PRELIMINARY RECONSTRUCTION OF DIET AT A NEOLITHIC SITE IN VIETNAM USING STABLE ISOTOPE AND BA/SR ANALYSES

Nathan W. Bower¹, Yuichiro Yasutomo¹, Marc F. Oxenham², Nguyen Lan Cuong³, Nguyen Kim Thy³

¹Chemistry Department, Colorado College, Colorado Springs, CO 80903, ²School of Anthropology and Archaeology, Australian National University, Canberra, ACT 0200, ³Institute of Archaeology, Hanoi, Socialist Republic of Vietnam

ABSTRACT

In order to better evaluate the role plant exploitation played at a Neolithic site (Con Co Ngua, ceramic context dated to 5000 years BP) in Vietnam, we measured the δ^{13} C in apatite from human teeth at this site as well as from a nearby Metal Period site (Nui Nap), with a carbon date of 1670 years BP, where δ^{13} C and δ^{15} N from collagen were also measured. Ba:Sr ratios at both sites were determined in order to estimate the importance of marine versus agricultural food sources. Results are consistent with incipient plant exploitation at Con Co Ngua, while dietary C_3 plants that might have included rice played a smaller role than at Nui Nap. Marine sources appear to be more significant at the earlier site.

INTRODUCTION

Vietnam is perhaps one of the least bioarchaeologically researched countries in Southeast Asia. While anatomically modern humans have resided in the region since at least 30,000 BP (Nguyen 1997), the earliest culturally distinct period is the Hoabinhian dating from between 18,000 and approximately 6000 BP. This period is characterized by foraging for a variety of vertebrates, shellfish and plants (Higham 1989). The Neolithic emerges from the Hoabinhian around 6000 BP in the form of a number of archaeological cultures including the Da But. The Da But culture period lasted for at least 1700 years and is characterized by numerous shell middens containing polished lithics, simple ceramics and apparently domesticated pigs and buffalo (Bui Vinh 1991).

While Da But communities are considered to be sedentary foragers, the relative importance of marine compared to terrestrial foods in their diet is unclear (Oxenham *et al.* 2005). Further, given that the Da But is traditionally seen as representing the beginnings of the Neolithic in the region, is there evidence of an agricultural component to their diet? The chief aim of this study is to assess the relative importance of terrestrial verses marine foods in the Da But diet and to determine whether plant exploitation (perhaps in the form of plant cultivation) might have played an important dietary role.

Little in the way of stable isotopic work has been carried out in Southeast Asia. Exceptions include King and

Norr's (2006) isotopic study of temporal changes in the diet at Ban Chiang, Thailand, between 4100 and 1800 BP, Bentley's (2004) strontium isotopic study of population movement at Khok Phanom Di, Thailand, between 4000 and 3500 BP, and Krigbaum's studies (2003, 2005) of Neolithic subsistence patterns in Borneo. Our own stable isotopic examination of dental remains from a Da But Period (Con Co Ngua) site in Vietnam in comparison with later Metal Period dental samples from the same region (Nui Nap) is a first step in clarifying the Neolithic dietary and subsistence picture, and it provides some of the first stable isotope analyses of material from Vietnam.

BACKGROUND

 $\delta^{13}C$ and $\delta^{15}N$ Stable Isotopes

Plants fall into three major metabolic groups: C₃, C₄ and CAM. Most plants (some 95%) fall into the C₃ group, including the common Southeast Asian food plants beans, tubers and rice. They fix atmospheric carbon dioxide using the enzyme rubisco to make a 3-carbon compound in the first step of the metabolic chain. Some food plants with Asian tropical climate origins fall into the C₄ group, including Job's tears (*Coix lacryma-jobi*), some millets and sugarcane. They produce a 4-carbon compound using PEP carboxylase during the first step of photosynthesis. Finally, CAM plants such as cactus have the crassulacean acid metabolism, which improves the photosynthetic performance in water and/or carbon dioxide limited environments (Voet *et al.* 1999). The CAM plants are not of concern in human diet studies in Vietnam.

