Ancient Maya Mobility at Caledonia, Cayo District, Belize: Evidence from Stable Oxygen Isotope Analysis

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Mobility among the ancient Maya at Caledonia, Cayo District, Belize was investigated using stable oxygen isotope analysis of 15 bone and five tooth enamel carbonate samples. While all samples exhibited values expected for the site area, some variation was detected. Three individuals from Burial 1 had the most positive bone oxygen isotope values, indicating they may have lived elsewhere before moving to Caledonia later in life. The difference between the enamel and bone oxygen isotope values suggests a non-local origin for one individual from Burial 3. Finally, the four individuals who may have consumed marine protein exhibited local bone oxygen isotope values and likely did not move to Caledonia from a coastal region in the last several years of their lives.

Introduction
Isotope analysis of human skeletal and dental tissues has become a common means of addressing archaeological questions of diet and mobility in past populations (see Katzenberg 2008). The stable isotopes of carbon and nitrogen in human skeletal and dental material from the ancient Maya site of Caledonia, located in the Cayo District of Belize, have been previously analyzed to investigate the diet of these individuals (Rand et al. 2013). Stable oxygen isotope values were also obtained as a by-product of analyzing stable carbon isotopes in bone and tooth enamel carbonate. This study examines the oxygen isotope values from 15 bone and five enamel samples from the Caledonia Maya to determine whether any of these individuals moved to the site before their deaths.

Site and Sample Description
Caledonia was a minor Maya centre consisting of four plazas situated at the intersection of the limestone-based Vaca Plateau and the granitic, pine-forested Pine Ridge region in the southern part of the Cayo District in Belize (see Figure 1). The site is located roughly 550 m above sea level and 80 km from the Caribbean coast (Awe 1985:15). Caledonia was constructed on the west bank of the Macal River, which would have provided drinking water and a variety of food resources for the site residents (Awe 1985:31). Although first settled during the Late Preclassic Chichanel phase around 100 C.E., Caledonia primarily dates to the Late and Terminal Classic periods (600 to 1000 C.E.; Awe 1985:388).

Five burials containing the remains of at least 22 individuals were encountered during the 1980 and 1984 excavations (Awe 1985). In order to investigate the diet using stable carbon and nitrogen isotope analysis (Rand et al. 2013), three individuals were sampled from Burial 1, a tomb located in Str. A-1 dating from 500 to 800 C.E. (Healy et al. 1998). An additional four
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individuals were sampled from Burial 3, a cist burial dating from 675 to 800 C.E. located in Str. C-2, and seven fibulae and three mandibles were sampled from Burial 4, a tomb located in Str. C-2 dating from 600 to 800 C.E.. Finally, the individual from Burial 5, a simple burial located in Str. C-1 dating from 300 B.C.E. to 250 C.E. was also sampled. A detailed discussion of Caledonia and the sampled individuals can be found elsewhere (Awe 1985; Rand 2012).

Stable Oxygen Isotope Analysis

Stable oxygen isotope analysis can be used to investigate human mobility because the values in skeletal tissues reflect those of water consumed during life (Longinelli 1984; Luz et al. 1984). The ratio of the stable isotopes $^{16}$O and $^{18}$O in a sample is measured using a mass spectrometer where it is compared to the $^{16}$O/$^{18}$O of the standard reference material Vienna Standard Mean Ocean Water (VSMOW) for oxygen isotopes in phosphate (PO$_4^-$) and carbonate (CO$_3^-$), although oxygen isotopes from CO$_3$ may also be presented relative to Vienna Pee Dee Belemnite (VPDB) (Coplen 1994). The results are presented as $\delta^{18}$O values in permil ($\%$).

