

# THE DEAD TELL TALES:

ESSAYS IN HONOR OF JANE E. BUIKSTRA

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# METHODOLOGICAL AND ETHICAL CONSIDERATIONS WHEN SAMPLING HUMAN OSTEOLOGICAL REMAINS

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**W**E HIGHLIGHT SEVERAL CONSIDERATIONS that must be made when embarking upon destructive analyses of human remains. We view this as a step toward the creation of widely implemented standards within this particular methodological branch of bioarchaeological research. Thus it is fitting that this work is situated in a volume dedicated to Jane E. Buikstra, who, with her colleague Douglas Ubelaker, published the indispensable and oft-cited book *Standards for Data Collection from Human Skeletal Remains* (Buikstra and Ubelaker 1994). *Standards*, as it is known in shorthand throughout the discipline, integrated relevant methodological techniques compiled by a multidisciplinary team of experienced researchers and graduate students. That standardized compilation has contributed to more reliable comparisons of skeletal data sets collected by different researchers.

In its nine printings, *Standards* has found its place in anthropology and criminal justice departments in North, Central, and South America; Europe; and the Middle East (Deborah Sabo, Arkansas Archeological Survey Publications, personal communication 2010). We do not presume that this short paper is within the realm of the comprehensive volume created by Buikstra and Ubelaker. Yet we have taken our cue from those impressive scholars, noting that standardized methodological rigor will aid in improving our discipline. In this chapter we suggest research considerations and

procedural practices intended to limit bone and tooth destruction and to maximize research potential and long-term posterity.

## BACKGROUND ON DESTRUCTIVE SAMPLING

Gross observation of diagnostic features on human skeletal and dental remains, including natural, pathological, and anthropogenic traits, provides a means to reconstruct demographic profiles, population morbidity, biological relationships within and between populations, and frequencies of violence and cultural practices such as bodily expressions of identity (for example, cranial modification and tooth ablation) (Buikstra and Beck 2006; Walker 2005). Further details of one's health status and lifeways can be obtained through biochemical and molecular tests, which typically require the extraction and destruction of a small skeletal and/or dental sample. Those samples can provide a wealth of data for dietary reconstruction (Lambert et al. 1984; Schoeninger 1989), radiocarbon dating (Piotrowska and Goslar 2002), assessing migration (Knudson and Price 2007; Price et al. 1994), and documenting physiological adaptations (Bridges 1996), ontogenetic patterns (Dean 2006), demographic details (Wittwer-Backofen et al. 2004), and genetic variation within and among populations (Pääbo et al. 2004).

Because a sample of bone or tooth must be destroyed or altered, a cost-benefit analysis regarding the amount of destruction required for the amount of information to be gained must be evaluated. Sampling procedures should minimize destruction and enhance the quality of data obtained. There is a conspicuous absence of presenting such procedures in research articles. This leaves such vital considerations and procedures to individuals with a broad range of backgrounds, not all of whom will be specialists in bioarchaeology.

## RESEARCH CONSIDERATIONS

Given the significant ethical concerns involved in the destructive analysis of human skeletal remains (Larsen and Walker 2005), serious consideration should be given to the potential insights to be gained vis-à-vis the material that will be lost and the concerns the process may bring to descendant communities. In the many world regions where indigenous descendant populations reside, there are conflicting views regarding the utility of the scientific study of human skeletons. As Larsen and Walker (2005:111) note:

The traditional perspective of scientists who study ancient remains has been to consider human remains as valuable objects full of research potential. Many descendants of the people whose remains bioarchaeologists study, in contrast, view ancestral remains as objects of veneration that should be protected from what they see as the indignity of examination by scientists whose motivations they consider suspect at best and immoral at worst.

In light of these potentially disparate perspectives regarding the treatment of human remains, and after consultation with appropriate descendant communities (and see questions 7 and 8, below), the authors suggest that at least the following questions be considered before conducting any kind of destructive analysis:

### 1. Do I have a clear research question that can be addressed by this analysis?

A research question should always be articulated prior to data collection and analysis, especially if the data collection procedures lead to the destruction of the material under study. If any of the destructive analyses are outside the scope of the research questions to be addressed, they should be avoided. If it is concluded that destructive analysis will be conducted, consider ways to maximize the amount of information to be gained by the sample (see question 2).

