The Reconstruction of Ancient Diets and Environments

Mr. Jorge A. Natal, Secondary Educator, East Lake High School, Tarpon Springs, Florida
Mr. Mark W. Dignan, Secondary Educator, James S. Rickards High School, Tallahassee, Florida

PURPOSE:

The purpose of the laboratory research is to reconstruct ancient diets and environments using stable carbon and oxygen isotopes in fossil teeth.

Analysis of the fossil tissues of ancient animals using carbon isotopes has enabled scientists to determine the food ancient animals consumed and to infer what type of habitats they lived in. Since most living organisms spent a substantial amount of time trying to replenish lost energy, determining how this energy was obtained is crucial. Information about ancient diets and environments is also important for understanding the evolution of Earth's climate system and how organisms responded to past changes in the environment. This data enables us to discover the evolutionary changes that may have occurred due to dietary differences.

Morphologically, teeth are shaped to process the type of food the organism eats. Scientists now know that foods rich in hard fruits or grasses leave microscopic traces on teeth as well as distinct damage patterns on enamel surfaces (Fig. 1). Yet, not all teeth adaptations lead to the actual behavior of the animal.

So scientists turned to another investigative approach probing for the information locked in the crystal structure of fossil tooth enamel. Tooth enamel is a crystalline mineral made up mostly of calcium and phosphate along with other small amounts of other ions, including carbonate. These ions are then preserved in the tooth when it was forming. In addition, these carbonate ions inside the enamel have specific carbon and oxygen isotope ratios that are determined by the carbon and oxygen isotope ratios in food and water consumed by the animal. These isotope ratios are what we will be investigating to determine environmental and historical conditions of particular species (Fig. 2).

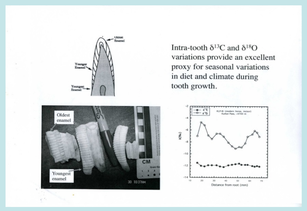

Figure 1
Enamel coated shown in white and stripes.

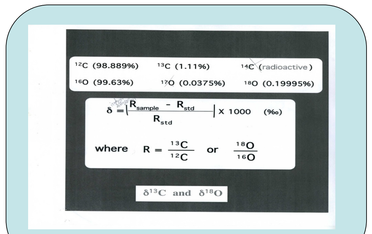

Figure 2
Formula used calculate the 12C/13C ratio

INTRODUCTION:

The ratios of the two stable isotopes of carbon (distinguished only by their atomic mass, ^{13}C and ^{12}C), provide a natural tracer for the chemical and biochemical reactions of the carbon cycle. In our research, the fossil teeth are about 50,000 years old and were from a Late Pleistocene fossil cave (Baxian Cave) in Guangxi Province, South China. The study area is currently located within the subtropical evergreen forest zone which is dominated by plants using the C3-photosynthetic pathway – C3 plants, with only a minor amount of C4 plants using the C4 photosynthetic pathway (Fig 3). Some plant examples for C3 plants are trees, shrubs, forbs, and cool season grasses. The plant example for C4 plants are warm season grasses such as corn, amaranth, sugarcane, sorghum, and some of the millets. Because they use different photosynthetic pathways to fix carbon, these two groups of plants have very distinct $^{13}C/^{12}C$ ratios. Animals incorporate the plant carbon they eat into their tissues, which then directly reflect proportions of C_4 grasses and C_3 plants eaten (Kohn et al., 2005). In order to adequately retrieve the isotope ratios of our enamel samples, a gas spectrometer must be used to separate each isotope variation. The way this is done is by first positively ionizing the elements into ion beams that are run through the mass spectrometer. Then the spectrometer increases the speed of the ions so that each variation is at the same speed. The mass spectrometer then deflects the moving ions by magnetism. Ions with different isotopes are separated into categories based upon their mass and is then recorded electronically to determine the ratio of different isotopes (Clark, 2014).