The heavier isotopes of elements like carbon (C) and nitrogen (N) will be enriched in plant tissues over ambient levels during photosynthesis. $\delta^{13}C$ and $\delta^{15}N$ are the indices used to express the shift of these stable isotopes relative to their respective reference standards. Calcium carbonate from the PeeDee Limestone Formation (PDB standard) is used as the ^{13}C reference standard, and atmospheric nitrogen is used as the reference standard for ^{15}N . The $\delta^{13}C$ and $\delta^{15}N$ indices are defined as $(R_{sample}/R_{standard}-1) \times 1000$ per mil, where R_{sample} and $R_{standard}$ are the ratios of ^{13}C to ^{12}C (^{15}N to ^{14}N for $\delta^{15}N$) in the sample and standard, respectively. Because of metabolic enrichment, animals that are higher in trophic level tend to have higher $\delta^{13}C$ and $\delta^{15}N$ values. This is because the body retains heavier

isotopes preferentially during normal metabolic processes. Therefore, predators have higher concentrations of ¹³C and ¹⁵N than their prey (van der Merwe 1982).

For similar reasons, understory vegetation (typically C_3) that is shaded is expected to have a more negative $\delta^{13}C$ than vegetation that has ready access to light and atmospheric CO_2 , as plants in this environment will recycle more of the carbon (Jackson *et al.* 1993). Therefore foragers who collect from the understory of heavily forested regions would also be expected to have a more negative $\delta^{13}C$ signature compared to those who forage in more open regions, and C_3 consumers should have a more negative $\delta^{13}C$ signature than C_4 consumers.

There are two distinct groups of $\delta^{15}N$: legumes and non-legumes. Legumes utilize both atmospheric nitrogen and ammonium ions in the soil, while non-legumes utilize only soil nitrogen. Soil nitrogen has higher $\delta^{15}N$ values than atmospheric nitrogen. As a result, non-legumes have higher δ^{15} N values than do legumes (DeNiro 1987). Marine animals have higher values for both δ^{13} C and δ^{15} N than terrestrial animals because seawater is enriched in heavier isotopes compared to the atmosphere for both nitrogen and carbon dioxide, and marine life is in equilibrium with the ocean in which it resides. Because human beings will concentrate the heavier isotope from the foods we eat, the δ^{13} C and δ^{15} N values in humans will be about +5 higher than the values found in the things we consume. Generally, browsing animals eat herbs, shrubs and trees (mostly C₃ plants), while grazing animals will consume grasses (mostly C₄ plants), and they will add their own positive metabolic shift to varying degrees with ruminants (e.g., cattle) having more positive shifts.

C and N may be obtained from any compounds that have survived in unadulterated form, but each may have slightly different $\delta^{13}C$ and $\delta^{15}N$ values due to their different synthetic pathways. Because bone or tooth apatite can survive over very long time periods, it is usually the component of choice if only $\delta^{13}C$ is to be measured, but it contains no nitrogen. To measure both $\delta^{13}C$ and $\delta^{15}N$, collagen extracted from teeth is widely used, though it is not a single pure molecule. Because collagen's isotopic ratios are preferentially impacted by the sources of dietary protein while apatite reflects a broader spectrum of dietary carbon energy sources, both are of value even though their isotopic ratios may be impacted by both diagenesis and preparation method (Garvie-Lok *et al.* 2004).

If $\delta^{13}C$ values from the 5000 BP Con Co Ngua site are significantly greater than those from the 1670 BP Nui Nap Metal Period site, then people from 5000 BP may have eaten more C_4 plants such as wild millet, coupled with foraging from coastal, riverine and marine foods. This would match the material culture expectations. If the $\delta^{13}C$ values from the two sites are close to each other, then the Da But diet contains significant percentages of C_3 plants. Such congruence might imply plant (rice?) cultivation had been developed or transmitted here relatively early for the region, though other C_3 sources are also possible.

Ca. Ba and Sr Ratios

Although $\delta^{15}N$ is most useful for determining the relative contribution of marine sources to the diet, nitrogen-rich compounds such as collagen may not survive over long time periods of time. In such instances, the barium to strontium (Ba:Sr) ratio may be a useful complementary method (Burton and Price 1990), especially when coupled with other elemental methods that differentiate terrestrial and marine sources.

