Diet and Lifestyle in the First Villages of the Middle Preceramic: Insights from Stable Isotope and Osteological Analyses of Human Remains from Paloma, Chilca I, La Yerba III and Morro I

Supplemental Text 1 – Materials and Methods

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## Chronology

Paloma’s chronology is defined by 19 radiocarbon dates (Benfer 1984) across occupation over six broadly defined stratigraphic levels: ‘Layer 100’ at the surface through to ‘Layer 600’ at its foundation (Engel 1980: 105, see also Quilter 1989: 11, Benfer 2008: 371).

The unusual archaeological contexts and isotopic results of two individuals at Paloma discussed further below prompted us to directly date their bone collagen (see Supplemental Table 1): Burial 2 (PAL 01) from its uppermost Layer 100; and Burial 110 (PAL 13), in a rubbish midden beyond the main area of excavated house structures at Paloma and reported to be from ‘Layer 300?’ (Quilter 1989: 123). Together all these Paloma radiocarbon dates are calibrated here using the ShCal13 atmospheric curve (Hogg *et al*. 2013), and then modeled using OxCal’s Bayesian analysis following Bronk Ramsey (2009, 2017), in a sequence of phases, according to those layers. Certain of the published Paloma radiocarbon dates were originally highlighted as intrusive or otherwise problematic by Benfer (1984). A few others show poor agreement indices in the OxCal analysis. These are therefore set as outliers to model boundary limits for Paloma’s stratigraphic layers (see Supplemental Table 2).

Chilca I’s stratigraphy is defined by six radiocarbon dates, according to five stratigraphic layers (Engel 1988: 14 & Fig. 3) and a house context (XIII, dated by Kaplan & Lynch 1999: 266). These are also all calibrated here using ShCal13 (Hogg *et al*. 2013)(see Supplemental Table 3). Chilca I’s lower contexts have Middle Preceramic archaeological assemblages (Engel 1988) identical to those of Paloma’s Layers 300 and 400 and La Yerba III.

La Yerba III’s chronology is defined by six radiocarbon dates from two house contexts (Engel 1991: 56, Beresford-Jones *et al*. 2018: 401), all calibrated here using ShCal13 (Hogg *et al*. 2013)(see Supplemental Table 3). The three individuals analyzed from La Yerba III in this paper derive from a single house context (SU 7006, Cateo 1, see Chauca & Beresford-Jones 2016).

Morro I’s chronology, meanwhile, is defined by a total of 25 radiocarbon dates (Arriaza 1995, Standen 2003) covering over two millennia. Of the 28 Morro I burials analyzed osteologically in this paper only five have been directly dated (one, T10b, with two dates). Following Standen (1991), Arriaza (1995), Standen (2003) and Arriaza & Standen (2009), however, we can date the others by association with varying degrees of precision (see Supplemental Table 5, which also shows Morro I burials analyzed isotopically by King *et al*. 2018). All the dates from Morro I are calibrated here using ShCal13 (Hogg *et al*. 2013). The calibrated date ranges shown in Supplemental Table 5 suggest that seven of the Morro I individuals analyzed osteologically here (including directly dated T10b) likely date to Standen’s (2003) Phase 1 of Morro I and are therefore approximately contemporary with the Middle Preceramic contexts of the Peruvian villages Paloma, Chilca I and La Yerba III. Meanwhile, 14 (including three directly dated: T19C1, T23C5, T23C12) likely date to Phase 2; and five (including one directly dated: T28C9) to Phase 3 and therefore postdate the Peruvian villages by one to two millennia, while the remaining two can only be assigned to the broad Preceramic time frame 6250 – 3850 cal BP.

Four of the five burials from Morro I previously analyzed for stable isotopes (T4, T6, T10a and T12) are assigned to Morro I Phase 1 (T10a being associated with burial T10b, directly dated to 6285 – 5999 cal BP), and therefore correspond chronologically with the Middle Preceramic Peruvian village contexts (see Supplemental Table 5). A fifth (T23C13), meanwhile, is associated with Morro I Phase 2 and therefore postdates the Peruvian villages by one to two millennia.