### 2. Have I consulted with other specialists who plan to obtain data from this extracted sample?

Because several kinds of data may be obtained from one specimen, consulting a histologist, an archaeological chemist, and a molecular anthropologist, among others, is advised. These specialists can assess the feasibility of sectioning the sample for different studies. For example, it is typically possible to use a dental sample for multiple studies such that casts are made for microwear analysis, enamel is used for isotopic analyses (of both light and heavy isotopes, which may need to be conducted in different labs, thus requiring detailed planning), and roots are used to obtain ancient DNA. The order in which the specialists handle the samples is an important consideration. For instance, ancient DNA studies are vulnerable to modern contamination from handling, so it may be wise to conduct DNA extraction before other analyses. (In our experiences, removal of the root does not significantly interfere with making casts of the enamel crown.)

### 3. Is an experienced person or lab providing and analyzing the data?

Many laboratories will conduct analyses of your samples for a fee. Before consulting a lab, consider doing a background check on its reputation and the qualifications of its personnel. Review publications produced from the lab. Be explicit when discussing fees and author order (if applicable) for the resultant publications. It is strongly advised to employ a method of quality control (see below).

### 4. Is a system in place for quality control?

There are two effective approaches for quality control: the independent lab strategy and the blind sample strategy.

The independent lab strategy is standard for high-profile ancient DNA studies, but the strategy applies equally well to most destructive analyses. Duplicate samples are sent to independent (unaffiliated) laboratories, which report the results directly to the primary investigator, who then assesses whether results from the two labs are consistent.

The blind sample strategy is best used when the laboratory requires no identifying contextual information, only an identification code number. Each sample, regardless of whether it is a duplicate, has a unique identification number that can be linked to the contextual information only by the primary investigator. The laboratory is never given any information regarding which samples are duplicates. In fact, the laboratory may be unaware that duplicate samples are included.

Consequently, the primary investigator assesses whether results from the duplicate samples are consistent.

The independent lab strategy and the blind sample strategy work equally well when the primary investigator of the project and the director of the laboratory conducting the analysis are the same individual. Laboratory directors may wish to include duplicate blind samples to evaluate whether their lab is producing consistent results. Alternatively, laboratory directors may wish to send duplicate samples to a colleague for independent tests.

#### **5. Will I be able to contribute to the conservation of the remaining skeletal material?**

Often, monetary support for conservation material is limited. Researchers should consider including a budget for skeletal conservation. At the most basic level, human remains should be stored in an acid-free environment, with padding for the more delicate elements, and all elements should be stored in sturdy, well-labeled containers for long-term preservation.

The environmental conditions of the storage space are a critical aspect of proper conservation. In particular, humidity can lead to decay of skeletal elements. Removing moisture from the air using a dehumidifier will aid in protecting the bones. Pollutants in the air can also contribute to degradation or contamination of the skeletal material. In particular, insecticides, pollution from cars and factories, and even cleaning supplies will hinder conservation efforts. Researchers can protect human remains from contaminants by placing bones in rooms that are adequately sealed.

#### **6. Are there additional samples or contextual information that would complement the skeletal samples?**

In addition to the skeletal material, nonhuman samples and contextual information may be required to address questions posed in the study. For instance, samples from local soils, flora, or fauna may be needed for isotope studies on dietary reconstruction or residential mobility (Price 1989; Schoeninger 1989). For molecular studies, the principal investigator may need to obtain permission to acquire biological samples from project members who have handled remains, as they may be sources of modern contamination (Sampietro et al. 2006). Such samples would require informed consent from the participants.

#### **7. Have I obtained the proper permission to conduct a destructive analysis?**

National and local guidelines related to repatriation and cultural property rights must be met, and all relevant

permissions should be obtained in writing. In addition to getting permission from the person or institution in charge of the material, you may need permission from the original excavator, the repository holding the material, the close relatives or descendants of the individuals studied, and relevant government agencies, such as those involved in cultural resource management. Additional permits from government agencies may be required for shipping samples out of the country from which they originated.

