

STABLE ISOTOPE ANALYSIS OF HUMAN REMAINS: A TOOL FOR CAVE ARCHAEOLOGY

CARMEN C. TRIMBLE AND STEPHEN A. MACKO

Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22903 USA

Stable isotope analysis of human remains is a research tool that can provide paleodiet information for archaeological sites, such as caves, where traditional evidence may be missing or out of context. Unlike other lines of evidence, the stable isotopes of carbon and nitrogen in human bone reflect the chemistry of the diet and therefore provide a direct measure of the foods consumed. As an example, the data from isotopic analyses of bone from the Mer site (44LE280), a cave in Lee County, Virginia, are presented. Although this site lacks faunal and floral remains to provide basic information about the availability and potential utilization of food resources, the stable isotope data from other sites throughout Virginia and North Carolina provide a basis for comparison. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the cave burials suggest a diet composed of primarily C_4 plant proteins and some terrestrial animal proteins.

To understand a past civilization, knowledge about its subsistence strategy is necessary. Unlike other paleodietary indicators, the stable isotopes of carbon and nitrogen in human bone reflect the chemistry of the diet and therefore provide a direct measure of the foods consumed. Traditional methods of diet determination focus on an often incomplete and sometimes misleading archaeological record of faunal and floral remains, artifacts, or other cultural evidence associated with a site to provide information on available food resources, procurement strategies, and processing methods. However, population mobility and differential artifact preservation make quantification of the relative inputs of foods difficult. Ethnohistoric accounts generate a general outline of potential food items and their relative importance, but such accounts are usually biased by the observer and present an idealized view of past cultures. Observations of dental attrition, caries, and general health also provide information about what may have been consumed.

Stable isotope analysis of human remains is an especially valuable research tool in archaeological sites, such as caves, where traditional dietary evidence may be missing or out of context. For example, the Mer site, a cave in the extreme southwest corner of Virginia, lacks faunal and floral remains to provide basic information about the availability and potential utilization of food resources. Comparison of the stable isotope data for human bone recovered at the site with isotope data from other sites throughout Virginia and North Carolina provides specific information about the primary dietary components. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the Mer site burials suggest a diet composed of primarily C_4 plant proteins and some terrestrial animal proteins.

In the first half of this paper we present stable isotope theory, interpretation, and methodology. Stable carbon and nitrogen isotope data for burials recovered from the Mer site are

presented and discussed in the second half. Comparison to data from two other archaeological sites, Parker (31DV4) in Davidson County, North Carolina, and Shannon (44MY8) in Montgomery County, Virginia, facilitates interpretation of the Mer site data.

STABLE ISOTOPE THEORY AND INTERPRETATION

Isotopic compositions of carbon and nitrogen are expressed as

(1)

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

(2)

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

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are expressed relative to the international standards Pee Dee Belemnite (PDB) and atmospheric nitrogen (N_2), respectively. A substance with an isotope ratio less than that of the standard will have a negative δ value, and is said to be depleted in the heavy isotope relative to the standard. A substance that is enriched relative to the standard will have a positive δ value.

Isotopic fractionations that occur during the uptake and conversion of CO_2 into plant carbon influence the carbon isotope ratios of terrestrial plants. It is well established that C_4 plants have average $\delta^{13}\text{C}$ values around $-12.5‰$ relative to PDB, whereas C_3 plants have average $\delta^{13}\text{C}$ values around $-26‰$ (Bender, 1968; van der Merwe & Vogel, 1978; Vogel, 1980; van der Merwe, 1982; Matson and Chisholm, 1991). Most terrestrial plants are C_3 plants. Most of the edible C_4

plants in North America are species that have been domesticated, such as maize, sugar cane, sorghum, millet, some grains belonging to *Amaranthus* sp., and some members of the *Chenopodiaceae* family (e.g., *Atriplex* sp. and *Kochia* sp.). The domesticated *Chenopodium berlandieri* found at some archaeological sites is a C₃ plant (Smith, 1985; Spielmann, et al., 1990). Plants, such as succulents, that use the Crassulacean acid metabolism (CAM) photosynthetic pathway can have $\delta^{13}\text{C}$ values similar to either C₃ or C₄ plants, depending on environmental factors (O'Leary, 1981, 1988).

