APPLICATION OF DENTAL CEMENTOCHRONOLOGY IN UNIDENTIFIED MIGRANTS IN SOUTH TEXAS

by

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DEDICATION

This thesis is dedicated to the unidentified migrants who died during their attempt across the US-Mexico border and their families.

I dedicate my passion for learning and striving for greater things to my grandfather, Arne, who taught me my love for archaeology and bones. I miss you every day.

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LIST OF ABBREVIATIONS

Abbreviation	Description
ADBOU	Anthropological Database at Odense University
ADD	Accumulated Degree Day
CODIS	Combine DNA Index System
DCIA	Dental Cementum Increment Analysis
DNA	Deoxyribonucleic Acid
FACTS	Forensic Anthropology Center at Texas State
FBI	Federal Bureau of Investigation
NamUs	National Missing and Unidentified Persons System
NGO	Non-Government Organization
OpID	Operation Identification
PCOME	Pima County Office of the Medical Examiner
PMI	Postmortem Interval
ROI	Region of Interest
RPM	Revolutions Per Minute
SOD	Season-of-death
TBS	Total Body Score

I. INTRODUCTION

The number of reported deaths of undocumented border crossers along the United States-Mexico border has been steadily increasing despite a decrease in apprehensions since the early 1990's (De Leòn, 2015). This is due to the "Prevention Through Deterrence" policy (1994), implemented by the Clinton administration and enforced by the United States Border Patrol (De Leòn, 2015). This policy has inadvertently pressured undocumented migrants away from urban communities and into the hostile desert environment in which many individuals succumb to exposure to dehydration, heat, and hunger. The humanitarian effort to put names to the unidentified migrant remains that are found in Texas, is referred to as Operation Identification (OpID). OpID is directed by Dr. Kate Spradley and is based at Texas State University. As of May 15, 2018, a total of 279 individuals have been transferred to Texas State University for anthropological analysis. Twenty-eight of these individuals have been positively identified and four potential identifications are currently pending.

One of the critical factors to consider when comparing potential identification matches between missing persons reports and forensic anthropology reports, is an accurate age-at-death estimate. This allows researchers to narrow down the potential matches. The OpID cases present a distinct obstacle, as these individuals have a high degree of skeletal diversity in which many of the common anthropological methods used have not been established for this population (Meckel et al., 2016). Previously, the OpID cases have had rib samples taken for histological age-at-death analysis; however, in many of the cases the ribs are not well preserved or are absent all together. Additionally, femoral histological aging has been attempted using the Thompson (1979) method;

however, it was discovered that these estimates were not accurate when compared to known ages of identified migrants from the OpID cases (Mavroudas et al., 2015). The obstacles outlined above have encouraged the need for alternative aging techniques for the OpID cases.

A. AIMS OF RESEARCH

In this study, dental cementochronology is utilized to estimate age-at-death of unidentified migrants that were found in South Texas. The cementochronology age estimates are compared to rib histology and skeletal age estimates based on the Anthropological Database at Odense University (ADBOU) scoring system and software Boldsen et al., 2002). The two questions that will be addressed by this research include:

1) How do cementochronology, rib, and ADBOU age-at-death estimates correlate?

2) Can cementochronology be used in teeth associated with skeletonized remains to accurately estimate season-of-death (SOD)?

Once more positive identifications are made of individuals included in the study sample, two other questions that could potentially be examined but are beyond this thesis include:

1) Is cementochronology an accurate and reliable aging technique for an unidentified migrant population?

2) Which aging technique is the most accurate and precise based on identified individuals?