#### **8. Have I considered the impact of this study on related individuals or communities?**

While most destructive analyses can be conducted on relatively small bone samples and/or on teeth, any destructive analysis on human bone involves a serious ethical decision. The impact of your study requires careful consideration of the long-term conservation of the skeletal material. Importantly, the impact the study will have on living individuals closely connected with the material, both biologically and culturally, must be carefully considered. There are several well-written reviews on this subject (Larsen and Walker 2005; Walker 2000, 2005).

#### **9. Have I attempted a pilot project to assess the feasibility of the study?**

It is possible that postdepositional processes have made certain destructive analyses unfeasible. This is especially a concern for many ancient DNA studies. As a proof of principle, it is wise to begin with a pilot project on a limited number of individuals. The researcher first gauges what level of DNA preservation would be considered acceptable to continue the project. Applying a simple probability calculation can be informative. For example, if the researcher decides that a 50 percent DNA recovery rate is acceptable to continue the project, then, all things being equal, the probability of having a successful DNA extraction within three, four, or five samples is .875, .937, and .968, respectively, calculated as  $p = 1 - (1 - r)^n$ , where  $r$  is the hypothetical success rate and  $n$  is the number of samples examined. Thus, if after five samples no DNA preservation is observed, it is likely that the overall recovery rate will be less than 50 percent.

## PROCEDURAL PRACTICES

The following procedural standards are intended for someone well trained in identifying a variety of dental

and skeletal lesions and standard skeletal and dental traits. Such identifications are best made through hands-on experience rather than photographs.

### *Handling Bone*

Destructive analyses for ancient DNA are particularly sensitive to modern contamination. As a result, minimizing direct contact with the skeleton is ideal. Nevertheless, there are in-house laboratory techniques that reduce modern DNA contamination (Kemp and Smith 2005; Yang and Watt 2005). Consequently, a permanent record of the skeletal material outweighs the potential added complication of having to purge modern contamination from a sample. Still, there are standard procedures that reduce modern contamination during the handling of human remains.

One should wear gloves to avoid passing oils, salts, and other contaminants to the specimen. Disposable sterile nitrile gloves are ideal. These gloves greatly reduce contamination when compared to reused cotton gloves, and they are smoother than disposable latex gloves, so they are less prone to snagging.

If possible, do not wash the skeletal material or treat it with any preservatives before sampling. If a consolidant needs to be applied to a skeleton to excavate it, treat one side only or at least leave a small portion clean for future testing.

In many cases, researchers may want to use museum specimens for isotope studies, and those are sometimes covered in PVAc glue (polyvinyl acetate with acetone solution). A recent study showed that if the PVAc is removed with organic solvents, researchers will still get reliable results for the analysis of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopes in bone collagen, for carbon isotopes in carbonate from bone hydroxyapatite, and for oxygen ( $\delta^{18}\text{O}$ ) isotopes from phosphate in hydroxyapatite (France et al. 2011). However, oxygen isotopes from carbonates exhibit variable results and should not be used in investigations (France et al. 2011).

### *Data Collection Before Destructive Analyses*

Osteological and pathological data should be recorded before samples are cut or extracted. We recommend using the data collection standards as outlined by Bulkstra and Ubelaker (1994). This work should include at least the following:

1. Recording of:

- Dental calculus
- Enamel hypoplasias, hypocalcifications

- Dental caries
  - Dental wear
  - Dental chipping
  - Dental and skeletal measurements
  - Dental and skeletal nonmetric traits
  - Dental and skeletal modifications
  - Cut marks or other anthropogenic alterations to the specimen
  - Stains from copper or other elements (for example, ochre, cinnabar)
2. Observations of pathological lesions (for example, periostitis, osteoarthritis, bone fractures, porotic hyperostosis)
  3. Taphonomic descriptions (for example, color, sun or water damage, burning, rodent or other animal chew marks)
  4. Creation of dental casts or a digital rendering of the element
  5. Photographs of all views of dental and skeletal specimens (mesial, distal, lingual, buccal, occlusal, and inferior views of teeth; anterior, posterior, medial, and lateral views of bones), with a metric scale in the photos
  6. Additional photos after sample extraction (for example, of the alveolar bone where a tooth was removed)

