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STABLE ISOTOPE INTERPRETATIONS OF BONE ORGANIC MATTER: AN ARTIFICIAL DIAGENESIS EXPERIMENT AND PALEOECOLOGY OF THE PLEISTOCENE AND HOLOCENE OF NATURAL TRAP CAVE, WYOMING

By

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ABSTRACT

STABLE ISOTOPE INTERPRETATIONS OF BONE ORGANIC MATTER: AN ARTIFICIAL DIAGENESIS EXPERIMENT AND PALEOECOLOGY OF THE PLEISTOCENE AND HOLOCENE OF NATURAL TRAP CAVE, WYOMING

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The presence of original organic matter and the retention of an indigenous isotopic signal in fossils have been under dispute for many years. To address this issue, an experiment was conducted to evaluate the influence of diagenesis on bone protein isotope values. Collagen and non-collagenous proteins (NCP) were extracted and their isotopic and elemental composition were characterized.

An analysis of Holocene and Pleistocene fossils from Natural Trap Cave, Wyoming (NTC) suggested good preservation due to their high protein yields, C:N, and realistic trophic structure based on isotope values. Isotopic data showed that carnivores, such as the North American cheetah, *Miracinonyx trumani*, have high nitrogen and carbon isotope values, while herbivores, such as the pronghorn, *Antilocapra americana,* have lower nitrogen and carbon isotope values. Herbivore nitrogen isotope values appear to reflect digestive physiology, distinguishing ruminants from non-ruminants. This difference may be related to the more efficient absorption of the ^{15}N enriched microbial protein by ruminants relative to monogastric herbivores. These data emphasize that isotopes have the ability to provide information on trophic relationships, digestive tract physiology and other ecological attributes of ancient assemblages.

This thesis is dedicated to my grandfathers, William J. McNulty Sr. and Col. George T. Larkin, who both passed away during the completion of this work. I can not find words to express the love I feel for them. Both of these men achieved significant accomplishments during their time on earth. I hope to be able to carry on the honor and courage that both these men symbolized. I was fortunate to spend the first twenty-three years of my life learning from them. I will always remember and miss you both.

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KEY TO ABBREVIATIONS AND SYMBOLS

KUVP= University of Kansas: Museum of Natural History and Vertebrate Paleontology Collections, Lawrence, Kansas

MSU= Michigan State University; East Lansing, Michigan

NTC= Natural Trap Cave

NCP= Non collagenous proteins

AN= *Antilocapra americana* (pronghorn antelope)

BI= *Bison bison* (bison)

BR= *Ursus* sp (bear)

BV= *Bos taurus* (modern cow)

CA= *Camelops* sp (camel)

CH= *Miracinonyx trumani* (American cheetah)

FX= Unspecified genus and species (fox)

HS= *Equus* sp (horse)

LI= *Panthera atrox* (lion)

MO= *Bootherium bombifrons* (musk-ox)

RB= *Lepus* sp (rabbit)

SH= *Ovis catclawensis* (bighorn sheep)

WL= *Canis* sp (wolf)

WV= *Gulo gulo* (wolverine)

Introduction

Stable isotopes are a long-established tool for interpreting the diet and ecological relationships of modern organisms (Schoeninger & DeNiro 1984; Schoeller *et al.* 1985; Ostrom & Fry 1993; Cormie & Schwarcz 1994; Gould *et al.* 1997; & Schoeninger *et al.* 1999). This approach is based on the observation that an organism's isotopic composition is directly related to its diet. The isotope values of consumers differ from those of their diet by ~1‰ in $\delta^{13}C$ (DeNiro & Epstein 1978) and ~3-4‰ in $\delta^{15}N$ (Schoeninger & DeNiro 1984, Minagawa & Wada 1984). By measuring naturally occurring isotopic ratios, information can be obtained concerning the organism's diet, trophic level, climate and habitat (Peterson & Fry 1987, Ambrose 1991, Jacoby *et al.* 1999).

Carbon and nitrogen isotopes have been used to determine the type of primary producer at the base of the food web and establish trophic level within many different environments (Peterson & Fry 1987; Mizutani & Wada 1988; Hobson & Clark 1992). For example, carbon isotopes values can distinguish between the consumption of C_3 and C_4 plants, marine versus terrestrial dietary sources, browsing versus grazing herbivores, and forest versus prairie conditions (DeNiro & Epstein 1978; Krueger & Sullivan 1984; Schoeninger & DeNiro 1984; Ambrose & DeNiro 1986; Ambrose, 1991; Szepanski *et al.* 1999). The 3-4‰ increase in $\delta^{15}N$ values between trophic

levels is of particular interest because it is a robust indicator of food web structure (DeNiro & Epstein 1981; Minagawa & Wada 1984; Schoeninger & DeNiro 1984; Peterson & Fry 1987). Successful use of $\delta^{13}C$ and $\delta^{15}N$ values to establish food web relationships requires that the potential dietary sources are isotopically distinct, and that variables which can influence isotope data are taken into account along with diet and trophic level such as climate, starvation, and digestive strategy (Ambrose & DeNiro 1986; Ambrose 1991; Hobson 1993). In such cases, carbon and nitrogen isotope values can be sensitive indicators of material flow.

A good example is the use of carbon and nitrogen isotope ratios of bone collagen to determine diet and habitat selection of the larger mammals of East Africa (Ambrose & DeNiro 1986). The $\delta^{13}C$ data showed differences between grazers and browsers in savanna grasslands, forest floor and savanna grassland herbivores, and forest floor and forest canopy species. In addition, herbivores and carnivores, forest and savanna grassland herbivores, and water-dependent and drought tolerant herbivores could be distinguished on the basis of nitrogen isotope values. It has also been observed that organisms from hot and arid environments have a tendency to yield higher nitrogen isotope ratios when compared to cool and wet climates (Heaton *et al.* 1986; Sealy *et al.* 1987; Ambrose 1991).

If organic matter within fossils remains isotopically constant through geologic time, $\delta^{13}C$ and $\delta^{15}N$ values should provide information on paleoecological relationships, such as trophic structure and the primary producers at the base of the food web. In cases where diagenetic alteration of bone collagen has been discounted

(e.g. using data on C:N, collagen concentrations, and carbon and nitrogen concentrations in collagen), isotopic analysis provided insight into paleoenvironments and climates (Heaton *et al.* 1986; Katzenberg 1992; Koch *et al.* 1994, Bocherens *et al.* 1996, 1997; Hilderbrand *et al.* 1996; Johnson *et al.* 1997; Iacumin *et al.* 1997). A good example is the use of carbon and nitrogen isotope ratios of ancient bone collagen to determine prehistoric climate and habitat conditions of herbivores from Kenya (Ambrose & DeNiro 1989). In this study, a comparison between modern and prehistoric organisms suggested that modern climate in Kenya was very similar to that of 5365 years B.P. (Ambrose & DeNiro 1989). The later Holocene dry phase produced herbivores with higher nitrogen isotope values associated with water stress and the earlier Holocene wet phase produced lower nitrogen isotope values which suggests a greater availability of water. Carbon isotopes show a change associated with altitude shifts of relative abundances of C_3 and C_4 plants . C_3 plants grow in cool, moist and shaded environments typical of high altitudes, where C_4 plants grow in hot and dry environments typical of low altitude open savannas in Kenya. Therefore, carbon isotopes indicated that prehistoric hunter-gathers preferred herbivores associated with open habitats who had a large portion of their diet associated with C_4 plants (Ambrose & DeNiro 1989).

The application of stable isotopes to paleodietary studies is dependent on the ability to isolate a portion of the original organic material from fossils and to demonstrate its isotopic integrity. Loss of organic matter and introduction of contaminants may obscure the original isotope value of collagen (Ostrom *et al.* 1993).

One method for evaluating the stability of geochemical characteristics of organic matter associated with skeletal material is through an artificial diagenesis experiment (Hare 1980; Qian *et al.* 1995; Andrews 1998).

The primary objectives of this research was to first evaluate the geochemical characteristics of bone proteins during artificial diagenesis, and to understand how the results might relate to fossils. The aim of the artificial diagenesis experiment was to mimic the process in which proteins are degraded through time by heating modern bone at 100°C in an inert atmosphere. Thermal alteration of bone collagenous and non-collagenous proteins (NCP) influenced protein yield, carbon and nitrogen isotope values, and the C:N ratio for collagen. A second objective was to apply information from the artificial diagenesis experiment, to assess the preservational state of collagen within fossils. Specifically, the geochemical characteristics of fossils from Natural Trap Cave (NTC), a late Pleistocene to Holocene vertebrate fauna of north central Wyoming, were analyzed. These fossils exhibit excellent preservation, which suggests that NTC is a suitable choice for an isotopic study (Martin & Gilbert 1978a). Consequently, the isotopic composition of NTC fossils should provide information on trophic relationships of the late Pleistocene and Holocene of Wyoming.

Methods

In the artificial diagenesis experiment samples were heated with excess water at 100°C under an inert atmosphere. Prior to the experiment, modern cow (*Bos taurus)* bone was first mechanically cleaned to remove any external tissue and broken into small pieces. To remain consistent with methods used for fossils, the bone shards were then acid etched with 4°C 1N HCl. Approximately 3.0 g of bone shards were placed into a quartz tube purged with helium (99.999% purity) and sealed. Samples were heated with enough ultra pure de-ionized water (Barnstead E-Pure) to completely cover the bone (~3.0mL). The tubes were heated for various lengths of time for up to ~240 hours at 100°C. After heating, the aqueous portion of the sample was separated from the bone shards and frozen. The bone was dried in an evaporatory oven at room temperature (25°C) for approximately 24 hours. The dried bone shards were powderized using a SPEX CertiPrep 6750 Freezer/Mill. To mimic the procedure used to remove the humic substances from fossils, the bone powder was stirred for one hour in excess sodium buffer solution (1N NaH_2PO_4, pH = 6-7) at 4°C (Gundberg *et al.* 1984). The pellet was centrifuged (4000 rpm) and rinsed with e-pure water. This rinsing procedure was performed three times in order to remove any remaining salts. The bone powder was then demineralized with 1N HCl for 18 hours at 4°C. In order to separate the collagen from the NCP fraction, the demineralized bone was centrifuged at 12000-14000 rpm. The collagen pellet and NCP supernatant were pipeted into 3500 molecular weight cut-off dialysis membrane.

