample and cause less of the collagen to go into solution. Not only was the time frame reevaluated but also the amount of acid used to decalcify the collagen. Forty milliliters of 0.6 M HCl was being used, but that was an excessive amount compared to the amount of sample that was being processed, which was ~200mg. The extra protons from the excessive amount of acid may also contribute to the breaking down of the collagen structure. Therefore we lessened the amount of acid to 5mL and shorten to the time from 30sec. to 5mins

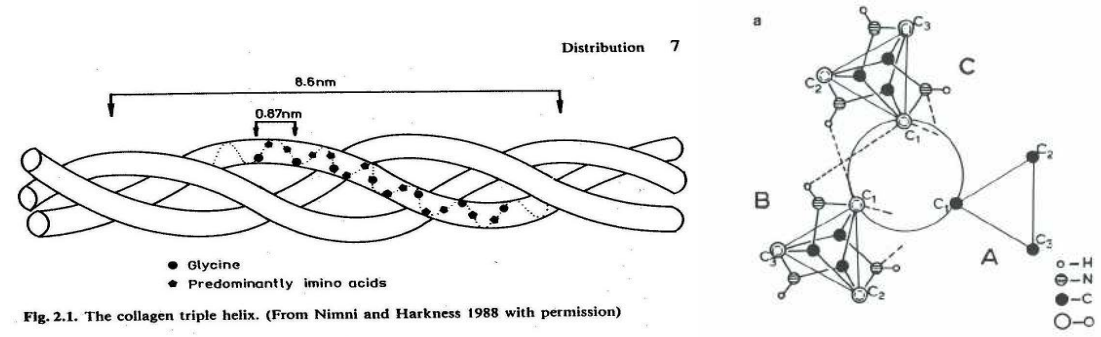


Figure 10: Helical structure and hydrogen bonding in collagen (Kucharz, 1992 and Ramachandran, 1976).

Diagenesis

The fossil bone collagen may have been exposed to a number of post-mortem processes that can alter its elemental and isotopic compositions. These include subaerial weathering, leaching by groundwater, microbial attack, and incorporation of sedimentary organic matter (Hare et al., 1991). Any alteration will most likely result in the breakdown of collagen and the subsequent release of nitrogen from the residual organic matrix. Based on the results of collagen C/N analyses from a variety of animals from the three quarries, it appears that all animal bones from the Watkins and Ladds quarry deposits are altered.

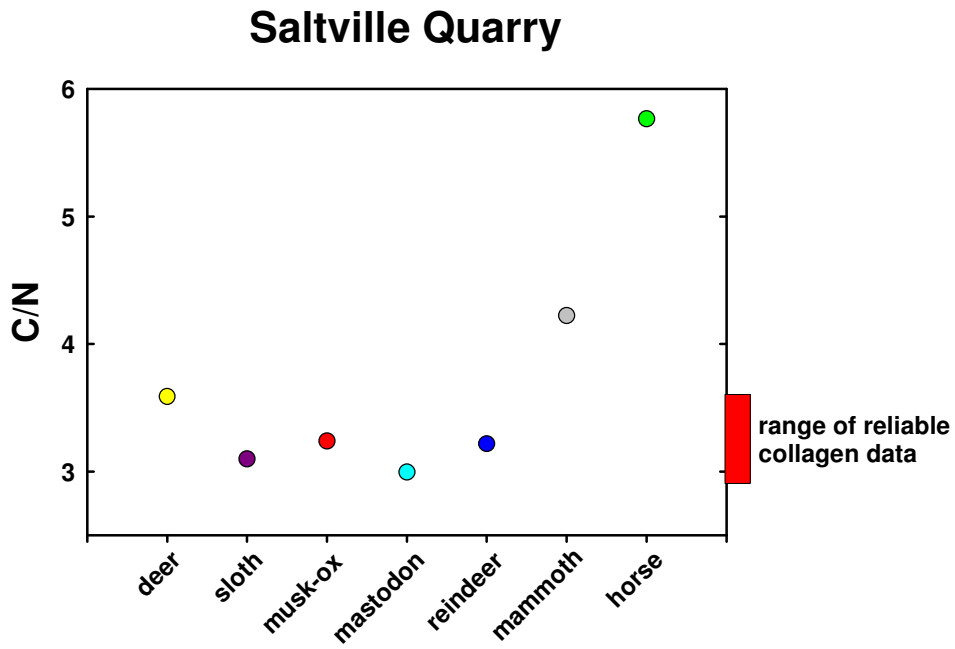


Figure 11: C/N values of bone collagen from animals of the Saltville Quarry. Size of individual point markers encompasses statistical error which is ± 0.07 2σ.

