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An evaluation of fecal stanols as indicators of population change at Cahokia, Illinois

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ABSTRACT

Fecal stanols deposited in sediment provide evidence of trace human waste products and have been proposed as a proxy for measuring population change. Despite its potential to contribute to paleodemographic studies, the method has not been evaluated against conventional archaeological population reconstructions to determine its fidelity in identifying changes in ancient populations nor has it been applied in an environmental setting outside of the Arctic, where low temperatures enhance stanol preservation. We studied sediment cores recovered from a lake adjacent to Cahokia, the largest and most well-studied prehistoric mound center in North America. We found fecal stanol data closely track independently established population reconstructions from multiple sources, confirming the utility of the method and demonstrating its viability in temperate climates.

1. Introduction

Understanding a region's demography is crucial to understanding its history. Population size and density are the consequences of subsistence and settlement strategies and are impacted by the effects of climate change, warfare, disease, migration, famine, and political, social, and economic instability. For example, the impacts of technological advancements and increasing globalization are made clear through the meteoric rise in world population toward the present (Lam, 2011). Indeed, demography is the human story (Tuljapurkar, 2011).

Demographic reconstructions in archaeology frequently rely on indirect evidence, such as summed calibrated date probability distribution of radiocarbon dates (SCDPD), artifact densities, architectural data, or midden volume to infer changes in population (Naroll, 1962; Hassan, 1978; Meindl and Russell, 1998; Peros et al., 2010; Downey et al., 2014). Such proxies, however, are based on generalizations and difficult-to-constrain variables, such as archaeological sampling, the decay of archaeological material, settlement density, settlement duration, and average dwelling occupancy. Fecal stanol analysis is an emergent method in geoarchaeology that provides a relatively direct proxy of population change by identifying variations in the relative amount of trace human waste products retained in the sediment of a specific watershed.

Fecal stanols are recalcitrant organic molecules that persist in sediment for hundreds to thousands of years (Bull et al., 1998). The most prominent human stanol is coprostanol (5β-cholestan-3β-ol), which is formed through microbial degradation of cholesterol in the intestinal tract. Although other mammals, including dogs, donkeys, horses, goats, and cattle, produce coprostanol, only sheep and pigs are known to generate sufficient quantities that could mask changes in human stanol concentration (Leeming et al., 1996; Bull et al., 2002; Prost et al., 2017). Once introduced into the environment as a component of feces, coprostanol is typically buried *in situ* or transported and deposited in a basin, such as a lake or marsh (Fig. 1). With time, coprostanol will degrade to its derivative form, epicoprostanol (5β-cholestan-3α-ol) (Bull et al., 2002). Thus, the abundance of coprostanol and epicoprostanol can be directly linked to the relative size of a population in the environment.

Fecal stanol analysis originated in modern sewage studies (Green et al., 1992), before being employed by archaeologists to identify human presence on a specific landscape (Bethell et al., 1994; Bull et al., 2001; Sistiaga et al., 2014). D'Anjou et al. (2012) were the first to connect changes in the amount of recovered stanols over time to changes in the population of a small settlement north of the Arctic

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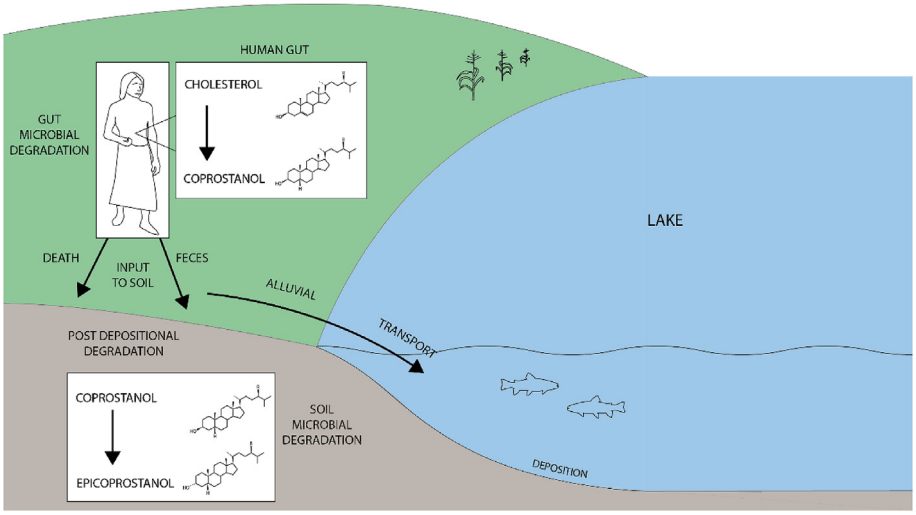

Fig. 1. Schematic depicting the formation, deposition, and degradation of human fecal stanols.