### *Selecting the Specimen*

*Selecting a Tooth.* Teeth are among the most informative parts of a skeleton. Gross observation alone can provide insights into dental health (caries, abscesses, periodontal disease, antemortem tooth loss), developmental health status (linear enamel hypoplasias and metrics), diet (caries, dental wear, and calculus), genetic relationships (metric and nonmetric traits), and cultural affiliation (dental modifications). As noted above, all teeth should be photographed and an accurate cast or digital rendering should be made prior to sampling. When casting teeth, it is important to use a high-resolution casting material such as vinyl polysiloxane.

There are a number of reasonable tips for selecting a tooth to sample. Choose a tooth that has a lateral counterpart (left and right present) to reduce loss of valuable information in future studies. Lateral counterparts may differ somewhat, particularly in terms of pathological status, and while some studies prefer bilateral comparisons, selecting a tooth with a lateral counterpart is the most sensible.

Teeth with carious lesions or severe dental wear should be avoided as samples because of their potentially useful cultural information and, with respect to

molecular studies, the increased risk of modern contamination. Of course, this advice does not apply if the research question itself relates to related studies on caries or dental wear.

Researchers should avoid destroying teeth that exhibit rare nonmetric traits in a particular population (for example, a protostylid), even if the tooth was caste or digitally rendered. Consult an osteologist with in-depth knowledge of the skeletal population to ensure that you are not destroying the one example of a rare trait in that study group.

Teeth with calculus (plaque) should be avoided because they can be used for noninvasive studies such as phytolith analysis (see Fox et al. 1996 for an example). If a tooth with calculus is selected, bag a sample of the calculus, noting its origin and the reason for removing it. Leave the labeled calculus sample with the original skeleton or arrange to have a phytolith specialist analyze it.

Selecting the appropriate tooth is a negotiation between the questions to be addressed, the teeth available, and the information stored in that tooth. Teeth are not equal in terms of the information they yield. For example, canines are much more likely to exhibit enamel hypoplasia and are well suited for histological analysis. In contrast, molars provide many more nonmetric traits than do canines. Nevertheless, it is straightforward to document dental traits before tooth destruction. Consider the pros and cons of each potential tooth sample before the final selection is made.

*Selecting a Bone.* The type of bone sample necessary for the destructive analysis may vary; consultation with the laboratory conducting the analysis is essential. As with the selection of dental specimens, select a sample that is free of pathological lesions (unless the research question is centered on analysis of such a pathology), and select a bone element that is present on both the left and right sides. As always, photograph and record the presence or absence of nonmetric traits and record all metric data.

There is a better chance of DNA preservation in denser material, such as long bone shafts. Yet long bones provide a wealth of morphological data. If long bones are sampled, every attempt should be made to keep the bone intact while taking the smallest excision required for the analysis. Although, from a conservation standpoint, it might be good to select an already fragmented piece of bone, this might not be ideal for the laboratory because exposed broken edges (especially exposed trabecular bone) often have soil and other contaminants integrated into the trabeculae, requiring additional

cleaning. Bone freshly cut with a clean blade (see below) might be better. Ribs and phalanges are common alternatives to long bones in DNA studies and also work very well for isotope studies. For isotope studies, if comparisons are being made within and between skeletal populations, every effort should be made to sample the same element. Standardization is essential for reliable intra- and interpopulation comparisons because isotopic incorporation varies from bone to bone. Thus the sampling of a rib or a phalanx is encouraged because there are 24 ribs and 56 phalanges from which to choose. It is also worth noting that permitting agencies are typically more willing to allow destructive analysis of ribs and phalanges than femora and tibiae.

### *Sample Extraction*

If a sample needs to be cut, do so with a sterilized tool. We suggest manual saws or multiple-speed rotary drills (Dremel, for example) with mini-carbon disks and engraving cutters. Always wear gloves, goggles, and a mask to protect both yourself and the sample. A rotary drill typically has disposable attachments (such as disks and cutters) that must be changed for each sample extraction to avoid cross-contamination. When using a rotary drill, check voltage requirements (110 or 220 volts) in the country where you are conducting the extraction. While manual saws disperse less bone dust, automated saws typically require less direct pressure on fragile materials. For molecular studies, one concern with rotary tools is the heat they generate, which may reduce DNA preservation (Adler et al. 2011); Adler et al. found that using lower speeds (100 rpm) resolves much of this issue.

