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Abstract

Given that sampling strategies and protocols in bioarchaeology are rarely discussed in the literature, this paper is an attempt at reflecting upon the skeletal sampling process (e.g., preparation period, development of strategies and protocols, decision-making process, collaboration with those involved) as well as provide some considerations that may be useful to other junior researchers carrying out their sampling within the realm of bioarchaeology (also may be applicable to other research fields that engage in sampling specimens from museum collections). I provide the considerations about human bone and teeth as it pertains to stable isotope analysis from the literature and then move to discuss my sampling process experience: the preparation period, the sampling process, and the sampling map I developed as an initial guide in the field. Finally, I discuss the main considerations I found helpful in the field which overall involve: 1) Familiarity with the skeletal collections; 2) Constant communication and participant collaboration with those involved in the process; 3) Establishing a feasible sampling protocol well-founded on research questions and biochemical analysis planned as a guide in the field but flexible and open to changes; 4) Handling administrative and logistical aspects of the process well in advance of the sampling visit, and 5) Continual awareness that while as researchers we value skeletal collections in a scientific manner, these also may have other kind of value to others so we must treat these collections with outmost respect at all times (i.e., when discussing, sampling, analyzing, interpreting, and disseminating our research).

Keywords

bioarchaeology, stable isotope analysis, skeletal sampling process, sampling strategies and protocol, participatory collaboration, Templo Mayor Museum

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Given that sampling strategies and protocols in bioarchaeology are rarely discussed in the literature, this paper is an attempt at reflecting upon the skeletal sampling process (e.g. preparation period, development of strategies and protocols, decision-making process, collaboration with those involved) as well as providing some considerations that may be useful to other junior researchers carrying out their sampling within the realm of bioarchaeology (and also may be applicable to other research fields that engage in sampling specimens from museum collections). I provide the considerations about human bone and teeth as it pertains to stable isotope analysis from the literature and then move to discuss my sampling process experience: the preparation period, the sampling process, and the sampling map I developed as an initial guide in the field. Finally, I discuss the main considerations I found helpful in the field which overall involve: 1) Familiarity with the skeletal collections; 2) Constant communication and participant collaboration with those involved in the process; 3) Establishing a feasible sampling protocol well-founded on research questions and biochemical analysis planned as a guide in the field but flexible and open to changes; 4) Handling administrative and logistical aspects of the process well in advance of the sampling visit, and 5) Continual awareness that while as researchers we value skeletal collections in a scientific manner, these also may have other kind of value to others so we must treat these collections with outmost respect at all times (i.e., when discussing, sampling, analyzing,

interpreting, and disseminating our research).

Introduction

My proposed PhD research project involves the use of stable carbon, nitrogen, and oxygen isotope analysis to study the diets and geographical origins of humans from the Aztec capital city of Tenochtitlan (present-day Mexico City) and adjacent archaeological sites in the Basin of Mexico dating to the Postclassic period (A.D. 1200-1519). The collections include adult and subadult sacrificial offerings from the Templo Mayor of Tenochtitlan and Tlatelolco's Templo R, as well as non-sacrificial burials from nearby Aztec communities. These isotopic analyses will allow us to gain insight into the life histories of these individuals and improve our understanding of how these subjects were chosen for sacrifice by the Aztec priests. This study will also help document the diversity in dietary and migratory patterns within and between sacrificial subject groups as well as in comparison with the local Aztec people living across the Basin of Mexico.

As I undertook this research project I designed a protocol for the sampling process. However, early on I noticed that bioarchaeological sampling strategies are rarely discussed in the literature. Based on this lack of discussion, and given that I have gone through my first sampling experience, the goal of this paper is to take a moment to reflect upon the strategies and decision-making involved in the sampling process and provide some considerations that may be useful for other bioarchaeologists preparing for the sampling process. I first discuss considerations regarding human bone and teeth as they pertain to stable isotope analysis. I then discuss the sampling preparation process and reflect on strategies I found useful in the field. Next, I provide a sampling map I developed, and lastly,

summarize the key considerations for bioarchaeological sampling processes.

Considerations for the Sampling of Bone for Stable Isotope Analysis

Before thinking about sampling strategies, I identified a set of research questions in relation to the skeletal collections proposed for my project and followed the appropriate administrative channels to obtain official approval to carry out my proposed research in two phases. Phase I or the “pilot study phase” involved sampling a sacrificial offering from the Templo Mayor of Tenochtitlan (Offering 48) onto which consolidants were applied at the time of excavation¹, as well as sacrificial offerings at Templo R in Tlatelolco without consolidants.