ADBOU, rib histology, and cementochronology have not been cross-validated with one another to test their correlation. Having multiple methods to estimate age, sex, and ancestry of an unknown skeleton is crucial in circumstances where there is limited skeletal material to increase accurate estimates. Rib histology has been used in several OpID cases. The age estimates provided by the rib histological analysis were accurate when compared to identified individuals in previous research (Mavroudas et al., 2015). If cementochronology age estimates are correlated with the other methods, it is likely that it can be used at a similar accuracy. The main goal of this study is to test the correlation among these methods to validate the use of cementochronology in the OpID cases. More precise and reliable age-at-death estimates of unidentified migrants will improve the cost in time and resources associated with positive identifications. This research also serves as a validation study of the user-friendliness and reliability of cementochronology for estimating age-at-death and season-of-death (SOD) in unidentified migrants. The sample size for this research consists of 48 individuals, two of whom have been positively identified. There are currently three additional OpID cases included in this sample set that are pending possible identifications. More positive identifications from this sample, will aid in establishing cementochronology as an accurate aging technique.

B. CEMENTOCHRONOLOGY

Cementochronology is the microscopic study of the alternating bands in dental cementum called tooth cementum annulations (Naji et al., 2014). Cementochronology has typically been researched using a known age sample. The current research is based on an unknown sample with remains that have been exposed to different taphonomic processes. The results of this research will allow for comparison of the consistency and bias associated with rib histology, ADBOU, and cementochronology aging methods. If this research can provide insight into which histological method provides the most accurate and reliable age-at-death estimate range, minimal samples can be taken from each individual.

i. AGE-AT-DEATH

Cementochronology is used to observe dental annulations in the cementum to estimate age-at-death. Annulations can be defined as the light and dark bands that are slowly deposited in a circannual pattern in tooth cementum. The number of pairs of annulations are input in a simple equation (# of paired annulation + age of root completion) to get these age-at-death estimates. The definition of "root completion" is based on the root length completed with parallel ends (AlQahtani, 2012). Root completion is used here as the base for age estimation as it is likely that annulations will begin depositing once the root is developed. Currently, there is a gap in the literature for accurate and comprehensive dental eruption rates for a migrant population such as the OpID cases. The overall literature is focused on American and European populations. Because dental eruption rates need to be known for estimating age with cementochronology, the London Atlas is used here to estimate the age at which the tooth root is completed (AlQahtani, 2012). These ages are based on tooth type and sex (which is established by anthropological analysis and DNA for the OpID cases).

ii. SEASON-OF-DEATH

Season-of-death can be estimated based on the opacity of the outermost annulation. Sectioning the teeth for age-at-death also made it feasible to observe the outermost annulation for possible correlation with the estimated season-of-death. Only individuals that were fleshed or in bloat at time of exhumation or recovery were used for this study. The postmortem interval (PMI) estimate is more accurate during the early decomposition stages. These cementum observations may help establish whether cementochronology can be used to estimate SOD in an unidentified migrant population and in naturally decomposed skeletal remains.

C. BROADER IMPACTS

The OpID cases are suspected to belong to Latin American migrants as these remains were found in common migrant corridors near the United States-Mexico border. Once more identifications are made and it is established they are in fact Latin American, the data collected from their remains can provide anthropologists with a better understanding of the potential microstructural and morphological variation present in this population. Furthermore, the data collected for this research can be used in future studies to examine whether ancestry and decomposition processes are reflected in cementochronology age-at-death and SOD estimates. However, it should be noted that it cannot be assumed that all the skeletal remains belong to Latin American migrants until proper anthropological and DNA analyses are completed. Recently, there has been an increase in research focusing on Latin American populations; however, there has not been many studies on age estimation methods that are best suited for these populations. Therefore, the data collected from the OpID cases provide an exceptional opportunity for future histological research on a Latin American population.

Age ranges for macroscopic aging techniques, such as ADBOU, are broader in comparison to dental histological age ranges. Narrow age ranges provide better opportunity for identification when information is uploaded to databases such as National Missing and Unidentified Persons System (NamUs), in which families and local officials can match missing person reports to case reports. Some of the OpID cases that have been recovered do not have sufficient skeletal material to complete macroscopic age-at-death estimates because the features required for analysis are not observable or present. Alternative aging techniques are crucial for providing age estimates for these individuals to aid in identification. Many of the individuals in the OpID cases have similar skeletal identifiers such as ancestry, sex, and stature. Age estimates allow for researchers to narrow down potential matches even further.