The samples were dialyzed for five days against 4.0 L of distilled water and then lyophilized. During dialysis, the water was changed approximately two times daily.

Samples of collagen and NCP (3.0 - 6.0 mg) were prepared for isotopic analysis by modified Dumas combustion (Macko 1981). In this method, purified gases were obtained by cryogenic gas separation and subsequent isotopic measurements were performed on a PRISM stable isotope ratio mass spectrometer (MicroMass). A Carlo-Erba elemental analyzer interfaced to the Prism mass spectrometer was also used to determine the isotopic and elemental ratios (Wong *et al.* 1992). The carbon and nitrogen isotopic values are expressed as:

$$\delta X = \{(R_{sample} / R_{standard}) - 1\} \times 1{,}000$$

where X represents ^{13}C, ^{15}N, and R represents $^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively. The isotopic values determined by the two methods were identical, and the associated precision was 0.2‰ or better.

Fossils from NTC were obtained from Dr. Larry D. Martin of the University of Kansas. NTC has tight stratigraphic layers with three lenses of volcanic ash. Ages of some fossils were determined by radiocarbon dating of collagen and other dates represent biostratigraphic assignments. The stratigraphy of NTC is well constrained with ages determined from fission-track dating of the volcanic ash or associated faunal elements (Martin & Gilbert 1978a).

Prior to isotopic analysis, fossils were mechanically cleaned and acid etched in order to remove any visible contaminants. Microscopic analysis was employed to assist in the removal of contaminants. Subsequent extraction and isolation of

collagen and NCP from fossils was identical to that described for the artificial diagenesis experiment.

Results & Discussion

Artificial Diagenesis Experiment

The percent yields of the collagen and NCP fractions, expressed as weight of protein per weight of bone, are shown in figures 1 and 2. The collagen yield for unheated bone was 18 %, which is similar to previous estimates for modern collagen (DeNiro & Weiner 1988). The collagen yields decreased 9% after heating for 190 hours. At ~240 hours of heating nearly all of the collagen was lost from the bone. The yields of NCP did not change appreciably up to 190 hours. Like the collagen, heating for ~240 hours resulted in almost total loss of the NCP fraction. In that large losses of collagen were not balanced by substantial increases in NCP yield, collagen degradation products were likely incorporated into the aqueous phase.

A question to address was whether or not changes in protein yield could influence the C:N data and isotopic values of heated bone. An emphasis was placed on collagen rather than NCP C:N because these values are used as an indicator of collagen preservation. Several studies suggest that C:N values of well-preserved collagen should fall within a range of 2.9-3.6 (DeNiro 1985). Recently, van Klinken (1999) established a confined range for well-preserved collagen of 3.1-3.5 for archaeological samples. The C:N value of modern, unheated bone from the current

work (2.9) is consistent with the lower range reported in earlier studies. The majority of C:N values for heated samples were within a confined range of 2.9-3.2 (Figure 3). This is the case despite a loss of approximately half of the collagen over 190 hours of heating.

Despite large decreases in collagen yield (~18% to ~9%), nitrogen isotope values deviated by less than 0.5‰ over 190 hours at 100°C (Figure 4). In this and subsequent figures, a dashed line represents a 0.5‰ difference from unheated bone (solid line). The large isotopic shift, 1.7‰, observed for samples heated beyond 200 hours was likely due to isotopic fractionation during degradation reactions such as ammonification or deamination (Sutoh *et al.* 1987). These data emphasize that $\delta^{15}N$ values of collagen are quite resilient even up to 200 hours of heating and losses of approximately half of the bone's collagen. The large increases in $\delta^{15}N$ that were observed with extensive heating provide a perspective for what may occur in diagenetically altered fossils.

By comparison to the reference lines, the $\delta^{13}C$ variation relative to unheated bone (-12.7‰) was approximately 0.6‰ or less with up to 190 hours of heating (Figure 5). Most variation in isotope values occurred between 90 and 240 hours of heating, when collagen yields were less than 14%. It is unlikely that this variation resulted from collagen inhomogeneity because the $\delta^{15}N$ values did not change over the same heating interval. The depletion in $\delta^{13}C$ after 90 hours of heating was likely related to hydrolysis and associated reactions such as de-carboxlyation (Keeling *et al.* 1999).

Although NCP are not currently used for paleodietary reconstruction, the artificial diagenesis data suggest that this protein fraction may also retain important geochemical information. The NCP nitrogen isotope values deviate by less than 0.4‰ from unheated bone for up to 190 hours at 100°C (Figure 6). At extended heating times, nitrogen isotopes become enriched by 0.8‰ from that of the unheated bone value (6.3‰). The NCP $\delta^{13}C$ values demonstrated more variability than nitrogen isotope data (Figure 7). The $\delta^{13}C$ values vary by approximately 0.5‰ over ~120 hours of heating when compared to that of unheated bone.

Paleoecology and Site Description of Natural Trap Cave

Natural Trap Cave (NTC) is located in northwestern Wyoming and contains a late Pleistocene (Sangamonian-Wisconsinan) to Holocene vertebrate fauna (Martin & Gilbert 1978a). The cave is a 26-meter deep karst sinkhole on the west side of the Big Horn Mountains. NTC contains a detailed faunal record that begins before 111,000 years BP, as determined by fission track dating of volcanic ash contained within the stratigraphic sequence (Martin & Gilbert 1978a). NTC comprises a serially deposited record of the fauna in the area since the Sangamon interglacial period, 75,000-125,000 years BP (Chomko & Gilbert 1987). In 1978, Martin and Gilbert first described the excavation of NTC, and how information from the site could be used to examine Late Pleistocene climatic change and its possible

relationship to large mammalian extinction (Martin & Neuner 1978). These geologic age stages are well differentiated owing to tight biostratigraphic control at NTC.

The fossil assemblage at NTC is an excellent candidate for an isotopic study. The well-constrained biostratigraphy provides a reliable framework to evaluate the ages of the samples. In addition to established ages, the fossils from NTC exhibit superb preservation, owing to minimal weathering and seasonal snow cover. NTC also offers a taxonomically and ecologically diverse fauna (Table 2). Faunal diversity was enhanced by the cave's location along a natural game trail leading to the Big Horn Basin and was used during annual migrations by large grazers and their predators (Wang & Martin 1993). Since there were large numbers of individuals that fell to their death in the cave, the assemblage includes a diverse group of organisms that filled a variety of trophic levels. For example, the American Lion, *Panthera atrox*, is the dominant carnivore of NTC and is the largest known felid in North America (except for the cave lion, *Panthera spelaea*) (Martin & Gilbert 1978b). The most likely prey of the lion and other top carnivores was the giant Pleistocene bighorn sheep, *Ovis catclawensis*, which was the most common herbivore at the site (Wang 1988). Prior knowledge of predator/prey relationships helps to validate trophic structure based on $\delta^{15}N$ values. The geochemical characteristics of collagen (e.g. C:N) isolated from the fossils also allows us to evaluate organic matter integrity and validate the use of stable isotope data to derive paleoecological relationships.

Geochemical Characteristics of Natural Trap Cave Fossils

The C:N values of the majority of NTC samples fell between 2.9-3.3, which was similar to that observed in the artificial diagenesis experiment and in the range for well preserved collagen (Figure 8). Similar to the trend observed in the artificial diagenesis experiment, C:N was independent of collagen yield. The large range (2-14%) of collagen yields suggested that factors beyond organic matter yields must be evaluated to determine the integrity of collagen. Furthermore, if C:N is an acceptable indicator of protein integrity, these data suggest that low yields of collagen do not necessary suggest poor preservation potential. For example, AN-1 had a collagen yield of 2.1% but a C:N value of 3.1, which suggested well preserved collagen (Table 2; Figure 8). The possibility also exists that a sample with high collagen yield might be poorly preserved or contaminated and exhibit deviant C:N values; e.g., SH-4 had high collagen yield (7.9%) and a high C:N value (5.0).

Ultimately an important goal was to use C:N data as an indicator of samples that could be poorly preserved and, therefore, suspect with regard to their isotope values. A comparison of C:N and isotope values showed no correlation and indicated that the majority of the variation observed in $\delta^{15}N$ and $\delta^{13}C$ values appeared to be a function of trophic level (Figures 9 & 10). For example, top carnivores of NTC including the lion, *Panthera atrox,* and the cheetah, *Miracinonyx trumani,* had high $\delta^{15}N$ and $\delta^{13}C$ values relative to herbivores such as rabbits, *Lepus* sp, and horses, *Equus* sp. As will be discussed below, several other variables may influence the

dispersion in the isotope values. Among these, diagenesis was only apparent in two samples. In comparison to other sheep of the assemblage, the collagen of sheep SH-4 had a high $\delta^{15}N$ value and low $\delta^{13}C$ value ($\delta^{15}N$ ~12‰, $\delta^{13}C$ ~ -21‰). This sheep also fell outside the expected range of C:N (C:N ~ 4.3). The second sample, fox FX-1, also with a high C:N (C:N ~ 4), had a high $\delta^{15}N$ ($\delta^{15}N$ ~ 7‰) relative to all other samples except SH-4, and its $\delta^{13}C$ ($\delta^{13}C$ ~ -22.3‰) value was lower than any other sample in the data set. Although the nitrogen isotope value might be appropriate for an omnivore with a highly carnivorous diet, the sample was suspect owing to its anomalous C:N. Furthermore, high carbon isotope values would be expected for carnivores and this is not observed. Owing to their irregular C:N and isotope data, $\delta^{13}C$ and/or $\delta^{15}N$ values of the sample sheep SH-4 and fox FX-1 were not used in subsequent interpretations of the paleoecology of NTC.

