Migration and maize in the Virú Valley: Understanding life histories through multi-tissue carbon, nitrogen, sulfur, and strontium isotope analyses

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Abstract

Objectives: Stable isotope analysis can provide crucial insight into the function and development of early state-level societies on the north coast of Peru.

Materials and Methods: Multi-tissue (bone collagen, tooth enamel, hair, nail, skin, and tendon) stable isotope analyses (carbon, nitrogen, sulfur, and strontium) were conducted for 13 individuals from the lower Virú Valley.

Results: Non-seasonal changes in a predominantly C4-based terrestrial diet, with minimal inputs of marine foods were identified. One individual (Burial 5), however, had a stable isotope signature unlike any previously found on the north coast of Peru, indicating both a large contribution of C3-terrestrial resources to their diet and an 87Sr/86Sr value suggestive of highland residence during childhood.

Discussion: This research provides the first strong stable isotope evidence of a highland individual within a coastal burial in northern Peru, new insight into the ritual killing event at Huaca Santa Clara during the late middle horizon and supporting evidence of the importance of C4 terrestrial resources to the developing Virú polity during the early intermediate period.

Keywords

coastal-highland interactions, early intermediate period, north coast Peru, ritual killing event, stable isotope analysis

1 | INTRODUCTION

The early intermediate period (EIP) (200 BCE–600 CE) was an important time for early state development on the north coast of Peru. The development and expansion of agropastoral practices, increasing populations, and interactions between diverse groups of people shaped the history of this region, impacting the formation of complex societies. The social, political, and economic changes associated with the development of complex societies can have profound impacts on the history and cultural practices of people living in those societies. Stable isotope analysis can provide insight into the lives of individuals during this critical period and illuminate the complex mechanisms that promoted and supported early state societies.

The Virú Valley has a long history of research focused on the development of early states (Millaire, 2010). The sites of Huaca Gallinazo and Huaca Santa Clara were important centers of the Virú polity during the EIP as the polity’s capital and a regional administrative center, respectively (Figure 1). In addition to EIP burials at both sites, a unique ritual killing event exclusively involving young humans and camelids was performed at Huaca Santa Clara during the late middle horizon (LMH) (600–1000 CE) long after the site’s abandonment (Millaire, 2015). Understanding the dietary practices and potential places of childhood residence for these individuals can provide insight into the functioning of the Virú polity as an early state society, particularly with respect to interactions between coastal and highland groups during the LMH.
1.1 Geography and cultural history

The Andean Cordillera is a vast mountain range that produces distinct ecological zones at different altitudes that span from the western coastal deserts to the eastern Amazonian jungles (Pulgar Vidal, 1996). The north coast of Peru falls under the rain shadow produced by the Andean Cordillera and the cold Humboldt current which results in almost no rainfall along the coast and excellent preservation of organic remains. The Humboldt current supports one of the world’s most productive coastal ecosystems and marine resources were likely important for the development of the earliest societies along the coast (Moseley, 1975; Moseley, 1992). Complex cultures in this region resided in fertile river valleys fed by glacial meltwater and seasonal rainfall from the highlands. Distinct archaeological cultures developed within many of these river valleys, however, inter-valley trading networks resulted in a larger sphere of influence that produced unified north coast ideologies and worldviews (Burger, 2008; Jones, 2010).

By the end of the EIP exponential population growth ceased and the first archaic states had appeared in northern Peru (Millaire, 2010; Topic, 1982). These early states were characterized by tiered political structures, settlement hierarchies, and outposts in other valleys (Lau, 2004; Millaire, 2010; Strong, 1957; Tello, 1929, 1940; Topic, 1977, 1982). It was during the EIP that the Virú Valley’s population peaked, irrigation networks were expanded to support farming, and the Virú Polity established control over the valley’s resources (Millaire, 2010; Millaire et al., 2016). In contrast, during the MH the old states lost their influence as new cultures, such as Wari and Tiwanaku, rose to prominence (Isbell, 2008; Shimada et al., 1991). The strong connections with the Moche state declined in the Virú Valley as the influence of the Moche state waned (Bawden, 2008; Castillo & Rengifo, 2008; Chapdelaine, 2010). The rise of prominent highland states during the LMH and their influence on the north coast has been suggested in the Virú Valley by the increased frequency of “highland” cranial modifications and ceramic styles (Dillon, 2015).

1.2 Stable isotope analysis

Stable carbon (δ¹³C), nitrogen (δ¹⁵N), and sulfur (δ³⁴S) isotope analyses provide information about the types of foods people choose to consume. δ¹³C values best distinguish the contributions of C₃ and C₄ plants. In the Andes, δ¹³C values are especially useful for identifying the consumption of C₄ crops (particularly maize but also amaranth in contexts where this crop was economically important) compared to wild and cultivated C₃ plants such as legumes, fruits, peppers, and gourds (Szpak et al., 2013; Tieszen et al., 1992). δ¹³C values also tend to be higher in marine animals relative to terrestrial animals that consume C₃ plants (Chisholm et al., 1982). δ¹⁵N values increase with trophic level (Minagawa & Wada, 1984) and can be useful for distinguishing diets...
predominantly based on plants from diets that include meat and secondary animal products. While δ13C and δ15N values can be used in combination to estimate the contribution of marine foods (high δ13C and δ15N values) (Hansen et al., 2012; Schoeninger & DeNiro, 1984), δ34S values can further distinguish terrestrial and marine-based diets (Richards et al., 2003; Tieszen et al., 1992). Most marine animals have uniformly high δ34S values of +15 to 20 ‰ whereas plants and animals from terrestrial and freshwater ecosystems tend to have much lower δ34S values around ~0 to 10 ‰ (Nehlich, 2015).