Hydroxylapatite (Ca$_5$[PO$_4$]$_3$OH), or bioapatite (ap), is the mineral component of bone and tooth
enamel. Oxygen is found in PO$_4$ and hydroxide (OH) as well as in CO$_3$, which can substitute for both in bioapatite (LeGeros 1981). Because the P – O bond is strong, bioapatite PO$_4$ is chemically stable and is considered to be resilient to post-mortem chemical alteration (diagenesis) relative to CO$_3$ (Nielsen-Marsh and Hedges 2000a, 2000b). Given proper preparation techniques, CO$_3$ can also produce oxygen isotope values that reflect biogenic $\delta^{13}$C and $\delta^{18}$O values (Koch et al. 1997; Nielsen-Marsh and Hedges 2000b). There is also a direct relationship between the $\delta^{18}$O values of body water, PO$_4$, and CO$_3$ from the same individual, and thus these values can be converted using developed equations (Chenery et al. 2012). Any comparison of $\delta^{18}$O values between individuals and sites is, however, considered tentative as $\delta^{18}$O values for the same sample obtained from different laboratories can differ by several permil (Pestle et al. 2014).

**Stable Oxygen Isotope Analysis in Mesoamerica**

Drinking water oxygen isotope ratios ($\delta^{18}$O$_w$) are a function of the local climate; more negative $\delta^{18}$O$_w$ values are associated with higher amounts of precipitation, increasing distance from the sea, higher altitude, increasing humidity, and decreasing temperature (Rozanski et al. 1993). In Mesoamerica, atmospheric circulation is dominated by the north easterly trade winds, as well as moisture convergence in the Intertropical Convergence Zone (ITCZ) and the North Atlantic High (Bermuda-Azores High). These fluctuate (Hodell et al. 2005), and so this area experiences a dry season from December to May followed by a wet season from June to November, punctuated by a period of decreased precipitation known as the midsummer drought (Magaña et al. 1999). Seasonal variation in $\delta^{18}$O$_w$ values due to differential precipitation (i.e., seasonality) is present in Belize and Guatemala (Lachniet and Patterson 2009; Wright 2012:336); however, this should not affect this study as these values are homogenized as they are incorporated into human tissues (see Smith and Tafforeau 2008).

The majority of Mesoamerican studies that have utilized human $\delta^{18}$O values to assess mobility in the past have focused on high status individuals at major sites (e.g., Price et al. 2010; Wright 2012), although recent research has begun to focus on movement between smaller sites within a single region (e.g., Freiwald 2011 looked at movement within the Belize River Valley). These studies have found that mobility across Mesoamerica was characterized by variability with no apparent relationships between a person’s ability to move and his or her age, sex, social status, or time in which he or she lived.

**The Effect of Breastfeeding on Enamel Stable Oxygen Isotopes Values**

Oxygen isotope ratios become more positive when they are incorporated into the body from drinking water because $^{16}$O is preferentially lost in expired water vapour (Bryant et al. 1996). Breast milk is comprised of body water and therefore has elevated $\delta^{18}$O values, which are passed on to breastfeeding infants and reflected in the tooth enamel that forms during this period. As the infant is weaned, the $\delta^{18}$O values decrease as more water is ingested. Attempts have been made to counter the effect of breastfeeding by adjusting the $\delta^{18}$O$_e$ values of teeth that form during the breastfeeding period by -0.7‰ to -0.35‰ (e.g., Spence 2005; White et al. 2007). However, due to variability in the duration of nursing between individuals and sites (Wright 2012:341), time periods, and the amount by which researchers adjust the $\delta^{18}$O$_e$ values for different sites, the values obtained from the Caledonia individuals will remain unadjusted here.
Identifying Non-Local Individuals using Stable Oxygen Isotope Values

Enamel forms during infancy and early childhood (Moorrees et al. 1963), and does not remodel, thus preserving δ^{18}O_w values ingested during early life. In contrast, bone is remodelled throughout life (Parfitt 2001) and reflects the δ^{18}O_w values consumed several years before death. Therefore, an individual’s tooth enamel preserves the isotopic signature of the area in which they grew up, regardless of where they were buried. This childhood δ^{18}O_w value is temporarily reflected in her or his bone, which over time equilibrates with local δ^{18}O_w values. The relocation of this individual may therefore be detected if there is a large difference between the δ^{18}O values in his or her bone and tooth enamel bioapatite (Δ^{18}O_{bio}) values.