The observed isotopic fractionation that results from the assimilation of dietary carbon and nitrogen varies according to the tissue type sampled and the diet. Based on a study of mice and insects raised on known, monotonous diets, the $\delta^{13}\text{C}$ value for the homogenized whole body of a consumer is enriched by about 1‰ over the diet; bone collagen may be enriched by about 2.0‰ to 3.7‰ over a vegetarian diet (DeNiro & Epstein, 1978; Bender, et al., 1981). A study by Schoeninger and DeNiro (1984) of modern terrestrial and marine food webs showed an enrichment of about 5‰ between plants and the bone collagen of herbivores; the enrichment between the bone collagen of subsequent trophic levels (e.g., herbivore and carnivore) was only 0‰ to 1‰. Using an average collagen enrichment of about 2‰ for a consumer relative to the plant base, the bone protein of a terrestrial herbivore eating only C₃ plants should have $\delta^{13}\text{C}$ of about -26‰ plus 2‰, or about -24‰, whereas a consumer of only C₄ species should have a $\delta^{13}\text{C}$ of -12.5‰ plus 2‰, or -10.5‰. Intermediate $\delta^{13}\text{C}$ values for a vegetarian would indicate a mixed diet of C₃ and C₄ plants. The $\delta^{15}\text{N}$ values for bone protein consistently have been shown to be enriched about 3‰ relative to the food source (DeNiro & Epstein, 1981; Minagawa & Wada, 1984; Schoeninger & DeNiro, 1984). Terrestrial herbivores and carnivores have average $\delta^{15}\text{N}$ values of 5.3‰ and 8.0‰, respectively.

It has been demonstrated that the combination of stable carbon and nitrogen isotopes can be used to distinguish between terrestrial and marine food sources (DeNiro & Epstein 1978, 1981). Marine plants use dissolved bicarbonate, rather than atmospheric CO₂, during photosynthesis. Bicarbonate is about 8.5‰ more enriched in ¹³C than atmospheric CO₂ (Schoeninger & DeNiro, 1984). Marine plants utilize dissolved nitrate and ammonium and are about 7‰ to 10‰ more enriched in ¹⁵N than terrestrial plants. Thus, the $\delta^{15}\text{N}$ value for marine animals feeding on fish is 16.5‰, and the $\delta^{15}\text{N}$ value for marine animals feeding on invertebrates is 13.3‰ (Schoeninger & DeNiro, 1984). It follows that marine animals have higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than terrestrial animals, owing to the more positive $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of marine plants. This difference will be passed on to human consumers of marine foods (Norr, 1981; Tauber, 1981; Chisholm, et al., 1982, 1983; Schoeninger, et al., 1983; Hobson & Collier, 1984).

Isotopic studies of paleodiet are based on the observation that the stable carbon and nitrogen isotopes of an organism

appear to be maintained in its bone following death (DeNiro & Epstein, 1978, 1981). The earliest applications of stable isotope analysis to human dietary research utilized stable carbon isotopes and focused on the timing of the introduction of maize agriculture to various regions throughout North America. For example, isotopic analyses of human bones from archaeological sites in Ohio, Illinois, New York, and West Virginia revealed a gradual shift in diet from the Late Archaic period (around 2000 BC) to the Upper Mississippian period (around AD 1300). The $\delta^{13}\text{C}$ values (-21.9‰ to -21.1‰) for human bone collagen from 37 individuals from seven sites suggested a lack of C₄ plants (presumably maize) in the diet in that area prior to the Late Woodland period (Vogel & Van der Merwe, 1977; van der Merwe & Vogel, 1978; Bender et al., 1981). The $\delta^{13}\text{C}$ values for 72 individuals from 17 sites increase from -20.0‰ for the Late Woodland period to -9.1‰ for the Upper Mississippian period (around AD 1300) (Vogel & Van der Merwe, 1977; van der Merwe & Vogel, 1978; Bender et al., 1981; Farrow, 1986; Buikstra & Milner, 1991). It appears that reliance on C₄ plants increased steadily through this period. A similar shift in $\delta^{13}\text{C}$ values was seen in southeast Missouri and northeast Arkansas around AD 1000 (Lynott, et al., 1986). Late Archaic, Woodland, and early Mississippian bone samples from ten individuals from eight sites had $\delta^{13}\text{C}$ values of -21.7‰ to -19.9‰, whereas ten later Mississippian and Euro-American samples from seven sites had $\delta^{13}\text{C}$ values of -15.8‰ to -10.4‰.