Strontium isotope ratios may also provide some indication of the contribution of marine-based diets because the 87Sr/86Sr of the ocean (0.7092) (Elderfield, 1986; Veizer, 1989) can differ greatly from the 87Sr/86Sr value of the terrestrial environment (Sealy et al., 1991). The geological history of the Andes has resulted in longitudinal bands of bedrock with distinct 87Sr/86Sr values (Knudson et al., 2014; Scaffidi & Knudson, 2020) that can distinguish non-local individuals who lived at varying distances inland. This method is, however, less suited to distinguish north–south migrations between river valleys.

### 1.3 Multi-tissue approach

The preservation of a wide range of human tissue in the arid environment of the north coast of Peru allows for the creation of a longitudinal data set that can characterize how individuals’ diets changed over their lifetimes. As tissues grow and remodel they incorporate the isotopic signature of their diet and by analyzing tissues with different formation times and turnover rates, the changes in their diet or residency at different periods can be determined (Tieszen et al., 1983). Bone collagen grows and remodels quickly during childhood but then slowly continues to remodel throughout adulthood producing stable isotope values that represent long-term diet over years to decades, depending on the age of the individual (Hedges et al., 2007). Tendon and skin are also actively maintained but have a turnover rate that reflects only the last 2–3 months and 3.5–4 months respectively before death (Babraj et al., 2005; El-Harake et al., 1998; Tieszen et al., 1983). Human hair and nail are metabolically inert once formed, and their isotopic compositions reflect only the period of formation (Tieszen et al., 1983). Human hair and nail grow at approximately 1.0 cm and 2.1 mm per month (Saitoh, 1969; Yaemsiri et al., 2010) although an individual’s tissue growth rate may be impacted by illness, malnutrition, and the indiscriminate sampling of hair in both active and inactive growth phases (Harkey, 1993; L. J. Williams et al., 2011). Tooth enamel is inert once formed and represents the diet and environment of early childhood only (Harris & Buck, 2002; Reid & Dean, 2006). The range of tissue types analyzed in this research is rare and has provided a wealth of information about the individuals buried in the Virú Valley.

### 2 MATERIALS AND METHODS

#### 2.1 Materials

The carbon, nitrogen, sulfur, and strontium isotopic compositions of the tissues of 13 individuals were analyzed. Individuals from the sites of Huaca Gallinazo (n = 2) and Huaca Santa Clara (n = 11), dating to the EIP (n = 7) and LMH (n = 6) (Table 1) were included in this analysis.

| Table 1 | Age and sex estimation, and burial context for individuals analyzed in this research (Dillon, 2015) |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Burial  | Site                  | Period | Sex          | Age category | Estimated age | Burial Interpretation                                                                 |
| 1       | Huaca Gallinazo       | EIP    | Female       | Adult        | 30–35 years  | Was buried beneath the floor, but no conclusive decision on whether their burial was a dediatory offering |
| 2       | Huaca Gallinazo       | EIP    | Infant       | 12 ± 4 months | Reburial after being disturbed or a dediatory offering |
| 7       | Huaca Santa Clara     | EIP    | Female?      | Young adult  | 20–25 years  | Principal with retainer (HSC9)                                                        |
| 8       | Huaca Santa Clara     | EIP    | Male         | Young adult  | 30–35 years  | Perhaps warrior or ritual violence                                                     |
| 9       | Huaca Santa Clara     | EIP    | Female       | Young adult  | 20–25 years  | Retainer with principal (HSC7)                                                        |
| 13      | Huaca Santa Clara     | EIP    | Female?      | Middle adult | 45–50 years  | Potential retainer burial with principal (HSC14)                                      |
| 14      | Huaca Santa Clara     | EIP    | Male         | Middle adult | 35–40 years  | Potential principal burial with retainer (HSC13)                                      |
| 3       | Huaca Santa Clara     | LMH    | Child        | 4 years ±15 months | Retainer burial for Tomaval Period                                           |
| 4       | Huaca Santa Clara     | LMH    | Child        | 12 years ±21 months | Retainer burial for Tomaval Period                                          |
| 5       | Huaca Santa Clara     | LMH    | Child        | 7 years ±20 months  | Retainer burial for Tomaval Period                                           |
| 6       | Huaca Santa Clara     | LMH    | Child        | 10 years ±14 months | Principal burial for Tomaval Period                                           |
| 10      | Huaca Santa Clara     | LMH    | Child        | 7 years ±20 months  | Retainer burial for Tomaval Period                                           |
| 11      | Huaca Santa Clara     | LMH    | Child        | 12 years ±21 months | Retainer burial for Tomaval Period                                           |

Abbreviations: EIP, early intermediate period; LMH, late middle horizon.
Many of the EIP burials from Huaca Santa Clara are principal and retainer pairings, while all LMH burials from Huaca Santa Clara are children associated with a single ritual killing event that also included juvenile camelids (Dillon, 2015; Millaire, 2015). Additional information on the samples is available in Data S1, Table S1.

### 2.2 Sample preparation

Bone collagen was extracted as described previously (Szpak et al., 2014). The skin and tendon samples (n = 9) were prepared for stable carbon and nitrogen isotopic analysis following Finucane (2007). Approximately 100 mg of skin or tendon were extracted from the available remains using a scalpel cleaned in acetone. External debris was removed from the samples by sonication in 10 mL of Type I water (resistivity >18.2 MΩ-cm) for 20 min. The resulting solution was removed, and surface lipids were extracted by sonication in 10 mL of 2:1 chloroform: methanol (v/v) for 1 h three times. Following the lipid extraction, the samples were washed three times by sonication in 10 mL of Type I water. To extract the insoluble residue (“collagen”) from the samples, 3.5 mL of 0.01 M HCl was added to the samples, which were then heated at 75°C for 48 h. The samples were centrifuged, and the solution was transferred to clean vials, frozen for 24 h, and freeze-dried. Subsets of each sample were removed following each stage of the procedure and analyzed separately to determine the impact of each step on the isotopic composition of desiccated skin and tendon.