Research of cementochronology may provide another method for accurate and reliable age-at-death estimations in forensic and archaeological cases, specifically for the unidentified migrant remains. It is vital to have multiple methods to estimate age-at-death to accommodate trauma, taphonomy, and pathology that may obstruct other aging methods. Teeth preserve rather well due to the protection provided by the enamel (for the crown) and the alveolar bone (for the root). If age-at-death ranges are too broad or unknown, the other information provided in missing person reports and case reports might not be connected.

Finally, SOD can be estimated based on the sequencing of deposited annulations. This can provide researchers an approximate SOD which can be included in case reports to cross-validate with missing persons reports. In forensic anthropology and human rights investigations, the main goal is identification and repatriation to loved ones. These applications for cementochronology permits for minimal samples to be taken with the potential for maximum information extracted from the skeletal material. Estimation of season-of-death is important because it establishes a general timeframe in which a person was alive and potentially last seen. Informants that establish the missing persons reports may have seen their loved one alive and can provide this information to have matched to the estimated season-of-death. This information can further narrow down potential matches between anthropological reports and missing persons reports. Cementochronology permits the researcher to estimate both age-at-death and SOD, whereas the other aging techniques such as morphological traits and rib histology is limited to providing estimates for age-at-death. The opportunity to narrow age-at-death and provide SOD estimates can be invaluable in the overall assessment of each set of remains. A key purpose of this research is to aid in the identification process of unidentified migrant remains by providing narrow age-at-death estimates and SOD estimates for comparison to anthropological reports and missing person reports. It is sometimes difficult for people to identify their missing loved one’s ancestry or stature; however, it is likely they remember their age and the last time they were seen. This information is invaluable for the identification process of unidentified remains.

II. LITERATURE REVIEW

A. WHY CEMENTOCHRONOLOGY?

The occurrence of fragmentary casework in forensic medicine is a regular occurrence. Dependent upon the size and anatomical region of a bone fragment, there are challenges in constructing a biological profile (age, sex, ancestry, trauma, pathology, and stature). It is not atypical for one or more morphological trait(s) used to make these estimates to be absent or altered. Most of the macroscopic aging techniques require mostly complete or entire bones of the skeleton. Thus, the assessment of fragmentary remains is difficult because features used to estimate age-at-death are limited or missing all together (Tersigni, 2005). It is likely teeth are better preserved than other elements of the skeleton due to the durability of enamel for the crown and the alveolar bone for the roots (i.e. cementum).

Histological analyses are not as widely used as macroscopic methods for age-at-death estimations due to limited access to equipment, specialists, and ultimately because it is a destructive analysis. There continues to be limited understanding of how microscopic and macroscopic analyses compare to one another. Skeletal and dental histology are the only form of chronological aging in adult populations, whereas skeletal morphological aging techniques are based on degeneration. The use of histological analyses of bone can be used to facilitate a better understanding of bone modeling and remodeling (Hillier and Bell 2007). Histological analyses incorporate qualitative and quantitative methods to enhance understanding of age-related changes, pathological conditions, and taphonomic and environmental alterations on the skeleton (Hillier and Bell, 2007). Macroscopic age-at-death estimates are reliable, accurate, and widely accepted. However, these methods are not applicable to many forensic cases as the features scored may not be present, may be pathological, or taphonomically altered. Macroscopic age-at-death estimation methods require the researcher to consider multiple factors of the skeleton. Basing age of an unknown individual from just one feature such as the auricular surface, is not ideal and it is best to assess other features for an accurate age range. Whenever possible, it is recommended to use microscopic and macroscopic age methods in conjunction with one another.