*Obtaining Dental Samples.* Teeth are often free or loose in the alveoli (sockets). In these circumstances, extraction requires minimal invasive procedures. Unfortunately, this is not always the case. If a tooth is affixed in the socket, pull it gently to evaluate the degree of resistance. Sometimes a light wiggling of the tooth may free it. If not, determine whether an alternative tooth is available, assuming that your research question is not dependent on extracting that particular tooth type.

Evaluate whether curators or a previous researcher glued the teeth in their sockets. If so, the glue can sometimes be reversed with water or acetone (depending on the type of glue used) or by picking away glue chunks with tweezers and a drill. Obtain permission from the curator to reverse the gluing, and be sure to inform the laboratory if you used any water or acetone (or other reversing agent) to extract the tooth.

If the tooth is tightly affixed in the alveolar bone, additional tools will be required to extract it. Check with the curator, excavation director, or other responsible agent to ensure that you have permission to destroy some of the alveolar bone for extraction. Maxillary molars are much more difficult to extract, owing to their three-pronged root structure, so single- or double-rooted teeth may be better options. Using a drill with a cutting disk or engraving cutters, carefully cut away tiny portions of the alveolar bone surrounding the tooth. (This procedure is not advised if there is an abscess at the socket location.) After removing a small bit of bone, gently wiggle the tooth to extract it. Repeat this process until the tooth comes free. Do not yank the tooth, as excessive force will result in a fractured root, fractured enamel, and/or fractured bone.

*Obtaining Skeletal Samples.* Ribs and phalanges may be taken whole. For large bones, some cutting will likely be needed. If the bone is already broken, cut off a section from the broken end, ensuring that you have enough intact bone that is free of contamination. If a bone is intact, remove a rectangular piece, as if cutting a window into the skeletal element. This technique is preferred over cutting off the proximal or distal end of the bone, as that destroys the entire integrity of the bone. If you're using a rotary tool, you might need multiple cut disks to complete the removal of the bone sample, and the use of protective eyewear becomes even more essential.

### *Storing and Transport of Samples*

Bone samples that are to be used for radiocarbon dating should not be wrapped in paper; rather, they should be wrapped in aluminum foil. All extracted samples should be individually wrapped in sterile bags. Each sample bag should then be placed inside another bag or container with an accompanying sample tag. The tag should be labeled with a permanent marker. Each tag should be marked with identification information (site name/number, excavation unit, feature, burial number, specimen number, and so on). Excavators on different projects often use different coding systems, so it is the responsibility of the osteologist to learn each project's coding system. Be sure to include all identification information with the extracted sample. Also include details of the sampled element (for example, "shaft fragment from distal end of left femur" or "mandibular right molar 2") and the weight of the specimen in micrograms. Finally, leave a tag in the original bag noting the following information for researchers who may study the skeletal collection 5 or 50 years later: (1) sample taken; (2) tests

being performed and at which labs; (3) gross observations made on the element; (4) name; (5) date. Business cards are also good to leave with the original bag.

The loss or mixing of contextual information can have dire consequences for current and future studies of skeletal materials. It is a good practice to complete the sampling process one skeleton at a time. This helps prevent mislabeling samples or mixing skeletal elements from more than one individual. It is important that once someone begins the sampling process, that same person completes the process before moving on to another skeleton.

## CONCLUDING REMARKS

The considerations and procedures presented here provide a foundation for studies of human remains that require the destruction of samples. Inevitably, different types of analyses will involve unique challenges for sample extraction, quality control, and skeletal conservation. We ask that papers presenting novel destructive analyses include brief descriptions of how the research assured quality control and conservation efforts. We hope that this brief description helps in establishing and maintaining responsible research agendas and provides guidance to those wishing to add these novel approaches to their own research design.

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