A subsample of the nail from both the distal and proximal ends of the available nails (n = 2) was cut using acetone-cleaned nail clippers. The nail preparation procedure was informed by the protocols of O’Connell et al. (2001) and J. S. Williams and Katzenberg (2012). The nails were cleaned of any adhering sediment by three rinses in 10 mL of Type I water with sonication for 20 min each. The amount of 5 mL of 2:1 chloroform: methanol (v/v) was added to the culture tubes and the samples were placed on an orbital shaker for 30 min to remove any adhering lipids from the nails. Samples were then sonicated in 10 mL of Type I water for 20 min to remove any residual solvent. The samples were decanted of their second rinse of Type I water and dried in an oven at 60°C overnight. Once dry, approximately 500 µg of nail sample was cut and weighed into tin capsules for isotopic analysis.

The hair samples (n = 8) were prepared following a procedure based on Cooper et al. (2018) and L. J. Williams et al. (2011). Following the considerations suggested by L. J. Williams et al. (2011) large sample sizes of 30 or more hairs were extracted from each individual to reduce the impact of sampling hairs in an inactive growth phase. Hair was pulled in line with the hair roots using acetone-cleaned tweezers and placed in a clean surface of aluminum foil. The straightened hair was aligned, wrapped in aluminum foil, and 1 cm increments were marked and cut along the foil wrapping. Adhering dirt was removed by sonication in 10 mL of Type I water for 30 min. Lipids were removed with two rinses in 8 mL of 2:1 chloroform: methanol (v/v) solution sonicated for 30 min. Two rinses of 10 mL Type I water with ultrasonication for 30 min removed any remaining 2:1 chloroform: methanol (v/v) solution. The final Type I water rinse was decanted, and samples were dried overnight at 50°C. The hair samples were minced using an acetone-cleaned razor blade and weighed into tin capsules for stable carbon and nitrogen isotope analysis. A subset of hair samples was also analyzed for δ28S and for these, vanadium pentoxide was added to ~1 mg of hair in tin capsules.

Tooth enamel was prepared based on the techniques of S. Ambrose et al. (2018). External dirt was removed from the tooth by sonication in 10 mL of 0.1 M acetic acid for 2 x 20 min. Teeth were then cleaned of visible calculus/plaque using a dental drill. The tooth crown was separated from the root and the dentine within the crown was removed from large enamel pieces. The enamel pieces were powdered using an industrial mortar and pestle and 150–200 mg of enamel powder was weighed into perfluoroalkoxy alkane (PFA) vials. Additional technical information concerning the operation and conditions of the analytical equipment is available in Data S1.

### 2.3 Data treatment

To make direct intra-individual comparisons between different tissue types all sample δ13C and δ15N values were adjusted to be directly comparable to bone collagen using the proposed inter-tissue isotopic offsets determined by O’Connell et al. (2001). To directly compare hair keratin to bone collagen hair keratin isotopic compositions were adjusted +1.40 ‰ for δ13C and +0.86 ‰ for δ15N, and nail keratin results were adjusted +1.40 ‰ for δ13C and +0.21 ‰ for δ15N (O’Connell et al., 2001). Skin and tendon tissues are predominantly composed of collagen (Józsa et al., 1984; Kucharz, 1992) and are compositionally similar to that of bone collagen, so no inter-tissue isotopic adjustments were made (White & Schwarz, 1994). A Kruskal-Wallis test was performed to compare the mean isotopic ratios among individuals and groups. To examine trends in changing stable isotope values over time non-parametric Spearman’s r was performed.

### 3 RESULTS AND DISCUSSION

#### 3.1 Sample preservation

The bone collagen isotopic compositions included in this analysis passed all quality control criteria (wt% collagen >1%, 3.13 < C, Natomic < 3.38, wt% C > 13.0%, wt% N > 4.5%) (Table 2) (S. H. Ambrose, 1990; DeNiro, 1985; van Klinken, 1999). All hair segments had C:Natomic ratios between 3.36 and 3.95 (Table S2) which fit well with the range for modern human hair (2.9–3.8) determined by O’Connell and Hedges (1999). The range of C:Natomic for the nail samples was between 3.78 and 4.01 (Table S3) which is higher than the range observed by O’Connell et al. (2001) for modern nail samples (3.00–3.80). While the higher C:Natomic ratio could represent some residual contamination from the burial environment after the cleaning pre-treatment, there was no significant correlation (Pearson’s r, r = 0.68, p = 0.32) between δ13C and C:Natomic values, which would be
expected if samples were contaminated by soil humics or lipids (with low $\delta^{13}C$ values).

Changes in C:N$_{atomic}$, wt% C, and wt% N for soft tissue samples (skin and tendon) with each successive pre-treatment phase suggested that this procedure successfully removed some carbon-rich contaminants. For samples with initially high C:N$_{atomic}$ ratios (>5.00) after only the Type I water rinse, the gelatinization step further reduced the C:N$_{atomic}$ ratios by an average of $-1.68 \pm 1.51$ (n = 4) (Table S3). Four soft tissue samples had C:N$_{atomic}$ > 3.6 even after the complete preparation procedure, and these values were excluded from further analysis (Table S3). The skin and tendon samples with C:N$_{atomic}$ < 3.6 had the highest $\delta^{13}C$ values of all tissues sampled (skin: +11.68 ± 0.88‰, n = 2; tendon: +11.74 ± 0.71‰, n = 4; nail: +9.99 ± 0.92‰, n = 4; bone collagen: +9.8 ± 0.9‰, n = 13; hair: +9.09 ± 1.35‰, n = 114). When tissues from Burial 7 were adjusted for inter-isotopic offsets, tendon, and skin $\delta^{15}N$ values were higher than the nail and incremental hair $\delta^{15}N$ values that corresponded with the 2–3 months (tendon) and 3.5–4 months (skin) before death (Figure 2). In contrast, the $\delta^{13}C$ values of tendon and skin closely matched the $\delta^{13}C$ values of incremental hair 1 and 2 months before death, respectively (Figure 2). The higher $\delta^{15}N$ values of tendon and skin are likely the result of post-burial degradation or contamination, despite the excellent physical preservation of the skin itself. The $\delta^{15}N$ values of these tissues were not used for further analysis, but the $\delta^{13}C$ values were included.