Unfortunately the Watkins Quarry was the only site in which samples of *E. laurillardi* were available, so the original hypothesis (see Introduction) cannot be directly tested. On the other hand, collagen from bones collected in the Upland Bog Formation at Saltville Quarry did produce substantial nitrogen peak heights and C/N ratios that were within the 2.9-3.6 range of well-preserved collagen (Figure 11). Even though the Saltville Quarry did not contain *E. laurillardi*, it did contain another large sloth, *Megalonyx jeffersonii*. Usable data was recovered from this sloth, as well as from, *M. americanum* (American mastodon), *Rangifer tarandus* (reindeer), *Bootherium sp*. (musk-ox) and *Odocoileus virginainus* (white-tailed deer). The two other specimens from this quarry, *Mammuthus sp*. (mammoth) and *Equus sp*. (horse) had higher C/N values and were not considered in the analysis of trophic level and niche splitting.

Trophic Levels

Based on the analyses of the five animals with apparently well preserved collagen from the Saltville Quarry, it is possible to evaluate the trophic levels of these individuals by comparison of their nitrogen isotope compositions. Of the group, the sloth, *Megalonyx jeffersonii*, shows the highest $\delta^{15}N$ values with approximately a 3‰ increase from the known herbivores, including the reindeer, musk-ox, and deer (Figure 12).

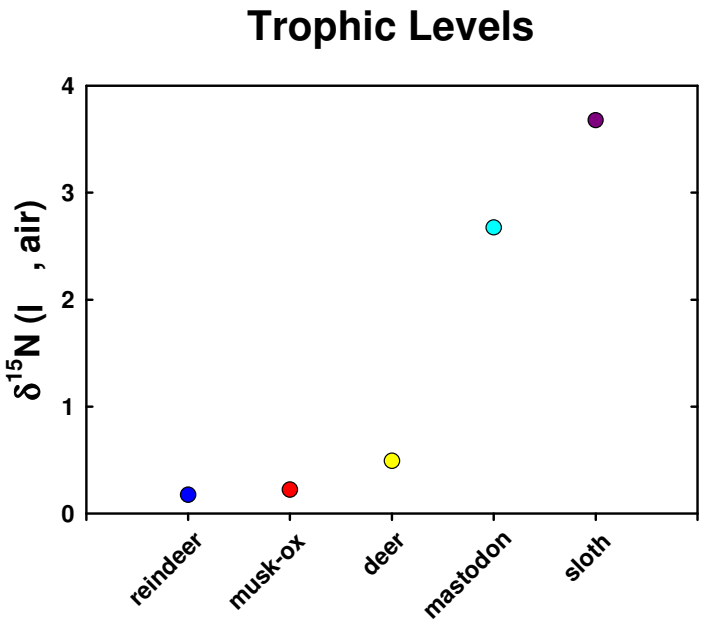


Figure 12: Nitrogen isotope compositions of bone collagen from animals of the Upland Bog Formation at Saltville Quarry. Size of individual point markers encompasses statistical error which is ± 0.16 2σ.

This increase in ^{15}N content of the sloth is consistent with this animal being higher on the food chain, indicating that the sloth likely consumed some meat and could be classified as an omnivore. Although similar analyses of *E. laurillardi* were not possible, the re-designation of this related sloth to a higher trophic level supports the view of Farina (1996) that these animals in general may have used their sharp claws to eat meat. Coltrain et al. (2004) also noted ^{15}N enrichment in another species of giant ground sloth, *Paramylodon harlani* (Table 4). Notably, this conclusion does not support my original hypothesis.

The mastodon bone collagen sample also shows a higher $\delta^{15}N$ than the other known herbivores. Insofar as no proboscidea of today consumes meat the ^{15}N enrichment in this sample may be an anomaly, although Coltrain et al. (2004) also saw higher $\delta^{15}N$ values in the mastodon (Table 4).

Fossil Animal	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Mammut americanum (American mastodon)	~ 4	~ -20
Paramylodon harlani (giant ground sloth)	~ 7.5	~ -21

Table 4: Data from Coltrain et al. (2004) taken from their Figure 4 showing ^{15}N enrichment in both sloth and mastodon bone collagen from Los Angeles Basin, California.