In the American Southwest, faunal and floral samples of potential food items from archaeological sites in Cedar Mesa, Utah, provided baseline $\delta^{13}\text{C}$ values of -9.9‰ for maize, -23.8‰ for pine nuts and rice grass, -17.0‰ for mountain sheep, and -20.6‰ for deer (Matson & Chisholm, 1991). All of the Anasazi Indian bone samples had $\delta^{13}\text{C}$ values ranging between -7.1‰ and -7.9‰, clearly indicating a heavy reliance on a C₄ plant such as maize. There was no evidence of other C₄ plants or CAM plants in the coprolite (paleofecal) and floral samples from these sites that could account for the high $\delta^{13}\text{C}$ values.

Other studies have focused on differentiating between the resources exploited by coastal and interior populations. For example, populations subsisting on a marine economy (Alaskan Eskimos and Northwest Coast Indians) had $\delta^{15}\text{N}$ values ranging from 17‰ to 20‰, whereas populations with an agricultural economy (manioc farmers from Columbia, South America; Mesoamerican maize agriculturalists; and grain growers from the Neolithic period in Europe) had $\delta^{15}\text{N}$ values ranging from 6‰ to 12‰. Groups utilizing a mixture of marine and terrestrial foods had intermediate $\delta^{15}\text{N}$ values (Schoeninger, et al., 1983).

Although most stable isotope studies isolate bone collagen for analysis, it is not clear that true bone collagen survives post mortem deposition (DeNiro, 1985; Masters, 1987; Tuross, et al., 1988). The organic fraction of fresh bone consists of 90% collagen, 5% noncollagenous proteins, and 5% lipids and carbohydrates. Lipids and carbohydrates leach rapidly from bone

METHODS

after burial. Collagen is relatively insoluble, owing to linkages between its triple helix polypeptide chains, and is not strongly bound to the inorganic matrix of the bone. Noncollagenous proteins are acidic polypeptides that adsorb strongly to the bone mineral matrix of hydroxyapatite. As bone degrades, the adsorbed acidic proteins and peptide fragments are preferentially retained, whereas the collagen is lost (Masters, 1987). Therefore, fossil bone most likely contains noncollagenous proteins and collagen in proportions different from fresh bone; highly degraded bone may contain only traces of collagen. As suggested by Masters (1987), the composition of these two proteins in the organic fraction of bone or dentin should not affect the isotopic ratios of carbon and nitrogen.

The most common method of sample preparation to isolate organic matter from bones is the method of DeNiro and Epstein (1981) as modified by Schoeninger and DeNiro (1984). Briefly, powdered bone is demineralized by soaking in 1 M HCl for 20 minutes, washed with distilled water, soaked in 0.125 M NaOH for 20 hours, washed, hydrolyzed by placing it in 0.001 M HCl at 90°C for 10 hours, filtered, and freeze dried. This process results in a gelatinous material that is equated with collagen. However, the amino acid profile of this material differs from true collagen standards and more closely resembles that of noncollagenous proteins (Tuross, et al., 1988; Schoeninger, et al., 1989; Weiner & Bar-Yosef, 1990). Interpretations of paleodiet based on samples prepared by this method may therefore be based on noncollagenous proteins, not collagen. Collagen from modern bone is characterized by high concentrations of the amino acids glycine and proline and the presence of hydroxyproline and hydroxylysine; noncollagenous proteins are characterized by high concentrations of glutamic acid and aspartic acid and little hydroxylysine (Wycoff, 1972; Hare, 1980).

