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## COMPARING BIOAPATITE AND COLLAGEN RADIOCARBON DATES FROM A 16TH CENTURY CEMETERY CONTEXT—EL JAPÓN, XOCHIMILCO, MEXICO CITY

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**ABSTRACT.** El Japón is a 16th century hamlet site in the marshlands of the southern Basin of Mexico in central Mesoamerica. Radiocarbon (<sup>14</sup>C) dating and OxCal modeling of human bone collagen (n = 11) identifies a range of burials at El Japón cemetery from 1550–1650 cal. CE. The refined chronology identifies use of this rural settlement well after the onset of colonial government-sponsored relocation of Indigenous people to larger settlements (congregaciones). Historically documented information in this work supports chronological modeling beyond stand-alone calibration. Stable isotopic study of bone samples demonstrates similar sources of dietary protein and carbohydrates. The similarity of carbon sources for bone apatite (bioapatite) and collagen offers security that both bone fractions are viable <sup>14</sup>C dating opportunities. Recent extension of this work examines bioapatite <sup>14</sup>C dates (n = 5) from the same bone samples when quality parameters are met—atomic carbon-nitrogen ratios of 3.2–3.3 and collagen yield of 10–20%. No significant difference is found between collagen and bioapatite dates of the same individuals (p = 0.17, Mann-Whitney U test). <sup>14</sup>C dates from human bone samples in this primarily terrestrial dietary context can be successfully acquired from either collagen or bioapatite fractions.

**KEYWORDS:** bioapatite radiocarbon, collagen radiocarbon, Colonial period, Mesoamerica.

### INTRODUCTION

El Japón is an archaeological hamlet site with extensive evidence of marshland agriculture in the Xochimilco area of the southern Basin of Mexico. Preliminary radiocarbon (<sup>14</sup>C) dating and OxCal modeling of human bone collagen (n = 11) identifies a range of burials at El Japón cemetery from 1550–1650 cal. CE (Alarcón Tinajero 2022; in preparation). Prior to this suite of <sup>14</sup>C determinations, dating of El Japón site was done contextually by archaeological material distribution (cf. González 1996). Archaeological remains of houses including stone foundations, daub, and utilitarian ceramics are found throughout the site of El Japón (González 1996). González (1996) attributed occupation of the household sites that surround the cemetery site of El Japón to the last Postclassic cultural period (900–1521 CE) and early Colonial cultural period (1521–1821 CE) based on the preponderance of Postclassic ceramic styles. The <sup>14</sup>C chronology presented here compares bioapatite and collagen dates from a sample of human burials from El Japón to demonstrate that a more precise estimate of cemetery use is possible via <sup>14</sup>C dating and modeling than what is offered by estimates based on stylistic and technical ceramic designations.

### <sup>14</sup>C Dating and Culture History

European colonization in Mesoamerica and North America more broadly had pervasive effects on indigenous communities. Indigenous societies in central Mesoamerica predating European contact generally formed part of state societies with extensive bureaucracies,

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cross-cutting ritual connections and a regional market system (Sanders et al. 1979; Parsons et al. 1983; Nichols 2017). For some communities, European colonization and settlement implied interruption or supplanting of existing political and economic structures without implying absolute cultural change (Conway 2014). Interpretation of how European colonization affected individual polities, settlements, or ethnic groups is abundant in historical and archaeological study (Jalpa Flores 2008; Zamudio Espinosa 2001; Santiago Cortez 2021). In that same approach, this study aims to contribute to the study of the early Colonial period chronology by cross-examining the utility of  $^{14}\text{C}$  dating and modeling of two related data sources: human bone bioapatite and bone collagen. Human remains are dated as opposed to material remains from the site due to the availability of human data from an archaeological salvage project (Ávila López 1995). Consideration of the cultural context of mortuary treatment that led to the El Japón cemetery skeletal assemblage permits a contextualized understanding of the chronological span of cemetery use.