Circle in Norway. Despite the success of D'Anjou et al., the method is yet to be evaluated in an archaeological setting with previous population reconstructions from archaeological studies. Additionally, it remains to be seen if fecal stanol analysis is a viable technique in climates other than those associated with high latitudes, where low temperatures may have enhanced preservation of the stanols.

To evaluate the efficacy of fecal stanols as proxies of human population change relative to other measures, we conducted a blind study on cores from Horseshoe Lake, Illinois, which contains in its watershed the Cahokia Mounds Historic Site (UNESCO No. 198), a massive prehistoric mound center and one of the most intensely studied prehistoric archaeological sites in the United States (Fig. 2) making it especially well-suited for fecal stanol biomarker analysis (Fowler, 1989; Pauketat and Emerson, 1997; Milner, 1998; Pauketat, 1998; Emerson, 2002). First, the site is situated adjacent to a large oxbow lake and coprostanol that was transported or deposited in the lake should be present in lake bottom sediment. Second, Eurasian livestock, notably pigs and sheep, were not introduced into the Americas until the 16th and 17th centuries, roughly two centuries after the abandonment of Cahokia, and therefore elevated pre-contact coprostanol levels are most likely attributable to a human presence (Leeming et al., 1996). Although low levels of coprostanol may derive from wild animals or microbial activity and form a background distribution (Holtvoeth et al., 2016), the large and fluctuating number of humans known to have inhabited the watershed is the best candidate for controlling major changes in the stanol record. Third, considerable effort has been directed toward reconstructing Cahokia's population dynamics, from early estimates conducted in the 19th century (McAdams, 1882) to modern population density calculations (Pauketat, 2003), thus providing multiple population reconstructions for comparison (Pauketat and Lopinot, 1997; Milner, 1986, 1998; Pauketat, 2003).

Existing population reconstructions for Cahokia are based on site architectural data and suggest that Cahokia began emerging as a large population center during the Edelhardt Phase (1000–1050 CE) (Table 1) (Milner, 1998; Pauketat, 2003). According to these reconstructions, the population of the site peaked during the Lohmann Phase (1050–1100 CE), before declining slightly in the Stirling Phase (1100–1200 CE) and declining significantly in the Moorehead Phase (1200–1275 CE). By the Sand Prairie Phase (1275–1350 CE), the region was largely abandoned (Pauketat and Lopinot, 1997), producing one of

