Investigating season of death using carbon isotope data from the hair of 500 year old Peruvian mummies

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The primary focus of bioarchaeology is to understand the life experiences of past populations using data from both biological and archaeological contexts (Larsen, 2002). Mortality and morbidity are universally experienced by all individuals but in seasonal environments, the frequency of each is not constant year round. In modern populations living in seasonal environments, winter is generally the period of highest mortality (Becker and Weng, 1998; van Rossum et al., 2001; Wyndham and Fellingham, 1978) with low temperature exhibiting a strong correlation to high mortality rates (Moran, Johnson and Johnson, 2000; Wyndham and Fellingham, 1978; Yan, 2000). Studies on modern populations demonstrate that summer is characterized by increased morbidity from such factors as: bacterial diarrhea (Lanata et al., 1992; Martin et al., 1996; Villa et al., 1999), cholera (Franco et al., 1997; Koelle et al., 2005; Lama et al., 2004); malaria (Guthman et al., 2001); and parasitic infection (Martinson et al., 2003; Ortega and Bonavia, 2003). However, in general these summer diseases have lower mortality rates than the acute respiratory diseases (e.g., pneumonia, asthma, otitis media, and bronchitis) that characterize the winter months. For example, in 2006, respiratory diseases accounted for 12% of all mortality in Peru and diarrhoeal diseases accounted for only 3% of all deaths (World Health Organization, 2006). Seasonal patterns in morbidity and mortality have also been observed in historic populations (Madrigal, 1994; Malina and Himes, 1977; Marcuzzi and Tasso, 1992; Moffat and Herring, 1999; Munoz-Tuduri, Garcia-Moro and Walker, 2006), although winter is not uniformly the most common season of death (see Herring and Hoppa, 1997; Landers and Mouzas, 1988; Sawchuk, 1993). With the epidemiological transition, seasonal fluctuations in mortality have steadily decreased (reviewed in Munoz-Tuduri, Garcia-Moro and Walker, 2006) due to a shift from exogenous to endogenous causes of death (Herring and Hoppa, 1997). As such, in prehistoric populations, seasonal fluctuations in morbidity and mortality should be even more pronounced than in modern or historic populations.

A promising method to reconstruct the season of death in archaeological populations relies on the isotopic analysis of sequential segments of hair (Fernández, Panarello and Schobinger, 1999; Sharp et al., 2003; White, 1993). Unlike bone, hair is not remodeled after formation; consequently its isotopic composition reflects diet at the time of tissue formation. Various studies demonstrate that, like bone, the carbon and nitrogen isotopic composition of hair reflects the individual's diet during life (DeNiro and Epstein, 1978, 1981; Minson, Ludlow and Troughton, 1975; Tieszen et al., 1983; Vogel, 1978). In environments where diet fluctuates with the seasons, and these dietary changes correspond to foods that are isotopically unique (i.e., plants utilizing different photosynthetic pathways), it is possible to use stable carbon isotope data to determine season of death from sequential segments of the individual's hair shaft. Although the rate of hair growth has not been extensively studied in humans, published studies show relatively good agreement, between 0.44 to 0.35 mm per day (reviewed in Saitoh, Uzuka and Sakamoto, 1967). Based on the growth rate of 0.35mm/day, one centimeter of hair corresponds to approximately one month of diet. Because hair grows incrementally, and is an inert tissue, its isotopic composition provides a short term record of diet for the weeks/months/years (depending on the length of hair) preceding death. Various studies on human and animals indicate that a change in diet is followed by a concomitant change in the isotopic composition of hair within 6-12 days (Jones et al., 1981; Katzenberg and Krouse, 1989; Nakamura et al., 1982).

The follicles that produce hair pass through controlled periods of growth including an active phase of hair growth (anagen) and a resting phase where the hair is not actively growing (telogen) (Ebling, Hale and Randall, 1991; Valkovic, 1977). The isotopic composition of hair in the telogen phase of growth will not reflect the newest diet until it resumes growth; conversely, hair in the anagen phase of growth will record diet virtually immediately (O'Connell and Hedges, 1999a). Recently, Lana Williams (2007) demonstrated that carbon and nitrogen isotope values differ between hairs in the anagen phase versus hairs in the telogen phase. This new research has important implications for paleodietary reconstructions using hair, since published studies to-date have sampled bulk hair regardless of growth phase (e.g., Fernández, Panarello and Schobinger, 1999; Knudson, Aufderheide and Buikstra, 2007; Macko et al. 1999; Sharp et al., 2003; White, 1993).