Alternatively, non-local individuals may be identified as those whose δ^{18}O values differ from the δ^{18}O values for local human remains. This centres on establishing the expected local δ^{18}O values, which can be difficult. One approach is to assign local status to burials based on the archaeological context. The mean δ^{18}O value of these “local” individuals is then considered representative of δ^{18}O values expected from the environment surrounding the site (e.g., Price et al. 2010; White et al. 2007). This is, however, problematic because sampled nonlocal individuals will unknowingly contribute to this “local” average (White et al. 2004). Therefore, researchers have also compared human δ^{18}O values with modern environmental water oxygen isotope values (δ^{18}O_w) near the site of interest (e.g., Freiwald 2011; Wright 2012). In the absence of δ^{18}O_w for the Vaca Plateau in Lachniet and Patterson’s (2009) study of ground water in Belize and Guatemala, the single value of -6.8‰ (VPDB) obtained for the Macal River (Freiwald 2011:248) is used as the environmental baseline for comparison with the Caledonia data. To do so, the original human Caledonia δ^{18}O values have been adjusted using the equation in Iacumin et al. (1996; δ^{18}O_{ap} = [δ^{18}O_w * .998] + 33.63) to reflect local drinking water values. It is recognized that the Caledonia skeletal δ^{18}O values may differ slightly from the baseline δ^{18}O_w value due to observed chronological changes in δ^{18}O_w in this region (Curtis et al. 1996), as well as the interlaboratory variation in δ^{18}O values (Pestle et al. 2014) discussed above.

Analytical Techniques

The original sex and age estimates of the Caledonia skeletons (Awe 1985) were confirmed and expanded upon using standard techniques (Buikstra and Ubelaker 1994; see Rand 2012). All sampled individuals were adults at the time of their deaths and the sex of all individuals, excluding the female from Burial 5, remained indeterminate due to the fragmentary condition of the collection.

Samples were prepared for isotope analysis at the Bioarchaeology Laboratory at Trent University. Bone bioapatite was isolated following Koch et al. (1997), where cleaned bone samples were powdered and treated with sodium hypochlorite (NaClO) followed by acetic acid (CH₃COOH) buffered with calcium acetate (C₄H₆CaO₄). Tooth enamel bioapatite samples were collected using a diamond-tipped dremel and prepared the same way as bone. Samples were analyzed on a Fison’s Optima dual inlet isotope ratio mass spectrometer (DI-IRMS) at McMaster University. The analytical precision was .1‰ and .08‰ or better for tooth enamel and bone bioapatite, respectively. Analytical accuracy could not be calculated because the lab standards were not provided. Details on sample preparation and analysis can be found elsewhere (Rand 2012).

The post-mortem alteration of δ^{18}O_b and to a lesser extent δ^{18}O_e values resulting from
diagenesis is well known (Nelson et al. 1986; Nielsen-Marsh and Hedges 2000a). Tooth enamel is considered to be less susceptible to diagenesis than is bone; however, diagenetic alteration of both tissues is possible. Sample integrity was testing by calculating the crystallinity index (CI) of as subset of 10 samples using Fourier transform infra-red (FTIR) spectrometry (Shemesh 1990). Acceptable CI values of bone mineral range from 2.8 to 4.4 (Wright and Schwarcz 1996) and two modern tooth enamel samples had values of 3.8 and 4.4 (Keenleyside et al. 2011). Samples were prepared following Wright and Schwarcz (1996) and were scanned using a Nicolet 6700 FT-IR Spectrometer

### Table 1: $\delta^{18}O_{\text{VPDB}}$ values and crystallinity indices for human bone and tooth enamel samples from Caledonia.