Postcontact Christian proselytism and religious conversions played a role in indigenous religious expression (Ricard 1966; Inoue 2007) as mortuary and burial practices shifted to be more similar to contemporaneous European Christian practices. The most prominent change is a shift towards burials in uniform body position and burial orientation similar to modern western cemeteries (Pugh et al. 2012; Price et al. 2012). This specifically includes individual supine body position and consistent orientation of burials on an east–west axis. In comparison, prehispanic burials varied greatly in body position and were often interspersed in household courtyards throughout settlements. Burials at El Japón cemetery site were postulated to postdate European contact in the area (1521 AD) prior to  $^{14}\text{C}$  dating due to the preponderance of a cemetery pattern. El Japón burials are oriented east–west (with a slight offset), consistent with European Christian cemeteries of the time. In other Mesoamerican contexts that postdate European contact, religious influence including proselytism and European settlement predate changes in burial style (Cohen 1994; Wright 2006; Graham 2011; Warinner et al. 2012; Price et al. 2012). El Japón burials occupy a small area: 360 m<sup>2</sup>.

### **$^{14}\text{C}$ Dating Human Bone**

$^{14}\text{C}$  dates give an estimate of the age since formation and renewal of the bone samples that then serve as a proxy of human lifespans. Measurement and calibration of bone samples from human individuals provides an estimate of age since death, and by extension, burial. Dating and calibration of burial samples from the cemetery allows for an estimate of the use of the cemetery site and by extension an estimate of the occupation of the community. Human bone collagen primarily incorporates carbon (including  $^{14}\text{C}$ ) from protein sources (Ambrose and Norr 1993) as opposed to carbohydrates or lipids in diet. Bioapatite, in turn, incorporates carbon from the amalgam of carbon sources in the total diet: all macronutrients including carbohydrates, lipids, and proteins (Ambrose and Norr 1993). Dietary proteins and carbohydrates are demonstrated to differentially route to bone protein (collagen) and mineral (bioapatite) (Ambrose and Norr 1993). Bone mass is acquired and conserved in the years leading to skeletal maturity which oscillates around 19–25 years of age (Hedges et al. 2007). Turnover rates vary within individuals when comparing bioapatite and collagen but potentially on a scale too small to be of influence for  $^{14}\text{C}$  dating (Tsutaya and Yoneda 2013).

Carbon dietary sources from human diets must be considered to adequately consider the trophic and geographic origin of foods. Archaeological evidence of substantial marine food consumption would require a  $^{14}\text{C}$  approach distinct from a terrestrial dietary context. As an

example: a cultural and material archaeological context in a coastal settlement (Naito et al. 2010) gives a preliminary indication that  $^{14}\text{C}$  dated individuals consumed marine foods. Conversely, dietary protein sources in Postclassic central Mesoamerica included domestic crops and animals, wild game, and wild terrestrial or freshwater foods (Valadez Azúa and Rodríguez Galicia 2014; Moreiras Reynaga et al. 2020). Stable isotopic study of bone samples from El Japón ( $n = 74$ ) demonstrates sources of dietary protein and carbohydrates similar to other central Mesoamerican samples (Alarcón Tinajero et al. 2022). At El Japón, more than 50% of dietary protein and more than 70% of dietary carbohydrates came from  $\text{C}_4$  sources (Alarcón Tinajero et al. 2022). El Japón residents had a very restricted diet in comparison to other agricultural communities of the Postclassic and Colonial periods, consuming largely domestic crops with resulting low trophic levels (Alarcón Tinajero et al. 2022). The same diet models (Alarcón Tinajero et al. 2022) support an interpretation that marine foods formed no major portion of diet for the sampled individuals.

Geologic origins of consumed foods and geologic contexts of taphonomic environments of archaeological samples must be considered in addition to diet-based variation in carbon sources. In other geologic regions, limestone contribution to sediments and their disintegration in bodies of water are known sources of dissolved inorganic carbon that affect either bone sample preservation or  $^{14}\text{C}$  age determinations of organisms procuring water or foods from bodies of water with dissolved carbon (Gustafsson et al. 2011; Schulting et al. 2015; Svyatko et al. 2017; Hadden and Cherkinsky 2017). It is unlikely that dissolved geological carbonate played a role in carbon cycling in Lake Xochimilco because most of the Basin Mexico lies on igneous bedrock (de Cserna 1989; López-Acosta et al. 2019). The soils at El Japón site where burials took place are primarily andosols with overlying technosols (McClung de Tapia and Acosta Ochoa 2015) and can be assumed to have low levels of carbonates in comparison to minerals and inclusions typical of those soil types. Perennially wet soils at El Japón may have supported gradual microbial decomposition without contributing to a particularly aggressive chemical deterioration of bioapatite. Terrestrial dietary carbon sources at El Japón established through dietary modeling together with consideration of carbon cycling and soil types offer security that both bioapatite and collagen fractions are viable  $^{14}\text{C}$  dating opportunities.