### 3.2 Major components of diet

The $\delta^{13}C$ values for both long-term (bone collagen) and short term (hair keratin, nail keratin, tendon, and skin) tissues were relatively high and support a diet predominantly based on C$_4$ plants (likely maize) for the burials at both Huaca Gallinazo (bone collagen: $-11.74 \pm 0.46$‰, n = 2) and Huaca Santa Clara (bone collagen: $-12.30 \pm 2.06$‰, n = 11) (Table 2 and Figures 2 and 3). The direct consumption of maize or the consumption of animals foddered with maize is supported by the abundance of maize in the archaeobotanical remains from both Huaca Gallinazo and Huaca Santa Clara (L. Masur, 2012; L. J. Masur et al., 2018). The individual from Burial 5 is an outlier and their low bone collagen $\delta^{13}C$ value of $-17.58$‰ suggests that the protein portion of their diet emphasized C$_3$ plants to a much greater extent than any of the other individuals (Table 2 and Figure 3).

The $\delta^{15}N$ values of the bone collagen of all the individuals analyzed were between +8.7 and +11.4‰ (Table 2 and Figure 3) while the $\delta^{34}S$ values from incremental hair samples ranged from +1.7 to +6.9‰ (Table 3, Figures 2 and 4), further supporting the notion that marine resources were not a quantitatively important part of their diet. If marine food sources were a significant component of the diet of these individuals, much higher $\delta^{15}N$ (c. +15 to +25‰) and $\delta^{34}S$ (c. +20‰) values would be expected (e.g., King et al., 2018; Santana-Sagredo et al., 2015; Tieszen et al., 1992; Tomczak, 2003) (Table 3 and Figure 4). The strontium isotope results also support a diet with minimal input of marine foods during childhood. Marine diets alter tooth enamel $^{87}$Sr/$^{86}$Sr signatures to approximate the oceanic $^{87}$Sr/$^{86}$Sr value of 0.70987 (Elderfield, 1986; Veizer, 1989), however the $^{87}$Sr/$^{86}$Sr ratios from Huaca Gallinazo and Huaca Santa Clara were all below the oceanic $^{87}$Sr/$^{86}$Sr value and ranged from 0.70569 to 0.70868 (Table 4).

Human bone collagen, tendon, skin, nail, and hair $\delta^{13}C$ and $\delta^{15}N$ values were adjusted for trophic enrichment and compared to the Virú Valley archaeological camelids (Szpak et al., 2014) and modern plants from the neighboring Moche Valley (Szpak et al., 2013) (adjusted $\delta^{13}C$ values for the Suess Effect, and $\delta^{15}N$ estimated as one trophic level below the Virú Valley camelid bone collagen) to determine the relative importance of these protein sources to their diet (Figure 5). The trophic enrichment factor (TEF) adjusted human $\delta^{15}N$ values were intermediate between camelids and the plants, which suggests that terrestrial herbivore protein, most likely camelid meat, and crops such as maize were the major components of the diets (Figure 5).

### Table 2: Isotopic and elemental compositions of bone collagen from this research

| Burial | ID #   | Site          | Period | wt% collagen | $\delta^{13}C_{VPDB}$ (‰) | $\delta^{15}N_{AIR}$ (‰) | wt% C | wt% N | C:N$_{atomic}$ |
|-------|-------|---------------|--------|--------------|----------------------------|--------------------------|-------|-------|---------------|
| 1     | AIS-312 | Huaca Gallinazo | EIP    | 11.6         | −11.41                     | +8.7                     | 45.7  | 16.7  | 3.19          |
| 2     | AIS-317 | Huaca Gallinazo | EIP    | 7.4          | −12.06                     | +9.9                     | 36.7  | 13.2  | 2.33          |
| 3     | AIS-532 | Huaca Santa Clara | LMH   | 13.6         | −10.32                     | +11.0                    | 45.4  | 15.6  | 3.38          |
| 4     | AIS-537 | Huaca Santa Clara | LMH   | 16.4         | −12.42                     | +9.0                     | 43.7  | 16.3  | 3.13          |
| 5     | AIS-541 | Huaca Santa Clara | LMH   | 14.5         | −17.58                     | +9.0                     | 41.3  | 14.7  | 2.37          |
| 6     | AIS-544 | Huaca Santa Clara | LMH   | 19.0         | −10.36                     | +11.0                    | 40.5  | 13.8  | 3.41          |
| 7     | AIS-547 | Huaca Santa Clara | EIP   | 12.8         | −10.99                     | +9.0                     | 42.8  | 14.9  | 3.34          |
| 8     | AIS-551 | Huaca Santa Clara | EIP   | 14.5         | −12.69                     | +10.0                    | 41.2  | 14.8  | 3.23          |
| 9     | AIS-555 | Huaca Santa Clara | EIP   | 24.2         | −11.56                     | +9.3                     | 46.0  | 16.5  | 3.24          |
| 10    | AIS-560 | Huaca Santa Clara | LMH   | 8.6          | −13.07                     | +8.9                     | 35.7  | 12.8  | 3.25          |
| 11    | AIS-565 | Huaca Santa Clara | LMH   | 7.3          | −10.66                     | +11.4                    | 36.1  | 13.2  | 3.19          |
| 12    | AIS-569 | Huaca Santa Clara | EIP   | 23.8         | −13.39                     | +9.6                     | 45.8  | 16.1  | 3.31          |
| 13    | AIS-573 | Huaca Santa Clara | EIP   | 4.8          | −12.23                     | +10.8                    | 38.1  | 13.4  | 3.31          |

Abbreviations: EIP, early intermediate period; LMH, late middle horizon.
3.3 | Isotopic changes in the months before death

The isotopic compositions of the hair, nail, skin, and tendon suggest that the individuals analyzed in this study consumed a predominantly C₄ terrestrial plant and animal-based diet, consistent with the bone collagen data. After hair, nail, skin, and tendon isotopic compositions were normalized relative to bone collagen (adjusted for inter-tissue isotopic offsets) there were only slight differences between long-term and short-term diet (Figures 2, 5, and 6). To some extent, this variation may also be driven by the uncertainty in the inter-tissue offsets that were used in this study.