Once the administrative process and the skeletal collections for sampling were agreed upon with the Museum’s research team, I prepared my first sampling trip by reviewing the literature on bone as it relates to sampling for biochemical analyses, specifically stable isotopes (e.g., Ambrose 1993; Ambrose and Norr 1993; Cox and Sealy 1997; Hiller et al. 2004; Kohn and Cerling 2002; Lambert and Grupe 1993; Lewis Jr. and Tung 2013; Price 1989; Sealy, Armstrong, and Schrire 1995). Topics I focused on, given their relevance to a stable isotopic study, included: human bone physical and chemical characteristics (e.g., bone growth, turnover rates, cortical vs. trabecular bone, collagen and bioapatite structure), common human bone pathologies, the application and removal of consolidants on bone, and the potential effects of all these aspects on bone stable isotopic values found in the literature (see for example Cerling et al. 2007; Cox and Sealy 1997; France,

Giaccari, and Cano 2011; Katzenberg and Lovell 1999; Olsen et al. 2014).

Bone is composed of 75% inorganic components consisting of a mineral base, mainly carbonate hydroxyapatite, enclosing 25% organic components (Hiller et al. 2004:1349; Sealy, Armstrong, and Schrire 1995). For the analysis of stable carbon and nitrogen isotopes in ancient skeletons, bone collagen is the most common tissue analyzed. This is given by the fact that bone is quite abundant in archaeological contexts since it generally preserves well in post-burial environments. Moreover, collagen is a strong fibrous structural protein and the main organic component in bone (25% by weight in modern bone, though this percentage fluctuates in archaeological bone based on preservation and taphonomic conditions) (Ambrose 1993). Bone collagen stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values provide us with a sense of an individual’s overall diet. For instance, carbon isotopes discriminate between 100% C_3 , 100% C_4 or marine foods, and C_3 and C_4 /marine (mixed) diets (Ambrose and Norr 1993; Froehle, Kellner, and Schoeninger 2010; Tieszen and Fagre 1993), while nitrogen isotopes discriminate between trophic levels along terrestrial and marine food webs (DeNiro and Epstein 1981; Schoeninger and DeNiro 1984; Schoeninger, DeNiro, Tauber 1983). This allows us to position an individual on the local food web and learn about the individual’s diet in terms of the contribution of protein from different food sources (for in depth discussions about these isotopes see: Ambrose 1991, 1993; Ambrose and Norr 1993; Froehle, Kellner, and Schoeninger 2010; Hare et al. 1991;

¹ Samples with consolidants will be pretreated and processed with the consolidant-free samples. Once results are available, they will be assessed to determine if the isotopic compositions of samples with consolidants were affected or influenced by the consolidant itself. This will ensure that future isotopic analyses can be carried out on other Templo Mayor Museum collections where consolidants were applied to skeletons following their recovery from the field between the late 1970s and 1990s.

Kelly 2000; Lee-Thorp 2008; Macko, Lee, and Parker 1982; Reitsema 2013).

The mineral component in bone and tooth enamel, known as bioapatite, is analyzed to obtain $\delta^{13}\text{C}$ and oxygen ($\delta^{18}\text{O}$) isotopic values from the structural carbonate and/or phosphate components of bioapatite (White, Price, and Longstaffe 2007; White, Spence, and Longstaffe 2004; Wright and Schwarcz 1996). Carbon isotope values from structural carbonate provide us with further dietary information about an individual, particularly the whole composition of one's diet, including lipids, carbohydrates, and protein (Ambrose and Norr 1993; Froehle, Kellner, and Schoeninger 2010; Wright and Schwarcz 1996). These isotopic data can be evaluated in complement with bone collagen carbon isotope data to derive the proportion of C_3 , C_4 , and marine protein as well as the proportion of C_3 , C_4 , and marine whole diet (Ambrose and Norr 1993; DeNiro and Epstein 1978; Froehle, Kellner, and Schoeninger 2010). When carbon isotope data from both these materials are obtained, it provides the researcher with a more complete picture of an individual's diet (Froehle, Kellner, and Schoeninger 2010).