## **MATERIALS AND METHODS**

### **Sampling**

$^{14}\text{C}$  dating in this study was carried out on a random stratified sample of human bone taken from burials of lower cemetery strata. The cemetery strata were defined at the time of excavation in approximately 10-cm increments. Layers 11–8 are grouped as lower layers, and these begin lower than the foundations of the only architecture in the cemetery area (Figure 2). Burials in upper layers (7–4) are far fewer in number and generally occupy space open between earlier burials. Samples were taken for both  $^{14}\text{C}$  dating (this work) and stable isotope analysis (Alarcón Tinajero et al. 2022). Fibulae, radii, or ribs were selected from each individual because sufficiently thick cortical bone could be taken with minimally invasive methods. These samples were existing fragments or sampled from portions of bone that are not diagnostic for sex and age estimates, or for evaluation of pathologies. This work specifically examines bioapatite  $^{14}\text{C}$  dates ( $n = 5$ ) from concomitant collagen samples with adequate quality parameters—atomic carbon-nitrogen ratios of 3.2–3.3 and collagen yield of 10–20%.



Figure 1 Location of El Japón archaeological site in the southern Basin of Mexico. Maximum estimated extent of anthropogenic agricultural islands (chinampas) in the southern Basin of Mexico is filled iconographically (Armillas 1971). Map modified from an image with a Free Art License (YAVIDAXIU 2007). Physical Map of Mexico marking the general area of the southern Basin of Mexico (Addicted04 2011).



Figure 2 Lower stratigraphy burials (Layers 11–8), shallower burials in Layers 7–4 are not mapped here. Burials in ellipses are <sup>14</sup>C dated individuals. The map is adapted from Ávila López (1995).

### Collagen Sample Preparation

Sample preparation began with inspection for adhesives using UV light. Samples were sonicated in three consecutive acetone baths then rinsed and soaked in (Milli-Q) ultrapure water to neutrality. Cortical bone surface was removed by abrasion. Collagen isolation follows a modified Longin (1971) procedure. Procedure modification introduces a base step (Haynes 1968; Gurfinkel 1987). The acid-base-acid procedure dissolves adsorbed organic material and bone mineral to isolate biogenic collagen. Stable isotope samples ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) were analyzed in a Thermo Fisher elemental analyzer isotope ratio mass spectrometer. Carbon-nitrogen atomic ratios and percent collagen yield (van Klinken 1999) were calculated as a measure of collagen preservation. Those results supported a preliminary dietary interpretation that contextualize  $^{14}\text{C}$  interpretation (Alarcón Tinajero et al. 2022). Carbon-nitrogen atomic ratios (C:N ratio; Ambrose 1990) were calculated to gauge success of collagen purification. Purified collagen samples were selected for  $^{14}\text{C}$  dating when atomic C:N ranged 3.2–3.3, indicating low diagenetic alteration to molecular structure in the depositional environment (DeNiro and Hastorf 1985).

### Bioapatite Sample Preparation

Bioapatite  $^{14}\text{C}$  samples were selected from collagen  $^{14}\text{C}$ -dated bone with adequate collagen preservation (10–21% yield and 2.9–3.5 C:N) making the sample a systematic sample. Bone samples for bioapatite analysis were sonicated three times in (Milli-Q) ultrapure water in 30-minute increments to remove any sediment not removed by abrasive cleaning. The remaining bioapatite pretreatment procedure follows Cherkinsky (2009). The procedure uses acetic acid to remove secondary carbonates that may otherwise react during acidification. Samples were acidified with 100% phosphoric acid ( $\text{H}_3\text{PO}_4$ ) and heated for 12 hours at 50°C before analysis in a Thermo Gas Bench coupled to a Delta V isotope ratio mass spectrometer (IRMS) at the Center for Applied Isotope Studies (CAIS). Separate aliquots of solid, pretreated sample were acidified in evacuated reaction vessels and the reaction products were cryogenically purified to recover  $\text{CO}_2$ .  $\text{CO}_2$  samples were cryogenically purified from the other reaction products and catalytically converted to graphite using the method of Vogel et al. (1984). Graphite  $^{14}\text{C}/^{13}\text{C}$  ratios were measured using the CAIS 0.5 MeV accelerator mass spectrometer. Sample ratios were compared to the ratios measured from the Oxalic Acid I standard (NBS SRM 4990).