The solubility of BaSO₄ is much less than that of SrSO₄, and in seawater where there is an excess of sulfate ions compared to Ba and Sr, the Ba:Sr mass ratio equals 0.004 (Bowen 1966). However, in freshwater the concentrations of these cations are almost equal, as unpolluted freshwater does not typically contain high levels of SO₄². As a result, the ratio of Ba:Sr is close to one in terrestrial or freshwater species while in marine plants and animals the Ba:Sr is usually <0.1 (Burton and Price 1990). It should be noted that the Ba:Sr ratio is also expected to decrease as trophic level increases, as Sr better mimics calcium (Ca). Therefore, for populations where agriculture is more developed and where it is expected to play a greater role compared to marine food sources, the Ba:Sr ratios should be relatively higher. Both Ba and Sr may be ratioed to Ca to gain insights into the relative roles of trophic level and marine versus terrestrial sources in the diet, as Sr:Ca generally decreases with increasing trophic level while Ba:Ca decreases with increasing amounts of marine food sources as well as with increasing trophic level. Thus, Ba:Sr focuses on the marine component.

SAMPLE DESCRIPTIONS

Teeth (see Table 1) from four individuals were sampled from Nui Nap: 78NN-M10Kb, F, age 20-29, mandibular M1 with small piece of adhering mandible; 78NN-13Aka, F?, 30-39, maxillary I1, I2, M1, and M2; 78NN-MX2, F, 40-49, M2; and 76NN-M3, age and sex unknown, left mandibular M1 portion of crown only. From individual 78NN-13Aka four teeth were sampled to provide variation with age (generally speaking: permanent incisor crowns are complete by 3 to 4 years and erupt between 7 and 9 years; M1 crowns are completed during the 3rd year and emerge between 6 and 7 years; M2 crowns are completed between 6 and 7 years and erupt around 12 years). Another four individuals were sampled at Con Co Ngua: 80CCN-M64, age and sex unknown, mandibular M1; 80CCN-MY, female?, adult, maxillary M1; 80CCN-M44, male, 30-39, mandibular M1; and 80CCN-M9, sex unknown, 20-29, mandibular M1.

Nui Nap is a Metal Period site with the majority of its individuals radiocarbon dated to 1670±85 years BP with two individuals from as early as 2500 to 3000 BP. It is located 8 km east of present day Than Hoa about 8 km from the coastline (Figure 1). Situated on the side of a low limestone bluff, the site contained a variety of pottery and ceramic vessels as well as many bronze implements. The bioarchaeological and historical records suggest the inhabitants subsisted on mixed terrestrial (possibly including plant cultivation) and marine foraging.

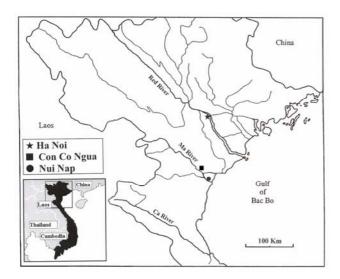


Figure 1. Sample site locations in Thanh Hoa Province, Northern Vietnam

Individuals at this site exhibit evidence of the beginnings of infectious diseases, which is consistent with the beginnings of massive population movements into the region at this time (Oxenham *et al.* 2005)

Con Co Ngua is a Da But (Neolithic Period) site about 30 km inland from the current coastline and 3.6 km north of the Ma River. It would have been much closer to the ocean 5000 years ago as the river has deposited significant quantities of sediment over this period. Con Co Ngua lies in a small valley surrounded by 300 m high limestone hills about 20 km north of Thanh Hoa in northern Vietnam at 20° N and 104°45" W (Fig. 1). It consists of two pits that are thought to be temporally discontinuous with 28 burials in pit 1 and 78 burials in pit 2. Ground, polished, and flaked stone artifacts, bone implements, mat-impressed pottery fragments, and marine, riverine and estuarine remains of fish, oyster and mussels as well as bones of pig, deer, and buffalo were found at the site. The ceramic record is consistent with other sites that date to about 5000 years BP. The latter date is preferred to the clearly much too recent dates obtained from marine shell (3020 \pm 100 years BP) and human bone (2740 \pm 1050 years BP). There is no biological evidence that agriculture or horticulture was practiced at this site, but the human assemblage suggests the inhabitants lived a sedentary life that was, overall, healthier than the average Southeast Asian populations of the period with the exception of an elevated frequency of traumatic injury (Oxenham et al. 2001; Oxenham et al. 2005).