<table>
<thead>
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<th>Sample #</th>
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<tr>
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<td>-</td>
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<td>-6.3</td>
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</tr>
<tr>
<td>A1-1-LM3</td>
<td>4.2</td>
<td>-6.6</td>
<td>3.6</td>
<td>-</td>
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</table>

Note: All values are reported relative to the VPDB standard and bolded values have been removed from this study (see text).

*Data from Rand et al. (2013)

$^a$ $\delta^{18}O$ values have been adjusted using the equation in Iacumin et al. (1996; see text).

$^b$ Values were reported for the wrong individuals in Rand et al. (2013) and have been corrected here.
in the Biomaterials Research Laboratory at Trent University.

Finally, relationships among the isotope data were tested using the non-parametric Spearman’s rank order correlation ($\rho$) and Mann-Whitney U ($Z$) statistical tests due to small sample size. All statistical analyses were run using the IBM SPSS Statistics, Version 20.0.

**Results and Discussion**

**Preservation of the Caledonia Samples**

The $\delta^{18}$O and CI values for the Caledonia individuals can be found in Table 1. The average CI for the bone and enamel samples were $3.7 \pm .1$ and $3.8 \pm .3$, respectively, and all values were within acceptable ranges (Rand et al. 2013). If the original biogenic $\delta^{18}$O values were altered by post-mortem recrystallization they would be correlated with their CI values, which was the case for neither bone ($\rho = .8; p > .1$) nor enamel ($\rho = .5; p > .1$). The $\delta^{13}$C value in the permanent maxillary first molar (M1) of C2-3-A was removed from a previous analysis because it was an extreme outlier (Rand et al. 2013) and the $\delta^{18}$Oe value for this tooth was similarly removed here.

**Identifying Non-local Individuals at Caledonia**

The $\delta^{18}$Oe values from Caledonia range from $-8.3\%$ to $-6.3\%$ (mean $-7.3 \pm .5\%$), while the $\delta^{18}$Ob values ranged from $-7.9\%$ to $-6.5\%$ (mean $-7.2 \pm .7\%$) which encompass the $\delta^{18}$Ow value for the Macal River ($-6.8\%$; see Figure 2). While this implies that all individuals have values consistent with the local environment, variation among the samples is present. This variability may be explained by fluctuations in $\delta^{18}$Ow values related to chronological changes in precipitation (e.g., Curtis et al. 1996), as the Caledonia burials were interred during different periods. Alternative explanations are explored in more detail below.

**Outlying $\delta^{18}$O Values**

None of the $\delta^{18}$O values fell beyond one standard deviation of the mean $\delta^{18}$Oe value. Therefore these individuals likely consumed drinking water available around Caledonia, and thus resided near the site during the formation of these teeth. However, four individuals (C2-4-F1, A1-1-LM1, A1-1-LM2, and A1-1-LM3) had outlying $\delta^{18}$Ob values that fell beyond one standard deviation of the mean $\delta^{18}$Oe value. Three of these individuals were interred in Burial 1 and had $\delta^{18}$Oe values slightly more positive than the value from the Macal River (see Figure 2) and significantly more positive than those from Burial 4 ($Z = -2.4; p < .05$) and Burial 3 ($Z = -2.2; p < .1$). While the Burial 1 remains may have been affected by diagenesis due to soil hydrology (Hedges and Millard 1995; Wright and Schwarz 1996), this is unlikely because the burial was located atop a low hill above the water table and the single individual assessed using FTIR had an acceptable CI value. In contrast, the individuals from Burial 1 may have participated in different cultural practices than others at Caledonia. For example, they may have consumed stored water, which can have more positive $\delta^{18}$Oe values due to evaporation (Lachniet and Patterson 2009; Wright and Schwarz 1996), or plant foods with fluids with much more positive $\delta^{18}$O values as a result of evapotranspiration (Bariac et al. 1990). Finally, it may be that the Burial 1 individuals moved from an area with more positive $\delta^{18}$Ow values and their bones were in the process of equilibrating with more negative $\delta^{18}$Ow values around Caledonia. Such positive $\delta^{18}$Oe and $\delta^{18}$Oe values occur at several major Maya sites (Williams et al. 2009; Wright 2012). However, because the majority of Maya mobility likely occurred over short distances (Freiwald 2011) the most probable origin location for these individuals are sites in the nearby Belize River Valley (see Figure 1) although this is difficult to
Figure 2: $\delta^{18}O$ values in human bone and tooth enamel samples from Caledonia. The Macal River baseline value of -6.8‰ is from Freiwald (2011) and the tooth enamel sample from C2-3-A was removed from this analysis.

