From The Fauna's Mouth: Assessing Seasonality using CO_2-Laser Ablation on Prehistoric Tooth Enamel from Island Southeast Asia

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Accurate Paleoclimate reconstruction in paleoanthropology and archaeology is integral in understanding past human subsistence and settlement. However, fine-grained details of paleoclimate and paleoecology in Southeast Asia tropical rain forest environments are hampered by the coarseness of the record and limitations in analytical equipment. Here, the use of Laser Ablation Continuous-Flow Isotope Ratio Mass Spectrometry (LA-CR-IRMS) has been utilized to sample incrementally 10 mammalian teeth recovered from Niah Cave, located in Northern Borneo, from late Pleistocene and Holocene. Due to the nature of enamel formation, $\delta^{18}O$ values produced from sequentially sampling each tooth along the enamel surface provide insight into seasonal climate patterns.

INTRODUCTION

The Site: Niah Cave

This study focuses on applying methods of LA-CR-IRMS to provide information about sub-annual weather patterns during the late Pleistocene and Holocene. Findings will be considered with respect to prior knowledge and information gathered about the region, and it is thereby necessary to detail the site context. Niah Cave is located within the district of Miri in Sarawak, Malaysia, on the island of Borneo. The environment of the region is tropical and lush with vegetation, as approximately one-fourth of the world's existing tropical forest is located in Indo-Australia (Heaney, 1991). During the late Pleistocene, however, global climatic changes influenced the landscape of the region, and induced increased seasonality and rainfall (Heaney, 1991). During the last glacial maxima in particular, changes in climate impacting sea levels, flora, and regional weather patterns would in turn have had significant impact on the human populations living in the area at the time (Krigbaum, 2001). The effects of the South East Asian Monsoon are also key in understanding dynamics of climate, such as heavy rains and drier seasons, as these changes in weather would affect human activity, subsistence, and dispersal (Stephens et al. 2008).

Mammalian Enamel

In this study, $\delta^{18}O$ and $\delta^{13}C$ values were obtained from prehistoric mammalian tooth enamel collected from Niah Cave in order to add to the paleoecological record of the region. While the tooth enamel samples used in this study are from various species, mammalian enamel formation has several fundamental properties that allow for sub-annual assessment from isotopic values of each tooth. There is a rhythm to enamel formation as it mineralizes, and there are layers that correspond to the formation of each tooth. At the beginning of amelogenesis, enamel is laid out in successive, dome-like increments until the ameloblasts at the apex of the dome reach maturation. After this point, enamel formation switches to sleeve-like increments, and proceed down the tooth to the cervical part of the crown (Hillson, 1986), as shown in Figure 1. The nature of this incremental growth allows for discrete sampling along the surface of the tooth enamel from crown apex down to the Cementoenamel Junction, providing successive snap shots of the animal's surrounding environment during this period of enamel formation.

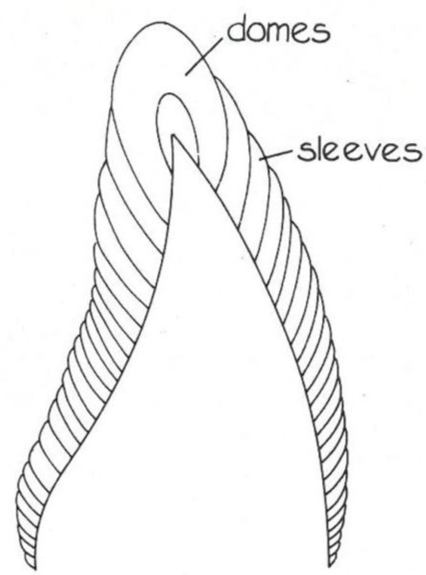

Figure 1. Structure of Enamel Formation (Hillson, 1986)

Previous Regional Paleoclimate Studies

Although the record on South East Asian Paleoclimate is limited, several studies that utilize various methods have been published that provide useful data on this topic. In Northern Borneo, for example, isotope analysis of Stalagmites has yielded information about millennial climatic changes over the last 27,000 years (Partin et al. 2007). Although the scaling of this data cannot be refined enough to provide any insight into sub-annual seasonality, it is useful to view the data gathered from this research within the context of their findings to better understand sub-annual weather patterns within the scope of larger-scale climatic changes.