METHODS OF ANALYSIS

$\delta^{13}C$ and $\delta^{15}N$ Analysis

Each tooth was first physically cleaned by removing the surface with a rotary carbide tool, followed by sonication in deionized water (18 M-ohm). The enamel covered portions of the teeth were crushed and pieces of enamel free of dentin were hand picked under a microscope (30X) for the apatite analyses. Tooth pieces that were cleaned and

subsequently used for $^{13}\text{C}/^{12}\text{C}$ and elemental analyses were prepared by pulverizing them in a tungsten carbide ball mill to < 0.05 mm. Enamel-protected dentin was similarly prepared for use in subsequent collagen analyses.

A preliminary analysis of N and C (Table 1) revealed the earlier Da But site had little detectable N and only 0.5 to 2% C left in the enamel-protected material, indicating no useful collagen remained. The later, Metal Period samples had 1.5% N and 5% C (9-12% collagen), suggesting they were well preserved (Stafford et al., 1988; Ambrose 1990). A number of different collagen preparation methods have been developed (Katzenberg 2000). In our case, demineralization of the samples was accomplished by soaking in 1 M HCl (trace metal grade) at 4°C for 3 days with gentle swirling, followed by washing with 18 M-ohm water. Lipid in the samples was removed with 2 mL of chloroform/methanol (3:2) (Tuross et al. 1988; Liden et al. 1995) followed by drying under vacuum. Collagen purity was verified by analysis of the collagen (Fig. 2) using FTIR (PE Spectrum RX 1) and by obtaining the expected C:N ratios (Carlo Erba NC2100 combustion analyzer). Collagen samples were subsequently analyzed for $\delta^{13}C$ and δ¹⁵N using a stable isotope mass spectrometer (Thermo-Finnegan Delta Plus) in continuous flow mode.

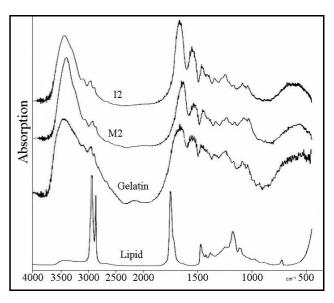


Figure 2. FTIR spectra of collagen extracted from sample 78NN13-Aka-II and M2 teeth, collagen (gelatin), and lipid reference samples.

Although the Da But site samples lacked enough collagen to be reliably analyzed for $\delta^{13}C$ and $\delta^{15}N$, the apatite in the enamel could be analyzed for $\delta^{13}C$. Following the recommendations of Koch *et al.* (1997), 100 mg samples of powdered tooth enamel from each site (Table 1 – all tables at end) were prepared for analysis of the inorganic, apatite carbon. Hydrogen peroxide (H₂O₂) was used to destroy the organic matter by swirling (200 rpm) the enamel powder in 5 mL of 30% H₂O₂ for a day, followed by vortexing five times with 5 mL aliquots of 18 M-ohm water and centrifuging (Beckman J2-HS) to

separate a pellet from the liquid. The powders were then swirled in 5 mL of 1 M acetic acid/calcium acetate buffer (pH=4.3) for a day to remove any carbonate contaminants. The samples were washed a final five times with 18 M-ohm water and dried at 40° C followed by analysis of their δ^{13} C as described above. The enamel powders were analyzed by thermogravimetry (Shimadzu TGA-50) and x-ray diffraction (Philips PW1830/1710 XRD) before and after cleaning to check for residual contamination or alteration and to estimate crystallite sizes using the [002] peak at 26° (2θ).

Elemental Analyses

Because diagenetic alteration of Ba and Sr can be significant, care must be exercised in preparing samples and in interpreting results (Burton *et al.* 1999). Samples (100 mg) of powdered enamel were placed in virgin polyethylene vials and cleaned with 1 M acetic acid/calcium acetate buffer and washed as described above. The enamel was analyzed for calcium (Ca) and trace elements such as uranium (U) before and after cleaning by x-ray fluorescence (Philips PANalytical Epsilon 5 XRF) to monitor the efficacy of the cleaning procedure. Cleaned samples were dissolved with trace metal grade nitric acid (Fisher) and analyzed by ICP-OES (Thermo-Jarrell Ash Atomscan 16) to obtain adequate detection limits for the trace metals of interest.