Isotopic Paleoecology of Natural Trap Cave

To evaluate paleoecological relationships among NTC consumers based on isotope values, it is necessary to understand the factors, which influence these data. The isotopic composition of primary producers at the base of the food chain and trophic level are important factors that affect the $\delta^{13}C$ and $\delta^{15}N$ values of consumers (DeNiro & Schoeninger 1983; Peterson & Fry 1987; Ostrom & Fry 1993; Cormie & Schwartz 1994). For example, the $\delta^{13}C$ values of herbivores that consume C_3 plants can be distinguished from those that consume C_4 grasses (Ambrose & DeNiro 1989)

while $\delta^{15}N$ has shown small differences between browsers and grazers (Gröcke & Bocherens 1996). Increases in $\delta^{13}C$ and $\delta^{15}N$ values with trophic level result from respiration of ^{12}C enriched carbon dioxide and excretion of ^{14}N enriched urine, respectively (Abelson & Hoering 1961; DeNiro & Epstein 1978; Steele & Daniel 1978; Peterson & Fry 1987). As a consequence, a consumer's isotope value is higher than its diet and herbivores have lower $\delta^{13}C$ and $\delta^{15}N$ values when compared to carnivores (Minagawa & Wada 1984; Schoeninger & DeNiro 1984).

In addition to diet and trophic level, an organism's nitrogen isotope value can be influenced by changes in urea excretion associated with heat stress or water deprivation (Heaton *et al.* 1986; Ambrose 1991). As the organism experiences an increase in water stress, the concentration of urea in the urine increases (Livingston *et al.* 1962). Heat stress and water deprivation also cause an increase in daily urine excretion rates and a reduction of total food intake (Maloiy 1973). The increase in the excretion of highly concentrated urea in urine is believed to produce the pronounced increase in $\delta^{15}N$ values of organisms that inhabit arid environments relative to those of animals from moist environments (Heaton *et al.* 1986; Ambrose 1991; Cormie & Schwarcz 1994). Alternatively, the influence of aridity on consumer $\delta^{15}N$ may be associated with protein deprivation (Sealy *et al.* 1987). Starving or protein-limited organisms may metabolize amino acids to obtain energy (Waterlow 1978; Hobson *et al.* 1993). Associated deamination removes amine groups that are enriched in ^{14}N (Gaebler *et al.* 1966; Steele & Daniel 1978). Because this pool of ^{14}N

enriched nitrogen is excreted and not replaced by dietary protein, an increase in $\delta^{15}N$ values occurs during the course of starvation.

Isotopic data sets, based on the analysis of collagen, can also be influenced by dietary routing (Ambrose & Norr 1993; Tieszen & Fagre 1993; Gannes *et al.* 1997, 1998). This is because the isotopic composition of collagen frequently reflects that of dietary protein rather than that of bulk diet. This phenomenon is most problematic in the case where the relative abundance of protein within the diet varies within individuals of the same feeding strategy (Gannes *et al.* 1997, 1998). For example, this would be the case for omnivores but less for carnivores.

It is important to recognize that each of the factors described above can vary with the geologic age of the fossil. Isotope values of individuals within the same taxa from NTC show similar values despite large differences in the age of the sample (Table 2). For example, sheep SH-1 is 14000 years BP and has $\delta^{15}N$ and $\delta^{13}C$ values of 6.9‰ and –18.6‰ respectively, while sheep SH-3 is 18000-21000 years BP and has $\delta^{15}N$ and $\delta^{13}C$ values of 6.2‰ and –19.1‰ respectively. Similarly, the rabbit RB-1 is 20000 years BP and has $\delta^{15}N$ and $\delta^{13}C$ values of 2.3‰ and –20.6‰ respectively, while rabbit RB-2 is 110000 years BP and has $\delta^{15}N$ and $\delta^{13}C$ values of 2.3‰ and –20.0‰ respectively. This suggests that the isotope data are not confounded by factors that change with geologic age.

As indicated earlier, isotopic variation for collagen isolated from NTC fossils appears to primarily reflect trophic structure (Figure 11). Based on their isotope values, carnivores are at the top of the trophic structure, while the herbivores with

lower isotope values are separated into two groups. The difference in the $\delta^{15}N$ values between the cheetah (CH-1) and pronghorn (AN-1) are close to the expected range for a carnivore and its prey. This interpretation is consistent with earlier ideas on the paleoecology of the site (Chorn *et al.* 1988). The primary food sources of the lion (LI-1) were sheep (SH) and camel (CA) (Martin & Gilbert 1978b). The difference in isotope values between the lion and the sheep or camel (~ 2.5 for $\delta^{15}N$, <1‰ for $\delta^{13}C$) was less than expected between an organism and its diet. This suggests that the diet of this particular lion also included organisms with lower values, such as horses. This dietary preference is consistent with the feeding behavior of modern savanna lions, which also eat perissodactyls such as zebras. The wolverine (WV-1), *Gulo gulo,* has lower $\delta^{15}N$ and $\delta^{13}C$ values when compared to the cheetah and lion. This difference may be a function of diet. Whereas the prey of lions and cheetahs consists of larger herbivores, the diet of the wolverine was thought to include various portions of smaller mammals (e.g. marmots and rabbits), birds, fruit, and carrion (Kurten 1980). Unlike lions and cheetahs, wolverines store large quantities of food (Kurten 1980). In order to detract predators, the wolverine urinates on the carrion. This contribution of ^{14}N enriched urea could decrease the $\delta^{15}N$ values of wolverines.

There are two isotopically distinct herbivore groupings in the NTC fauna: (1) rabbits and horses and (2) sheep, pronghorn, and bison. The observation that two selective grazers, horse and sheep differ by ~3.0‰ and ~1.0‰ in $\delta^{15}N$ and $\delta^{13}C$, respectively, suggest that diet alone does not completely explain the isotopic separation between these groups. If the isotopic difference was a result of aridity, this

would imply that horses and rabbits experience a moister habitat in comparison to other herbivores.

As an alternative to diet and water stress, nitrogen isotope value of herbivores may be influenced by digestive strategy (Sealy *et al.* 1987; Bocherens *et al.* 1996). Most herbivores cannot produce the enzymes necessary to digest cellulose (Janis 1976). Instead they rely on microorganisms to digest cellulose and other fermentable substrates. An important difference between the two isotopically distinct herbivore groups at NTC is the position of their fermentation vat relative to the stomach and small intestine (Stevens and Hume 1998). In the bison, sheep, and pronghorn, fermentation is conducted in portions of the forestomach (a multicompartmentalized organ between the esophagus and true stomach) called the rumen and reticulum. In contrast, among non-ruminants such as the rabbit and horse, fermentation predominates in a branch off the large intestine called the cecum. Because nitrogen from dietary protein is absorbed as amino acids in the small intestine, the large quantity of microbial protein generated in the hindgut of non-ruminants is not assimilated (Janus 1976; Van Soest 1982).

The amount of microbial protein assimilated may be a critical factor in determining the isotopic values of an herbivore. Ruminants derive a large portion of their nitrogen from microbes, which have higher $\delta^{15}N$ values than their substrate (Steinhour *et al.* 1982; Sutoh *et al.* 1987). These simple observations suggest that nitrogen isotope values of ruminants will be higher than non-ruminants feeding on isotopically similar vegetation.

Ruminal microbial flora must be assimilating a pool of ^{15}N enriched nitrogen and/or eliminating nitrogen that is depleted in ^{14}N. Ammonia is the primary form of nitrogen assimilated by ruminal bacteria (Dziuk 1984; Stevens & Hume 1998). It can be produced in the rumen through bacterially mediated protein degradation and it can be formed from urea by bacterial urease (Beitz & Allen 1984; Wallace 1996). The latter case is an important mechanism occurring in ruminants where a portion of the urea formed in the liver is retained by the organism instead of being excreted by the kidneys (Stevens & Hume 1998). In this case, the urea would represent a residual pool of nitrogen that would likely be enriched in ^{15}N (Marriotti 1981). Similarly, the ammonia assimilated by bacteria may represent a residual nitrogen pool that has not been converted to urea and subsequently excreted. Thus, fundamental differences in nitrogen metabolic pathways and digestive physiology between ruminants and monogastric organisms likely play important roles in the isotopic separations observed between these two groups at NTC.

A pattern of elevated $\delta^{15}N$ values for ruminants relative to non-ruminant herbivores is not a conspicuous feature in previous studies. It was not observed in studies of modern South African consumers (Sealy *et al.* 1987; Ambrose 1991) or in a study of Upper Pleistocene herbivores from Siberia (Bocherens *et al.* 1996). However in both these cases, data were obtained from several locations where a number of variables in addition to digestive physiology (e.g. variation in the degree of aridity and vegetation type and protein content) likely influence the nitrogen isotope data.

Among the factors that could influence herbivore isotopic data sets, isotopic routing deserves particular attention (Gannes *et al.* 1997, 1998). Variation in dietary protein content and associated isotopic routing can occur among herbivores due to the fact that C_3 plants have a higher protein content that C_4 vegetation (Caswell *et al.* 1973). Thus low carbon isotope values among herbivores may reflect dietary routing rather than dominant consumption of C_3 plants.

Relative to non-ruminants, foregut fermentors are believed to mix the nitrogen of all-dietary components and even body protein through nitrogen recycling (Gannes *et al.* 1997). Even though the diet of ruminants is generally thought to be higher in protein than that of non-ruminant organisms (Janis 1976; Gannes *et al.* 1998; Stevens & Hume 1998), nitrogen mixing by ruminants might ameliorate isotopic differences between the two groups. In hindgut fermenting herbivores the potential for dietary routing is high. In these organisms, the degree of isotopic routing of dietary constituents is dependent on many factors including degree of fermentation, urea recycling, and nitrogen balance (Gannes *et al.* 1997). Clearly, digestive physiology and dietary nitrogen balance work in concert to influence collagen isotope values.