The isotopic compositions of the soft tissues were quite variable (δ¹³C max range: 6.74 ‰ for Burial 7 hair samples; δ¹⁵N max range: 3.85 ‰ for Burial 8 hair samples) and showed that the diet of these individuals changed within the months before their death (Figures 2 and 7 and Table S3). The change in incremental hair δ¹³C, δ¹⁵N, and δ³⁴S values did not repeat over a yearly cycle (assuming ~1 cm of scalp hair growth per month) nor was it patterned in any way to suggest that the processes causing these changes were seasonal (Figure 7). Changes in δ¹³C and δ¹⁵N values of incremental hair samples occurred in phase with each other for almost all hair samples analyzed, but most prominently in Burials 7, 8, and 13 (Figure 7). The synchronous changes in hair keratin δ¹³C and δ¹⁵N values suggest sporadic periods in which individuals consumed variable amounts of a food source with high δ¹³C and δ¹⁵N values.

The contributions of variable amounts of marine protein would fit this requirement, however the δ³⁴S values were consistently low for all of the individuals analyzed (range: +1.65 ‰ to +6.85 ‰) (Table 3 and Figure 4) and did not change in phase with δ¹³C or δ¹⁵N values for many individuals (Burials 2, 4, and 10). This pattern would be expected if marine resources were the food with high δ¹³C and δ¹⁵N values driving the pattern of temporal variation observed in the hair. The potential exceptions are Burials 7 and 8 in which δ³⁴S and δ¹⁵N values were significantly correlated (Spearman’s ρ, ρ = 0.68, p < 0.01; Spearman’s ρ, ρ = 0.86, p < 0.01) (Figure 7). A diet that varies in the

**FIGURE 2** Intra-individual multi-tissue comparison of δ¹³C and δ¹⁵N values of burials from Huaca Santa Clara (EIP). All δ¹³C and δ¹⁵N values are adjusted for direct comparison to bone collagen δ¹³C and δ¹⁵N values.

**FIGURE 3** Bone collagen δ¹³C and δ¹⁵N values by site and period with labels indicating burial number.
contribution of camelid manure-fertilized maize and non-fertilized C₃ plants (e.g., legumes) would better account for the isotopic signatures observed and provide a more parsimonious interpretation for Burials 2, 4, 10, and 13. Maize fertilized by camelid dung would be characterized by relatively high δ¹³C, high δ¹⁵N, and low δ³⁴S values, while non-fertilized C₃ plants, such as legumes or wild C₃ plants, would be characterized by relatively low δ¹³C, low δ¹⁵N, and low δ³⁴S values (Szpak et al., 2019; Szpak & Chiou, 2019).

Burial 7 and 8 have evidence for non-dietary changes in their δ¹³C, δ¹⁵N and δ³⁴S values involving a potential pregnancy and short-term migrations, respectively. Burial 7 showed a steady decrease in δ¹⁵N values between 34–25 months before death while their δ¹³C and δ³⁴S values remained relatively stable (Figures 7 and 8). This approximately nine-month period matches the isotopic changes that have been documented during modern pregnancies (Fuller et al., 2004). This individual was identified as a probable female (20–25 years old) based on the osteological analysis (Dillon, 2015) and the isotopic variation in the hair of Burial 7 suggests a pregnancy may have occurred approximately 2 years before her death. This potential pregnancy is one of only two periods in which changes in δ¹³C and δ³⁴S values for Burial 7 were not synchronous with changes in δ¹⁵N values (Figure 7). Burial 8, who could have been a warrior based on the osteological evidence of a perimortem cutmark on a rib (Dillon, 2015), has changes in their hair isotopic compositions that could reflect migration between isotopically distinct regions (Figures 7 and 8). From 43–31 months before death Burial 8 had an isotopic signature of −13.82 ± 0.28 ‰ for δ¹³C, +10.02 ± 0.27 ‰ for δ¹⁵N, +4.63 ± 0.53 ‰ for δ³⁴S which then quickly changed to a signature of −12.35 ± 0.29 ‰ for δ¹³C, +11.52 ± 0.18 ‰ for δ¹⁵N, +5.73 ± 0.33 ‰ for δ³⁴S for 30–22 months before death (Figures 7 and 8). This pattern suggests a sustained shift in diet and could be the result of a change in residency, however, the differences between these diets are not large enough to suggest a change of residency from the coast (C₄-rich environment with available marine resources) to the highlands (rare marine resources and a predominantly C₃ environment). The subtle isotopic variation between these two long periods of stability is likely the result of dietary variation while the individual resided in the lower Virú Valley. Perhaps this individual moved between areas of the coast with subtly different food sources as a part of their role as a warrior (i.e., between two of the coastal river valleys). The lack of comparative data from other sites in the Virú Valley and on the north coast in general, makes it difficult to assess how much systematic variation in diet may have existed among settlements within and among valleys.