RESULTS AND DISCUSSION

Integrity of Samples

The relative standard deviation of the %N and %C (Table 1) between individuals is comparable to that found within one individual's teeth (78NN13Aka-incisor 1 and 2, right molar 1 and 2). This individual exhibits slightly better preservation in the molars than the incisors, as expected. The mandible fragment and the loose crown fragment (76NNM3) both had very poor preservation for the period and they were different tissue types than the enamel-protected material used for the remaining analyses so they are excluded from the rest of this discussion.

The cleaning procedure lowered Fe, Mn, and U to 69, 25, and 0 ppm, respectively, in the Da But samples. These levels are near modern enamel values (Kohn *et al.* 1999) and are slightly higher than in the Metal Period samples (35, 8, and 0 ppm). No contamination or any alteration products after cleaning were observed using XRD (Fig. 3). The XRD spectra and crystallite sizes are comparable to enamel data from recent human skeletons (data not shown). The [002] FWHM (full width at half maximum) were not significantly different (P = 0.46) between the two sites (Table 2), and relative peak height data (not shown) are consistent with archaic human apatites reported by Koch *et al.* (1997).

Elemental Analyses

The logs of the ratios of Ba:Sr for the tooth samples from 5000 BP are significantly lower (two-group t-test, P < 0.001) than those from 2000 BP (Table 3). Thus, people from 5000 BP appear to have had greater percentages of

marine foods in their diet than people from 2000 BP. This interpretation is supported by the somewhat higher levels of Cu (8 versus 6 ppm, P=0.31) and lower levels of Zn (65 versus 123 ppm, P=0.07) found at Con Co Ngua compared to Nui Nap using ICP-OES. Similar levels (10.5 versus 6.8 ppm for Cu, and 97.2 versus 104.6 ppm for Zn) were found in pre-Hispanic Canary Island populations with respectively higher and lower amounts of marine food in their diet (González-Reimers $et\ al.$, 2001). The trend for the individual with multiple teeth analyzed also suggests that marine sources played an increasing role with age.

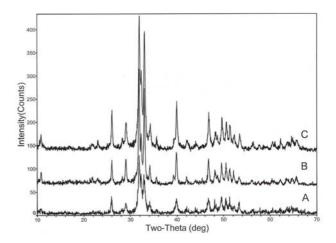


Figure 3. XRD spectra of tooth enamels for (A) Da But CCN-M64 before cleaning; (B) Metal Period NN-Aka13-I2 before cleaning; and (C) Metal Period NN-Aka13-M2 after cleaning.

$\delta^{13}C$ and $\delta^{15}N$

The δ^{13} C values for apatite (-16.54 ± 0.42%) were significantly lower (two-group t-test, P < 0.001) than the collagen values (-19.77 \pm 0.58%) for the Metal Period site. Collagen tracks the dietary protein sources while apatite follows the whole diet. In addition, collagen from dentin will reflect a later period in the individual's life than apatite from tooth enamel. A comparison with the stable isotope data from Lee-Thorp et al. (1989) suggests these Metal Period individuals probably obtained their dietary energy primarily from C₃ sources, and their protein from either marine or C₃ sources. Temporal (±1‰) and spatial $(\pm 1.5\%)$ variations in the sources can blur these distinctions (Heaton, 1999). Jim et al. (2004) conducted controlled feeding studies with rats and the data presented here best fits a C₃ protein source coupled with a C₃ energy source based on their findings. This conclusion is further supported by the $\delta^{15}N$ (10.20 ± 0.34‰) data, as this value fits primarily with a terrestrial source of protein (6-10%), albeit on the marine end of that continuum. Chisholm and Koike (1996) have obtained $\delta^{15}N$ and $\delta^{13}C$ values from inland and coastal sites for Japanese dating to the Jomon and Yayoi Periods, and a comparison of our Nui Nap, Metal Period collagen data with their values fits best with an inland site where rice cultivation has been well established and where marine resources are present but play a minor role.