### $^{14}\text{C}$ Date Calibration and Modeling

Demonstrated low-trophic level terrestrial diets of El Japón individuals (Alarcón Tinajero et al. 2022) justified use of an atmospheric calibration curve to calibrate human bone  $^{14}\text{C}$  dates: both collagen and bioapatite. Dates are calibrated using the North American IntCal20 Curve (Reimer et al. 2020) using OxCal 4.4 (<https://c14.arch.ox.ac.uk/>) software. OxCal 4.4 software was also used to create and test chronological models. Bayesian statistics express the degree of belief in models created with contextual information independent of the  $^{14}\text{C}$  age determinations (Hamilton and Krus 2017). Contextual information may include stratigraphic information that relates dated objects or individuals to each other. OxCal agreement indices ( $A_{\text{agreement}}$  and  $A_{\text{overall}}$ ) are used to evaluate the agreement between the  $^{14}\text{C}$  data and the model, with  $A_{\text{agreement}} = 60$  and  $A_{\text{overall}} = 60$  being the thresholds of acceptable agreement (Bronk Ramsey 1995). The following OxCal functions are used to constrain the date distributions: Phase, Sequence, and C\_Date. A C\_Date, or calendar date within a Sequence constrains modeled calibrated dates (Thompson et al. 2018) to postdate or predate a calendar year. A C\_Date is selected and justified by information independent of the  $^{14}\text{C}$  age determinations. A typical

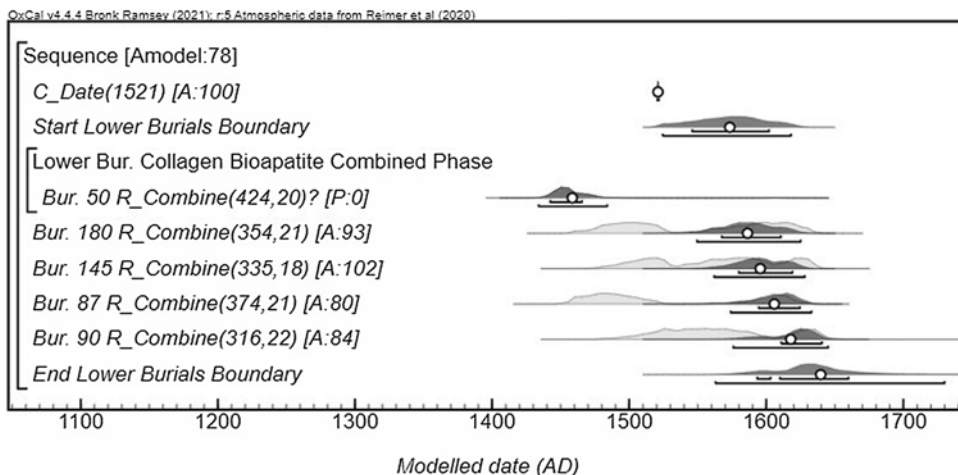


Figure 3 Model 3 Plot. Mean values are marked with “o”. 68.3% and 95.4% ranges are marked with brackets.

example may include a dated coin in an archaeological deposit providing the earliest date a deposit of associated artifacts may have taken place.

The year 1521 is used as a Calendar date in the included models to constrain modeled dates not tied to a particular dateable object but to the region-wide pattern of European arrival and dispersal beginning with armed conflict in the Basin of Mexico in 1521 CE (Díaz del Castillo 2003). Burial treatment of all undisturbed burials at El Japón are consistent with European Christian cemetery style—individual supine burials arranged with an east–west orientation. This pattern is not common in postclassic sites. El Japón’s mortuary treatment pattern is consistent with a period postdating European influence in Mesoamerica. Models 1 and 2 (Supplemental Material) calibrate collagen and bioapatite dates separately. Model 3 (Supplemental Material) combines bioapatite and collagen dates testing the agreement of bioapatite and collagen dates on the same individuals using a  $\chi^2$  test as implemented in the R\_Combine function.