B. DENTAL CEMENTUM MICROSTRUCTURE

Dental cementum is made of organic and inorganic compounds like bone, yet it does not remodel like bone (Cool et al., 2002). Dental cementum consists of cellular and acellular material, as well as extrinsic fibers, called Sharpey's fibers, which run perpendicular to the cementum surface (Cool et al., 2002) (Figure 2.1). Dental cementum anchors teeth to their sockets via the periodontal ligament (Wedel et al., 2013). Dental cementum has alternating opaque and bright circumferential bands with "varying crystallite orientations" (Cool et al., 2002: 386). Renz and Radlanski (2006) suggest banding is caused by changes in the orientation of mineralization and collagen. Tooth cementum is developed by "mineralization during the growth of the desmodontal fibroblasts during lifetime" (Wittwer-Backofen et al., 2012: 130). This mineral crystal orientation is recorded in the circannual rhythm of dark and bright layered bands (Wittwer-Backofen et al., 2012). Cold stress is considered a physiological stress that is caused by reduced availability of calcium which interferes with cementogenesis (Dirks et al., 2002; Pike-Tay, 1991; Lubinski and O'Brien, 2001). This results in hypomineralization and arrested growth of collagen fibers in the newly formed layers

(Dirks et al., 2002; Pike-Tay, 1991; Lubinski and O'Brien, 2001). Under polarized light microscopy, the collagen-leached zones appear opaque or dark (Dirks et al., 2002; Pike-Tay, 1991; Lubinski and O'Brien, 2001). These are classified as the winter annulations.

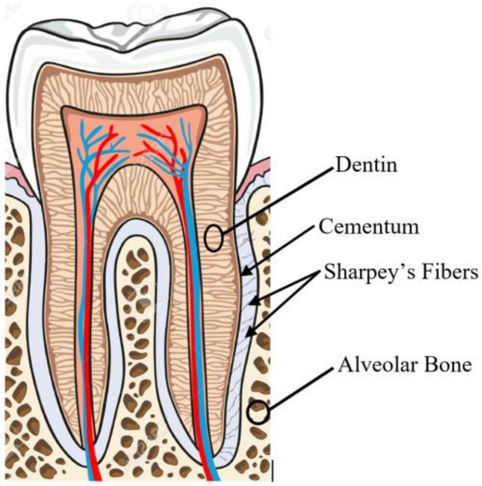

Figure 2.1 Anatomy of a Tooth (modified from Wikimedia Commons)

There are three kinds of cementum that are classified by their composition and function, which are named 1) Acellular Extrinsic Fibers Cementum (AEFC), 2) Cellular Intrinsic Fibers Cementum (CIFC), and finally 3) Cellular Mixed Stratified Cementum (CMCS) (Figure 2.2). The acellular region of the cementum is typically used for cementochronology because it is deposited slowly and in an ordered manner which allows for clearer observation of the banding (Cool et al., 2002). The results found by Cool et al. (2012) states that the lack of change in the birefringence pattern suggests the layered appearance of the cementum was due to the mineral phase. Other researchers, such as Broucker et al. (2015), propose the birefringence of the annulations is due to the orientation of the Sharpey's fibers. The varying orientation in mineral crystals could possibly explain the cause of birefringence patterns seen in tooth cementum (Cool et al., 2002). This requires further investigation and will not be addressed in this research. Ideally, cementum is deposited in a semiannual banding pattern. When this is interrupted in a specific way, it may indicate physiological stresses, diet patterns, reproductive history, seasonality, or other aspects of life history (Wedel et al., 2013). Additional research on diseases and disorders that affect bone and teeth are needed, along with developing a method to put these diseases and disorders into the cultural context. Dental cementum annulations have been studied in populations from many geographic origins, however the etiology of them is not currently understood (Naji et al., 2015).