The process used in this research isolates the high molecular weight (HMW), organic fraction from the bone or tooth, without discriminating against any specific protein (Ostrom, et al., 1990). This method requires less sample material and results in a greater yield of organic matter than the hydrolysis procedure (Schoeninger, et al., 1989). The amino acid profiles of modern HMW extracts are similar to collagen (Hare, 1980); the profiles for highly degraded bone, although consistent with a collagenous origin, are most likely derived from both collagenous and noncollagenous proteins (Schoeninger, et al., 1989; Ostrom, et al., 1990). As suggested by Masters (1987), the composition of these proteins in the organic fraction of bone or dentin should not affect the isotopic ratios of carbon and nitrogen. Although the amino acid compositions for collagen and noncollagenous proteins differ, the averaged isotopic contributions of the major amino acids in each protein to the total isotopic composition of the protein are the same. Therefore, an enrichment of 3‰ for nitrogen is expected per trophic level for the HMW extracts from bones and teeth; for carbon, an enrichment of about 2‰ between plants and herbivores and 1‰ between subsequent trophic levels is expected for the HMW fractions.

Dialysis was used to collect the HMW organic fraction of each bone sample. First, each bone sample was washed in distilled water, scrubbed with a soft brush, if necessary, and scoured to remove surficial contaminants. The sample was then etched in 30% cold HCl, rinsed thoroughly in distilled water, dried, and powdered. A portion of the cleaned, powdered bone was dissolved in cold 6 N HCl, placed in dialysis tubing (molecular weight cut off of 8000), and dialyzed at low temperature (2° to 5° C) against distilled water to separate the HMW fraction from the low molecular weight fraction. The HMW material was lyophilized prior to analysis. Floral samples were cleaned surficially, acid etched in 10% cold HCl, rinsed thoroughly in distilled water, dried, and powdered.

Each sample was combusted in the presence of copper and copper oxide while under vacuum. The resulting gases were cryogenically separated; carbon dioxide and nitrogen were analyzed on a V.G. PRISM isotope ratio mass spectrometer for the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. Approximately every fourth sample was replicated to verify the reproducibility of the measurements.

SITES

The Mer site (44LE280) is a cave located in a limestone hillside near a tributary of Indian Creek in Lee County, Virginia. A looted site reconnaissance survey in May 1993 by Dave Hubbard for the Marginella Burial Cave Project revealed disturbed human skeletal material under a rock ledge. The burials were approximately 15-18 m from the cave entrance. The location of this material in the dark zone and under a ledge suggests that the burials were intentionally placed and not the result of an accident or carnivore activity. Osteological analysis by Donna and Cliff Boyd, Radford University, identified a minimum of eight individuals: three male adults, three female adults, one subadult of undetermined sex, and one child aged 2½ to 4 years (Boyd & Boyd, 1997, Table 2).

The Shannon site (44MY8) is located on a ridge overlooking the floodplain of the North Fork of the Roanoke River approximately five miles east of Blacksburg in Montgomery County, Virginia. This site was excavated by Joseph Benthall in 1966 and 1967 (Benthall, 1969). The postmold pattern showed a 98 m (322 ft) by 64 m (210 ft) palisaded village comprised of a central plaza and 11 circular houses 2.5-7 m (8-23 ft) in diameter. Fire and refuse pits were associated with each house. Artifacts associated with these pits included ceramics, projectile points, refuse animal bone, bone tools (awls, fish hook, and chisel), turtle shells, mussel shells, shell and bone beads, and charred corn. Nearly all of the 100 human burials were individual burials in the flexed position. Grave goods included shell and turkey bone beads, mussel shells, turtle shell containers, bone tools (awls, chisels, fish hooks, flakers, hairpin, projectile points), stone tools (celts, drills, hammerstone, knife, projectile points), animal teeth (bear, eagle, elk, wolf),

animal claws (mountain lion), ceramics (sherds, pipes), copper fragments (of native copper), and burned corn and hickory nuts.

Based on the ceramics (New River, Clarksville, and Radford) and projectile points (Caraway, Peedee, and Randolph), the Shannon site was occupied most intensively during the Late Prehistoric around A.D. 1400 to A.D. 1600. An absence of European artifacts suggests that occupation probably did not continue into the Historical Period. The presence of Palmer, Big Sandy, and Savannah River projectile points indicate that the site may have been occupied periodically during the Archaic Period.

The Parker site (31DV4) is located on Horseshoe Bend of the Yadkin River in Davidson County, North Carolina. The site was excavated in 1971 and 1972 by J. Ned Woodall of Wake Forest University (Newkirk, 1978). Numerous trash deposits, artifacts, human burials, and evidence of one substantial structure were observed in this small village. Lithics include triangular projectile points, drills, scrapers, blades, and cores. Other artifacts recovered were bone fish hooks, shell disks, worked antler, and worked turtle shell. The 25 burials were all primary burials in the flexed or semi-flexed position. Only one burial pit contained more than one individual. There were no definite grave goods associated with the burials.