Incremental hair δ¹³C and δ¹⁵N values from ritually killed children have occasionally revealed significant isotopic evidence for changes in diet leading up to their deaths (Turner et al., 2013; Wilson et al., 2007). For example, there is isotopic evidence that children selected for ritual killing during the time of the Inca (Late Horizon) were assembled before their deaths and provisioned with foods that differed from their habitual diet (Andrushko et al., 2011; Wilson et al., 2007). However, the diets of individuals from Huaca Santa Clara dating to the LMH showed no evidence of dietary manipulation as a

| TABLE 3 | Summary of the δ¹³C, δ¹⁵N, and δ³⁴S values from 1.0 cm incremental hair samples |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Burial ID | Site | Time period | N | Range δ¹³C | Mean δ¹³C | Range δ¹⁵N | Mean δ¹⁵N | Range δ³⁴S | Mean δ³⁴S |
| TEAL 1874 | Huaca Gallinazo | EIP | 3 | −16.77 to −14.27 | −15.55 | −8.50 to +9.33 | +9.00 | +5.60 to +6.06 | +5.46 |
| TEAL 1877 | Huaca Santa Clara | LMH | 3 | −16.90 to −13.72 | −13.81 | −8.12 to +8.80 | +8.43 | −11.32 to +12.41 | +12.35 |
| TEAL 2097 | Huaca Santa Clara | LMH | 2 | −16.03 to −14.96 | −15.49 | −7.01 to +7.56 | +7.29 | +8.12 to +8.80 | +8.12 |
| TEAL 2104A | Huaca Santa Clara | EIP | 33 | −18.06 to −11.32 | −12.41 | +7.49 to +9.84 | +8.39 | +7.01 to +7.56 | +7.29 |
| TEAL 2104B | Huaca Santa Clara | EIP | 3 | −17.79 to −13.50 | −16.99 | +6.49 to +8.37 | +7.94 | +8.08 to +9.01 | +8.53 |
| TEAL 2110 | Huaca Santa Clara | LMH | 43 | −14.40 to −11.02 | −12.77 | +6.05 to +11.90 | +10.20 | +8.08 to +9.01 | +8.53 |
| TEAL 2128 | Huaca Santa Clara | EIP | 6 | −15.08 to −12.91 | −13.56 | +7.66 to +7.99 | +7.94 | +8.08 to +9.01 | +8.53 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Abbreviations: EIP, early intermediate period; LMH, late middle horizon. |
FIGURE 4  Incremental hair $\delta^{34}S$, $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values from Huaca Gallinazo and Huaca Santa Clara compared to the expected range for a marine-based diet: $\sim +20\%$ for $\delta^{34}S$, $\sim -15$ to $\sim -8\%$ for $\delta^{13}C$ (a), and $\sim +15$ to $+25\%$ for $\delta^{15}N$ (b).

TABLE 4  $^{87}$Sr/$^{86}$Sr data from Huaca Gallinazo and Huaca Santa Clara

| Burial # | Arch site   | Time       | Tooth                  | Lab ID# | $^{87}$Sr/$^{86}$Sr | SD   |
|----------|-------------|------------|------------------------|---------|---------------------|------|
| 1        | Huaca Gallinazo | EIP       | Third Permanent Molar | 1871    | 0.70618             | 0.00006 |
| 2        | Huaca Gallinazo | EIP       | First Deciduous Molar | 2098    | 0.70755             | 0.00004 |
| 8        | Huaca Santa Clara | EIP       | Third Permanent Molar | 2123    | 0.70853             | 0.00004 |
| 9        | Huaca Santa Clara | EIP       | Third Permanent Molar | 1875    | 0.70585             | 0.00005 |
| 13       | Huaca Santa Clara | EIP       | Third Permanent Molar | 2100    | 0.70477             | 0.00008 |
| 14       | Huaca Santa Clara | EIP       | Third Permanent Molar | 2126    | 0.70741             | 0.00008 |
| 4        | Huaca Santa Clara | LMH      | Second Permanent Molar | 2249    | 0.70597             | 0.00006 |
| 5        | Huaca Santa Clara | LMH      | Second Permanent Molar | 2102    | 0.70868             | 0.00016 |
| 6        | Huaca Santa Clara | LMH      | Second Permanent Molar | 2256    | 0.70569             | 0.00007 |
| 10       | Huaca Santa Clara | LMH      | Second Permanent Molar | 2118    | 0.70639             | 0.00005 |
| 11       | Huaca Santa Clara | LMH      | Third Permanent Molar | 2111    | 0.70778             | 0.00007 |

Abbreviations: EIP, early intermediate period; LMH, late middle horizon.

FIGURE 5  $\delta^{13}C$ and $\delta^{15}N$ values of human bone collagen (a) and soft-tissues (b) adjusted for TEF and compared to archaeological camelid bone collagen (Szpak et al., 2014) and adjusted modern plant (Szpak et al., 2013) $\delta^{13}C$ and $\delta^{15}N$ values from the Moche Valley (mean values ±1σ).
means of preparing these individuals for their ritual deaths. The hair samples from the LMH ritual killing event at Huaca Santa Clara did not show a consistent shift in diet or convergence on a similar diet in the months leading up to death (Figure 9). Neither was there a consistent change in the short-term diet, represented by hair samples, relative to the long-term diets, represented by bone collagen (Figure 6). There is, therefore, no isotopic evidence for a change in diet in the months before the ritual killing event. There may, however, have been some significant dietary changes that occurred for these individuals before death that were simply not detectable using these isotopic methods. Stable carbon and nitrogen isotope analysis can only distinguish changes in diet that relate to isotopically distinct food sources. For example, if individuals began consuming maize beer (chica) instead of maize or higher quality cuts of camelid meat rather than low-quality cuts, this would not be visible isotopically.