Finally, we used OxCal to model all 25 calibrated radiocarbon dates for Paloma’s Layers 300 and 400, Chilca I, La Yerba III and the earliest tomb contexts at Morro I as a single, contiguous phase to test the hypothesis that components of these sites were contemporaneous (see Main Paper, Figure 4). Four dates for Paloma’s Layer 400 and one for Chilca I’s Layer 600 showed poor agreement indices of less than 60% and were therefore set as outliers. The remaining 20 dates give an overall model Agreement Index of 97, thereby supporting the hypothesis (see Supplemental Table 3).

## Stable Isotope Analyses

Human bone samples were analyzed from 29 individuals from Paloma (1, 7, 15, 3 and 3 individuals from Layers 100, 200, 300, 400 and 500, respectively); three bone and two dentine samples were analyzed from 3 individuals from La Yerba III, and one bone sample analyzed from an individual from Chilca I (see Main Paper, Table 1).

Collagen was extracted following the method described in Privat *et al*. (2002). All collagen samples were analyzed in triplicate using a Costech elemental analyzer coupled in continuous-flow mode to a Thermo Finnigan Delta V mass spectrometer. Carbon and nitrogen stable isotope values are expressed as delta values (for example δ13C) relative to international scales (VPDB and AIR, respectively; Hoefs 2004). Repeated measurements on international and in-house standards showed that the analytical error was less than 0.2‰ for both carbon and nitrogen.

Measured collagen is deemed to be of good quality if it fulfills the following criteria: an atomic C:N ratio of 2.9 to 3.6 (De Niro 1985); a 'collagen' yield of >1% by mass; final carbon yields of >13%; and final nitrogen yields of >4.8% (Ambrose 1990). Twenty-one samples (72%) from Paloma and all three bone samples from La Yerba failed to yield reliable collagen. The remaining eight samples from Paloma, two dentine samples from La Yerba and one sample from Chilca I produced collagen with atomic C:N ratios between 3.1 and 3.6, collagen yields greater than 1%, carbon yields greater than 24%, and nitrogen yields greater than 8%.

Statistical analyses were performed using Rstudio version 1.0.143 for Macintosh. The code is available from the University of Cambridge data repository [link]. Samples were tested for normality using histograms and Shapiro-Wilk tests, and correlation was investigated using linear regression. Outliers are identified as samples that lie more than 1.5 times the inter quartile range (IQR) below quartile 1 (Q1) or above quartile 3 (Q3).

## Human Osteology

The sample composition for the osteological analyses is summarized in Supplemental Table 4. Only adults were studied (epiphyseal fusion of major long bones excepting the clavicle).

For 62 individuals from Paloma stature was calculated from maximum lengths of the femur, tibia, humerus, ulna and/or radius using sex-specific equations derived for coastal Andean and highland archaeological skeletons (Pomeroy & Stock 2012)(see Skeletal Data at https://doi.org/10.17863/CAM.63764). The need for population-specific stature estimation equations due to inter-population variation in body proportions is widely acknowledged (e.g., Raxter *et al*. 2007, Auerbach & Ruff 2010, Pomeroy & Stock 2012). Bone measurements and sex estimates were made by RAB, and the final stature estimate for each individual was selected based on the order of preference for different bones specified in Pomeroy & Stock (2012).

For the 28 individuals from Morro 1, stature estimates were based either on the revised Fully method (Raxter *et al*. 2007) or using the equations provided by Pomeroy & Stock (2012) following the same procedure for Paloma, depending on skeletal preservation (Pomeroy 2012)(see Skeletal Data at https://doi.org/10.17863/CAM.63764 and Main Paper Table 2). Sex estimates for Morro 1 skeletons were taken from mummy dissection records or skeletal assessments conducted at the *Museo Arqueológico* San Miguel de Azapa, *Universidad de Tarapacà*, Chile.

Cross-sectional geometric properties of the femur were derived for adult individuals from Morro 1 (Pomeroy 2012; Pomeroy 2013) using the external mould method (see Stock & Shaw 2007), with biomechanical properties calculated in ImageJ (NIH) using the MomentMacroJ plugin (Ruff 2006). Cross-sectional geometric properties for Paloma adults were kindly provided by Professor Alexander G. Robling (pers. comm. to EP 2010 and 2019). Details of the methods used to derive these properties are in Robling (1998). Data were available only for the femoral midshaft for Paloma. We analyzed femoral midshaft *Imax/Imin* because these data were available for both Paloma and Morro 1, the values do not need standardizing to body mass and bone length (for which some data are missing), and because these ratios have been used in numerous studies as an indicator of habitual mobility (Ruff 2008).