If the mastodon data is correct, the lower $\delta^{15}N$ values of the fossil reindeer, musk-ox, and deer may represent the anomaly. Comparison of my data with other studies of fossil reindeer (Bocherens et al., 1991) and fossil deer (DeNiro and Epstein, 1978) shows significant differences. The reindeer in this study is up to 6‰ less enriched than those analyzed in the earlier study by Bocherens et al. (1991) (Table 5). This may be due to regional variability in the food source, or that the ancient bones are diagenetically altered even while the extracted collagen retains expected values of C/N. On the other hand, the wide range of variability within the analyses of reindeer may reflect problems in their techniques.

Fossil Animal	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Rangifer tarandus (reindeer)	0.9	-20.2
Rangifer tarandus	3.1	-19.7
Rangifer tarandus	3.3	-19.9
Rangifer tarandus	6.1	-20.1
Rangifer tarandus	3.5	-19.0

Table 5: Data from Bocherens et al. (1991) Table 2. Isotopic compositions of fossil reindeer bone collagen from Marillac, France.

Similarly, the single deer sample from this study is 4 to 5.5‰ less enriched in ^{15}N than *Odocoileus virginainus* is from the earlier study by DeNiro and Epstein (1985) (Table 6). In that study the variability of $\delta^{15}N$ analyses is not as great, so the differences most likely highlight regional food source issues or unrecognized problems with their technique. Clearly, additional replicate analyses from a variety of individuals of the same species from the Saltville Quarry will be required to substantiate the present results.

Fossil Animal	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Odocoileus virginainus (white-tailed deer)	4.6	-20.4
Odocoileus virginainus	4.6	-20.6
Odocoileus virginainus	5.5	-20.5
Odocoileus virginainus	6.1	-19.2
Odocoileus virginainus	5.3	-18.6

Table 6: Data from DeNiro and Epstein (1985) Table 1. Isotopic compositions of bone collagen from fossil deer from Palmer and Wightman, Florida.

Niche Splitting

The carbon isotope compositions of the well-preserved collagen samples from the Upland Bog Formation may additionally be used to evaluate niche splitting between the herbivores. Based on these analyses, two niches appear to be represented here. The grazing animals (reindeer) that consume more C_4

biomass have higher $\delta^{13}C$ values as predicted (Figure 13), while known browsers (like the deer and musk-ox) that consume C_3 biomass have lower $\delta^{13}C$ values. Both the mastodon and sloth have carbon isotope compositions similar to the deer and musk-ox, suggesting that these were also browsers, which correspond to Coltrain et al. (2004) for the sloth *Paramylodon harlani*.

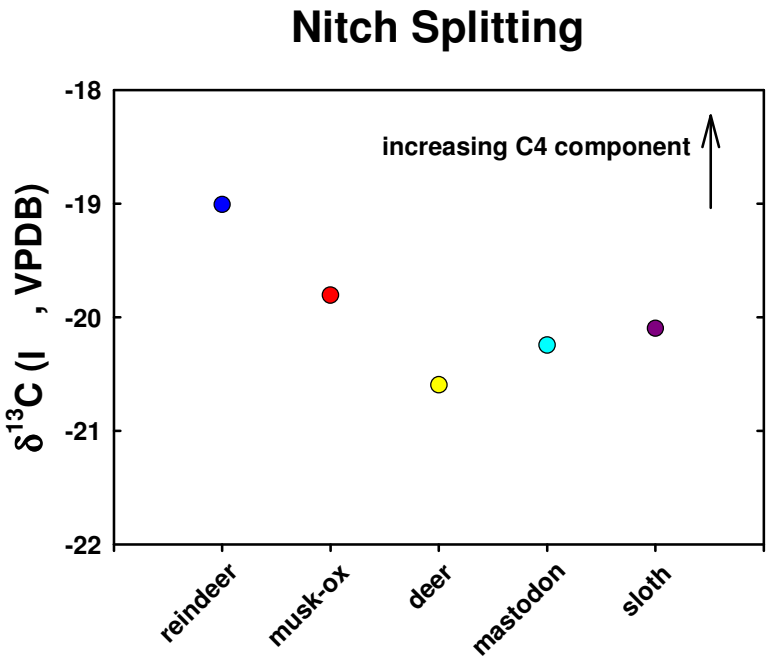


Figure 13: Carbon isotope compositions of bone collagen from animals of the Upland Bog Formation at Saltville Quarry. Size of individual point markers encompasses statistical error which is ± 0.24 2σ.

From my analyses it appears that the sloth and mastodon from the Upland Bog Formation occupy the same browsing niche, but these animals may not directly be competing with each other if the sloth also consumed meat.