Structural carbonate and phosphate oxygen isotope values aid in determining the residence or location of origin of an individual. In brief, meteoric precipitation, along with its corresponding $\delta^{18}\text{O}$ (and $\delta^2\text{H}$) value, is integrated into the landscape as surface water and it percolates into the soil to become groundwater. This local surface water ends up in water bodies (e.g., rivers, lakes) and the local groundwater is taken up by plants. As such, the plant and drinking water—along with their O and H isotopic compositions—are introduced into the local food web, and eventually, are incorporated into consumers' tissues (Bowen, Wassenaar, and Hobson 2005:338; Kirsanow and Tuross 2011:10). These water oxygen isotope values are reflected in plants, as well as the

bioapatite of consumers living in particular environmental regions. Thus, we can discriminate between local and non-local individuals in a particular region of study (for in depth discussions see: Bryant et al. 1996; Bryant and Froelich 1995; Kirsanow and Tuross 2011; Kohn and Cerling 2002; Longinelli 1984; Luz and Kolodny 1985; Luz, Kolodny, and Horowitz 1984; Podlesak et al. 2008).

Even though the chemical composition of bone has become established for isotopic studies for the most part, it is also important to understand how bone grows, develops, and changes throughout an individual's lifetime. This is mainly because the stable isotopic signal of an individual's tissues may vary due to dietary and locality changes at different points in his/her life, but also due to internal variation based on the analysis of different bone types and skeletal elements (Sealy, Armstrong, and Schrire 1995). As a result, we must first consider what skeletal element and type of bone, either cortical or trabecular, will be best to sample consistently throughout the skeletal collections under study, depending on age at death and the research questions we are hoping to answer by the stable isotope method.

Cox and Sealy (1997:212) have defined bone growth as “the process through which bone increases in size by increasing the number of cells and the intercellular material between them” and this growth primarily takes place during childhood and is completed by adulthood. Besides growth, bone remodels or “turns over” (i.e. it is resorbed and replaced) throughout the course of an individual's lifetime (Sealy, Armstrong, and Schrire 1995). This remodelling involves the resorption of older bone and the formation of new bone throughout life, and while exact turnover rates for healthy humans are not known, these vary depending on age and bone type (Sealy, Armstrong, and Schrire 1995). According to Cox and Sealy

(1997:212) and others (Price et al. 2010; White, Spence, and Longstaffe 2004; White et al. 2000), children have high turnover rates of 100-200% at the age of one, 10% between the ages of three and seven, and 1% at the age of eight. Adolescents experience little turnover, and adults between the ages of twenty and sixty have turnover rates ranging from 0.3 to 3%. Additionally, the turnover rate of cortical bone—predominant in long bones—is much slower (~2.5% per year) than that of trabecular bone since this bone type turns over 3-10 times as fast (~10% per year) (Cox and Sealy 1997; Sealy, Armstrong, and Schrire 1995). Understanding different turn-over rates is crucial in a stable isotopic study since it determines the period of dietary and movement history that we will observe in the skeletal isotope results. Thus, planning what skeletal element and type of bone to sample for analysis from the start of the research process is essential.

Some research has been carried out exploring the influence of human pathologies, observable in bone tissue, on stable isotopic values. Katzenberg and Lovell (1999), for example, analyzed the carbon and nitrogen stable isotope compositions of bone samples from modern humans with known pathological conditions, and from control individuals with no known pathologies to assess the effect of injury and repair, periostitis, atrophy, and osteomyelitis on stable isotope ratios. This study found that, with the exception of periostitic bone, the carbon and nitrogen isotope compositions of pathological bone samples exceeded normal variation. Particularly, bone from the individual with osteomyelitis had the greatest variation in the stable nitrogen isotope values (2 permil [‰]) between segments (healthy: +11.3‰; lesion: +12.9‰; healed: +11.0‰) of the same bone (Katzenberg and Lovell 1999). This suggests that some bone pathologies, especially those caused by

common infectious diseases, produce a different isotopic signal than the healthy or “normal” bone from the same individual. In a more recent study by Olsen et al. (2014), isotopic variations in stable nitrogen isotope values were observable on bone from individuals who had suffered an osteomyelitic lesion or a fracture, while stable carbon isotope values were also different in all the pathology categories studied, except for periostitis. It is important to be aware that changes in human metabolic processes due to disease may affect stable isotopic ratios, and understand the possible implications and issues if a bone lesion is chosen for analysis (Reitsema 2013).