A two-group t-test of the apatite δ^{13} C values (Table 2) indicates the Da But site (-14.5 \pm 0.6%) has significantly higher (P < 0.001) δ^{13} C than the Metal Period site (-16.54) \pm 0.42‰). The δ^{13} C alone does not tell us whether this difference is due to an increased use of marine sources, to a greater use of plants from more open areas, or to a greater use of C₄ plant sources. However, a plot of the Ba:Sr ratio versus the δ^{13} C values (Fig. 4) suggests that some of the dietary difference is due to a greater use of marine sources at Con Co Ngua. The average of the apatite δ^{13} C values for Con Co Ngua are more negative than the -13.2 \pm 0.8% reported for a coastal Neolithic site (Niah Cave, median age 3080 BP) in Borneo (Krigbaum 2005), but are equal to values (-14.4±0.19‰) for their inland, late Neolithic/Metal Period site (Lubang Angin, median age 1960 BP). This may indicate a lesser reliance on marine sources or a lesser use of C₄ plants (such as sugarcane) than at the coastal site in Borneo, or that the C₃ plant, rice, was beginning to play a role in the diet at Con Co Ngua.

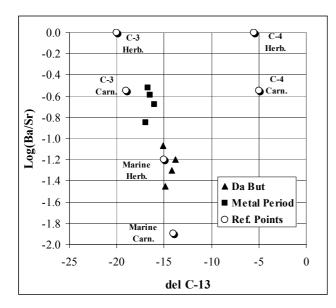


Figure 3. Comparison of Da But and Metal Period sites using apatite δ¹³C and Ba/Sr ratios. Average values for C-3, C-4, and marine herbivores and carnivores are also plotted. (Data for the reference points are based on DeNiro (1987) and Burton and Price (1990).)

CONCLUSIONS

While the preliminary nature of these results and the small sample sizes need to be considered, some interesting results have been found that will form testable hypotheses for future isotopic work in Vietnam. The people at Nui Nap appear to have relied heavily on C₃ plants (probably rice) with less use of marine sources than people from the earlier Da But, Con Co Ngua site. This is consistent with expectations that agriculture was well established in the Metal Period. Intriguingly, while the Da But diet contained significant marine foodstuffs, there was also more of a C₃ component compared to values found for an earlier Neolithic site in Borneo. This suggests that rice may have begun to play a role in the mid-Holocene diet in northern

Vietnam, though other C₃ plants were also consumed. At present there is a lack of archaeological evidence for rice cultivation between 5,000 and 6,000 years BP in northern Vietnam. Future work will focus on clarifying this issue.

ACKNOWLEDGMENTS

We thank the Colorado College Natural Science Division and the McKee Family Trust for funding, and instrumentation used in this study was purchased through grants from the Margaret and Otis Barnes Trust and the Fairchild and National Science Foundations (ILI-9352208).

REFERENCES

- Ambrose S. H. 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. *Journal of Archaeological Science* 17: 431-451.
- Bentley R.A. 2004. Characterising human mobility by strontium analysis of the skeletons. In Higham C.F.W. and Thosarat R. eds. *The Excavation of Khok Phanom Di: a Prehistoric Site in Central Thailand* Volume VII: Summary and Conclusions, pp. 169-166. London: Society of Antiquities.
- Bowen H. 1966. Trace Elements in Biochemistry. New York: Academic Press
- Bui Vinh 1991. The Da But Culture in the Stone Age of Vietnam. Bulletin of the Indo-Pacific Prehistory Association 10: 127-131.
- Burton J. and Price T. 1990. The ratio of barium to strontium as a paleodietary indicator of consumption of marine resources. *Journal of Archaeological Science* 17: 547-557.
- Burton J.H., Price T.D. and Middleton W.D. 1999. Correlation of bone Ba/Ca and Sr/Ca to biological purification of calcium. *Journal of Archaeological Science* 26: 609-616.
- Chisholm B. and Koike H. 1996. Reconstructing prehistoric Japanese diet using stable isotopic analysis. In Omoto K. ed. *Interdisciplinary Perspectives on the Origins of the Japanese*, pp 199-222. International Research Center for Japanese Studies Kyoto.
- DeNiro M. 1987. Stable isotopy and archaeology. *American Scientist* 75: 182-191.
- Garvie-Lok S.J., Varney T.L. and Katzenberg M.A. 2004. Preparation of bone carbonate for stable isotope analysis: the effects of treatment time and acid concentration. *Journal of Archaeological Science* 31: 763-776.
- González-Reimers E., Velasco-Vásquez J., Arnay-de-la-Rosa M., Santolaria-Fernández F. and Galindo-Martín L. 2001. Palenutritional analysis of the pre-Hispanic population from Fuerteventura Canary Islands. The Science of the Total Environment 264: 215-220.
- Heaton T.H.E. 1999. Spatial species and temporal variations in the ¹³C/¹²C ratios of C₃ plants: implications for palaeodiet studies. *Journal of Archaeological Science* 26: 637-649.
- Higham C.F.W. 1989. *The Archaeology of Mainland Southeast Asia*. Cambridge: Cambridge University Press.
- Jackson P.C., Meinzer F.C., Goldstein G., Holbrook N.M., Cavlier J. and Rada F. 1993. Environmental and physiological influences on carbon isotope composition of gap and understory plants in a lowland tropical forest. In Ehleringer J.R. Hall A.E. and Farquhar G.D. eds. Stable