The paucity of data on modern herbivore isotope values limit our ability to separate the relative importance of digestive physiology, nitrogen balance and other variables on herbivore isotope values. Among NTC herbivores, the clear isotopic separation between ruminants and non-ruminants exists even between selective feeders with different digestive physiology's (e.g. horses and sheep). In addition, previous studies have indicated the majority of plants associated with NTC were C_3

plants prior to glaciation (Martin & Gilbert 1978a). This appears to limit the possibilities that dietary routing is the sole variable that influences the carbon isotope values of herbivores at NTC and supports the suggestion that digestive physiology is an important determinant of NTC herbivore $\delta^{13}C$ and $\delta^{15}N$ values.

Conclusions

This thesis has demonstrated that stable isotopes are resistant to thermal alteration even after a large percentage of protein loss. The artificial diagenesis data suggest that the isotopic and elemental ratios of proteins can withstand change despite long exposure to extreme heating (nearly 100 hours at 100°C) and losses of more than 50% of the original bone protein. This provides an important perspective for our understanding of the isotopic integrity of fossils. The geochemical characteristics of Pleistocene fossils from Natural Trap Cave, Wyoming suggest that most of the samples from this assemblage are well preserved. Although a variety of factors can influence isotopic paleoecological reconstructions, data from NTC appear to reflect trophic structure and digestive physiology. In this study, the origin of the samples from a single collection locality is an important factor that may have assisted with interpretation.

TABLE 1 - Artificial Diagenesis: mean and standard deviation (SD) of carbon and nitrogen isotope values. All values without SD had n = 1 or 2.

Specimen #	Hours	Collagen wt (%)	δ^{15}N Collagen (Mean ± SD)	δ^{13}C Collagen (Mean ± SD)	C:N Collagen	NCP wt (%)	δ^{15}N NCP (Mean)	δ^{13}C NCP (Mean)
BV.99.BH.500	0	17.8	6.3 ± 0.2	-12.7 ± 0.1	2.9	1.2	6.3	-13.7
BV.99.BH.510	11	16.4	6.5	-12.6 ± 0.1	3.2	0.9	6.3	-13.8
BV.99.BH.515	14.5	14.7	6.5	-13.1 ± 0.2	3.1	0.5	6.4	-14.4
BV.99.BH.524	23.5	14.1	6.3	-13.1 ± 0.3	3.2	1.2	6.6	-13.4
BV.99.BH.550	45.5	14.2	6.4	-13.0 ± 0.2	3.1	1.1	6.4	-13.5
BV.99.BH.560	68.25	13.0	6.6± 0.1	-13.0	2.9	1.5	6.3	-13.5
BV.99.BH.580	89	13.2	6.8	-12.2 ± 0.1	2.9	1.3	6.4	-13.3
BV.99.BH.596	95.5	12.3	6.7± 0.1	-12.3	2.9	1.7	6.5	-13.2
BV.99.BH.619	119	13.5	6.2	-13.4	3.2	2.0	6.4	-14.0
BV.99.BH.700	191.5	9.8	6.1	-13.3	3.2	1.0	6.4	-14.5
BV.99.BH.740	237.5	0.1	7.9	-16.8	n/a	0.2	7.1	-14.6