At the site of Niah Cave, Stephens et al. (2008) have been able to assess seasonality from the incremental isotope analysis of modern and early Holocene harvested bivalves. Sawtooth-like patterns in $\delta^{18}O$ values of the modern bivalves were correlated to seasonal changes in rainfall. Isotope analysis of prehistoric bivalves revealed similar cyclic shifts in $\delta^{18}O$ values, which were inferred to reflect the changing seasonality of the monsoon rains (Stephens et al. 2008). While Stephens et al. provided high resolution and intra annual data, a disconnect remains between the aquatic and terrestrial records. The goals of this study are to:

1. evaluate the ability of LA-CF-IRMS to sample sequentially small-sized tooth samples.
2. analyze $\delta^{18}O$ values for possible signs of seasonality.
3. add to the terrestrial record by documenting Paleoclimate of South East Asia.

METHOD

Samples

Ten mammalian teeth from four different species were used in this study. The teeth date from the late Pleistocene to the early and mid Holocene, and were all recovered from Niah Cave. The enamel sampled was from various teeth, including molars and incisors. Several of the teeth used in this study were also incomplete fragments, while the entire tooth was available for other. Table 1 shows the characteristics of the ten teeth used.

Table 1. Sample Characteristics

Sample	Taxon	Approximate Age (years old)	Tooth
NC 142	*Sus barbatus*	15,365 +/- 60	Incisor
NC 138	*Sus barbatus*	~3,000	Incisor
NC 120	*Bos*	~20,000	Molar Fragment
NC 122	*Bos*	15,365 +/- 60	Molar Fragment
NC 121	*Bos*	~3,000	Molar Fragment
NC 175	*Pongo*	~15,000	Molar Fragment
LM 2	*Bos*	15,365 +/- 60	Molar Fragment
LM 1	*Bos*	~15,000	Molar Fragment
BOS 34	*Bos*	34,180 +/- 230	Molar Fragment
LM 4	*Rhinoceros*	~20,000	Molar Fragment

*Radiocarbon dates given for NC 142, NC 122, LM 2, and BOS 34. Dates for remaining samples are approximate.

Sample Preparation

Selected teeth were prepared for laser ablation using a consistent cleaning process. The enamel surface of each tooth was first cleaned using a diluted ethyl alcohol solution, and then rinsed with deionized water. Teeth were then placed in a beaker filled with distilled water and placed in a sonicator for ten minutes. Each beaker was then emptied and refilled with fresh deionized water, and sonicated again for a period of ten minutes. The beaker was then drained and the sample was air dried for approximately thirty minutes. Samples were then placed in an evaporating oven set at 60° C for fifteen minutes, or until completely dry. In the cases when teeth were too large as a whole to fit into the laser cell chamber, a portion of enamel, from crown apex to the cervix, was removed using a Dremel drill. The dentine was then removed from the backside of these pieces using a stainless steel pick, leaving a sample of pure enamel. The same procedure was followed when tooth samples were fragments rather then full teeth. Samples were then submitted to the same methods of preparation as previously stated.

Laser Ablation Continuous Flow Isotope Ratio Mass Spectrometry

Teeth were sampled used Laser Ablation Continuous Flow Isotope Ratio Mass Spectrometry (LA-CF-IRMS). While isotope analysis of tooth enamel is not new, the use of laser ablation to produce CO_2 gas for isotope analysis allows for finer grained sampling of the specimen, which in turn allows for analysis of changing seasonality during enamel formation for the samples used in this study (Spotl et al. 2006, Cerling et al. 1996, Passey et al. 2006).