- *Isotopes and Plant Carbon-Water Relations*, pp. 131-140. San Diego: Academic Press.
- Jim S., Ambrose S.H. and Evershed R.P. 2004. Stable carbon isotopic evidence for differences in the dietary origin of bone cholesterol collagen and apatite: implications for their use in paleodietary reconstruction. *Geochimica et Cosmochimica Acta* 68: 61-72.
- Katzenberg M.A. 2000. Stable isotope analysis: A tool for studying past diet demography and life history. In Katzenberg M.A. and Saunders S.R. eds. *Biological Anthropology of the Human Skeleton*, pp. 305-327. New York: Wiley-Liss.
- King C.A. and Norr L. 2006. Palaeodietary change among pre-State Metal-Age societies in Northeast Thailand: a study using bone stable isotopes. In Oxenham M. and Tayles N. eds. *Bioarchaeology of Southeast Asia*, pp. 241-62. Cambridge: Cambridge University Press.
- Koch P.L., Tuross N., and Fogel M. L. 1997. The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite. Journal of Archaeological Science 24: 417-429.
- Kohn M.J., Schoeninger M.J. and Barker W.W. 1999. Altered states: Effects of diagenesis on fossil tooth chemistry. Geochimica et Cosmochimica Acta 63: 2737-2747.
- Krigbaum J. 2003. Neolithic subsistence patterns in northern Borneo reconstructed with stable carbon isotopes of enamel. *Journal of Anthropological Archaeology* 22: 292-304.
- Krigbaum J. 2005. Reconstructing human subsistence in the west mouth Niah Cave Sarawak burial sites using stable isotopes of carbon. Asian Perspective 44: 73-89.

- Lee-Thorp J.A., Sealy J.C. and van der Merwe N.J. 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite and their relationship to diet. *Journal of Archaeological Science* 16: 585-599.
- Liden K., Takahashi C. and Nelson D.E. 1995 The effects of lipids in stable isotope analysis and the effects of NaOH treatment on the composition of extracted bone collagen. *Journal of Archaeological Science* 22: 321-326.
- Nguyen K.S. 1997. In the framework of the maritime culture of Vietnam: the prehistoric maritime culture of the northeast. *Vietnamese Studies* 1231: 87-116.
- Oxenham M.F., Walters I., Nguyen L.C. and Nguyen K.T. 2001.

 Case studies in ancient trauma: mid-Holocene through Metal Periods in northern Viet Nam. In Henneberg M. and Kilgariff J. eds. *The Causes and Effects of Biological Variation*, pp. 83-102. Adelaide: Australasian Society for Human Biology, University of Adelaide.
- Oxenham M.F., Nguyen K.T. and Nguyen L.C. 2005. Skeletal evidence for the emergence of infectious disease in bronze and iron age northern Vietnam. *American Journal of Physical Anthropology* 126: 359-376.
- Stafford T.W. Jr. Brendel K. and Duhamel R. 1988. Radiocarbon ¹³C and ¹⁵N analysis of fossil bone: removal of humates with XAD-2 resin. *Geochimica Cosmochimica Acta* 52: 2257-2267.
- Tuross N., Fogel M.L. and 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* 70: 596-606.
- Voet D., Voet J.G. and Pratt C. W. 1999. Fundamentals of Biochemistry. New York: John Wiley and Sons.

Table 1. Initial determination of organic and inorganic carbon and nitrogen.