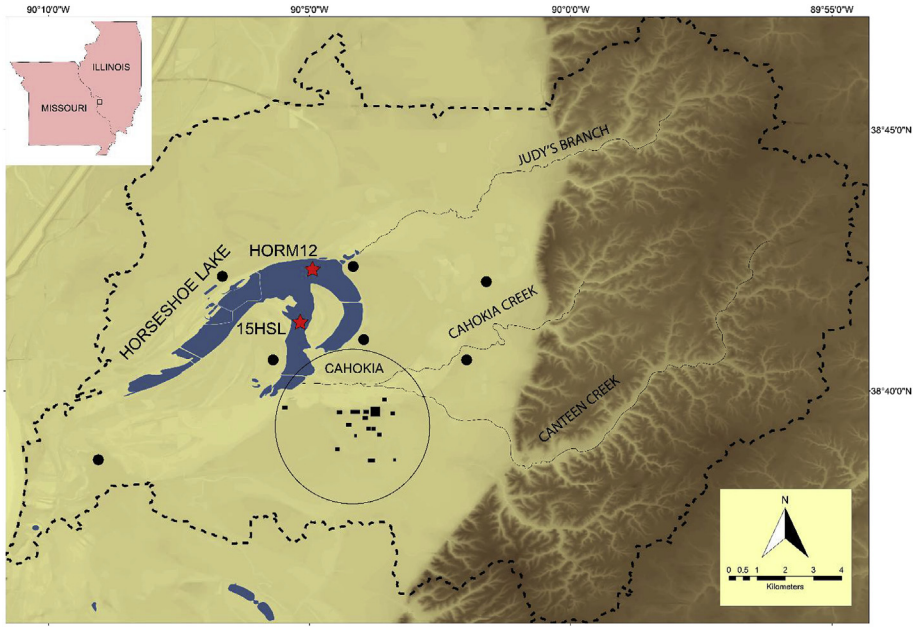

Fig. 2. Cahokia regional vicinity and Horseshoe Lake watershed, shown as the black dashed line. Coring sites are indicated by red stars. Cahokia largely consists of deposits that are within the large circle; black rectangles indicate the location of major Cahokian mounds. Black dots show the locations of small archaeological deposits (< 2 mounds) occupied contemporaneously with Cahokia (approx. 1000–1400 CE; Milner, 1998). Base map elevation data are derived from the National Elevation Dataset (Gesch et al., 2002). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Cahokia region chronology, after Fortier et al. (2006).

Calibrated Chronology Years CE	Phase
1275–1350	Sand Prairie
1200–1275	Moorehead
1100–1200	Stirling
1050–1100	Lohmann
1000–1050	Edelhardt
Approx. 975 - 1000	Merrell
Approx. 950 - approx. 975	Lloyd
900 - approx. 950	Collinsville

the most enduring puzzles in American archaeology and generating numerous hypotheses as to the cause of Cahokia's demographic decline and eventual abandonment (Milner, 1990, 1998; Emerson, 2002; Woods, 2004; Kelly, 2009; Benson et al., 2009; Munoz et al., 2015). The exact timing of the changes in occupation, however, is made somewhat problematic by the inherent error associated with calibrated radiocarbon ages (Milner, 1998). Although absolute population levels are not in agreement, Cahokia population reconstructions show similar trends over time (Pauketat, 2003). We can evaluate the degree to which these demographic reconstructions for Cahokia align with data derived from fecal stanols as indicators of ancient population change.

2. Materials and methods

We created a blind study of sediment from Horseshoe Lake core (HORM 12) (Munoz et al., 2014, 2015) to compare the ability of fecal stanol analysis to reconstruct relative population changes relative to the population history inferred from architectural data. We measured samples without age or depth reference to reduce laboratory bias. To address the issue that a single core may not represent the entire watershed, we obtained and analyzed a second sediment core (15HSL) approximately 2 km south of HORM12 closer to the input of Cahokia Creek (Fig. 2). Both cores were recovered using a modified Livingstone piston corer at water depths of approximately 1 m. Munoz et al. (2014) identified two broad distinctions in the stratigraphy of HORM12: a unit of dark brown fine silty clay (0–199 cm) and a unit of brown-grey sandy fine silt and abundant gastropod shells (218–320 cm). These homogenous to poorly stratified units are interrupted by multiple distinct silty clay layers, which Munoz et al. (2014, 2015) interpreted as overbank deposits from Mississippi River flood events. 15HSL is structurally and stratigraphically very similar to HORM12, although its units are slightly thicker due to its closer position to the lake's input. Munoz et al. (2014) used the software Clam 2.2 (Blaauw, 2010) and dates from nine terrestrial accelerator mass spectrometry (AMS) samples to build an age model for HORM12. This age model also provided the basis for our analyses of Core 15HSL, and we linked the two cores using stratigraphic correlation of flood events identified in both cores (Munoz et al., 2015).

We processed 29 HORM12 sediment samples with dry weights ranging from 2 to 6 g and 13 15HSL sediment samples with dry weights ranging from 4 to 11 g. We freeze-dried sediment samples and homogenized them prior to extraction. Stanols were extracted from the sediment by overnight soxhlet extraction with 200 mL of dichloromethane (DCM) in cellulose thimbles (Whatman). We then concentrated samples to a final volume of 0.5 mL using rotary evaporators (Büchi) and evaporation under a gentle nitrogen stream. Stanols were derivatized into their trimethlysilyl (TMS) ethers through a reaction with N,O-Bistrifluoroacetamide (BSTFA) for 30 min at 70 °C.