Three major techniques for micro sampling specimen at high spatial resolutions currently exist, and while the two other methods require a separate handling step, the technique employed in this study, laser ablation, does not. When using a microdrill or micromill, it is necessary to transfer the sample powder created to a carbonate preparation system linked to a mass spectrometer. Laser ablation allows for direct linkage to a mass spectrometer via continuous gas flow (Spotl et al. 2006). Here we used continuous He flow to interface the CO_2 gas produced from laser ablation directly onto the sample with an isotope ratio mass spectrometer. CO_2 gas is produced from a designated spot on tooth enamel controlled by computerized laser software, and then carried to the mass spectrometer by the flowing He gas, producing carbon and oxygen isotope ratios. Figure 2 displays the LA-CF-IRMS set up used.

Procedure

After each tooth was cleaned and prepared for sampling, they were transported to the Stable Isotope Lab in the Department of Geological Sciences at the University of Florida. Each tooth/tooth fragment was sampled individually, starting at the apex of the sample and then proceeding downward towards the cementoenamel junction. Each incremental sample consisted of a designated line of laser shots along the horizontal axis of the tooth (same growth axis). Because of the variance in sample availability, shape, and size, laser settings were determined specifically for each tooth in order to produce the most CO_2 gas possible from the enamel without polluting the isotopic values with gas from the dentin or other components of the tooth besides enamel. As many individual samplings as could fit moving down the growth axes of each tooth sample were taken, and each individual sample on a tooth was consistently 1 mm apart. Carbon and oxygen isotopic values were recorded from the CO_2 gas produced.

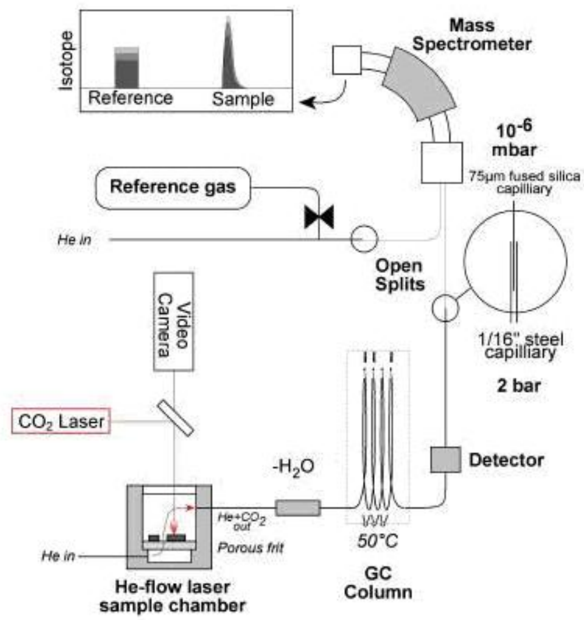

Figure 2. LA-CF-IRMS Set-up in the Stable Isotope Lab at the University of Florida, Department of Geology.

Table 2. Laser Variable Settings for Each Sample

Sample	Power	Pulse Width	Scan Speed	Pit Size (µm)	Length (µm)	Pits/Line	Lines (#)	Dist. btw (mm)
NC 142	25	180	150	160	2641.1	17	25	1
NC 138	20	120	150	160	2313.6	14	22	1
NC 120	30	140	150	160	1985.8	12	40	1
NC 122	30	140	150	160	1976.6	12	16	1
NC 121	30	140	150	160	1974.6	12	19	1
NC 175	30	140	150	160	1976.5	12	4	1
LM 2	30	140	150	160	1987.6	12	15	1
LM 1	25	120	150	160	1982.3	12	25	1
BOS 34	20	120	150	160	982.7	6	40	1
LM 4	25	140	150	160	1594.7	9	10	1

RESULTS

$\delta^{18}O$ and $\delta^{13}C$ values were obtained for each incremental sample on all ten teeth. A simple correction formula was then applied to the recorded isotopic values, derived from sampling a substance with known carbon and oxygen isotope ratios, Carrara Marble, and finding the difference between the known and recorded values. The number of samples taken on each tooth ranged from 4 to 40. Due to differences between each sample, each tooth was analyzed individually. Table 3 reports the $\delta^{18}O$ value statistics obtained and Table 4 the $\delta^{13}C$ value statistics obtained.