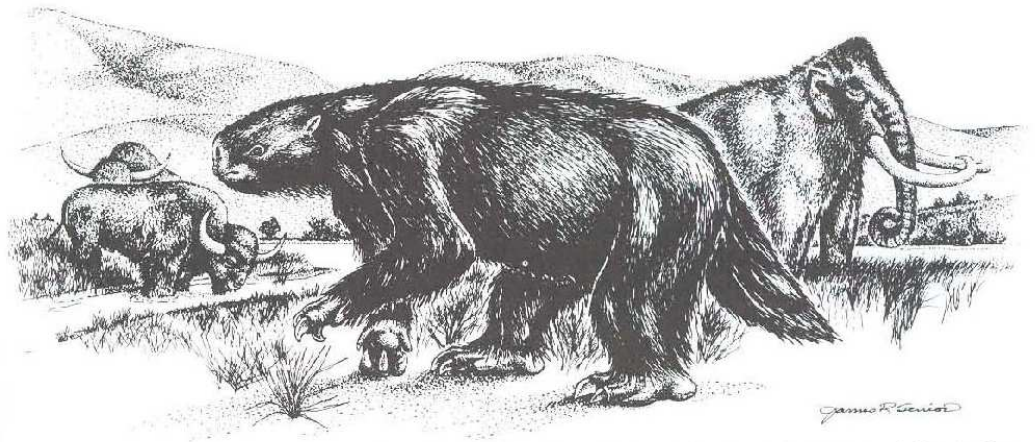
Conclusions

This study was designed to test the trophic structure of the Rancholabrean North American Land Mammal Age ecosystem that contained *Eremotherium laurillardi, Mammut americanum*, and *Mammuthus columbi*. In the initial hypothesis it was proposed that *E. laurillardi* was a herbivore that shared the same megaherbivore niche as *M. americanum*, and *M. columbi.* Diagenetic alteration of bone collagen from animals at the Watkins and Ladds quarries, however, made direct tests of this hypothesis impossible. However, measurements of similar animals from the apparently well-preserved Upland Bog

Formation at the Saltville Quarry were used to test the trophic structure and niche splitting in a similar Rancholabrean ecosystem.

In analyzing the three different quarries we recognized that diagenesis is a major obstacle in the effort to get reliable isotopic data, even with relatively young specimens. The ease with which bone collagen can be altered calls for a more direct way of choosing locations for sampling, and patience. Sedimentalogical and diagenetic investigations of the deposits that contain fossil bone must accompany isotopic studies, which should have a statistical number of analyses of each type of animal. This is often difficult given the reluctance of museum curators to allow destructive techniques on their specimens.

With the limitations of our sample set, however, it appears that bones from the Saltville Quarry did provide interesting clues to the trophic position of a giant ground sloth, which was different than our initial expectations. The sloth seems to be occupying a slightly higher tropic level than the strict herbivores. This would be consistent with the sloth consuming some meat in its' diet. If true, it is most likely that this would be in the form of insects or carrion scavenged from previous kills. Insofar as these giant ground sloths were very large and probably moved slowly, I cannot envision one would be able to actively hunt (Figure 14).



4.5 In the Rancholabrean near American Falls, Idaho, *Bison latifrons*, *Megalonyx jeffersonii*, and *Mammuthus columbi* were members of the large-mammal fauna. Note the plantigrade hind feet of *Megalonyx*.

Figure 14: Reconstruction of a giant sloth sneaking up on its prey. Being too slow, the prey has gotten away.

Appendix 1: Description of specimens (a, b, c) and sample list (d).

a. Watkins Quarry

Species	**Common Name**	**Extant**	**Diet**
Eremotherium laurillardi	Giant ground sloth	no	uncertain

b. Saltville Quarry

Species	**Common Name**	**Extant**	**Diet**
Megalonyx jeffersonii	Jefferson's sloth	no	herbivore
Mammut americanum	American Mastodon	no	herbivore
Equus sp.	Horse	yes	grazing herbivore
Rangifer tarandus	Reindeer	yes	grazing herbivore
Bootherium sp.	Musk ox	no	browsing herbivore
Odocoileus	Deer	yes	browsing herbivore
Mammuthus	Mammoth	no	herbivore

c. Ladds Quarry

Species	**Common Name**	**Extant**	**Diet**
Megalonyx sp.	Giant ground sloth	no	herbivore
Dasypus bellus	Beautiful armadillo	no	omnivore
Urus americanus	American black bear	yes	omnivore
Tremarctos floridanus	American speckled bear	no	omnivore
Panthera onca	Jaguar	yes	carnivore
Tapirus veroensis	Tapir	no	browsing herbivore
Mylohyus nasutus	Peccary	no	omnivore