We injected the derivatized samples using an autosampler (7683B series, Agilent Technologies, Santa Clara, California, USA) and an Agilent gas chromatograph (GC; 6890N series) equipped with a mass selective detector (MSD; Agilent 5973 inert series). We employed a Supelco fused silica capillary GC column (0.25 mm ID x 30 m × 0.25 μm film thickness). We programmed the temperature profile of the GC oven to rise from 45 °C to 225 °C at 25 °C/min, then to 285 °C at 1.5 °C/min and held for 12 min. We set the injector temperature at 285 °C. We used helium as a carrier gas, and we used the MSD in the Electron Ionization (EI) mode. The source and quadrupole temperatures were set at 230 °C and 150 °C, respectively.

We identified stanol and sterol compounds (coprostanol, epicoprostanol, 5α-cholestanol, cholesterol, and stigmasterol) by comparing characteristic mass spectra fragmentation patterns and gas chromatographic retention times of samples and blank spikes with the chemical standard solutions of these compounds (Sigma Aldrich). We analyzed stanol compounds (coprostanol, epicoprostanol, and 5α-cholestanol) in both cores to evaluate population change and analyzed sterol compounds (cholesterol and stigmasterol) in HORM12 to compare trends between compounds of different origins. We generated data for analysis using the Agilent Environmental Chem Station software. We determined concentrations by comparing peak areas with a calibration curve and the relative response factor of an internal standard (anthracene-d10 and benzo(g,h,i) perylene-d12; AccuStandard). We were unable to quantify epicoprostanol in several samples as the peak coeluted with a currently unidentified molecule (Fig. 3). Consequently, we only quantified coprostanol and 5α-cholestanol.

3. Results

Coprostanol concentrations ranged from 16.4 to 115.1 ng/g dry sediment in HORM12 (Table 2) and from 12.0 to 173.5 ng/g dry sediment in 15HSL (Table 3). A plot of HORM12 sterol and stanol concentrations shows that coprostanol displays similar trends to the other molecules for the majority of the record, but it declines from 1000 to 1300 CE while the other molecules increase over this period. The period of 1000–1300 CE is when the watershed is known to have supported a robust human population at Cahokia, which supports the interpretation that human input is the dominant contributor to the coprostanol signal (Fig. 4).

To account for variations in degradation rate and low stanol

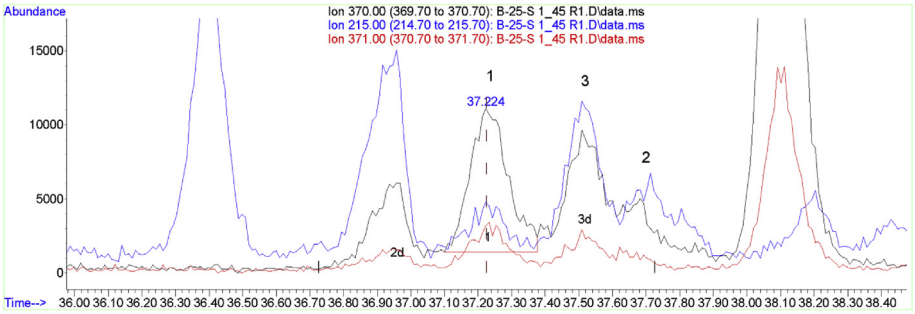

Fig. 3. Representative chromatogram showing coprostanol (1) and epicoprostanol (2) coeluted with an unidentified molecule (3). Displayed ions are 215 (blue), 370 (black), and 371 (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
HORM12 fecal stanol data summary. Concentrations of stanols represent ng/g of dry sediment. The ratio (1) refers to coprostanol/(coprostanol + 5α-cholestanol).