### 3.4 Isotopic evidence of non-locals

The strontium isotope data provide evidence for the inclusion of non-local individuals in the lower Virú Valley burials. Within this research the term “highland” refers to extremely high elevation zones above the suni/jalca (3500–4000 masl) while “coastal” is used in reference to the chala zone (0 to ∼500 masl) (Pulgar Vidal, 1996). Additionally, ethnicity is assumed to be innate based on the area of childhood residence. A comparison to modeled $^{87}$Sr/$^{86}$Sr values for this region (Scaffidi & Knudson, 2020) revealed that Burials 5, 8, and 11 had $^{87}$Sr/$^{86}$Sr values higher than 0.70770 which was likely the product of a highland origin, outside of the Virú Valley (Table 4 and Figure 10). All other Burials had $^{87}$Sr/$^{86}$Sr consistent with a childhood spent below the suni/jalca in the lower Virú Valley (0.70384–0.70770) (Table 4 and Figure 10).

While Burials 5, 8, and 11 had $^{87}$Sr/$^{86}$Sr ratios which suggest a childhood residence in the highlands (Figure 10), only the $\delta^{13}$C and $\delta^{15}$N values of Burial 5 support a potentially highland diet. Burials 8 and 11 had $\delta^{13}$C, $\delta^{15}$N, and $\delta^{34}$S values that were very similar to other individuals at Huaca Gallinazo and Huaca Santa Clara, who likely had “coastal” diets (Figure 3 and Table 2). Burial 8 was identified as a potential young adult male warrior dating to the EIP and may have been born in the highlands based on his high $^{87}$Sr/$^{86}$Sr ratio (Table 4 and Figure 10). He appears to have spent most of his adult life living on the coast or moving between different coastal areas, based on his isotopically coastal diet (Table 2 and Figures 3 and 8). Burial 11, however, was a younger individual (12 years ±21 months) and either similarly spent a short amount of their childhood in the highlands before permanently moving to the coast, or consumed a coastal diet throughout their life while living in the highlands. The rapid rate of collagen turnover during growth experienced by adolescents (Hedges et al., 2007) precludes the identification of one of these scenarios as more or less likely on the basis of the isotopic data.

Burials 4 and 6 had cranial modifications suggestive of highland origins (Figure 11), but enamel $^{87}$Sr/$^{86}$Sr values consistent with a childhood residence in the lower Valley and $\delta^{13}$C and $\delta^{15}$N values similar to a “coastal” diet (Table 2 and Figures 3 and 10). Burials 4 and

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**FIGURE 6** Intra-individual change in diet over time before death based on $\delta^{13}$C and $\delta^{15}$N inter-tissue corrected values for burials from Huaca Gallinazo EIP Burial 2 (a) and Huaca Santa Clara LMH: Burial 3 (b), Burial 4 (c), Burial 10 (d)
6 could represent individuals living in the intermediate elevations of the Virú Valley, (e.g., yungas or quechua) an area where ceramic evidence suggests that communities formed which were variably influenced by highland and coastal populations (personal communication Amedeo Sghinolfi). Little is currently known about the Virú Valley population that lived in these intermediate elevation zones. Specifically, it is unclear whether $^{87}$Sr/$^{86}$Sr values and dietary differences visible through $\delta^{13}$C, $\delta^{15}$N, and $\delta^{34}$S would differentiate these individuals from those living in the lower Virú Valley. The highland style cranial modifications and “coastal” isotopic signatures may suggest that Burials 4 and 6 were from this intermediate zone where the population had ties with the polities located in the Carabamba Plateau and the Huamachuco area, while also interacting with the coastal Virú Valley population (Amedeo Sghinolfi, unpublished data). Additional baseline $^{87}$Sr/$^{86}$Sr values from archaeological sites in the lower, intermediate, and upper portions of the Virú Valley are required to establish the local range of $^{87}$Sr/$^{86}$Sr values clearly and determine the ability for strontium isotope analyses to distinguish between these cultural zones. Oxygen isotope analysis may also provide useful distinctions based on differences in elevation, although $\delta^{18}$O values in human tissues are challenging to interpret as they may be impacted by evaporative processes during the preparing of drinks such as the boiling of water to produce chicha (maize beer), obscuring geographic patterning in groundwater $\delta^{18}$O values (Gagnon et al., 2015; Knudson, 2009).

3.5 | Huaca Santa Clara ritual killing event

While only one child with strong strontium isotope evidence for highland residency was identified out of the seven EIP children analyzed, half of the children ritually killed at Huaca Santa Clara may have lived outside of the lower Virú Valley during their life based on their upright burial position and cranial modifications. At the very least, the upright burial position and cranial modifications for these children suggest exchange of cultural practices from the highlands to the coast or the
maintenance of highland cultural practices by immigrant populations. The inclusion of both coastal, intermediate and highland populations in the LMH burials supports that coastal and highland groups were interacting during this period (L. Masur, 2012; Millaire, 2015). A child with highland cranial modifications (Burial 6) was selected as the principal burial (Dillon, 2015) and the retainer burials represented children from potentially intermediate, highland, and coastal populations. Juvenile llamas that were interpreted as having been raised locally on the coast accompanied these burials (Millaire, 2015; Szpak et al., 2014) and may have been selected to further establish the importance of coastal-highland cooperation in this ritual. Previous interpretations of this ritual killing event suggested that it may have been enacted at Huaca Santa Clara after the site's abandonment to establish and legitimize the power of the ruling elites over the population (Dillon, 2015; Millaire, 2015). The inclusion of children from a mixture of highland, intermediate, and coastal areas may have functioned to establish the elites’ power over the entire population of the Virú Valley in addition to linking their legitimacy to the past strength of the Virú Polity.

3.6 | Why fish when you could farm?

Contrary to expectations, all the individuals analyzed from the lower Virú Valley relied heavily, perhaps nearly exclusively, on terrestrial resources. Archaeological sites along the coast cannot be assumed to have relied on marine resources (Carmichael et al., 2014; Falabella & Sanhueza, 2019), however the Humboldt current supports one of the world’s most productive marine ecosystems (Chavez et al., 2008). So why were the people of the Virú Valley, who lived so close to this rich source of marine food, relying so heavily on agro-pastoral resources during the EIP?