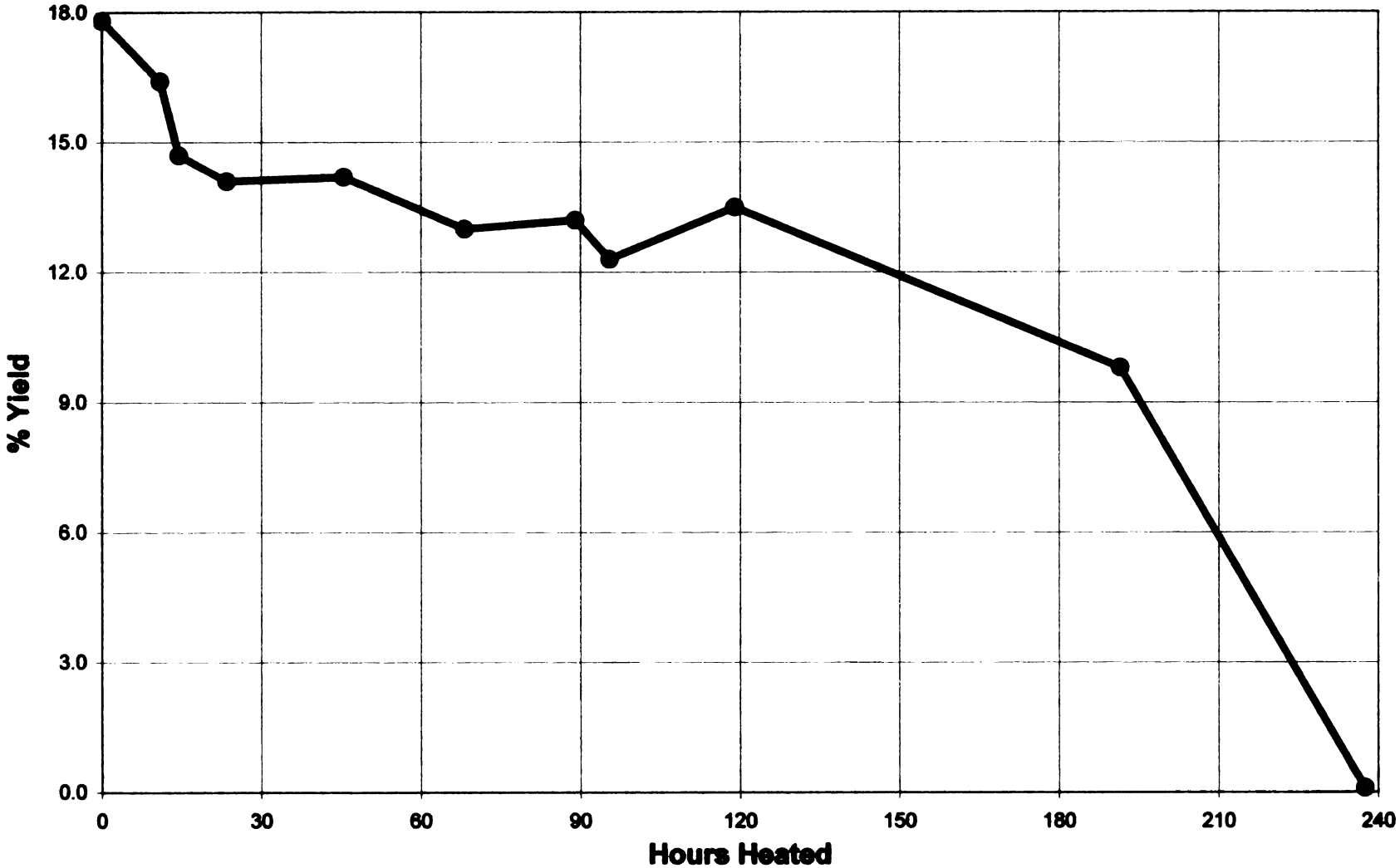

Figure 1 - Artificial Diagenesis Collagen Yields

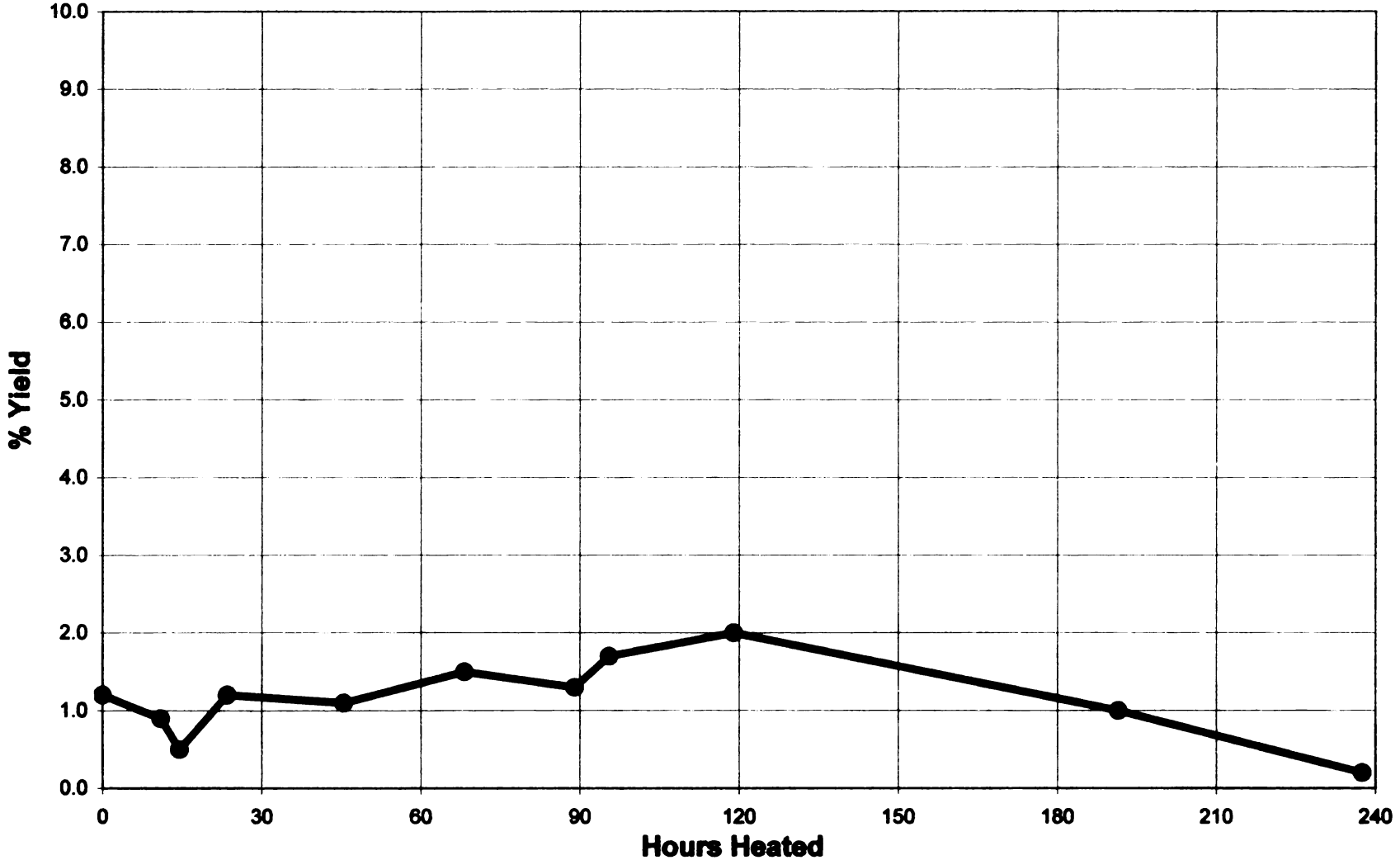

Figure 2 - Artificial Diagenesis NCP Yields

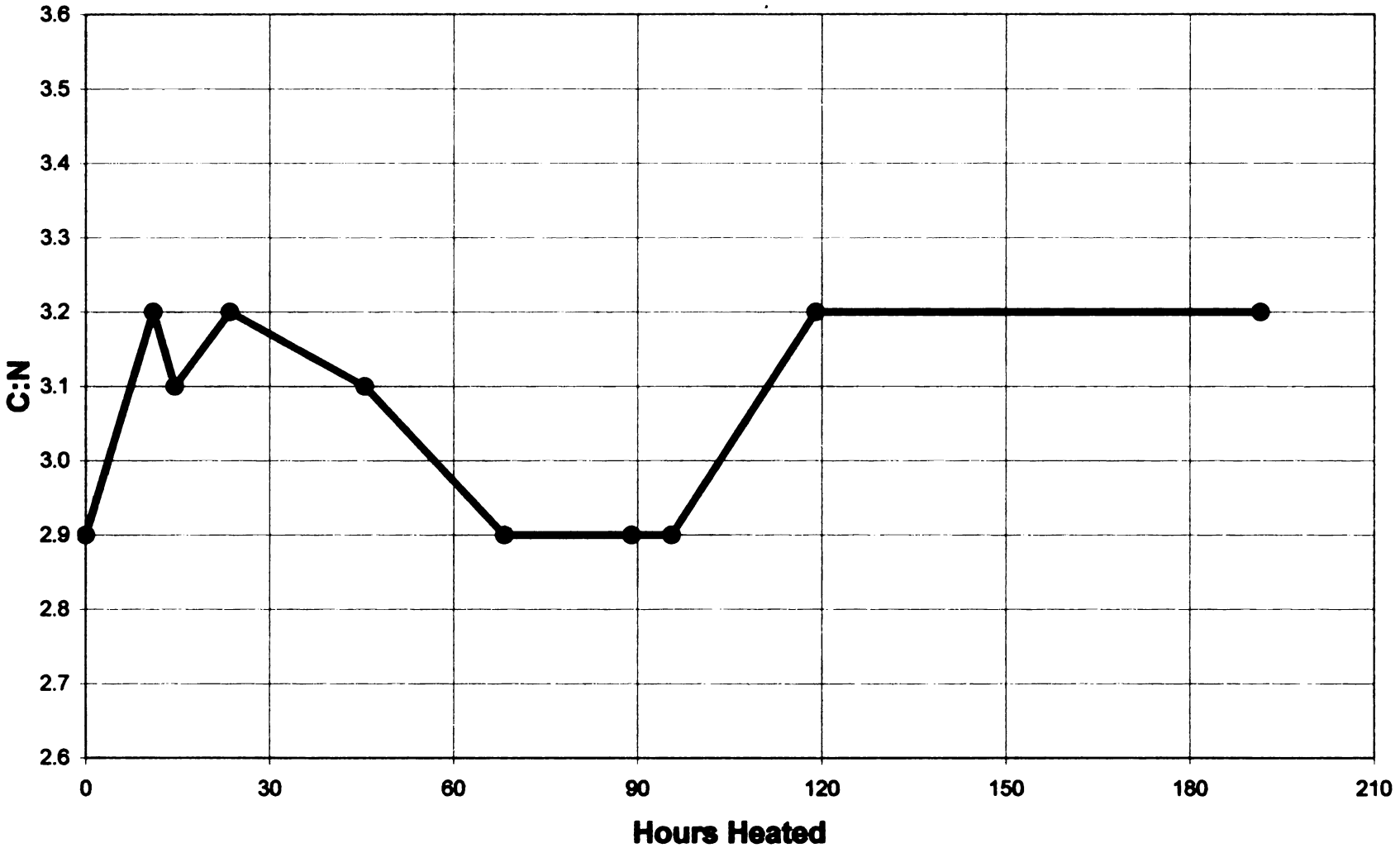

Figure 3 - Artificial Diagenesis Collagen C:N Values

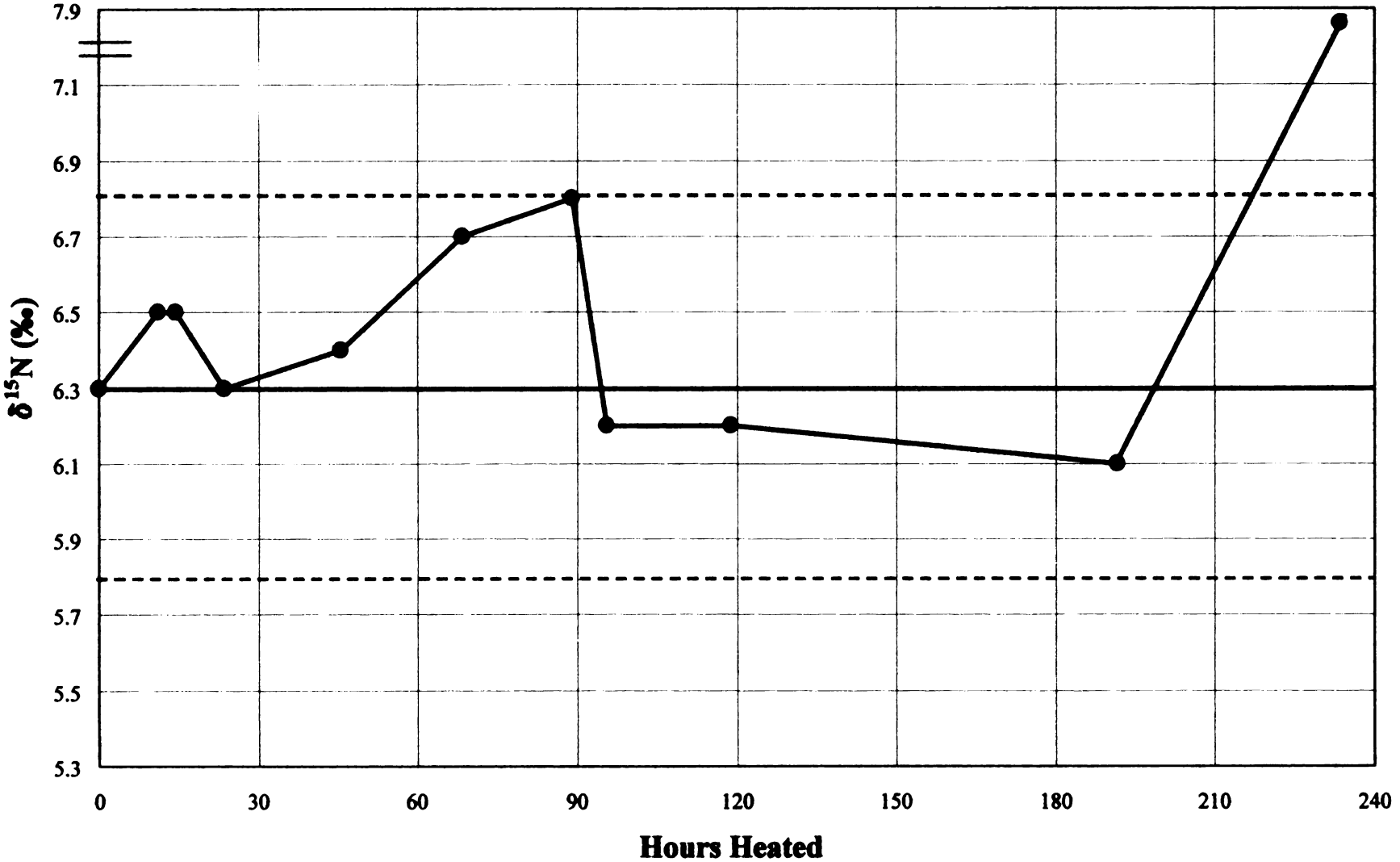

Figure 4 Artificial Diagenesis Collagen $\delta^{15}N$ Values

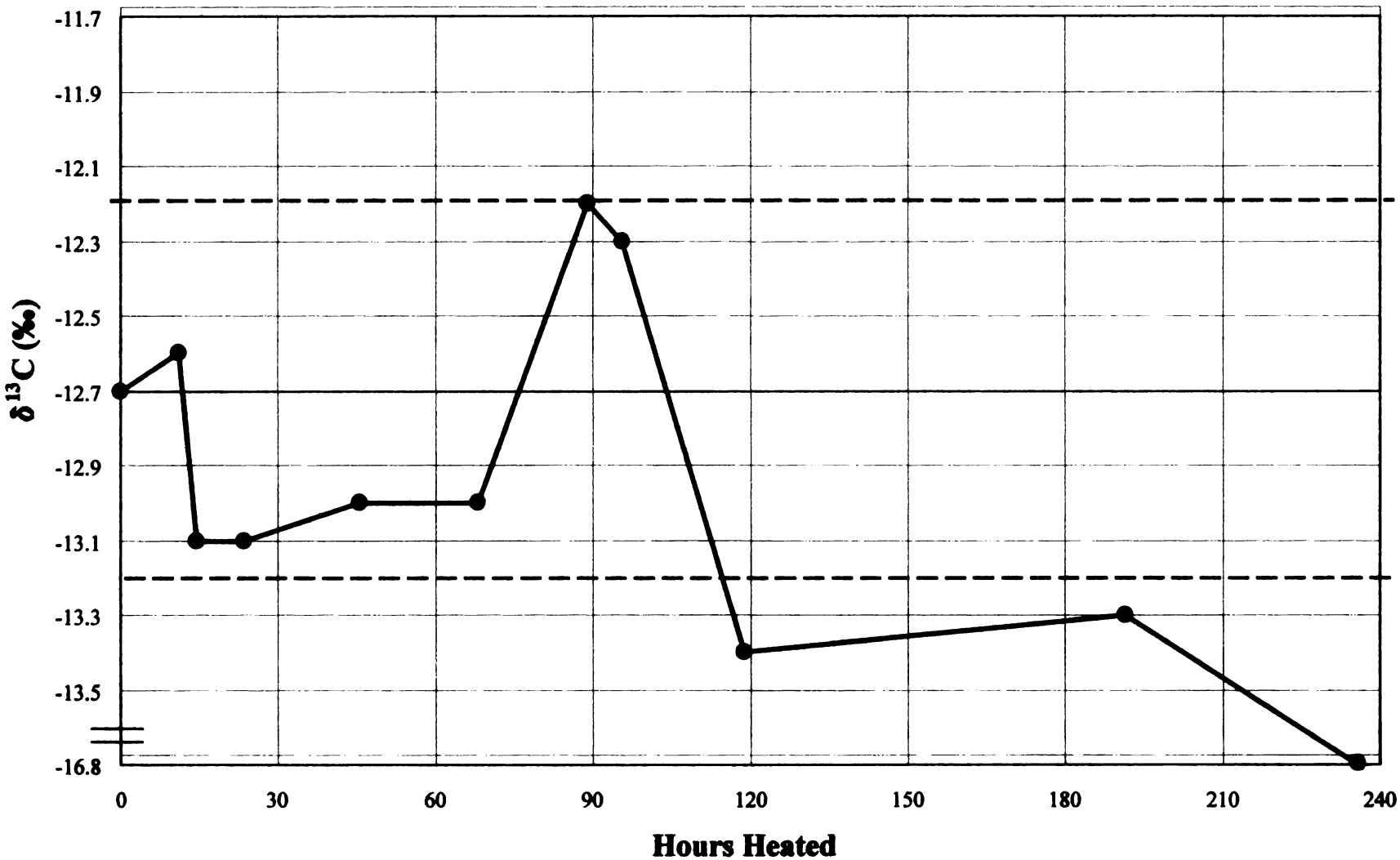