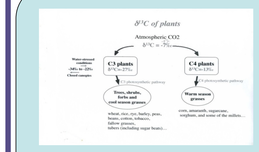

Figure 3
Categorization of isotopic variation of C3 and C4 plants

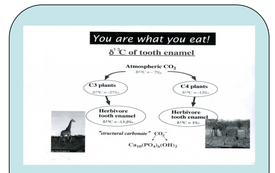

Figure 4
Diagram of diet variation for tooth enamel of various species

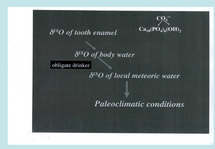

Figure 5
Categorized animals based on their food intake

EXPERIMENTAL PROCEDURES

1. Drill the serial sample teeth by taking weighing paper and folding it in half to collect the enamel powder, once cleared of debris found on the teeth (Fig. 6a).
2. Once 24 samples have been collected, **add 1 mL of 5% sodium hypochlorite solution to oxidize organic material** in each sample, mix them, and let it sit to react over night.
3. Rinse each sample with distilled water after pipetting the previous solution out by placing the samples in a centrifuge to separate the sample and the solution, and then adding the 1 mL of distilled water to each sample. Repeat this process 3 times.

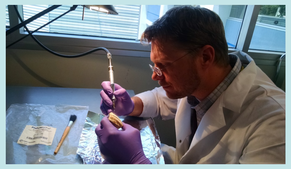

Figure 6a
The process of cleaning and grinding the enamel off a teeth.

Figure 6b
Centrifuge is used to rid the sample of impurities

4. Rinse each sample with acetic acid to extract any non-structural carbonates by adding 1 mL of the acid, shaking it, and centrifuging it to remove the reacted to acetic acid. Then an additional 1 mL of acidic acid is added to each sample for it to sit over night (Fig. 6b).
5. Repeat the process for **rinsing each sample with distilled water** to remove the solution in sample, as demonstrated in step 3.
6. Freeze-dry each sample by covering each sample with aluminum foil and poking a hole in it. Then place the samples in the freezer to sit overnight, so those frozen samples can be taking into the freeze dryer to remove any moisture (Fig 6c).

Figure 6c
Placing samples in the freeze dryer.

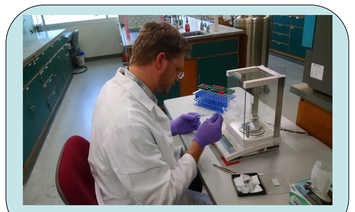

Figure 6d
Weighing the samples and carbonate standards in the microbalance

7.Weigh each sample and corresponding carbonate standards *on a microbalance. This will provide a basis of comparison to the flushed samples in the future use of the mass spectrometer* (Fig. 6d).
8.Bake each sample overnight to remove any additional moisture.
9. Flush each sample and standard with Helium to remove air from each sample and any carbon dioxide that exists in the air.
10. **Add 8-10 drops of 100% phosphoric acid** to each sample and let it sit over night to be able to react with the structural carbonate when the mass spectrometer runs through the sample (Fig. 6e).
11. **Run each sample through the mass spectrometer** to collect the isotopic variation data of oxygen and carbon (Fig. 6f).

Figure 6e
Pipetting 100% phosphoric acid to the samples

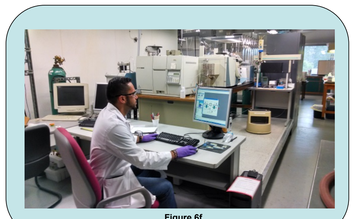

Figure 6f
Observing and analyzing the results of the samples.

RESULTS:

Table 1 Calibrated and Raw Data of Sample BXC-08

Lab #	Distance From Crown	$\delta^{13}C$	$\delta^{18}O$	$\delta^{13}C$ avg	stdv	$\delta^{18}O$ avg	stdv
BXC-08-01a	1 mm from crown	-15.9	-7.9	-19.1129	0.049727	37.6779	0.081528
BXC-08-01b	4 mm	-16.2	-9.4	-19.3574	0.038699	36.0781	0.073248
BXC-08-02a	6 mm	-16.1	-6.7	-19.274	0.067877	38.9108	0.090989
BXC-08-02b	8 mm	-16.3	-10.2	-19.4437	0.074886	35.30214	0.093328
BXC-08-03	12 mm	-15.9	-9.7	-19.0709	0.08996	35.80643	0.096196
BXC-08-04	16 mm	-16.1	-10.3	-19.2695	0.094165	35.1685	0.08971
BXC-08-05	19 mm	-16	-7	-19.1714	0.069455	38.53467	0.104645
BXC-08-06	21 mm	-16.2	-7.4	-19.3736	0.05673	38.205	0.092081
BXC-08-07	23 mm	-16.1	-6.8	-19.2556	0.08105	38.7958	0.052004
BXC-08-08	25 mm	-16.1	-6	-19.2856	0.070872	39.60356	0.073628
BXC-08-09	27 mm	-16.3	-7.1	-19.5268	0.052198	38.4744	0.051632
BXC-08-10	30 mm	-16.5	-7.6	-19.706	0.058195	37.9839	0.072212
BXC-08-11	32 mm	-16.3	-6.8	-19.5195	0.058735	38.7546	0.081097
BXC-08-12	34 mm	-16.6	-7	-19.7939	0.026227	38.5684	0.061778
BXC-08-13	36 mm	-16.6	-7.3	-19.8194	0.056875	38.2985	0.063588
BXC-08-14	38 mm	-16.5	-6.9	-19.7135	0.059584	38.6417	0.067562
BXC-08-15	40 mm	-16.9	-6.8	-20.0649	0.073194	38.7941	0.082936
BXC-08-16	42 mm	-16.7	-6.1	-19.8671	0.091957	39.5274	0.081594
BXC-08-17	44 mm	-16.8	-6.5	-19.977	0.039758	39.0607	0.068474
BXC-08-18	46 mm	-16.8	-6.5	-20.0182	0.057281	39.1301	0.049357

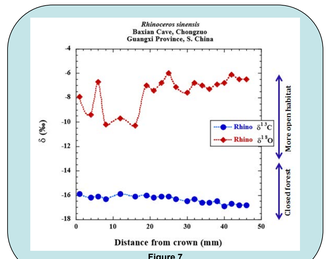

Figure 7
Graph representing calibrated data from the Mass Spec

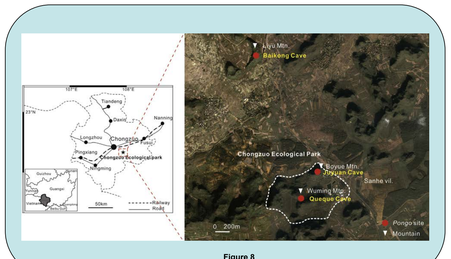

Figure 8
This is the region of the Chongzuo Ecology Park where the fossils were collected.

The data from the *Rhinoceros sinensis* show that there is no significant intra-tooth carbon isotopic variations (Fig. 7). This indicates that there is little or no seasonal variation in its diet. Based on the carbon isotope data, the *R. sinensis (*BXC-08) was a species that consumed C-3 plants (Figs. 4 and 7). In addition, the oxygen isotope variation is correlated with the standard seasonal variation, with the exception of the BXC-08-05 sample. The oxygen isotope variation data, associated with water consumption of the *R. sinensis*, suggests that water was obtained from two sources: meteoric/surface drinking water and leaf water.

CONCLUSION

The *Rhinoceros sinensis* was a species that existed during the Pleistocene Epoch in the Baxian Cave area located in the Guangxi Province in South China. A serial sample was taken from the enamel of the species to determine its isotopic variation of carbon and oxygen intake during development. The data confirmed that the species did consume C-3 plants and experienced a full seasonal cycle during it's development which enables us to be informed of the migration, location and identification of this species. The range in $\delta^{13}C$ values (−15.9‰ to −16.9‰) indicates that R. sinensis fed on solely C3 biomass, and lived in dense forest habitats, and not open country or savannas.

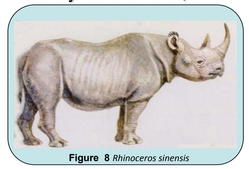

Figure 8 *Rhinoceros sinensis*

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