The primary occupation of Parker was earlier than that at Shannon. Radiocarbon dates of A.D. 960 \pm 86 and A.D. 634 \pm 64 were obtained from two charcoal samples. Dan River ceramics recovered from the site suggest that it may have been reoccupied as late as A.D. 1600 (Egloff, 1992).

RESULTS AND DISCUSSION

The Mer site has not been excavated. All that is available to investigate the activities at this site and the people who participated in them are disturbed human remains recovered from the surface of the cave floor and information about the site's location. With the exception of a 6 inch "spear point" reportedly removed from the cave by a looter, there are no artifacts from which to infer any cultural activities. There are no floral or faunal remains to aid in diet determination. Osteological analysis of the bone material provides information about the age, sex, number of individuals, physical trauma, disease, and health of the population. Stable isotope analysis provides information about their diet.

Bone samples from the femur shafts of four individuals from the Mer site were analyzed for their stable carbon and nitrogen isotope composition. The HMW organic fraction was isolated from each bone. The $\delta^{13}\text{C}$ values range from -12.7‰ to -9.2‰ (mean -11.0‰) and the $\delta^{15}\text{N}$ values range from 6.9‰ to 8.2‰ (mean 7.5‰) (Table 1).

To assess the diet, the isotopic compositions of several potential dietary sources must be determined. In the absence of faunal and floral remains from this site, a food web was constructed using data from other sites in Virginia and North Carolina. Ecological resources that may have been exploited

Table 1. Summary of Stable Isotope Data for Each Site.

Site	No. individuals sampled	mean $\delta^{13}\text{C}$	mean $\delta^{15}\text{N}$
Mer	4	-11.0‰	7.5‰
Shannon	16	-11.0‰	9.3‰
Parker	10	-18.8‰	9.3‰

by the individuals interred at the Mer site can be divided into three isotopically distinct categories: C_3 plant based, C_4 plant based, and aquatic. The isotopic values for carbon and nitrogen for food resources from these three categories plus an appropriate trophic level shift related to the isotopic fractionation for metabolism can be compared to the isotopic compositions of the bones recovered to assess the probable composition of the diet. No CAM plants were available in the study area in sufficient quantity to be considered a significant dietary component.

A sample of maize (*Zea mays*) from Governor's Land (44JC308) was representative of C_4 cultigens, and has a $\delta^{13}\text{C}$ value of -9.8‰ and a $\delta^{15}\text{N}$ value of 4.8‰ (Trimble & Macko, 1994). A diet consisting solely of this C_4 plant would result in $\delta^{13}\text{C}$ value of -7.8‰ and a $\delta^{15}\text{N}$ value of 7.8‰ for the HMW extract of human bone. Hickory and walnut, C_3 species recovered from several sites in the Virginia Piedmont, had an average $\delta^{13}\text{C}$ value of -25.7‰ and $\delta^{15}\text{N}$ value of 3.0‰. The bone protein of herbivores such as deer, beaver, squirrel, and rabbit subsisting on C_3 plants would have a $\delta^{13}\text{C}$ of -23.7‰ and a $\delta^{15}\text{N}$ of 6.0‰; the HMW fraction of bones from humans eating these herbivores exclusively should have a $\delta^{13}\text{C}$ of -22.7‰ and a $\delta^{15}\text{N}$ of 9.0‰. The aquatic component might contain fish such as sunfish, bass, and sturgeon. Although the aquatic component could have $\delta^{13}\text{C}$ values similar to the C_3 plant based component, aquatic protein is more enriched in ^{15}N . Largemouth bass from the James River have an average $\delta^{13}\text{C}$ of -23.5‰ and $\delta^{15}\text{N}$ of 15.0‰ (Garman & Macko, in press). Consumers of aquatic protein exclusively might have a $\delta^{13}\text{C}$ of -22.5‰ and a $\delta^{15}\text{N}$ of 18.0‰. A mixed diet would result in intermediate values.