MATERIALS AND METHODS

Puruchuco-Huaquerones is a late Horizon (A.D. 1476-1532) cemetery located on the central coast of Peru, in the Rimac valley east of Lima. The Rimac river is approximately 150 km long and flows year-round, although run-off is greatest during the summer months (December to May). The site is ideally located to exploit a variety of biomes, it is within an hour's walk of the Rimac river, a four hour walk to the sea and a day's walk to the highlands. The area receives an average of 19.7 mm of precipitation through the year, mostly concentrated in the winter months (4.5 mm in summer vs. 15.2 mm in winter) when the coast is blanketed by *garua* (fog) (World Climate a). The average temperatures during the summer and winter are 21.1°C and 16.9°C respectively (World Climate b). Similar to most of the Peruvian coast, cultivation was facilitated by a series of pre-Hispanic irrigation canals fed by the Rimac river.

Samples of hair were obtained from a total of 59 individuals and bundles of 15-20 strands from each individual were cleaned with distilled water using an ultrasonic cleaner (following Aufderheide et al., 1994). The bundles of hair were then cut into sequential one centimeter increments, dried and analyzed in continuous flow using a Finnigan Mat Delta+Xl mass spectrometer employing elemental analysis-isotope ratio mass spectrometry technology. Analytical error for the carbon isotope analysis was ± 0.4‰. The accepted C/N ratio for modern hair is 2.9 to 3.8 (O'Connell and Hedges, 1999b); the average atomic C/N ratio of hair for this study was 3.7 ± 0.1. Two individuals were eliminated because their C/N ratio fell outside the accepted limits. In order to asses whether diet varied from month to month, maximum and minimum isotope values along an individual's hair shaft were identified (Table 1). If the maximum and minimum isotope values within the individual's hair shaft differed by at least 1‰, they were considered to exhibit dietary change. Seven individuals were excluded because they did not meet this criteria. Because some researchers have observed a trophic effect in carbon isotope ratios of nursing infants (e.g., Williams, White and Longstaffe, 2005; Wright and Schwarcz, 1998), individuals younger than six years (n = 12) were eliminated from these analyses. For 70% of the remaining sample (25/38), the maximum and minimum isotope values differed by 2‰ or more. The sample used in this analysis can be characterized as follows: 16 females (+4 probable female), 11 males (+1 probable male), six subadults (≥ 6 years), 10 young adults (20-35 years), 19 middle adults (35-50) and three old adults (50+). A chi-squared test was used to determine if the frequencies departed from the expected ratio and only p values less than .05 were accepted as significant.

The storage of foods for year-round consumption is a confounding factor for determining season of death, since it would be impossible to detect seasonal shifts in isotope values if foods were available regardless of the season of harvest. If individuals at Puruchuco-Huaquerones were storing crops for use year-round, there should be a fairly constant isotopic composition along an individual's hair shaft (i.e., from month to month) (see Schwarcz and White, 2004). The isotope values of the sequential samples of hair for most individuals from Puruchuco show variation from month to month (Williams, 2005), indicating they were not storing food in any significant quantity. A second complicating factor is the possibility that the people buried at Puruchuco-Huaquerones had recently migrated to the central coast from the highlands. This relocation would produce a concomitant change in the isotopic composition of sequential segments of hair since the coastal diet and the highland diet differs in the relative amounts of C_3 and C_4 resources. When the average isotope values for all the soft tissues are compared to bone collagen, they are virtually identical indicating the dietary pattern has been this way for at least 10-15 years (Williams, 2005). Since there is no evidence for recent migration or the storage of foods, the season of death should be accurate for this population.

Agricultural practices

Overall, there is very little specific information (e.g., timing for planting or harvesting) on ancient Andean agricultural practices in Peru, particularly for the coast. In prehistoric and modern times, Andean farmers divided the year into a wet season (December to March) and a dry season (April to November) (Gillin, 1947) (Fig. 1). The wet season corresponds roughly to the coastal summer (December to May) and is the period when the water run-off from the highlands is greatest and the Rimac river would be at its peak. The dry season corresponds roughly to the coastal winter (June to November), when the water run-off from the highlands is lowest and the Rimac river would be at its lowest (Fig. 1). On the north coast of Peru, and likely the central coast as well, it was possible for farmers to plant and harvest two crops per year of various plants including corn, bean, and squash (Gillin, 1947). The first crop of corn, bean and squash was planted in September and harvested in late April or early May (Gillin, 1947) (Fig. 1). If temperatures remained warm enough, and water levels high, a second crop of this triad was planted in July and harvested in January (Fig. 1). Tubers, such as potato, sweet potato and achira, would have been planted in December and harvested in June (Gillin, 1947) (Fig. 1).