Sample	% Carbon (Total)	% Nitrogen (Total)	Weight % Collagen ^a	Non-Collagen % Carbon ^b
Nui Nap (~2000 BP)				
78NN13-Aka-I1	5.80	1.53	9.5	2.53
78NN13-Aka-I2	5.82	1.48	9.2	2.65
78NN13-Aka-RM1	6.62	1.79	11.2	2.75
78NN13-Aka-RM2	7.26	1.86	11.6	3.26
78NNM-10KB-LM1	5.52	1.40	8.7	2.52
10KB (Jaw Bone)	1.90	0.17	1.1	1.52
78NN-MX2-LM2	5.54	1.31	8.2	2.71
76NN-M3-LM1-frag	0.80	≤0.072	≤0.5	≥0.63
Con Co Ngua (~5000 BP)			
80CCN-M64-LM1	0.64	≤0.019	≤0.1	≥0.60
80CCN-MY-RM1	0.95	≤0.082	≤0.5	≥0.77
80CCN-M44-LM1	0.58	≤0.035	≤0.22	≥0.50
80CCN-M9-LM1	0.60	≤0.039	≤0.24	≥0.52

^aThe amount of collagen was estimated from % Collagen = (% of Nitrogen)*6.25.

^bInorganic carbon was estimated from Total % C – (% Collagen/2.90).

Table 2. Results of $\delta 13C$ and $\delta^{15}N$ analysis of collagen and apatite, and Ba/Sr data for apatite. Instrumental variation was $\leq 0.1\%$, and replicate analyses of the same tooth were $\leq 0.3\%$ for the isotopic data, and ≤ 0.10 in the log (ratio).

Sample	Collagen				Apatite		Crystallite FWHM
	δ15N (‰)	Wt.% N	δ13C (‰)	Wt.% C	δ13C (‰)	Wt.% C	[002]
Nui Nap (~2000 BP)							
78NN13-Aka-I1	10.8	16.58	-19.3	45.25	-16.5	0.66	0.3
78NN13-Aka-I2	9.4	15.91	-20.5	44.31		0.6	0.24
78NN13-Aka-M1	9.9	14.64	-20.5	40.38	-16.7	0.72	0.34
78NN13-Aka-M2	9.8	16.12	-20.5	43.45		0.75	0.24
78NN13-10KB-M1	10.6	15.94	-19.1	43.72	-17	0.85	0.3
NNMX2	10	16.11	-20.1	43.94	-16	0.74	0.33
Avg	10.2	15.92	-19.77	43.66	-16.54	0.72	0.29
St Dev	0.34	0.2	0.58	0.32	0.42	0.08	0.04
Con Co Ngua (~5000 BP)							
80CCN-M64-LM1/2					-13.8	0.57	0.35
80CCN-MY-RM1					-15.1	0.84	0.28
80CCN-M44-LM1					-14.9	0.89	0.34
80CCN-M9-LM1					-14.2	0.73	0.28
Avg					-14.5	0.76	0.31
St Dev					0.61	0.14	0.04

Table 3. Results of elemental analyses using ICP-OES (Ba, Cu, Sr, and Zn in ppm) and XRF (Ca in %).

Sample Origin			Eleme	nt		Log
Nui Nap (~2000 BP)	Ca	Ba	Cu	Sr	Zn	Ba/Sr
78NN13-Aka-I1	26.12	32	6	124	116	-0.59
78NN13-Aka-I2	26.03	30	5	136	110	-0.66
78NN13-Aka-M1	25.34	32	6	105	139	-0.52
78NN13-Aka-M2	25.85	20	11	126	102	-0.80
78NN13-10KB-M1	26.53	18	7	123	144	-0.85
NNMX2	25.99	27	3	129	124	-0.68
Avg	25.98	26	6	124	123	-1
St Dev	0.39	6	3	11	16	0
Con Co Ngua (~5000 BP)						
80CCN-M64-LM1/2	26.03	7	4	112	29	-1.20
80CCN-MY-RM1	26.63	11	13	133	51	-1.07
80CCN-M44-LM1	23.47	12	9	336	57	-1.45
80CCN-M9-LM1	25.71	23	8	466	122	-1.30
Avg	25.46	13	8	262	65	-1.25
St Dev	1.38	7	4	169	40	0.16