Table 3. Statistical Data for $\delta^{18}O$ Values Obtained

Sample	Mean	Standard Deviation	Range ($^{0}/_{00}$)
NC 142	-19.2	3.47	9.29
NC 138	-28.4	2.34	6.78
NC 120	-14.1	1.23	4.05
NC 122	-17.2	0.63	2.17
NC 121	-12.7	1.65	5.27
NC 175	-22.2	4.83	10.3
LM 2	-19.1	2.46	9.21
LM 1	-15.0	3.10	9.62
BOS 34	-12.6	0.64	2.19
LM 4	-17.0	1.57	5.35

Table 4. Statistical Data for $\delta^{13}C$ Values Obtained

Sample	Mean	Standard Deviation	Range ($^{0}/_{00}$)
NC 142	-15.2	0.73	2.04
NC 138	-15.4	2.34	2.42
NC 120	-17.0	0.66	2.63
NC 122	-18.9	0.55	1.59
NC 121	-17.5	0.61	2.41
NC 175	-16.1	0.71	1.59
LM 2	-20.1	0.23	0.81
LM 1	-19.2	0.48	1.46
BOS 34	-18.8	1.08	7.59
LM 4	-18.6	0.19	0.53

Seasonal Analysis

To assess seasonality, the $\delta^{18}O$ values from each sample were plotted against distance down the tooth enamel, as shown in Figures 3-12. Balasse et al. (2011) have shown a sinusoidal pattern of $\delta^{18}O$ variation to indicate changing seasonality over the span of a year, with the lowest $\delta^{18}O$ values corresponding to dry, cold seasons and the highest $\delta^{18}O$ values to wet, warm weather. Several of the samples show signs of possible seasonality with observable swings in $\delta^{18}O$ graphs and large standard deviations from the intra-tooth mean.

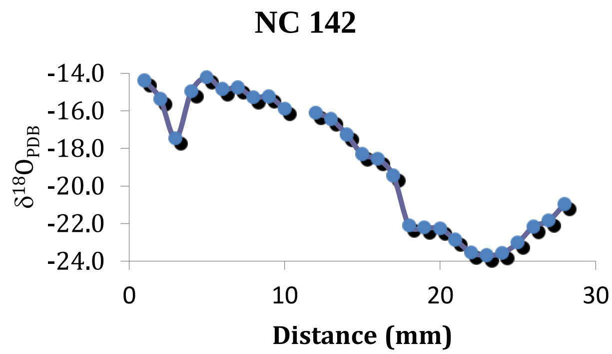

Figure 3. Sample NC 142: *Sus barbatus.* $\delta^{18}O$ values ranged from -23.69 to -14.40 with an average of -19.15.

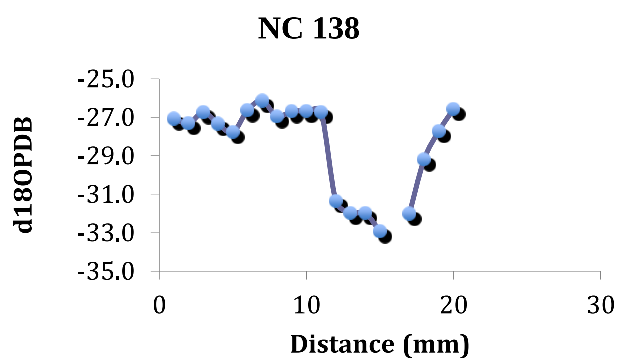

Figure 4. Sample NC 138: *Sus barbatus*. $\delta^{18}O$ values ranged from -32.93 to -26.15 with an average of -28.42.