Differences in stature and *Imax/Imin* between different levels at Paloma were tested by two-way ANOVA, revealing no significant differences between levels for males or females (p values for stature: sex <0.001; level = 0.1, sex x level interaction = 0.4; p values for *Imax/Imin*: sex = 0.01; level = 0.8, sex x level interaction = 0.1) justifying the combination of individuals from different levels in subsequent analyses. Similarly, as the dataset from Morro 1 included both individuals likely contemporary with Paloma (n = 7) as well as individuals dating to between one and two millennia later (n = 19, details in Supplemental Table 5), differences in mean stature and *Imax/Imin* between the earlier and later temporal groups were tested by two-way ANOVA, again revealing no significant difference between the two major temporal groups for males or females (p values for stature: sex = 0.001; temporal group = 0.8, sex x temporal group interaction = 0.4; p values for *Imax/Imin*: sex = 0.1; temporal group = 0.2, sex x temporal group interaction = 0.9) and justifying their combination in subsequent analyses. Sex-specific mean estimated stature and *Imax/Imin* were compared between sites using two-way ANOVA for the effects of site, sex and their interaction. All analyses were performed in SPSS for Windows v.25 (IBM, Inc.) (see Main Paper, Table 2).

Previous investigations, in contrast to the results presented here, suggested that stature significantly increased between levels 400 to 200 at Paloma, in line with evidence for decreased non-specific ‘stress’ indicators (cribra orbitalia and porotic hyperostosis: Benfer 1990). This was interpreted as indicating that adaptations to sedentism improved through time (Benfer 1990). Stature estimates in Benfer (1990) were based on equations from Genovés (1967) derived from recent Mesoamerican populations. The difference in results may arise from differences in the methods of stature estimation used, as in the present study, we applied equations specifically derived for archaeological populations from the central Pacific coast of South America (see Pomeroy and Stock 2012). Supplementary Figure 2A illustrates mean stature across different levels at Paloma and at Morro 1 based on stature estimates from the present study. While there does appear to be a substantial increase in stature among Paloma females between levels 300 and 200, the trend among males is limited and neither was statistically significant. The contrast between Paloma individuals from all levels and those from Morro 1 are particularly clear among males, but also evident among females.

Similarly, previous studies have suggested changes in activity through time at Paloma (Benfer 1990, Robling 1998, McNair 1988 in Robling 1998), but these are not supported statistically for the measure used here (Supplementary Figure 2B). Differences between studies may derive from the fact that different markers used in these studies may reflect different components of activity (e.g. upper vs. lower body use) and the influence of varying and relatively small sample sizes. Nonetheless, as with stature, the difference between Paloma individuals from all levels and Morro 1 individuals is evident, particularly among males.

Further comparisons of stature and femoral midshaft *Imax/Imin* were made between the Morro 1 sample and later agriculturalists from the Azapa Valley. The latter samples derive from agricultural populations residing 12-15 km inland within the same river valley as Morro 1, and dating to AD 900-1400 (Muñoz 2004). Data were collected by EP using the same methods as for Morro 1 (see Pomeroy 2012 for further details). Samples from the sites Az-71, Az-140 and Az-141 were combined as they demonstrate similar cultural, temporal and subsistence profiles (Muñoz 2004). Comparisons between adult males and females and between Morro and Azapa Valley sites using two-way ANOVA in SPSS v.25 (IBM, Inc.) show no significant differences between the samples in either stature or femoral midshaft *Imax/Imin* (see Tables 6 and 7 and Figure 3). This departs from patterns commonly observed in other parts of the world (e.g., Mummert et al. 2011) where the adoption of agriculture is commonly accompanied by decreased stature and habitual mobility as reflected by femoral midshaft *Imax/Imin* (see main text for further discussion). This result indicates that the quality of the growth environment and habitual activity levels were similar for the hunter-gatherers and agriculturalists in the Azapa Valley of Northern Chile.

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