When compared to this compiled food web, the $\delta^{13}\text{C}$ values for the individuals buried at the Mer site appear similar to the range expected for consumers of C_4 plants. The $\delta^{15}\text{N}$ values are between those expected for terrestrial herbivores and carnivores. These values strongly suggest that C_4 plants were a key component of the subsistence economy. Marine foods could have contributed to the $\delta^{13}\text{C}$ values observed, but the associated $\delta^{15}\text{N}$ values expected for marine resources are lacking in the Mer site samples. In fact, the $\delta^{15}\text{N}$ values are quite low, suggesting that the people themselves were primarily plant eaters and consumers of other grazers such as rabbit and deer. Although freshwater fish and mussels may have had a role in their diet, the isotopic evidence for this being a major component is also lacking.

The Mer site is an anomaly with respect to other sites with-

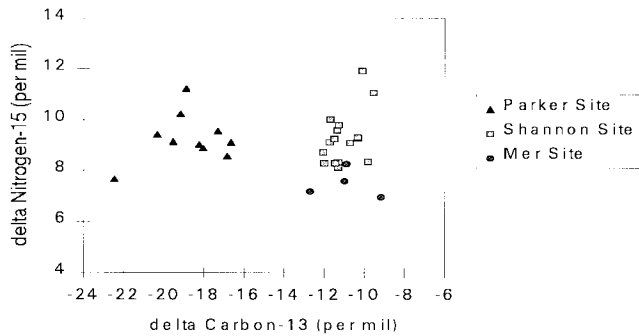


Figure 1. Stable carbon and nitrogen isotope values for the HMW fraction of human bone from the Mer, Shannon, and Parker sites.

in the Valley and Ridge physiographic province in terms of the stable isotope compositions. Out of over 20 sites in Virginia and North Carolina (Trimble, 1996), this site has the strongest evidence for C_4 plant consumption. Of those 20 sites, the Shannon site is the most similar.

Like the Mer site, bone protein from 16 individuals at Shannon has a mean $\delta^{13}C$ value of -11.0‰ (Figure 1). The mean $\delta^{15}N$ value, 9.3‰ , is higher than at the Mer site. This suggests that terrestrial animals may have been consumed in greater quantities at Shannon, or that more aquatic resources may have been utilized. The faunal and floral assemblages from Shannon provide evidence for a varied diet (Table 2). Plant remains included maize, beans, and nuts. Animal remains included mammals, birds, reptiles, mussels, snails, and fish.

In contrast, isotopic analysis of 10 individuals from the Parker site yielded mean $\delta^{13}C$ and $\delta^{15}N$ values of -18.8‰ and 9.3‰ , respectively (Figure 2). Fauna identified at Parker include deer, raccoon, fox, beaver, groundhog, squirrel, rabbit, wild turkey, gar, catfish, box turtle, and mussel. Flora identified include maize (*Zea mays*), hazel (*Corylus sp.*), hickory (*Carya sp.*), and a few other single seeds. The stable isotope compositions for the individuals at Parker are clearly within the range expected for consumers of C_3 plants. However, the presence of maize in the floral assemblage suggests that either maize agriculture was practiced at some time during the occupation of the site or contemporaneous trade networks existed to provide this resource. Alternatively, the maize may reflect a later occupation of Parker that is not associated with the burials examined.

Although it is unknown whether Shannon and Mer were contemporaneous, it seems that they shared a similar subsistence base. This information comes directly from the bones themselves and is independent of cultural or chronological affiliation. Given the small sample size for Mer, it is unclear whether the individuals recovered are representative of a specific social subset of a larger community or of the community at large.

Table 2. Faunal and floral remains recovered from the Shannon site.