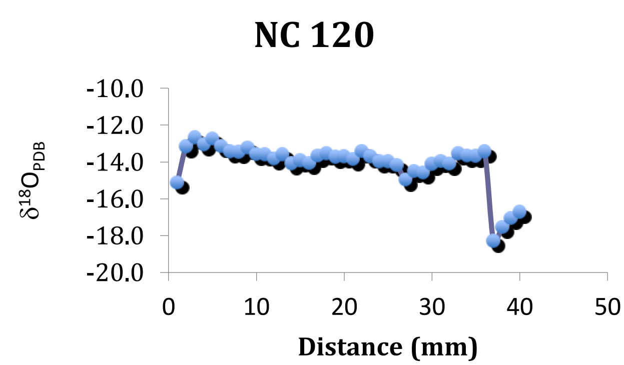

Figure 5. Sample NC 120*: Bos*. $\delta^{18}O$ values ranged from -16.71 to -12.66 with an average of -14.10

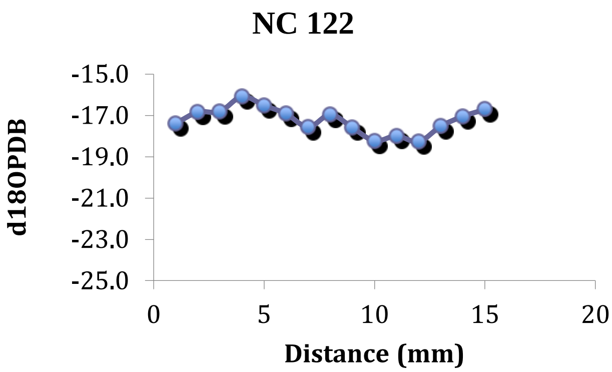

Figure 6. Sample NC 122: *Bos.* $\delta^{18}O$ values ranged from -18.27 to -16.10 with an average of -17.23.

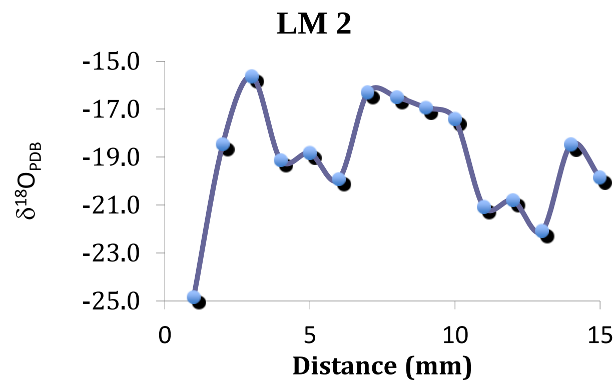

Figure 9. Sample LM 2: *Bos.* $\delta^{18}O$ values ranged from -24.85 to -15.64 with an average of -19.09.

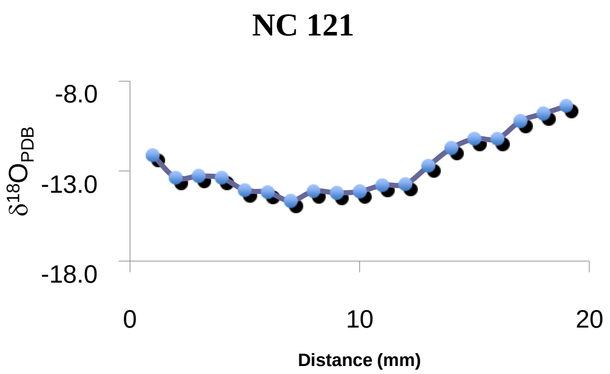

Figure 7. Sample NC 121: *Bos.* $\delta^{18}O$ values ranged from -14.66 to -9.39 with an average of -12.70.

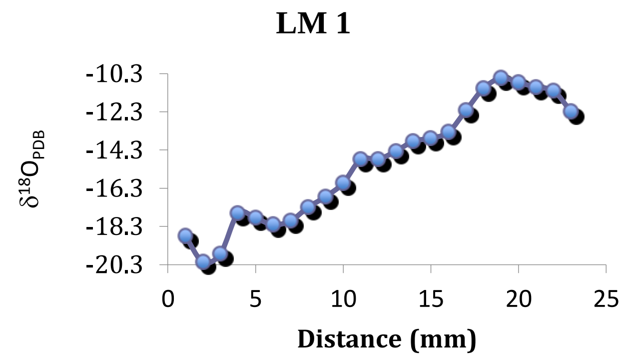

Figure 10. Sample LM 1: *Bos.* $\delta^{18}O$ values ranged from -20.16 to -10.54 with an average of -14.97.