## RESULTS

Quality indicators of all purified collagen samples—C:N ratio and collagen yields—are within acceptable range (Table 1). Burial 50 has the lowest collagen yield at 11% though this is still within acceptable range (cf. 2–22% collagen yield; Ambrose 1990). The role of Burial 50 in Bayesian models is discussed for each model. Unmodeled  $^{14}\text{C}$  dates are broadly consistent with a hypothesized late Postclassic/ early Colonial chronological origin (González 1996) based on the material archaeological remains and features of the archaeological site of El Japón prior to any  $^{14}\text{C}$  analysis.

## DISCUSSION

### The Bayesian Models

#### Model 1

Model 1 incorporates a Calendar date of 1521 in a Sequence prior to the burial Phase which includes collagen  $^{14}\text{C}$  dates only. A Calendar date command in OxCal is used in all models—

Table 1 <sup>14</sup>C dating results.

Burial	Bone fraction	Collagen yield (%)	Atomic C:N	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	<sup>14</sup> C age (BP)	Pairwise $\chi^2$ statistic <sup>a</sup>	Modeled calibrated dates <sup>b</sup>	
								68.3%	95.4%
50	Collagen	11.0	3.3	8.7	-7.6	399 ± 25	2.4	1448–1492	1440–1623
	Bioapatite	—	—	—	-2.0	460 ± 30		1427–1452	1412–1471
87	Collagen	20.9	3.3	8.2	-9.2	360 ± 25	0.9	1576–1623	1550–1634
	Bioapatite	—	—	—	-2.3	400 ± 35		1590–1620	1562–1631
90	Collagen	16.0	3.2	8.9	-7.5	299 ± 25	1.7	1554–1640	1534–1647
	Bioapatite	—	—	—	-1.9	360 ± 40		1580–1624	1554–1636
145	Collagen	12.0	3.2	8.7	-7.8	360 ± 25	2.0	1574–1622	1548–1635
	Bioapatite	—	—	—	-2.7	310 ± 25		1569–1635	1544–1643
180	Collagen	19.6	3.2	8.7	-8.9	330 ± 25	2.7	1560–1631	1541–1639
	Bioapatite	—	—	—	-3.2	400 ± 35		1589–1620	1562–1631

<sup>a</sup>5% critical value, 3.8. *df* = 1.

<sup>b</sup>Collagen dates modeled in Model 1 (Supplemental Material). Bioapatite dates modeled in Model 2 (Supplemental Material).

requiring the model to constrain individual dates after 1521 CE. 1521 is selected as the calendar year in which European contact and the earliest possible religious influence could have occurred. Burial 50 dates (bioapatite and collagen) are consistently older than other burials. Burial 50 is flagged as an outlier in Model 1 and subsequent models to calibrate and include the datapoint but without modeling the date to fall after 1521 or affecting the rest of the model. Model 1 produces satisfactory  $A_{\text{agreement}}$  and  $A_{\text{overall}}$ . Modeled dates span 1534–1647 (cal. CE 95.4%).

#### *Model 2*

Model 2 incorporates a Calendar date of 1521 in a Sequence prior to the burial Phase which includes bioapatite  $^{14}\text{C}$  dates only. These bioapatite dates are from the same individuals with dated collagen in Model 1. Like Model 1, Model 2 produces satisfactory  $A_{\text{agreement}}$  and  $A_{\text{overall}}$ . Modeled dates span 1544–1643 (cal. CE 95.4%).

#### *Model 3*

Model 3 incorporates a Calendar date of 1521 in a Sequence prior to the burial Phase which includes combined collagen and bioapatite dates. Dates from the same individual are paired using the R\_Combine function. Like Models 1 and 2, Model 3 produces acceptable  $A_{\text{agreement}}$  and  $A_{\text{overall}}$ . Modeled dates span 1545–1640 (cal. CE 95.4%).

### **$^{14}\text{C}$ Dating of Bioapatite and Collagen**

Previous work modeled the lower burial dates to 1550–1625 (cal. CE 95.4%) (Alarcón Tinajero 2022). This work uses similar modeling parameters (a calendar date of 1521) and  $^{14}\text{C}$  dates from bioapatite and collagen components of bone producing a slightly wider but comparable age estimate of 1545–1640 (cal. CE 95.4%). No significant difference is found between paired collagen and bioapatite dates. Burial 50 bioapatite and collagen dates differ from other burials despite similar dietary estimation as other individuals. The sample from Burial 50 could predate European contact but that interpretation would be unexpected as the burial orientation is consistent with all burials postdating European contact. Burial 50 was anatomically articulated at the time of excavation therefore lacking evidence of being a secondary burial. No current preservation parameters indicate insufficient preservation that may cause erroneous dating. Samples from burial 50 were marked as an outlier in models but not excluded.