Common name	Scientific name
Maize	<i>Zea mays</i>
Beans	<i>Prosopis sp</i>
Black walnut	<i>Juglans nigra</i>
Hickory nuts	<i>Carya glabra</i>
Virginia white tailed deer	<i>Odocoileus virginiana</i>
Elk	<i>Cervus canadensis</i>
Beaver	<i>Castor canadensis</i>
Groundhog	<i>Marmota monax</i>
Gray squirrel	<i>Sciurus carolina</i>
Black bear	<i>Ursus americanus</i>
Rabbit	<i>Sylvilagus floridana</i>
Muskrat	<i>Ondatra zibethicus</i>
Dog	<i>Canis familiaris</i>
Raccoon	<i>Procyon lotor</i>
Gray fox	<i>Urocyon cinereoargenteus</i>
Skunk	<i>Mephitis mephitis</i>
Bobcat	<i>Lynx rufus</i>
Mountain lion	<i>Felis concolor</i>
Turkey	<i>Meleagris gallopavo</i>
Bald eagle	<i>Haliaeetus leucocephalus</i>
Canada goose	<i>Branta canadensis</i>
Ruffed grouse	<i>Bonasa umbellus</i>
Bobwhite	<i>Colinus virginianus</i>
Passenger pigeon	<i>Ectopistes migratorius</i>
Terrapin	<i>Terrapene carolina</i>
Mussels	<i>Elliptio complanatus</i> (Roanoke River) <i>Villosa constricta</i> conrad (Roanoke River) <i>Cyclonaias turbiculata</i> Rafinesque (New River)
Snails	<i>Mudalia carinata variabilis</i> lea <i>Oxytrema symmetrica</i> Haldeman

Further research at the Mer site is necessary. It is imperative that the chronological affiliation of the site be determined. This will enable comparison with other sites of the same period. The date will also aid in tracing the rise and spread of maize agriculture. Archaeological excavation within the cave could reveal the extent of the burial location, the types of burials, associated grave goods, or other artifacts related to cultural activity. Additionally, the remains of contemporaneous plants and animals may be recovered. Isotopic analysis of such ecofacts would enable definition of a localized food web to use as the basis for diet determination at the Mer site and other nearby cave sites, such as Indian Burial Cave (44LE11) and Bone Cave (44LE169).

Stable isotope analysis is particularly useful for cave sites because, as demonstrated for the Mer site, it provides information about the subsistence economy of the occupants, even in the absence of all other traditional archaeological data. Furthermore, the information generated by isotope analysis may elucidate culturally or archaeologically significant facets

of a given site and may provide the impetus for further research.