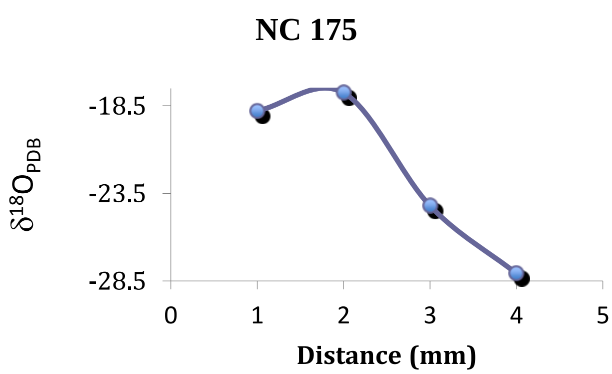

Figure 8. Sample NC 175: *Bos.* $\delta^{18}O$ values ranged from -28.08 to -17.76 with an average of -22.21.

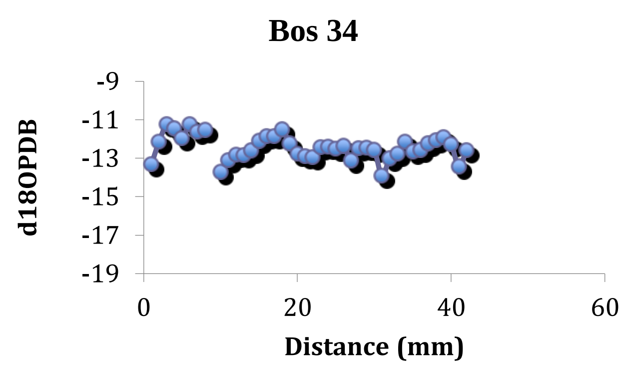

Figure 11. Sample: Bos 34: *Bos.* $\delta^{18}O$ values ranged from -13.92 to -11.24 with an average of -12.58.

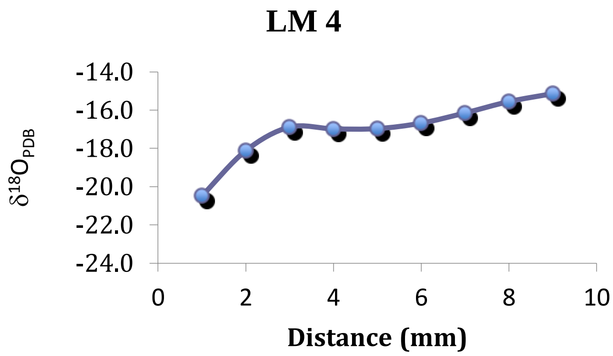

Figure 12. Sample LM 4: *Rhinoceros.* δ^{18}O values ranged from –20.49 to -15.14 with an average of -17.70.

DISCUSSION

The results of this study confirm LA-CF-IRMS as a successful method for incrementally and sub-annually analyzing small mammalian tooth enamel for δ^{18}O and δ^{13}C values. The isotopic ratios obtained reflect sub-annual data during enamel formation, regardless of consistency or variance. δ^{18}O graphs that do display sinusoidal curves may be due to changes in weather patterns occurring in the organism's surrounding environment, while δ^{18}O graphs displaying consistency may correlate to weather conditions that were unchanging throughout the year. However, analyzing each sample within a larger time context becomes difficult due to the variance in and uncertainty of age. While radiocarbon dates are available for some samples, estimates according to site stratification and the position of sample recovery are the best indication of age available. If more specific ages were available, the success of this in-situ laser ablation method of micro sampling faunal tooth enamel could prove useful in adding to the terrestrial record at Niah Cave during the periods of global and regional climate changes following the last glacial maximum, and ultimately provide insight into human patterns and behaviors during these times during which the record is incomplete.

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