The individuals analyzed in this study could represent a subset of the population that relied on terrestrial and agricultural resources to a greater extent than the population writ large. Agricultural products and camelid meat could have been high-status foods that were principally available at the larger centers of Huaca Gallinazo and Huaca Santa Clara, while marine resources were more significant to smaller commoners’ settlements (Reitz, 1979). Fishing may also have been practiced as a distinct economic specialization which limited its availability to predominantly agricultural communities (e.g., Tomczak, 2003). Different skillsets, knowledge, and technologies are required for agriculture and fishing, which might have prevented certain individuals or groups from practicing both.
successfully at larger scales (Moseley, 1992, p. 22). Ethnographic accounts from the north coast of Peru provide evidence that in the Late Horizon and post-contact periods, fishing and farming communities lived separately. Fisherfolk had a unique language distinct from the dialect of agriculturalists (Rabinowitz, 1983), members of the fisherfolk communities did not intermarry with agriculturalists, and fishing communities were governed by their own hereditary lords (Moseley, 1992-22; Netherly, 1977). Separate communities could have formed in the Virú Valley that specialized in marine and terrestrial resource exploitation, which would account for the terrestrial-based diets of those analyzed in this research.

The costly development and maintenance of an agropastoral mode of subsistence may also explain why communities in the Virú Valley became dependent on crops. During the EIP large investments were being made to expand irrigation networks throughout the Virú Valley which would have improved otherwise marginal land and resulted in greater yields of crops and the capacity to support both larger herds of camelids and the growing population of the lower Virú Valley (Millaire, 2010; Willey, 1953). The large investments required to maintain and expand irrigation networks may quickly have led to the dependency on agricultural food for large portions of the valley, especially once the population outgrew the carrying capacity of local marine resources.

The ability of local elites to control and monopolize agricultural resources may further explain why terrestrial resources were so important during the height of the Virú polity in the EIP. Land in the arid coastal valleys of the Andes requires irrigation and possibly fertilization to ensure its productivity (Billman, 2002; Masuda, 1985; Moseley, 1972) and the development and maintenance of irrigation systems in coastal valleys would have allowed subsets of the population to control this increased productivity (Moseley, 1972). For example, farmers of the southern Peruvian highlands went to great lengths to access offshore guano deposits to be used as fertilizer during early Colonial times, because without this important source of nutrients, their crops would not have produced adequate yields (Julien, 1985). In the Virú Valley, crop surpluses could be easily stored allowing those who controlled the irrigation systems to accumulate wealth and increase their power and influence (Lambert et al., 2012). Large storage rooms at the site of Huaca Santa Clara (Millaire, 2010) demonstrate the importance that the Virú Polity placed on the storage and redistribution of agricultural resources. The excess wealth could then have been used to further expand irrigation networks, feed growing local camelid herds, and increase the yield of surrounding lands. The strong connection between the development of the Virú Valley irrigation system and the rise of the Virú polity suggests that these leaders were likely those successful elites that monopolized access to agricultural resources.

The relatively low δ15N values observed among the individuals at Huaca Gallinazo and Huaca Santa Clara do not suggest crops were being extensively manured. Seabird guano produces extremely high δ15N values, similar to or higher than marine fish and mammals (Szpak et al., 2012) and macrobotanical remains from the Jequetepueque Valley that have been interpreted as having been fertilized with composted camelids manure have δ15N values that are, on average, several ‰ higher than the human bone collagen or hair keratin analyzed in this study (Szpak & Chiou, 2019). The low δ15N values therefore suggest a greater emphasis on irrigation rather than manuring to increase agricultural productivity (Austin & Vitousek, 1998), but additional isotopic data from both humans and botanical remains must be collected to test this hypothesis.

The consistently high contribution of C4 resources among individuals from both Huaca Gallinazo and Huaca Santa Clara lends further support to the characterization of the Virú polity as a true state-level society, as this represents an important system of redistribution (Millaire, 2010; Millaire et al., 2016). Local elites in the Moche Valley may have been similarly monopolizing agropastoral resources to establish their power and control during both the EIP and Middle Horizon as the early Moche state also intensified agricultural production during these periods (Billman, 2002; Lambert et al., 2012). This research supports the notion that the Virú polity represents one of the oldest functioning states along the north coast of Peru and highlights the importance of agricultural resources, especially maize, to the early state-level societies along the north coast of Peru (Millaire, 2010).
4 | CONCLUSION

Stable isotope analysis provided insight into the lives of 13 individuals as well as the functions of the societies in which they lived. The analysis of soft tissues and incremental hair provided a longitudinal dataset that generated insight beyond the scope of bone collagen as a marker of long-term average diet to reveal subtle changes in their diet through time. These individuals consumed a predominantly terrestrial diet based on C4 plants and camelid meat despite their proximity to the productive coast. Yet they also experienced large changes in their lives that were observable isotopically, including shifts in diet, changes in health, and both short and long-term migrations. These results demonstrate how dynamic individuals’ lives and mobility patterns were during the EIP. A combination of distinct farming and fishing communities, a developed dependency on agricultural resources, possibly due to population pressure, and the monopolization of crops by elites help explain why terrestrial resources were the staple foods of the early Virú Polity, which continued during the LMH. The strong highland influence in the LMH ritual killing event at Huaca Santa Clara highlights the need for further research into the interactions between coastal and highland populations and the role that those living in intermediate zones played as potential mediators of these interactions. Continued use of stable isotope analysis within the Virú Valley and along the north coast of Peru will provide enhanced insight into this important region of the Andes.

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AUTHOR CONTRIBUTIONS

Jean-François Millaire: Conceptualization; funding acquisition; resources; supervision; writing-review & editing. Paul Szpak: Formal analysis; funding acquisition; supervision; writing-review & editing. Corrie Hyland: Formal analysis; investigation; methodology; writing-original draft; writing-review & editing.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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