Depth (cm)	Age (Calibrated Year CE; Munoz et al., 2014)	Coprostanol (ng/g)	5α-Cholestanol (ng/g)	Ratio (1)	Cholesterol (ng/g)	Stigmasterol (ng/g)
7	1974	115.11	435.64	0.209	2763.1	980.64
24	1873	60.5	335.58	0.153	1265.19	484.16
27	1855	42.05	255.6	0.141	916.57	391.17
34	1824	31.97	168.58	0.159	488.47	257.12
47	1777	46.19	214.94	0.177	611.98	457.63
57	1727	51.67	225.53	0.186	787.62	685.32
67	1676	37.4	165.44	0.184	578.49	470.11
74	1637	32.13	138.23	0.189	479.56	390.26
87	1574	29.44	143.88	0.17	610.26	471.12
107	1506	16.38	128.28	0.113	153.09	235.16
117	1458	18.5	116.65	0.137	149.04	262.34
124	1428	16.79	134.78	0.111	174.73	294.65
127	1414	16.39	118.27	0.122	196.83	244.06
134	1400	16.51	121.35	0.12	183.2	99.16
148	1362	16.93	105.45	0.138	149.36	138.52
157	1321	31.87	148.87	0.176	236.71	269.14
167	1277	24.83	132.01	0.158	285.32	265.47
177	1230	30.9	122.13	0.202	233.69	248.63
227	1121	44.5	115.86	0.278	169.88	148.26
239	1068	44.82	103.51	0.302	181.26	143.77
248	1024	43	96.93	0.307	179.09	130.12
254	1003	50.1	108.94	0.315	183.5	140.25
274	924	18.11	56.59	0.242	125.6	89.1
291	842	30.41	101.59	0.23	276.77	190.27
297	813	24.28	67.98	0.263	136.34	86.35

Table 3
15HSL fecal stanol data summary. Concentrations of stanols represent ng/g of dry sediment. The ratio (1) refers to coprostanol/(coprostanol + 5α-cholestanol).

Depth (cm)	Age (Calibrated Year CE; Munoz et al., 2014)	Coprostanol (ng/g)	5α-Cholestanol (ng/g)	Ratio (1)
20	1896	173.51	663.57	0.207
45	1792	97.34	387.86	0.201
60	1722	53.96	265.18	0.169
75	1651	68.74	317.71	0.178
122	1458	26.41	148.95	0.151
150	1381	12.04	90.63	0.117
170	1296	15.76	76.13	0.172
205	1163	16.39	67.81	0.195
235	1130	31.68	113.82	0.218
245	1090	42.01	147.37	0.222
255	1048	35.32	106.01	0.25
265	1008	33.68	94.25	0.263
275	978	28	91.11	0.235

concentrations, we report our data using Grimalt et al.'s (1990) ratio of coprostanol to 5α-cholestanol:

$$\text{coprostanol} / \text{coprostanol} + 5\alpha\text{-cholestanol} \quad (1)$$

5α-cholestanol is formed from the degradation of cholesterol by soil microbial communities (Bull et al., 2002). By relating coprostanol to 5α-cholestanol, we make a comparison of stanol input and preservation in a specific environment (5α-cholestanol) to stanol input from feces (coprostanol). Thus, high values of ratio (1) indicate a large presence of humans in the region and low values indicate a small human presence. A plot of ratio (1) over the last 1200 years indicates three notable trends: an increase to a maximum at approximately 1000 CE, a decrease to a minimum at approximately 1400 CE, and an increase in ratio values to the present (Fig. 5). Both cores display these trends, although absolute values are higher in the prehistoric samples of HORM12. Except for a divergence in the 19th century, ratio values are similar between the cores in post-contact samples.

4. Discussion

The trends present in the stanol results are consistent with the population history inferred from demographic reconstructions of Cahokia based on architectural data (Fig. 6). Ratio (1) increases in the 10th century, when regional population nucleation and large-scale mound building at Cahokia began (Fowler, 1989; Milner, 1998). The steep positive slope of this ratio confirms that Cahokia population increased rapidly in the 10th century (Pauketat and Lopinot, 1997). The fecal stanol data indicate a population high during the 11th century, in alignment with previous population reconstructions that are based on architectural evidence (Pauketat, 2003), although the stanol maximum occurs slightly earlier than the population high of the archaeological reconstructions (Fig. 6). The offset in peaks may be explained in two ways. First, the uncertainty associated with radiocarbon dates for the sediment cores is large enough that the peaks may potentially align—thus there may be no offset. The second explanation is that the offset is real. Because the fecal stanol record represents population change at a watershed scale, the offset suggests many people were present in the Horseshoe Lake watershed prior to the centralization and major demographic expansion within the site of Cahokia. This latter explanation is supported by the presence of many Edelhardt Phase sites in the Horseshoe Lake watershed (Betzenhauser, 2011) and widespread and rapid land clearance that occurred between 450 and 900 CE (Munoz et al., 2014). Ratio (1) does not plateau at elevated levels and is in decline shortly after it reaches its maximum, indicating Cahokia supported its maximum population for less than a century.