Figure 5 Artificial Diagenesis Collagen $\delta^{13}C$ Values

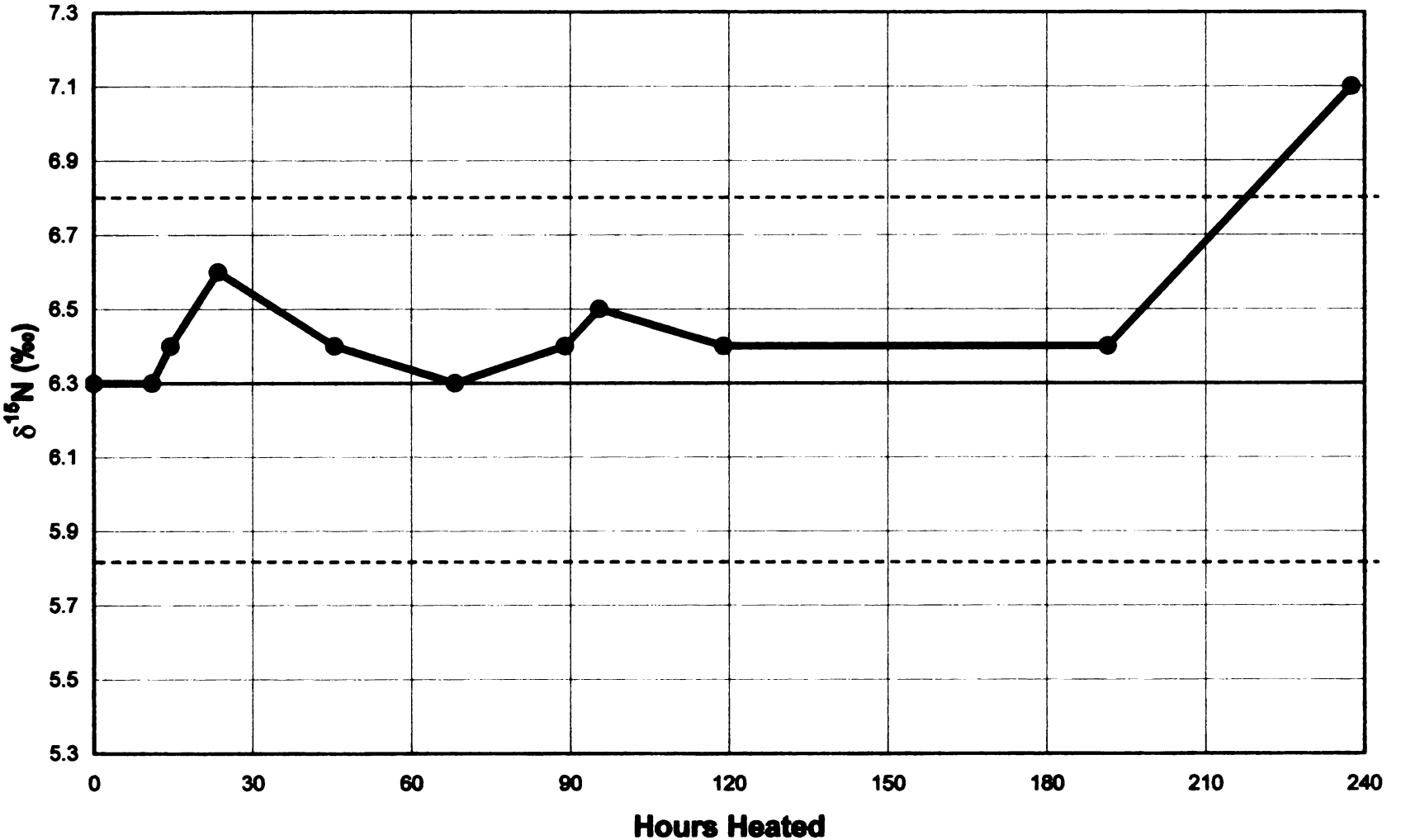

Figure 6 - Artificial Diagenesis NCP Nitrogen Isotope Values

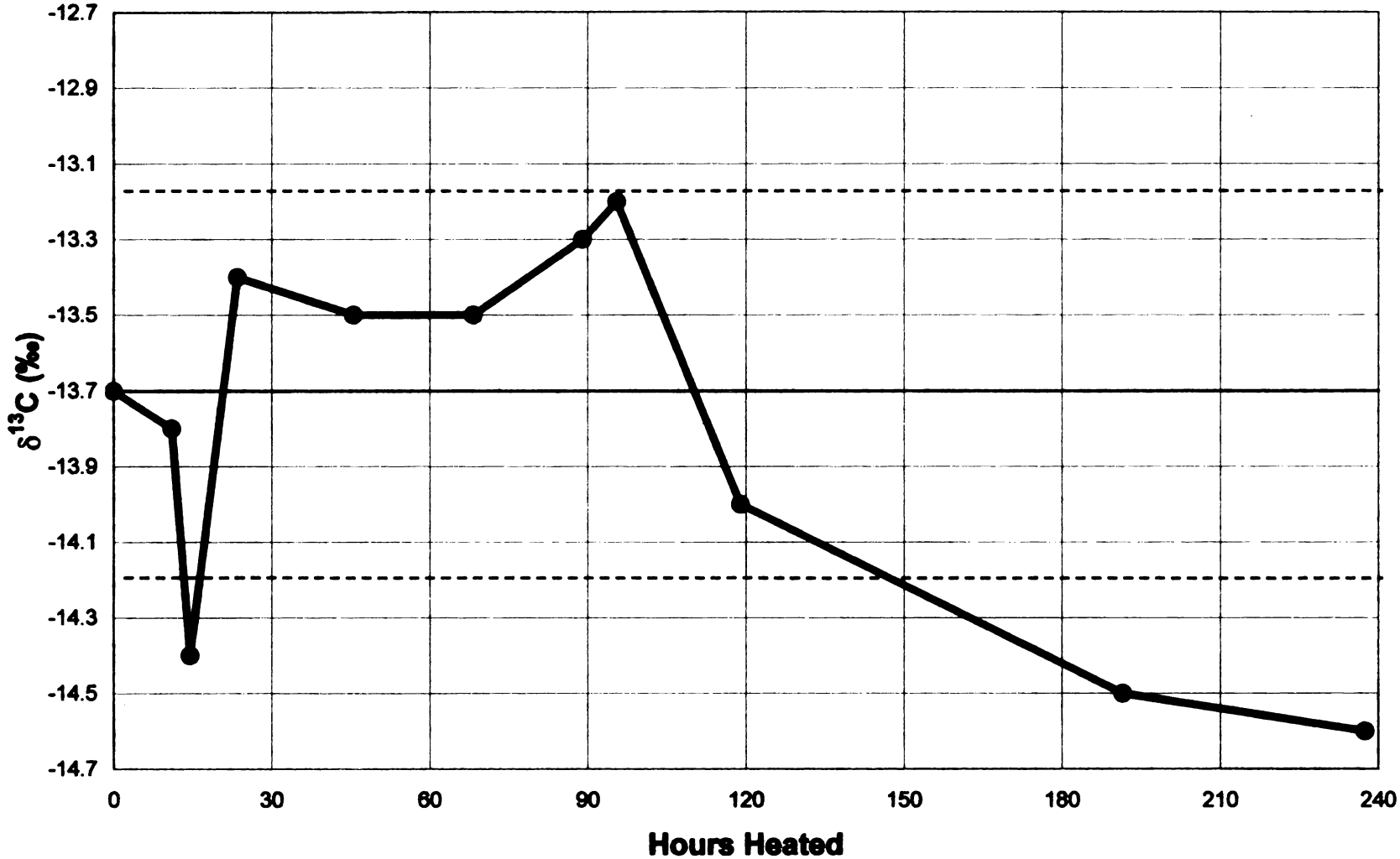

Figure 7 - Artificial Diagenesis NCP Carbon Isotope Values

TABLE 2 - NTC Taxon, sample age, collagen yield, and mean values of carbon and nitrogen isotope values. All values have an n = 1 or 2