confirm. Furthermore, the teeth of these individuals were unavailable for analyses, and therefore childhood $\delta^{18}O$ values could not be investigated.

Comparing $\delta^{18}O$ Values in Multiple Tissues from the Same Individual

As discussed above, the relocation of an individual can be detected if there is a sufficient difference between his or her $\delta^{18}O_b$ and $\delta^{18}O_e$ values ($\Delta^{18}O_{e-b}$). Due to poor preservation, only two individuals (C2-3-C and C1-5-A) were sampled for both bone and tooth enamel, and therefore these were the only two individuals for whom $\Delta^{18}O_{e-b}$ values could be calculated.

The permanent mandibular second molar ($M_2$) from C1-5-A, the older adult female from Burial S, produced a $\delta^{18}O_e$ value +.3‰ relative to her $\delta^{18}O_b$ value. The enamel of the $M_2$ crown begins to mineralize around three years and is completed around nine years of age (Moorrees et al. 1963). As discussed above, stable oxygen and nitrogen isotope analyses indicate weaning among the ancient Maya was a highly variable process, likely beginning around age one year and ending anywhere from ages three to six years (Williams et al. 2005; Wright and Schwarz 1998). Thus, the more positive $\delta^{18}O_e$ value from the C1-5-A’s $M_2$ may reflect some input from breast milk and this individual is considered to be local. Considering C1-5-A is likely one of the first inhabitants of Caledonia (Awe 1985:116), it may be that she moved to the site from a nearby area and/or one with similar $\delta^{18}O_w$ values.

The $\delta^{18}O_e$ value from C2-3-C’s permanent mandibular third molar ($M_3$) lies near that of the Macal River (Figure 2), and was not likely influenced by breastfeeding as the enamel of this tooth forms between the ages of 8 and 15 years (Moorrees et al. 1963). The $\delta^{18}O_b$ value for this individual was more negative than his or her $\delta^{18}O_e$, and is closer to the mean value from Caledonia (Figure 2). Although both could be argued to reflect local $\delta^{18}O_w$ values, the 1.2‰ difference between the sampled tissues suggests this individual consumed water with slightly more positive $\delta^{18}O_w$ values during late childhood compared to the water consumed in early adulthood. While this could reflect differential cultural practices, as was discussed above for the
Burial 1 individuals, it may be that C2-3-C moved to Caledonia from a nearby area with slightly more positive δ¹⁸O
values.