Ratio (1)'s steep negative decline at the start of the 12th century suggests the population decline in the Horseshoe Lake watershed was nearly as rapid as its increase. Additionally, the decline persists at approximately the same slope from 1100 to 1400 CE, signifying that demographic decline was not sudden or erratic, but was instead a long and steady process. The decline in Ratio (1) corresponds with the timing of palisade construction in the late 12th and early 13th centuries, a point in time in which archaeologists have suggested increased political factionalization and population decline (Fowler, 1989; Trubitt, 2000, 2003; Kelly, 2009). The fecal stanol data indicate that population reached its minimum at the end of the 14th century during the Bold Counselor Oneota Phase (1350–1400 CE; Pauketat and Emerson, 1997).

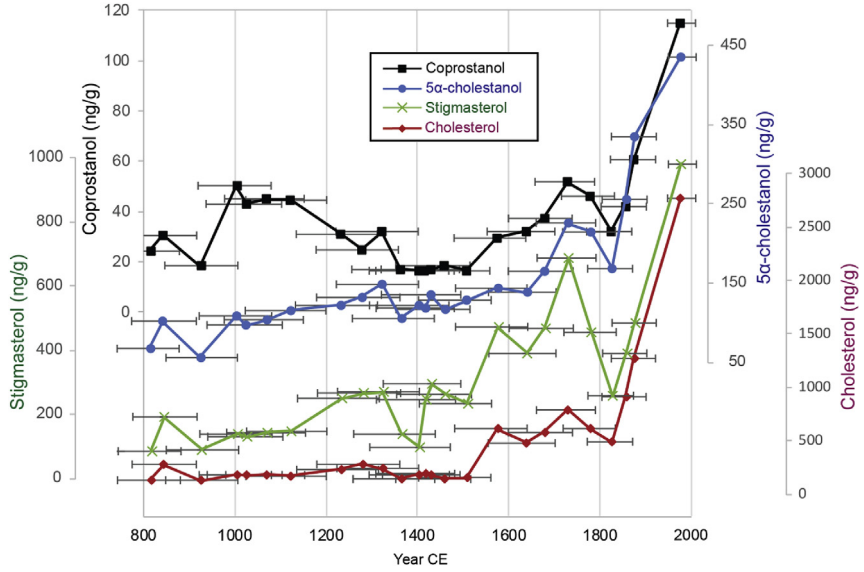

Fig. 4. HORM12 stanol and sterol concentrations (ng/g sediment). Error bars represent temporal uncertainty reported as 2-sigma (95%) confidence generated by the Clam 2.2 model (Munoz et al., 2014).

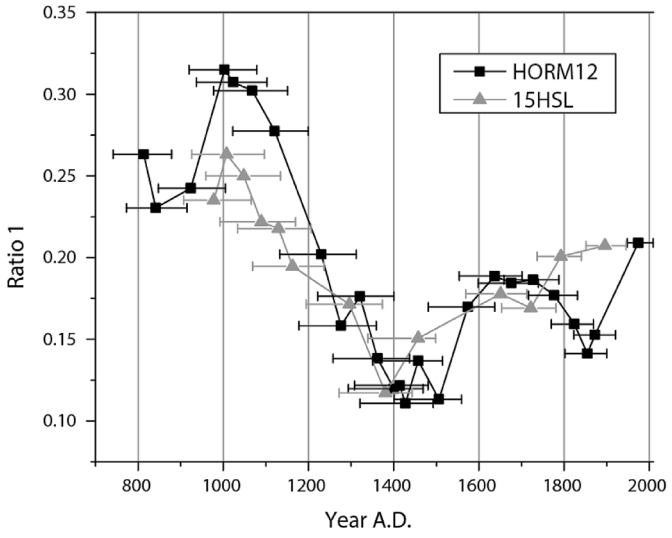

Fig. 5. Horseshoe Lake stanol data plotted as coprostanol/5α-cholestanol ratio. Error bars represent temporal uncertainty reported as 2-sigma (95%) confidence generated by the Clam 2.2 model (Munoz et al., 2014).