d. Sample list

Species	Sample number	Piece of bone	Bone type	Smithsonian Identification
Megalonyx jeffersonii	SQ-01	Left femur	Dense	Cabinet 47602 U. S. N. M. # 23737
Mammut americanum	SQ-02	Patella	Dense	Cabinet 47602 U. S. N. M. # 8071
Equus sp.	SQ-03	Left femur	Spongy and dense	Cabinet 47602 U. S. N. M. # 23703
Mammuthus columbi	SQ-04	Thoracic vertebral centrum	Mostly spongy	Cabinet 47602 Accn. # 56915
Equus sp.	SQ-05	Ankle bone	Spongy and dense	Cabinet 47602 Accn. # 66RS145
Rangifer tarandus	SQ-06	Skull fragment	Spongy and dense	Cabinet 47603 U. S. N. M. # 23700
Bootherium sp.	SQ-07	Right metacarpal III	Dense	Cabinet 45881 U. S. N. M. # 364320
Mammut americanum	SQ-08	Jaw bone	Spongy and dense	Cabinet 45879 Handwritten # 40
Odocoileus	SQ-09	Phalanx	Spongy and dense	Cabinet 45879 Handwritten # 51
Megalonyx sp.	LQ-10	Vertebrae	Spongy and dense	Cabinet 45900 U. S. N. M. # 24590
Megalonyx sp.	LQ-11	Left radius	Spongy and dense	Cabinet 45900 U. S. N. M. # 24591
Dasypus bellus	LQ-12	Left radius	Spongy and dense	Cabinet 45900 U. S. N. M. # 23315
Urus americanus	LQ-13	Right mandible	Spongy and dense	Cabinet 45900 U. S. N. M. # 24484
Tremarctos floridanus	LQ-14	Radius	Spongy and dense	Cabinet 45900 U. S. N. M. # 167656
Pantheria onca	LQ-15	Right humerus	Spongy and dense	Cabinet 45900 U. S. N. M. # 23688

Equus sp.	LQ-16	Limb bone	Spongy and dense	Cabinet 45900 8th drawer no number
Tapirus veroensis	LQ-17	Mandible	Spongy and dense	Cabinet 45900 U. S. N. M. # 24588
Mylohyus nasutus	LQ-18	Phalanges	Spongy and dense	Cabinet 45900 U. S. N. M. # 23328
Eremotherium laurillardi	WQ-19	Vertebrae	Spongy and dense	Cabinet 45907 # on bone G277
Eremotherium laurillardi	WQ-20	Vertebrae	Spongy and dense	Cabinet 45907 # on bone 800
Eremotherium laurillardi	WQ-21	Vertebrae	Spongy and dense	Cabinet 45907 # on bone G218A
Eremotherium laurillardi	WQ-22	Neural arch	Spongy and dense	Cabinet 45907 # on bone G234

Appendix 2: Elemental Analyzer settings.

Carrier kPa	65
Purge ml/min	80
O_2 vol	10
O_2 ΔP	30
Time sec.	5
SMP	8
Total run	320
Front oven combustion column	1020°C
Rear oven reduction	650°C
GC oven	115°C

Acknowledgements

I would like to thank…

The Smithsonian Museum of Natural History for donating the specimens for my project, and allowing destructive analysis of their valuable samples.

Kristina Brody for all of her help proof reading and for listening to me practice my presentations. For helping me to be proud of what I have accomplished.

Brendan Williams for helping me in the lab and with preparing my samples. For listening to all of the times I practiced my presentations and for listening to all of my complaining. For all the chauffeuring and every time he turned off the hot bath.

Dr. Thomas Holtz, Jr. for helping to develop the purpose of my thesis and obtaining the samples needed to conduct it. For providing me with an unlimited abundance of papers, books, his own personal knowledge, and a great enthusiasm for the project, helping me to see that sloths are exciting and interesting.

Dr. Alan Jay Kaufman for the use of all of his equipment and his laboratory. For his superb editing skills and the large amount of time he spend revising and proof reading my papers and presentations. For the importance he put into my project, he would drop what ever he was doing to help me. Also, for his patience and tolerance he showed me, even when I was in a panic and overly dramatic.

Christine France for everything. Without Chrissy's help there is no way I could have accomplished what I did. For the great amount of time she has given to me and the project. There is no way I can mention everything she has done, but I can't thank her enough for all of her help.

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