Successful Bayesian OxCal models were achieved by independent dating of collagen (Model 1), bioapatite (Model 2), and combined collagen and bioapatite dates (Model 3). Diagenetic alteration to the materials may have been minimal enough to be adequately addressed through standard pretreatment methods. The successful AMS results from both bone fractions concur with previous findings that bioapatite can be successfully  $^{14}\text{C}$  dated when quality indicators are sufficient (e.g., Cherkinsky 2009). Investment in preparation and dating of the two bone fractions however underscore estimation of dietary sources prior to  $^{14}\text{C}$  dating. In the case of El Japón, it is possible to rule out diagenetic alteration and marine diet as causes of  $^{14}\text{C}$  dates that are older than expected in a single sample: Burial 50 is not distinct in diet than any other dated individual (Alarcón Tinajero et al. 2022).

### **Significance**

Dating El Japón contextualizes a sample previously identified as important for the study of postcontact Indigenous population dynamics (Bullock et al. 2013). Bioarchaeological studies



highlight the high incidence of skeletal evidence for nutritional stress and infectious disease (Márquez Morfín and Hernández Espinoza 2016; Civera Cercedo 2018). Nutritional stress, epidemic disease, and mortality commonly grew in magnitude or ubiquity following European colonization (Pérez Zevallos and Reyes García 2003; Warinner et al. 2012) and as such, skeletal collections straddling or postdating European contact are important datasets. Bayesian models developed here from collagen and bioapatite fractions of bone contextualize the earliest burials of the El Japón skeletal collection and the reconstructed incidence of skeletal trauma, disease, and demography to only decades after European colonization in central Mesoamerica: 1545–1640 (cal. CE 95.4%, per Model 3). The decadal-scale modeling and discussion of Postclassic to Colonial period changes adds granularity to processes of cultural change that may otherwise be summarized as simply precontact and postcontact.

Bayesian modeling of post-contact sites in other regions of North America also elicit a more detailed image of Indigenous life and decadal-scale change following European contact. The granularity added to the chronology of El Japón fits with studies of colonial encounters elsewhere in North America that employ  $^{14}\text{C}$  methods to produce more precise chronological estimates. Schneider (2015), for example, uses mission period records (1776–1830 CE) with  $^{14}\text{C}$  dates and stable isotopes on shell to demonstrate that some Indigenous communities continued traditional subsistence practices after engaging with the mission system as religious converts. More importantly, Schenider (2015) uses the  $^{14}\text{C}$  methods to demonstrate Indigenous continuity in a landscape that may have been labeled as peripheral to Spanish colonial interests. Similarly, in northeastern North America, Bayesian modeling of  $^{14}\text{C}$  determinations contributes to reframing of the archaeological interpretation of Indigenous life immediately after European contact. Manning et al. (2019) cross-examine the historical record of French ventures into Iroquoian land in northeastern North America by building  $^{14}\text{C}$  chronologies of multiple Indigenous village sites.  $^{14}\text{C}$  determinations and modeling in turn facilitates interpretation of the timing of European trade goods that may not be possible without such  $^{14}\text{C}$  dating. Again, relying on  $^{14}\text{C}$  determinations, Birch et al. (2021) produce estimates of defensive palisade features in northern Iroquoian settlements.  $^{14}\text{C}$  dating and modeling clarify the relationship between violence, defensive structures, and European arrival.

At El Japón site, characteristics of burials indicated engagement with mortuary concepts not common during the Postclassic period thus justifying independent  $^{14}\text{C}$  determinations.  $^{14}\text{C}$  determinations and calibration of dates alone produced wide calendar age estimates due to the shape of the calibration curve. Bayesian modeling of calibrated human  $^{14}\text{C}$  samples from El Japón chronologically contextualizes the skeletal population sample that indicates continued use of the chinampa agricultural landscape and associated mortuary practices decades after European arrival and attempts at population relocation. Additionally, this study demonstrates the utility of Bayesian modeling of  $^{14}\text{C}$  samples for the late Postclassic to Colonial period transition of Mesoamerica despite the challenges from reversals and plateaus of the atmospheric calibration curve.

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### COMPETING INTERESTS

The authors declare none.

### SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit <https://doi.org/10.1017/RDC.2023.60>

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