Specimen #	KUVP #	Taxon (common name)	Age (B.P.)	Collagen wt (%)	$\delta^{15}N$ (‰) (Mean)	$\delta^{13}C$ (‰) (Mean)	C:N
BI.04.B.1	50605	*Bison bison* (bison)	17000-20000	9.02	5.9	-18.6	2.9
BI.04.B.4	52124	*Bison bison* (bison)	17000-20000	5.59	6.4	-18.2	2.9
BR.04.B.1	42728	*Ursus* sp (bear)	Pleistocene	13.74	4.2	-20.3	3.0
CA.04.B.1	35806	*Camelops* sp (camel)	17000-20000	12.66	4.9	-18.9	3.0
CH.04.B.1	82405	*Miracinonyx trumani* (American cheetah)	Pleistocene	11.87	8.6	-17.5	3.1
FX.04.B.1	36354	Undetermined sp. (fox)	12000-14000	0.66	7.5	-22.3	4.2
FX.04.B.3	52884	Undetermined sp. (fox)	14000	5.71	4.3	-20.6	3.3
HS.04.T.1	44010	*Equus* sp (horse)	20000	2.50	3.5	-19.8	3.1
HS.04.B.2	79057	*Equus* sp (horse)	Pleistocene	2.51	3.4	-20.7	3.1
HS.04.B.3	82525	*Equus* sp (horse)	Pleistocene	10.97	3.1	-19.8	3.2
LI.04.B.1	26749	*Panthera atrox* (lion)	17870	6.52	8.4	-18.3	2.9
MO.04.B.1	61699	*Bootherium bombifrons* (musk-ox)	Pleistocene	10.51	7.4	-19.5	3.1
RB.04.B.1	42698	*Lepus* sp (rabbit)	20000	10.42	2.3	-20.6	3.2
RB.04.B.2	-	*Lepus* sp (rabbit)	110000	7.22	2.3	-20.0	3.1
RB.04.B.3	26534	*Lepus* sp (rabbit)	Pleistocene	8.50	4.4	-20.0	3.2
SH.04.B.1	-	*Ovis catclawensis* (bighorn sheep)	14000	2.83	6.9	-18.6	3.0
SH.04.B.2	52362	*Ovis catclawensis* (bighorn sheep)	17000	6.59	7.1	-19.2	3.1
SH.04.B.4	25987	*Ovis catclawensis* (bighorn sheep)	11000-12770	7.92	12.9	-20.7	5.0
SH.04.B.7	84918	*Ovis catclawensis* (bighorn sheep)	Pleistocene	9.70	6.0	-18.5	3.0
WL.04.B.1	39131	*Canis* sp (wolf)	17000-21000	12.85	7.6	-18.8	3.1
WV.04.B.1	44130	*Gulo gulo* (wolverine)	17000-21000	4.05	7.2	-19.2	3.2

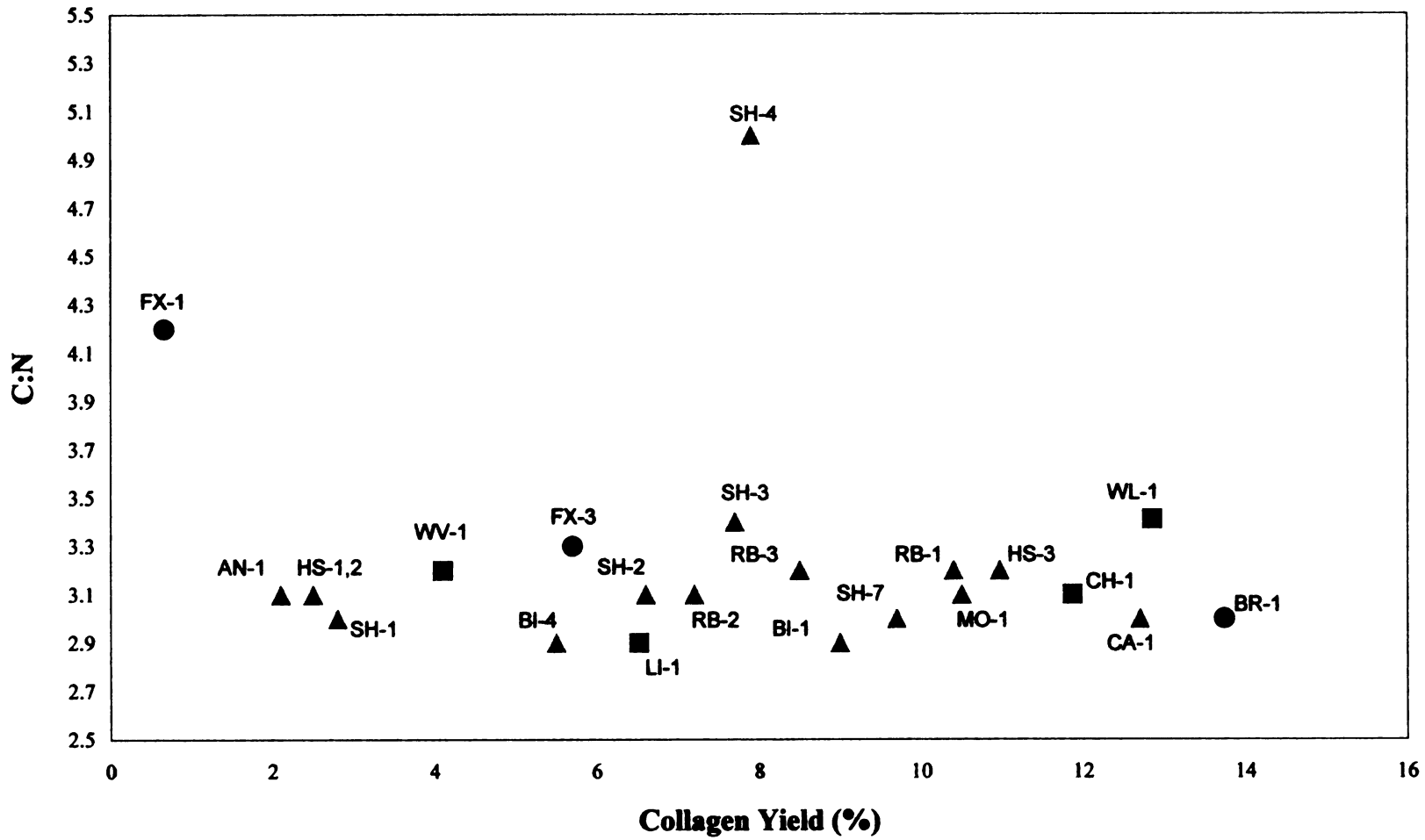

Figure 8 - NTC C:N vs. Collagen Yield; triangles =herbivores, circles = omnivores, squares = carnivores