Some of the skeletal collections at the Templo Mayor Museum were treated with consolidants such as polyvinyl acetate (Mowilith[®]DM1H) and acrylic resin (Paraloid[™] B72) shortly after they were recovered from the field during the 1980s. It was therefore extremely important to evaluate the potential effects that commonly used consolidants such as polyvinyl acetate (PVAc) and acrylic resin can have on stable isotope ratios. While few studies have been conducted on this subject, available publications provide some insights about consolidants as these relate to stable isotope studies. The earliest study, by Moore et al. (1989) provided an evaluation of carbon and nitrogen stable isotope ratios in bone collagen from consolidated (Alvar[™] and PVAc) and unconsolidated bone. The consolidants were removed with a number of solvent treatments and the authors concluded that since most consolidants are soluble, a removal treatment with organic solvents (e.g. acetone, methanol) can be employed before the stable isotope analysis is carried out without major implications. France et al. (2011) assessed PVAc and its derivatives and came to the same conclusions as Moore et al. (1989), adding the recommendation that PVAc be removed using acetone followed by

drying in an oven at 80°C. Also, France et al. (2011) found that the application of the PVAc treatment prior to the isotopic analysis affected the oxygen isotope values of bone structural carbonate suggesting that the isotopic variation was due to chemical alteration during processing (see also France et al. 2015). Keeping this in mind, it is possible to sample human bone that has been previously treated with consolidants as long as a removal treatment is established and tested prior to conducting the stable isotope analysis (Metcalf and Longstaffe 2008). As a result, I am establishing a treatment procedure with the consolidated bone samples from the Templo Mayor collections (Moreiras, Millaire, and Longstaffe, forthcoming).

Considerations for the Sampling of Teeth for Stable Isotope Analysis

Tooth tissues, particularly enamel and dentin, are important sources of dietary and residential information given that they record isotopic compositions at the time of tooth formation. These isotopic compositions remain unchanged for the rest of the individual's life² in contrast with bone (Cox and Sealy 1997; White et al. 2000; Wright and Schwarcz 1998, 1999:1161). Tooth dentin isotope values can be compared with bone collagen isotope values from the same individual to infer dietary changes between infancy and adulthood. Analyzing tooth enamel alongside bone bioapatite allows for comparisons between an individual's possible place of residence during infancy and possible movement or migration to a

different location during adulthood (White et al. 2000). Since teeth provide additional isotopic information different from bones, I decided to consider sampling teeth along with bone where possible during my first sampling trip. To assess which tooth would be the most relevant for my study—based on my research questions about dietary and mobility patterns of sacrificed individuals at the two Aztec temples—I first reviewed human tooth growth and development as well as common dental pathologies that I could potentially encounter, and preferably avoid, during sampling. I concluded that it would be best to refrain from sampling teeth with any of the following pathologies: enamel hypoplasias, caries, abscesses, tooth cracks, severe dental micro-wear, and dental modifications and alterations due to cultural and/or habitual practices (these may be important to preserve for future bioarchaeological research) (Buikstra and Ubelaker 1994).

I became familiar with the Templo Mayor Offering 48 and the Tlatelolco skeletal collections and noticed that the majority of the skeletons were identified as subadults³ (100% and 85%, respectively). I considered it important to look at the growth and eruption timelines for deciduous teeth as well as the first and second permanent molars since these reflect distinct isotopic values at different points *in-utero* and during childhood (Price et al. 2010). For this purpose, I referred to the sequence of formation and eruption of teeth among American Indians in Buikstra and Ubelaker (1994:51, Figure 24) which has been established as comparable to other

²Although there is debate surrounding this idea as it may be possible for remodeling to happen in teeth as well (Dolphin, personal communication, 2014). An example of this is the formation of secondary dentin during adulthood, thus providing different C and N isotopic composition compared to primary dentin formed during childhood. There is research being carried out currently to better understand how our skeletal tissues develop, grow, and remodel throughout life (e.g., Maggiano et al. 2016a and b).

³ I considered a "subadult" to be any individual aged chronologically between birth and 12 years, based on who would have been considered children in Aztec society (Berdan and Anawalt 1997; Joyce 2000; Román Berrelleza and Chávez Balderas 2006).

indigenous populations, including Mesoamerica. As such Mexican researchers have commonly used this aging sequence in bioarchaeological studies. According to this growth sequence, deciduous tooth crowns develop *in-utero* and eruption of the first teeth (incisors) begins around 9 months (± 3 months).