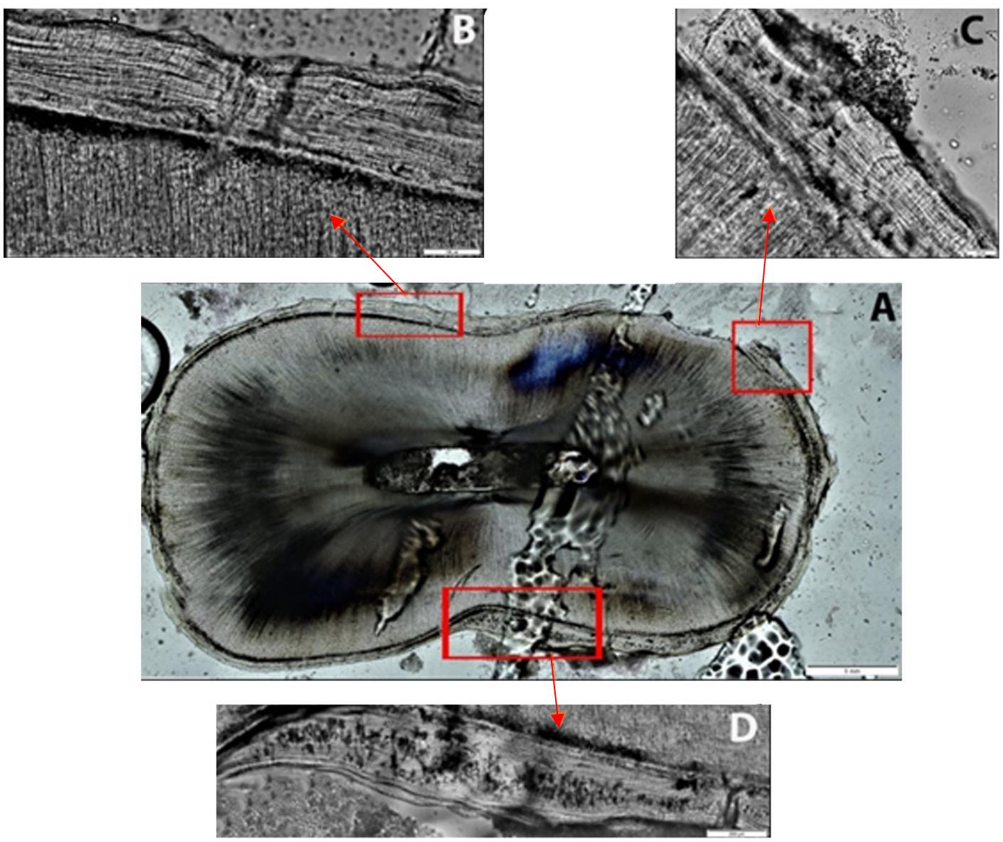

Figure 2.2 Types of Cementum Found in Dental Microstructure. (A) Cross section (1 mm) of maxillary right first premolar with example of (B) acellular cementum (100 μm), (C) mixed cementum (50 μm) and (D) cellular cementum (200 μm).

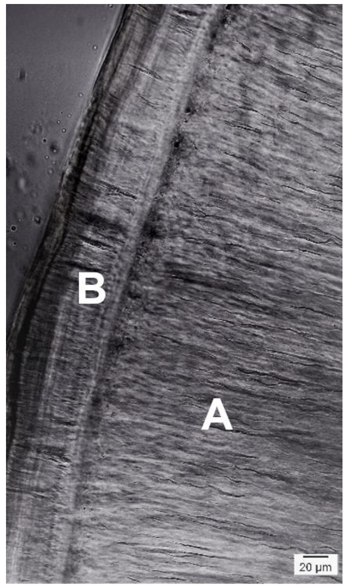

Figure 2.3 Composited Image of Tooth Thin Section. Dentin (A) and Acellular Cementum (B) at 40x Objective