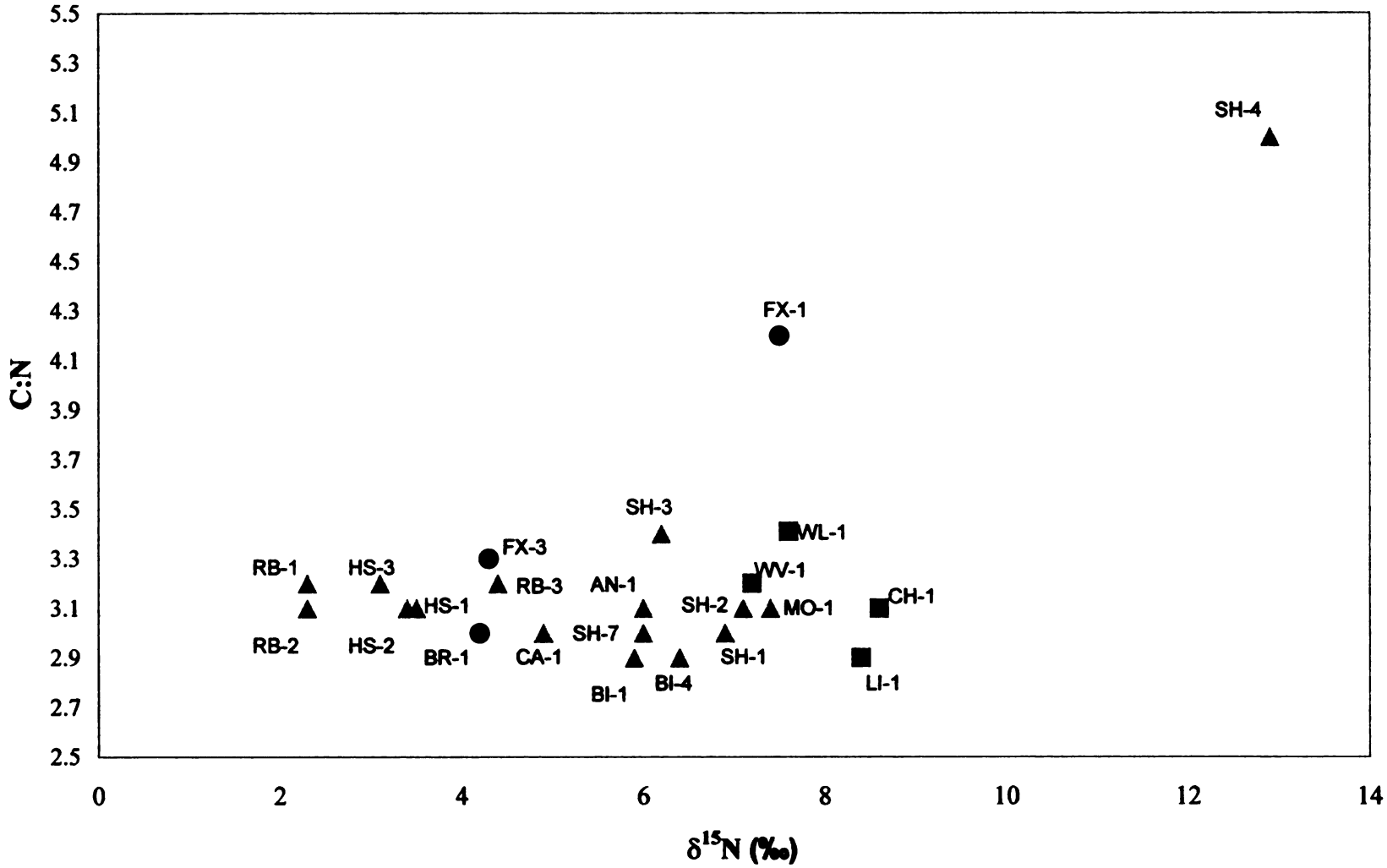

Figure 9 - NTC C:N vs Nitrogen Isotope Values; triangles = herbivores, circles = omnivores, squares = carnivores

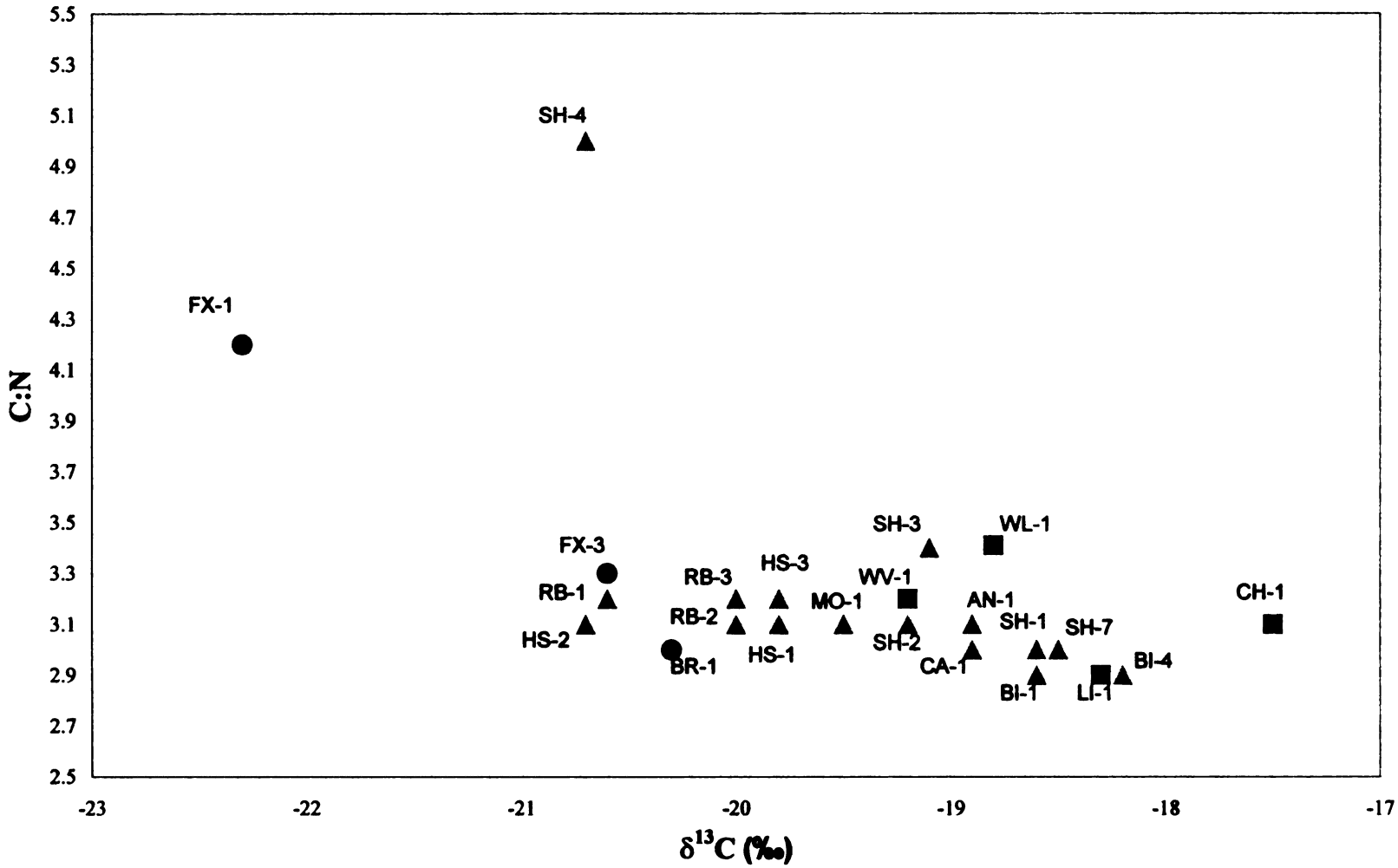

Figure 10 - NTC: C:N vs Carbon Isotope Values; triangles = herbivores, circles = omnivores, squares = carnivores

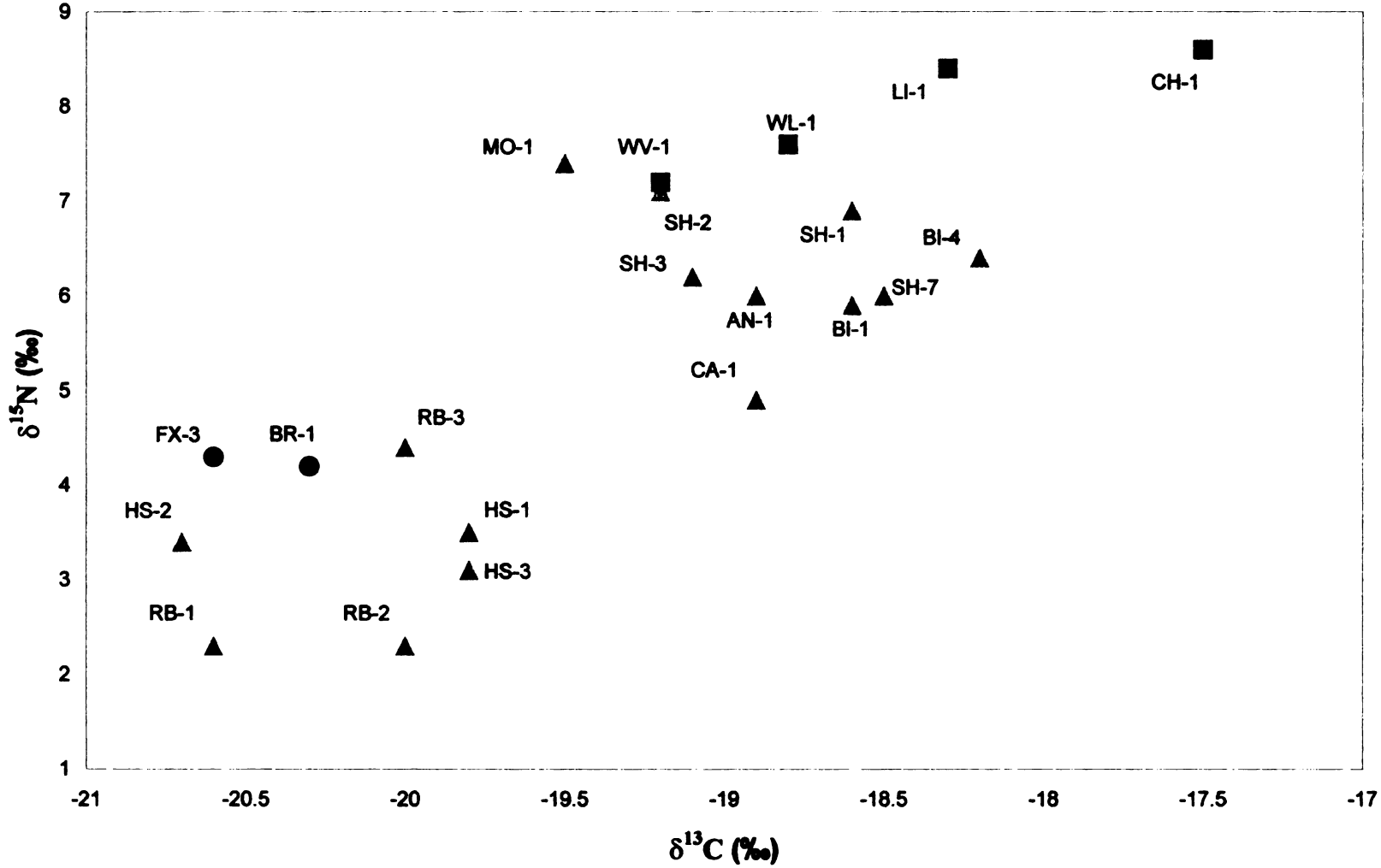

Figure 11 - NTC Trophic Structure; triangles = herbivores, circles = omnivores, squares = carnivores

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