Because of the two plantings, and its ease of preservation (roasting, grinding, brewing), maize would have been available year round. Squash and beans, which would have been cultivated with the maize, would have been plentiful around the harvest and beans may have been dried and consumed year-round as well. Because of the humidity and warm temperatures, relative to the highlands, it is unlikely that it would have been possible to store tubers for year-round consumption. As such, the carbon isotope composition of hair grown during the winter months would be depleted of ^{13}C (more negative) relative to the summer months (Fig. 1). The minimum isotope value should follow the tuber harvest (July) and the maximum isotope value should follow the second maize harvest (February) (since the tubers were likely exhausted by this time) (Fig. 1).

RESULTS

In order to asses how the isotopic composition of hair was changing in the period before death, the general trend in the sequential isotope values along an individual's hair shaft was examined from distal to proximal. The proximal isotope values correspond to the period immediately preceding death. If the proximal isotope values tended to decrease relative to the distal isotope values (i.e., depleted of ^{13}C relative to the distal isotope values), the individual was consuming C_3 resources (e.g., tubers) at the time of death, indicating death during the winter. If the proximal isotope values tended to increase relative to the distal isotope values (i.e., enriched in ^{13}C relative to the distal segments), the individual was consuming C_4 resources (e.g., maize) at the time of death, indicating death during the summer. If the most proximal isotope value (0-1 cm) showed a shift in the opposite direction as the general trend, the individual may have just changed their diet (i.e., right after the harvest) and their body had not yet equilibrated to the new food(s). As such, these individuals were assigned 'fall' or 'spring' as their season of death (Table 1).

For 37% (14/38) the isotope values for the proximal hair segments were depleted of ^{13}C relative to the distal segments, reflecting the consumption of C_3 resources (e.g., tubers) at the time of death and indicating death during the fall/winter. For 63% of the individuals (24/38) the proximal hair segments were enriched in ^{13}C relative to the distal segments, reflecting minimal consumption of C_3 resources at the time of death and indicating death during the spring/summer.

The difference in mortality rates between the seasons was not statistically significant (X^2 = 2.632; p = .104). Adult mortality by age was similar between the seasons (X^2 = 3.639; p = .662), although more young and middle adults died during the summer than the winter (Tab. 2). An equal number of subadults died during the winter and summer. Females experienced more mortality in the summer than the winter (Tab. 2) but this was not significant (X^2 = 3.200; p = .074). Male mortality was not significantly different between the seasons (X^2 = 0.333; p = .564) (Tab. 2).

DISCUSSION AND CONCLUSIONS

Although it was not statistically significant, mortality was higher in the summer months relative to the winter. This is in contrast to the majority of data from both modern and Historic populations and may reflect the small sample size used in this analysis. Alternatively, because temperature and precipitation remain fairly stable on the coast of Peru, seasonal trends observed in other climates (i.e., with marked fluctuations in temperature and/or rainfall) may not be readily applicable to Peru. For example, in the Atacama Desert of Northern Chile, which receives slightly less rainfall than central Peru, there were no seasonal fluctuations in mortality for three million deaths between 1945-1975 (Hajeck, Gutierrez and Espinosa, 1984). In the environments of central Chile and Gibraltar, mortality peaked in the summer months for both modern and historic populations respectively (Hajeck, Gutierrez and Espinosa, 1984; Sawchuk, 1993). As such, the seasonal mortality profile at Puruchuco-Huaquerones corresponds to data from tropical climates rather than strongly seasonal climates.

The summer peak in mortality may not be exclusively related to external factors of the environment (i.e., temperature, air quality, precipitation) but may be more closely related to the agricultural cycle. The first crop of maize and the crop of tubers are harvested in the fall and winter and the breadth of agricultural staples may be limited in the summer, this coincides with peak mortality. Additionally, if a second crop of maize was not planted (due to drought or a drop in temperature), it is possible that the population experienced food shortages during the summer.

Age does not appear to be a significant factor in season of death, although our data set did not contain enough old adults (50 years or greater) and subadults to adequately test this hypothesis. In Chile and Gibraltar, infants less than one year old and adults greater than 65 years (Chile only) experienced greater mortality than other ages during the peak season of mortality (Hajeck, Gutierrez and Espinosa, 1984; Sawchuk, 1993). At Puruchuco-Huaquerones, females were at a greater risk of dying during the summer than the winter. This could be related to a preference for childbirth (Malina and Himes, 1977) during the summer months when temperatures are at their highest, diseases associated with high mortality are at their lowest and the winter fog is abated. This is difficult to test however, since there are no differences in subadult seasonal mortality that would relate to a seasonal preference for childbirth.

Sequential isotopic analysis of one centimeter increments of hair from 38 individuals allowed for the reconstruction of diet at the time of death. Ethnographic information on Andean agricultural practices, climatic and isotope data then allowed the determination of season of death for these individuals. Although no seasonal differences in death reached statistical significance, general trends were still observable in the data. This research has provided important information about the inhabitants of Puruchuco-Huaquerones and their interactions with the local ecology. Diarrhoeal diseases, parasitic infection and possibly nutritional stress were important factors in their life experience and may have contributed to increased summer mortality during the Late Horizon (A.D. 1476-1532).