REFERENCES

- Bender, M.M. (1968). Mass spectrometric studies of carbon-13 variations in corn and other grasses. *Radiocarbon* 10:468-472.
- Bender, M.M., Baerreis, D.A. & Steventon, R.L. (1981). Further light on carbon isotopes and Hopewell culture. *American Antiquity* 46:346-353.
- Benthall, J.L. (1969). Archaeological investigation of the Shannon Site. Virginia State Library Publications, No. 32, Richmond, VA.
- Boyd, C.C. & Boyd, D.C. (1997). Osteological comparison of prehistoric Native Americans from southwest Virginia and east Tennessee mortuary caves. *Journal of Cave and Karst Studies* 60(1): 160-165.
- Buikstra, J.E. & Milner, G.R. (1991). Isotopic and archaeological interpretations of diet in the Central Mississippi Valley. *Journal of Archaeological Science* 18:319-329.
- Chisholm, B.S., Nelson, D.E. & Schwarcz, H.P. (1982). Stable carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. *Science* 216:1131-1132.
- Chisholm, B.S., Nelson, D.E. & Schwarcz, H.P. (1983). Marine and terrestrial protein in prehistoric diets on the British Columbia Coast. *Current Anthropology* 24:396-398.
- DeNiro, M.J. (1985). Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* 317:806-809.
- DeNiro, M.J. & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495-506.
- DeNiro, M.J. & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341-351.
- Egloff, K.T. (1992). The Late Woodland period in Southwestern Virginia. In Middle and Late Woodland Research in Virginia: A Synthesis, T.R. Reinhart & M.E.N. Hodges (eds.) *Special Publication No. 29 of the Archaeological Society of Virginia*: 187-224.
- Farrow, D.C. (1986). A study of Monongahela subsistence patterns based on mass spectrometric analysis. *Midcontinental Journal of Archaeology* 11:153-179.
- Garman, G.C. & Macko, S.A. (in press). Anadromous clupeid fishes as vectors of marine-derived organic matter within Atlantic coastal landscapes. *Journal of North American Benthological Society*.
- Hare, P.E. (1980). Organic geochemistry of bone and its relation to the survival of bone in the natural environment. In *Fossils in the Making*. A.K. Behrensmeier & A.P. Hill (eds.), University of Chicago Press, pp. 208-222.
- Hobson, K.A. & Collier, S. (1984). Marine and terrestrial protein in Australian aboriginal diets. *Current Anthropology* 25:238-240.
- Lynott, M.J., Boutton, T.W., Price, J.E., & Nelson, D.E. (1986). Stable carbon isotopic evidence for maize agriculture in southeast Missouri and northeast Arkansas. *American Antiquity* 51:51-65.
- Masters, P.M. (1987). Preferential preservation of noncollagenous protein during bone diagenesis: Implications for chronometric and stable isotopic measurements. *Geochimica et Cosmochimica Acta* 51:3209-3214.
- Matson, R.G. & Chisholm, B. (1991). Basketmaker II subsistence: carbon isotopes and other dietary indicators from Cedar Mesa, Utah. *American Antiquity* 56:444-459.
- Minagawa, M. & Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* 48:1135-1140.
- Newkirk, J.A. (1978). *The Parker Site: A Woodland site in Davidson County, North Carolina*. Unpublished Master's Thesis, Dept. of Anthropology, Wake Forest University.
- Norr, L. (1981). Prehistoric Costa Rican diet as determined from stable carbon isotope ratios in bone collagen. *American Journal of Physical Anthropology* 54:258-259.
- O'Leary, M.H. (1981). Carbon isotope fractionation in plants. *Phytochem* 20:553-567.
- O'Leary, M.H. (1988). Carbon isotopes in photosynthesis. *BioScience* 38:328-336.
- Ostrom, M.H., Macko, S.A., Engel, M.H., Silfer, J.A., Russell, D. (1990). Geochemical characterization of high molecular weight organic material isolated from Late Cretaceous fossils. *Organic Geochemistry* 16:1139-1144.
- Schoeninger, M.J. & DeNiro, M.J. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta* 48:625-639.
- Schoeninger, M.J., DeNiro, M.J. & Tauber, H. (1983). Stable isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric diet. *Science* 220:1381-1383.
- Schoeninger, M.J., Moore, K.M., Murray, M.L., & Kingston, J.D. (1989). Detection of bone preservation in archaeological and fossil samples. *Applied Geochemistry* 4:281-292.
- Smith, B.D. (1985). The role of *Chenopodium* as a domesticate in pre-maize garden systems of the eastern United States. *Southeastern Archaeology* 4:51-72.
- Spielmann, K.A., Schoeninger, M.J., & Moore, K. (1990). Plains-Pueblo interdependence and human diet at Pecos Pueblo, New Mexico. *American Antiquity* 55:745-765.
- Tauber, H. (1981). ^{13}C evidence for dietary habits of prehistoric man in Denmark. *Nature* 292:332-334.
- Trimble, C.C. (1996). *Palaeodiet in Virginia and North Carolina as determined by stable isotope analysis of skeletal remains*. Unpublished Master's Thesis, Dept. of Environmental Science, University of Virginia.
- Trimble, C.C. & Macko, S.A. (1994). Isotopic analysis of human teeth and bones from Virginia archaeological site 44JC308, Governor's Land at Two Rivers. Report submitted to the James River Institute for Archaeology, Inc., Williamsburg, VA.
- Tuross, N., Fogel, M.L., & Hare, P.E. (1988). Variability in the preservation of the isotopic composition of collagen from fossil bone. *Geochimica et Cosmochimica Acta* 52:929-935.
- van der Merwe, N.J. (1982). Carbon isotopes, photosynthesis, and archaeology. *American Scientist* 17:596-606.
- van der Merwe, N.J. & Vogel, J.C. (1978). ^{13}C content of human collagen as a measure of prehistoric diet in Woodland North America. *Nature* 276:815-816.
- Vogel, J.C. (1980). *Fractionation of the carbon isotopes during photosynthesis*. Springer-Verlag, Berlin, Heidelberg, New York.
- Vogel, J.C. & van der Merwe, N.J. (1977). Isotopic evidence for early maize cultivation in New York State. *American Antiquity* 42:238-242.
- Weiner, S. & Bar-Yosef, O. (1990). States of preservation of bones from prehistoric sites in the Near East: A survey. *Journal of Archaeological Science* 17:187-196.
- Wycoff, R.W.G. (1972). *The Biochemistry of Animal Fossils*. Williams and Wilkins, Baltimore, Maryland.