Since I needed to extract enough tooth enamel for multiple isotope analyses (C, N, and O) it was more appropriate to focus on sampling larger teeth, namely, deciduous molars and first permanent molars for subadults. The largest deciduous tooth with the most enamel, the second molar, begins development around 5 months *in-utero* and erupts between the ages of two and three, while the first permanent molar begins to grow at 6 to 9 months (± 3 months) after birth and begins to erupt between the ages of five and six (± 24 months). The second deciduous molar reflects the isotopic values of the mother while the child was *in-utero*, as well as the signal of the first two or so years of life (White, Spence, and Longstaffe 2004). Conversely, the isotopic values of the first permanent molar correspond to the period from birth up to the third year or so of life. Since the development, and hence the isotopic signals of these two teeth overlap somewhat, I decided that either of these teeth (mandibular or maxillary) would be appropriate for sampling. However, it would be preferable to sample the first permanent molar if it had erupted in most subadults since I do not need the mother's isotopic signal to answer my research questions. For the adults in the collection, the first permanent molar reflects the isotopic composition from early childhood as well as the second permanent molar (which grows from the age of three and erupts between the ages of eleven and twelve ± 36 months) (Buikstra and Ubelaker 1994:51). Thus, I determined that either the first or second permanent molars were good sample options to obtain childhood isotope

signals to compare with adult bone isotope values from the same individual.

The Sampling Preparation Process and Reflections on my Experience in the Field

In addition to reviewing the relevant literature prior to the sampling process, I had a very active sampling preparation program in the months prior to my museum visit. My first task was to compile as many details as possible about the two skeletal collections to become familiar with the individuals and the overall archaeological context. I found that having relevant information about the skeletal collections and their respective contexts was useful since I kept referring back to it during this preparation period. While it is not always possible to have very detailed information about the collections beforehand, I found this particularly convenient, especially when I discussed the sampling ideas and potential strategies with my supervisors, thesis committee advisors, and later on, with Juan A. Román Berrelleza, Physical Anthropologist in charge of the above mentioned skeletal collections at the Templo Mayor Museum.

In developing my sampling protocol, I engaged in frequent meetings with my supervisors and thesis committee advisors. Each advisor has a particular expertise and experience so they all provided a variety of suggestions and guidance about how to go about the sampling based on my research questions and the skeletal collections under consideration. I kept a journal with notes of these meetings, most of which I later turned into a field notebook (see below). I think that having this kind of support, and to receive feedback prepared me mentally and physically for this process as I began thinking ahead of time about a number of aspects including: options about how and what to sample (e.g., cutting bone, taking fragments, loose teeth); the materials needed and logistics involved, and how to strategize

based on different possible scenarios I could encounter (which I later turned into a sampling map).

A few weeks before my visit to the museum, I compiled the relevant information from the literature and the suggestions from my thesis committee into a single guide as a field notebook that broadly included the following: 1) logistical details (permits, contact information, materials and a supplies checklist); 2) information on the skeletal collections, dental formation and growth diagrams for aging, description of common pathologies, and 3) a sampling protocol that included sampling methods for bone as well as tooth samples and a sampling map with different scenarios that I could potentially find in the field.

One of my supervisors (Dr. Fred J. Longstaffe) and I travelled to Mexico City and met with Phys. Anth. Juan Román to carry out the sampling process in the museum's Physical Anthropology Laboratory (Appendix, Figure 1). He introduced us to the curators, restorers, and other researchers who were present at the time. I found that it was important to explain the purpose of our visit and our intentions with the skeletal collections. Given that I was about to cut fragments of bone and extract teeth from two skeletal collections, I had to put myself in the curator's shoes and be clear about how removing fragments of the nearly-complete and irreplaceable collections would be beneficial and allow us to tap into certain aspects—which would be otherwise unknown—of the lives of those individuals who were once sacrificed and offered to the Aztec gods. Hence, it was important to engage in constant communication during the sampling process since multiple stakeholders were involved and the benefit of this process had to be obvious to all parties.

Before even beginning the sampling process, Dr. Longstaffe, Phys. Anth. J. Román, and I looked at the collections from Templo Mayor and Tlatelolco for an initial assessment of the available skeletal elements, preservation status, and other characteristics specific to these collections. It was through this initial process that I was able to determine which skeletal elements I could potentially sample and which ones would be inaccessible due to unique morphological features, specific pathologies, and other traits held by particular individuals. For instance, included in the collection is a 'famous', very complete, and thoroughly studied child offering which was better to keep intact. There was another individual at Tlatelolco with a unique green stain on the bones of the arm (ulna and radius) from wearing a copper bracelet when offered to the Aztec god of wind, so these skeletal elements were better left intact (Appendix, Figure 2). Based on this assessment and with the help of the sampling map I developed, I began to discuss sampling possibilities with J. Román, explaining the reasoning behind why a specific bone fragment or tooth would provide certain type of data that could be beneficial for my study (Appendix, Figure 3).