**Consumption of Marine Protein and Mobility at Caledonia**

It has been previously proposed that four individuals from Caledonia (C2-3-B, C2-3-D, C2-4-F1, and C2-4-F6) may have consumed marine protein (Rand et al. 2013). This is based on the small (i.e., under 3‰) difference between the δ¹³C values in bone carbonate and collagen (δ¹³C_{ap} and δ¹³C_{col}, respectively), called the collagen-to-bioapatite spacing (Δ¹³C_{ap-col}) which can reflect the consumption of marine protein (Lee-Thorp et al. 1989; Williams et al. 2009). This was unexpected because Caledonia is located far inland and its inhabitants likely did not have sufficient political or economic influence to import marine foods. Marine shell ornaments were, however, found on the site (Awe 1985:362-371), and it was theorized that the Caledonia individuals with Δ¹³C_{bio-col} values below 3‰ may have moved to Caledonia from a coastal area, or a site where marine foods were available for consumption (Rand et al. 2013). Teeth were unavailable for these individuals and thus only their δ¹⁸O_{b} values were examined to assess whether this was the case. Interestingly, these individuals had δ¹⁸O values significantly more negative than individuals with Δ¹³C_{col-ap} values above 3‰ (Z = -18.4; p < .1), which is the opposite trend of what would be expected had they moved inland from a coastal site (Rozanski et al. 1993). Individual C2-4-F1 exhibited the most negative Δ¹³C_{ap-col} value from Caledonia, falling beyond one standard deviation of the mean. It is possible this individual moved to Caledonia from an area with more negative δ¹⁸O_{w} values (i.e., Guatemalan Highlands; Lachniet and Patterson 2009); however, the integrity of this sample is questionable as its CI value was not obtained.

The remaining individuals with low spacing values (C2-3-B, C2-3-D, and C2-4-F6) exhibited slightly more negative δ¹⁸O_{b} values than the Macal River, but similar to the mean value from Caledonia. It is therefore likely these individuals resided in Caledonia or a similar area rather than a coastal one several years before their deaths. However, only C2-3-B was assessed for diagenesis (see Table 1) and none of these individuals had associated teeth so their childhood δ¹⁸O values could not be assessed.

Finally, it is possible that the low Δ¹³C_{ap-col} values do not reflect the consumption of marine resources. Low values may, for example, represent extreme carnivory (Lee-Thorp et al. 1989), although this is unlikely among the Caledonia Maya because previous isotopic investigations at Caledonia suggest the consumption of various food resources (Rand et al. 2013) and isotopic studies of ancient Maya at other sites do not support the consumption of large quantities of meat (White 1999). Alternatively, Hedges (2003) has argued that dietary protein may only account for half of spacing values, and other influencing factors, such as non-equilibrated bone synthesis, may contribute to these low spacing values.

**Conclusion**

The origins of the individuals sampled from Caledonia, a minor Maya centre located on the Macal River on the Vaca Plateau, were investigated using stable oxygen isotope analysis. The sampled Caledonia individuals had similar mean δ¹⁸O_{b} and δ¹⁸O_{e} values that were both slightly more negative than a modern δ¹⁸O_{w} value from the Macal River. Variation in the values may be explained by chronological changes in δ¹⁸O_{w} values in drinking water available to site inhabitants, differential cultural practices, or the
arrival of nonlocal individuals at the site. The three individuals sampled from Burial 1 had the most positive $\delta^{18}O_b$ values indicating they may have relocated to Caledonia from elsewhere during adulthood. Both $\delta^{18}O_b$ and $\delta^{18}O_e$ values were analyzed in only two Caledonia individuals: C2-3-C likely moved to the site from a nearby area whereas C1-5-A is considered local. Finally, all of the individuals who may have consumed marine protein, excluding C2-4-F1, exhibited $\delta^{18}O_b$ values slightly more negative than the mean and Macal River values, the opposite of what is expected had they moved from a coastal area. Individual C2-4-F1 exhibited the most negative $\delta^{18}O$ value and may have moved to Caledonia from an area with more negative values (i.e., Guatemala Highlands). It should be kept in mind that enamel reflecting childhood $\delta^{18}O_w$ values and the affect of diagenesis were not assessed for all of these individuals and so these interpretations remain tentative.

This research supports the recent argument that mobility among the Maya primarily occurred over relatively short distances within a single region such as the Belize River Valley (Freiwald 2011), or in this case the Vaca Plateau. It also demonstrates how the analysis of $\delta^{18}O$ values in both bone and tooth enamel from curated skeletons excavated from minor Maya sites can contribute to theories about Maya mobility in general. The use of additional diagenetic indicators, sampling bone and enamel from the same individual, analyzing strontium isotopes in tooth enamel, and the collection of more environmental water samples from the area around Caledonia may further the interpretations presented here.

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