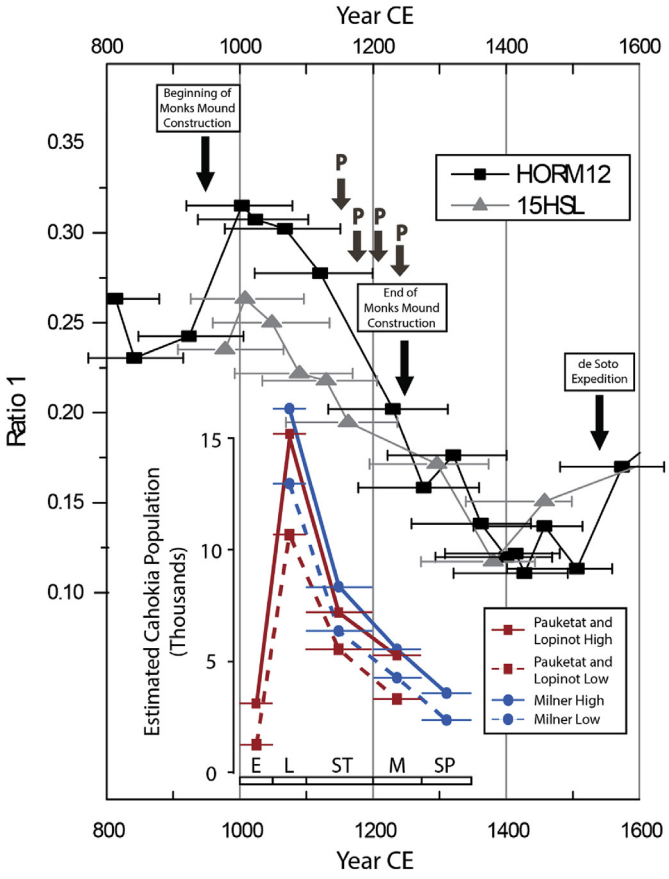

Fig. 6. Horseshoe Lake sediment stanols ratio against population estimates from Pauketat and Lopinot (1997) and Milner (1998), annotated with historical events (Fowler, 1989). The letter "P" indicates construction of a defensive palisade. The bar at the bottom of the figure indicates the temporal position of archaeological phases used in constructing population estimates: E = Edelhardt, L = Lohman, ST = Stirling, M = Moorehead, and SP = Sand Prairie.

This population minimum is later than those of previous reconstructions, which end in the Moorehead Phase (1200–1275 CE; Pauketat and Lopinot, 1997) or Sand Prairie Phase (1275–1350 CE; Milner, 1998). Ratio (1) is much lower in the 14th century than in the 10th century, indicating there was a net loss in the region's population from the period prior to Cahokia's nucleation to after its decline.

The period following Cahokia's demographic decline receives little academic attention and is poorly understood. Our data indicate the Horseshoe Lake watershed population was low throughout the 15th century, but population recovery began in the 16th century. This may represent the arrival of the Cahokia tribe, who were present at the time of sustained European interaction in the 17th century (Fowler, 1989). Ratio (1) plateaus with the onset of the historic period in the 17th century but never approaches the prehistoric maximum of the 11th century, indicating the Cahokia occupation was the largest population supported by the Horseshoe Lake watershed prior to the development of modern human waste disposal in the region. This study provides new insights to the large pre-Cahokia population present in the region prior to the demographic centralization at Cahokia and the later repopulation of the watershed following Cahokia's demographic decline. The correlation of this study and previous Cahokia population reconstructions

provides confirmation that fecal stanols are useful for tracking ancient population change in temperate environments.

5. Conclusion

The Horseshoe Lake fecal stanol record is consistent with proposed population histories at Cahokia that are based on architectural data, confirming the utility of the method to track ancient population change. The study also indicates the viability of this proxy in temperate climates and is promising for future use in other archaeological and environmental settings. Finally, the Horseshoe Lake fecal stanol record supports previous Cahokia population reconstructions while providing new detailed demographic information to the pre- and post-Mississippian time periods (< 1000 CE, > 1400 CE).

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