Throughout these discussions there was compromise on both ends. We both explained different aspects about skeletal elements and shared information with one another to come up with a jointly acceptable set of individuals and skeletal elements for sampling. This sample set would: 1) not alter the collections in a significant way, and 2) maintain relative sample consistency (i.e., using the same skeletal element for most samples) or from the skeletal elements and teeth I had identified as "good" candidates for isotopic analysis in my sampling map⁴. I consider that this type of communication and open

⁴ As a result of this collaborative process, I sampled fragments of left femoral shafts ($n = 24$) (posterior side by the *linea aspera*) and first permanent molars ($n = 15$) from the Templo Mayor (Offering 48) collection (Appendix, Figures

discussion is essential to develop strong and long-lasting collaborative relationships with the institutions and researchers in charge of the skeletal collections we are interested in studying scientifically.

Looking back at this sampling experience, I found some valuable insights throughout the process that benefitted me greatly when it came time to sample the collections at the Templo Mayor Museum. I practiced sampling bone and teeth from modern faunal mammal specimens⁵ at the Laboratory for Stable Isotope Science (LSIS) at The University of Western Ontario prior to my sample field trip. Becoming familiar with the tools and ways to sample different skeletal elements as well as having a sense of how much material I needed for multiple isotope analyses (and corresponding method duplicates) was very helpful. Thinking about the logistics and supplies I could potentially need during the sampling process was also very useful and it was very satisfying having all the tools and materials I could potentially need. Even when I did not end up using all of them just knowing I had them available made me feel more prepared.

Being familiar with the collections was instrumental prior to and during the sampling process. I must admit that even though I had compiled information on these collections, I was still unaware of many details such as the excavation process in each case and about each individual's particular mortuary context. The researcher discussed some aspects about each collection so I learned a lot more about these individuals during the sampling process itself. Additionally, he recommended specific books about the collections so now that I am analyzing the samples in the laboratory I am becoming

more knowledgeable about these individuals and their archaeological context.

I found it valuable and quite practical to have a field note book ready for the sampling process. The information about tooth formation and growth was particularly useful as we examined the tooth samples selected, and subsequently, estimated the individuals' ages. I also included a sample table with relevant columns such as: Sample ID, Collection, Sample Type, Provenience, Unique Features, Preservation, Age, Sex, and Weight (g). This facilitated the sampling process greatly making the process organized, practical, and timely.

As mentioned previously, I prepared myself by ranking preferred skeletal elements to sample based on my research questions and type of biochemical analysis to be carried out in the laboratory. This became my sampling map which I also included in the field notebook (see below). It was a great idea to set-up possible burial context scenarios I could encounter and pre-select certain skeletal element that would be potential candidates for isotope analysis. This was definitely productive during my sampling trip as an initial guide. However, at the same time I was aware and open to the possibility that my top sampling choices might not be available to sample in reality. Thus, while it is good practice to think about preferred skeletal elements for analysis, we cannot assume that our planned sampling strategies will be the "best" until we are able to actually look at the collections, observe their preservation status, and assess the skeletal elements in collaboration with the researchers and curators in charge of the collections in order to agree upon those deemed best to sample.

4-5) as well as ribs ($n = 24$), two first permanent molars, a second permanent molar, and two second deciduous molars from the Tlatelolco Templo R collection (Appendix, Figures 6-7).

⁵ I am thankful for the support and guidance from my colleagues Dr. Z. Morris, Dr. R. Schwartz-Narbonne, and T. Plint during this preparation period.

Below I include my sampling map, as a list of situations of preferred bone and tooth samples depending on the possible burial context in which the individuals from the Templo Mayor and Tlatelolco collections were recovered from. I separated sampling preferences for subadults and adults and incorporated the reasoning behind each sampling choice:

Subadult Samples

Situation 1: Single burial with complete skeleton:

- ❖ **Bone:** Occipital planum (back of skull) *AND/OR* right/left femoral fragment to a side of the *linea aspera* feature (posterior mid-point area).
 - Reasoning: Both bones have thick cortical bone which is less prone to post-mortem alterations than trabecular bone. Femoral diaphyses and other long bones are less likely to be contaminated by dirt or plant roots (DeNiro and Schoeninger 1983). Important features are avoidable in those areas. Two skeletal elements are ideal (but not crucial) to assess the isotopic signal of both cranial and post-cranial bones per individual due to different bone turnover rates.
- ❖ **Tooth:** Maxillary left or right deciduous second molar *OR* first permanent molar if erupted.
 - Reasoning: The second deciduous molar is the largest tooth during childhood with the most enamel for isotopic analysis and captures the isotopic composition from *in-utero* to 2.5 years of age. The first permanent molar captures the isotopic composition from around 4.5 months to approximately 7 yrs. of age. Can be

associated with the skull bone sampled per individual.