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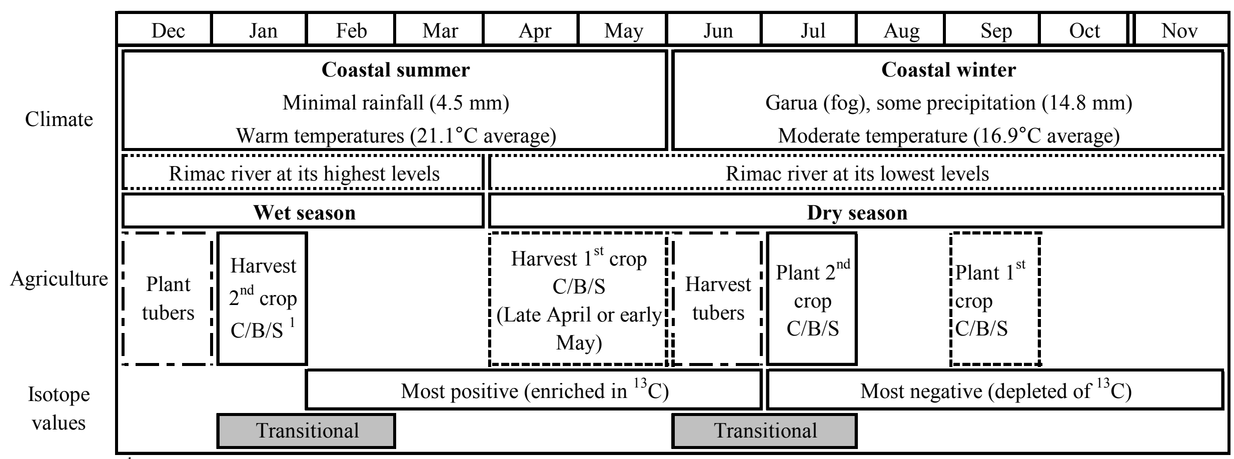
Figure 1



1. C/B/S corresponds to corn/bean/squash

Table 1

ID	Age	Sex	Max	Min	Δ[1]	0-1 [2]	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12
Spring season of death																	
16	35-50	M	-11.3	-15.0	3.7	-14.2	-15.0	-14.9	-14.1	-13.0	-12.0	-11.2	-10.9	-10.9	-11.4	-13.3	-15.1
Summer season of death																	
10	35-50	F	-13.8	-16.7	2.9	-14.7	-15.5	-16.2	-16.7	-16.6	-15.8	-15.2	-15.1	-14.8	-14.4	-14.2	-13.8
29	35-50	M	-13.2	-15.1	1.9	-13.2	-13.7	-15.0	-15.1								
Fall season of death																	
18	4-8	n/a	-10.4	-13.3	3.0	-11.2	-9.5	-10.7	-13.6	-13.0							
Winter season of death																	
68	8.5-13.5	n/a	-11.0	-14.1	3.1	-14.1	-13.0	-12.3	-11.8	-11.2	-11.0						
25	35-50	PF[3]	-12.2	-17.0	4.8	-14.2	-13.4	-12.9	-12.2	-12.3	-13.0	-11.1	-11.8	-12.1	-12.7	-13.1	-12.9

1. Δ corresponds to the difference between the maximum and minimum isotope values (i.e., max - min)

2. The values correspond to distance from the scalp (indicated by 0), unit is centimeters

3. Probable female is indicated by PF

Table 2

	Spring/Summer		Fall/Winter	
	Number	Percentage	Number	Percentage
Total number of individuals	*24*	*63*	*14*	*37*
Sex				
Female	11	52	5	45
Probable female	3	14	1	9
Combined [1]	14	67	6	55
Male	6	29	5	45
Probable male	1	5	0	0
Comined	7	33	5	45
Total [2]	21	100	11	100
Age				
Subadult	3	13	3	21
20-35 years	7	29	3	21
35-50 years	12	50	7	50
50+ years	2	8	1	7
Total	24	100	14	100

1. Sex and probable sex totals are combined for statistical tests

2. This total does not include subadults

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Figure Legends

Fig. 1 Annual cycle of climatic, agricultural and isotope data for the central coast of Peru. (Compiled from Gillin 1947, World Climate a, b and developed from a similar figure in Herring and Hoppa 1997: 123).

Table Legends

Tab. 1 Stable carbon isotope data for a sub-sample of individuals to show the typical patterns for each season.

Tab. 2 Summary table detailing the number, age and sex of individuals dying in the summer or the winter.