Situation 2: Cranium (no cranial and post-cranial association):

- ❖ **Bone:** Mandibular body fragment (between the gonial angle and the mental foramen).
 - Reasoning: Thick cortical bone and no important features in that area, although must be careful as subadults will have permanent teeth inside the mandibular area (prior to eruption). Bone can be associated with a mandibular tooth per individual.
- ❖ **Tooth:** Mandibular left or right deciduous second molar *OR* first permanent molar if erupted.
 - Reasoning: The second deciduous molar is the biggest tooth during childhood with the most enamel for isotopic analysis and captures the isotopic composition from *in-utero* to 2.5 years of age. The first molar contains the isotopic composition from around 4.5 months to 7.5 years. of age. Can be associated with the mandibular bone per individual.

OR

- ❖ **Bone:** Occipital planum (back of skull).
 - Reasoning: Has thick cortical bone and important features are avoidable.
- ❖ **Tooth:** Maxillary left or right deciduous second molar *OR* first permanent molar if erupted (Appendix, Figure 6).

- **Reasoning:** The second deciduous molar is the biggest tooth during childhood with the most enamel for isotopic analysis and contains the isotopic composition from *in-utero* to 2.5 years of age. The 1st molar contains the isotopic composition from around 4.5 months to 7.5 yrs. of age, and thus, the longer distance between adult and childhood isotopic compositions. Can be associated with the skull bone per individual.

Situation 3: no cranial remains present:

- ❖ **Bone:** Right/left femur fragment to a side of the *linea aspera* feature (posterior mid-point area) *OR* right/left rib fragments. Last choice would be to sample a phalanx (whole or fragment) (Appendix, Figure 4).
- **Reasoning:** These bones have thick cortical bone and are commonly sampled for isotopic analysis. Important features are avoidable and it involves minor loss of morphological information (Sealy, Armstrong, and Schrire 1995).

Adult Samples

Situation 1: Single burial with complete skeleton:

- ❖ **Bone:** Mandibular body fragment (between the gonial angle and the mental foramen) *OR* right or left femoral fragment to a side of the *linea aspera* feature (posterior mid-point area) *OR* right/left rib fragments (Appendix, Figure 6).
- **Reasoning:** These bones have thick cortical bone and important features avoidable in those areas. Ideal to

assess the isotopic signature of both cranial and post-cranial bones per individual due to different bone turnover rates. This bone can be associated with a mandibular tooth per individual.

- ❖ **Tooth:** Mandibular left/right permanent first *AND/OR* third molars.

- **Reasoning:** The first molar captures the isotopic composition from around 4.5 months to 7.5 yrs. of age, and thus, the longer distance between adult and childhood isotopic composition. The third molar contains the isotopic composition from the age of 8 to 16 or so. The ideal scenario would be to sample both molars for analysis as these include a childhood and adolescent isotopic composition to compare with the adult bone composition.

Situation 2: Cranium (no cranial and post-cranial association):

- ❖ **Bone:** Mandibular body fragment (between the gonial angle and the mental foramen) *OR* occipital planum (back of skull)/interior skull fragments (e.g., sphenoid bone).

- **Reasoning:** The mandibular fragment and occipital planum have thick cortical bone and important features avoidable in those areas. The interior skull fragments will not affect the outer integrity of the skull (no visual disruption). These bones can be associated with a mandibular or maxillary tooth per individual.

- ❖ **Tooth:** Mandibular *OR* maxillary permanent left/right first *OR* second molar (Appendix, Figure 7).

- **Reasoning:** The first molar contains the isotopic composition from around 4.5 months to 7.5 yrs. of age, and thus, the longer distance between adult and childhood isotopic compositions. The second molar contains the isotopic composition between ages 3 and 12 so there is still enough distance between the adulthood and childhood isotopic compositions.

Situation 3: No cranial skeleton present:

- ❖ **Bone:** Right/left femoral fragment to a side of the *linea aspera* feature (posterior mid-point area) *OR* right/left rib fragments. Last choice would be to sample a phalanx (whole or fragment).
- **Reasoning:** These bones have thick cortical bone and are commonly sampled for isotopic analysis. Important features are avoidable and it involves minor loss of morphological information (Sealy, Armstrong, and Schrire 1995).

Final Considerations on Sampling in Bioarchaeology

It is obviously one thing to establish a sampling strategy and another to carry out the sampling while navigating the practical, administrative, and ethical realities one inevitably encounters in the field. Bioarchaeologists are no exception and must always be aware that even when we are extremely prepared for our sampling process and plan to obtain the best sample set possible, it may not result how we originally planned. This is one of the main reasons why keeping an open mind and being flexible is crucial since the sampling process is based on constant compromise and collaborative efforts among the multiple parties involved. My sampling visit to the Templo Mayor

Museum was no exception. Below I provide a summary of key considerations that I have found useful and which may be relevant to other junior researchers preparing for their own sampling experiences:

- ❖ Familiarity with the collections proposed for sampling ahead of time as well as right before beginning the sampling process;
- ❖ Constant communication with the researchers involved prior and during the sampling process (e.g., researchers, curators, thesis supervisors and thesis committee advisors, relevant colleagues, etc.);
- ❖ Establishing a sampling protocol with corresponding methods and strategies to use as an initial guide in the field;
- ❖ Sampling skeletal fragments that will not damage the integrity of the skeleton, avoiding unique traits as well as important morphological, pathological, and cultural features in collaboration with the researcher(s) in charge of the collection(s);
- ❖ No sample is ever perfect in bioarchaeological research so while it is important to keep sample element consistency in mind one must be flexible and open to different sample possibilities;
- ❖ Addressing the logistical, administrative, and practical aspects ahead of the sampling process;
- ❖ It is quite exciting what we do as bioarchaeologists; we are able to reach into the past and tap into the lives of people in a very direct way. Nonetheless, the most important point is being aware and constantly conscious that we are

dealing with human remains. We must treat the skeletal collections with dignity and respect *at all times* (Larsen and Walker 2005; Walker 2000) recognizing the value of human remains not only as they provide bioarchaeologists with invaluable scientific information but as individuals who may possess other kinds of value to other people (e.g., descendent communities, general public, stakeholders, etc.); and finally,

- ❖ Each sampling experience will be unique based on the collections considered, preservation status, and research questions so while I have provided a sampling process (and its resulting protocol) that was beneficial to me, it is meant as an example of the preparation and decision-making process itself rather than as a 'one sampling guide fits all'.

We cannot assume that our sampling strategy is best until we are able to physically observe the preservation and burial contexts of the skeletal collections to be sampled. As such, it is essential to know the reasoning behind why the bone fragment or tooth we would like to sample is relevant or even necessary for a research project. I think this is the fundamental question that we all have to constantly ask ourselves and assess in collaboration with the researchers involved to evaluate if it is worth removing 'this' or 'that' bone fragment or tooth from a close-to-complete skeletal collection. It may be fruitful to consider the entire sampling process in the framework of participatory collaboration in which all parties are able to communicate, share ideas, provide suggestions and feedback, propose and weigh sampling options openly, compromise in certain aspects and agree to others, and learn from one another in a constructive and collaborative fashion. This will enable trust and open communication among the different

parties and strengthen the collaborative bond between those involved in the entire research and sampling process. Even though this is not discussed much in the literature (aside from ethical considerations), I think this is something we have to engage in constantly, given the nature of our work, and we will continue to do so throughout our careers. I propose we consider this collaborative engagement as one more set of skills we must develop and strengthen as part of our professional bioarchaeological repertoire.

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Appendix



Figure 1. Physical Anthropology Laboratory at the Templo Mayor Museum, Mexico City. Photograph courtesy of Juan A. Román Berrelleza.



Figure 2. Radius and ulna from an individual in the Tlatelolco collection with a unique trait: green stains from wearing a copper bracelet. Photograph by Diana Moreiras.



Figure 3. Diana Moreiras and Juan A. Roman in conversation about the sampling process. Photograph courtesy of Fred Longstaffe.



Figure 4. Close-up of femoral sample extraction by Diana Moreiras from the Offering 48 collection of the Templo Mayor of Tenochtitlan. Photograph courtesy of Juan A. Román Berrelleza.



Figure 5. Offering 48 collection of the Templo Mayor of Tenochtitlan: Cranium before extracting the right maxillary 1st permanent molar for isotope analysis. Photograph by Diana Moreiras.



Figure 6. Rib fragments taken for isotope analysis from an individual in the Tlatelolco collection. Photograph by Diana Moreiras.



**Figure 7. Loose second permanent molar taken for isotope analysis from the Tlatelolco collection.
Photograph by Diana Moreiras.**