C. AGE-AT-DEATH

Cementochronology requires destructive analysis in which transverse sections from the middle third of the root of a monoradicular tooth is recommended as they allow for more successful identification of regions of interest (Wittwer-Backofen et al., 2012). The process of analysis once sectioning, grinding, polishing, and mounting the tooth section to a glass slide, requires that the observer appropriately adjusts the magnification, light source, focus, and aperture settings accordingly to best observe the dental microstructure. Age-at-death estimations can be calculated by the following formula: Estimated Age = Total number of cementum annulation pairs + Age of root completion of that tooth (Alghonamy et al., 2015; Kaur et al., 2015; Wedel and Wescott, 2015; Wedel, 2007; Broucker et al., 2015). Root completion is based on the descriptions laid out in the London Atlas by AlQahtani (2012). Root completion is used here as it is understood that the cementum formation begins at the termination of the tooth root development. The error range associated with DCIA for age-at-death estimates is 0.8 years for young adults and 2-3 years for adults (Wittwer-Backofen et al., 2004; 2012). This error range is based on teeth extracted at several dental offices from a modern large known-age sample from Germany (Wittwer-Backofen et al., 2004; 2012). Dental eruption has sex, ancestry, and social factors that must be considered when calculating cementochronology age estimates. Therefore, it is recognized by the researcher that the London Atlas is not the most representative method for dental eruption rates in unidentified migrant populations; however, it is based on a modern clinical sample and has a user-friendly interface. The London Atlas is revered as the most comprehensive dental development/eruption method to date (Personal Communication with Dr. James P. Fancher, August 2017). However, it should be noted that the current version of the London Atlas does not provide a known error range for individual tooth eruption rates. This limits the ability to create realistic age ranges for individual tooth root formation to estimate age ranges based on cementochronology. In other words, the age ranges that are presented here for cementochronology are based on the Wittwer-Backofen et al. (2004; 2012) error range without the additon of individual tooth error ranges. Ideally, AlQahtani et al. (2010; 2012) will provide error ranges for age estimates to the web interface which will be necessary for future cementochronology age estimates using the London Atlas eruption rates.

It is important to note that the physiological and structural underlying factors influencing annulation deposition is not well understood (Colard et al., 2015). However, in recent decades, the use of cementochronology to estimate age-at-death has been thoroughly researched for the possible application to forensic and archaeological human remains (Gocha and Schutkowski, 2013; Wedel et al., 2013; Wedel and Wescott, 2015; Wittwer-Backofen et al., 2012). Cementochronology has been used to examine forensic cold cases (Wedel et al., 2013), thermally altered remains (Gocha and Schutkowski, 2013), and archaeological remains (Wedel and Wescott, 2015; Wittwer-Backofen et al., 2008). Cementochronology methods yielded significantly accurate age-at-death estimations in the forensic contexts and thermally altered remains in temperatures up to 600°C. Therefore, this lends support for cementochronology as a promising application to forensic and archaeological cases in which taphonomic alterations, thermal alterations, and limited skeletal material available.

D. SEASON-OF-DEATH

Cementochronology is also often referred to as Dental Cementum Increment Analysis (DCIA) when applied to estimating season-of-death. For the purpose of this research, the application of dental cementum increments will be referred to as cementochronology rather than DCIA. Cementochronology has been used to estimate season-of-death by previous researchers, using the outermost annulations (Wedel and Wescott, 2015; Wedel, 2007; Meckel, 2016). Dental cementum annulations are incrementally deposited in an alternating pattern according to season. This pattern is similar to the rings observed in a tree trunk cross-section. The light (also referred to as translucent or bright) bands are deposited between April and September and are categorized as the Spring/Summer increment (Wedel and Wescott, 2015; Kagerer and Grupe, 2001; Alghonamy et al., 2015; Gocha and Schutkowski, 2013). The dark or opaque bands are deposited between October and March and are categorized as the Fall/Winter increment (Wedel and Wescott, 2015; Kagerer and Grupe, 2001; Alghonamy et al., 2015; Gocha and Schutkowski, 2013). The outermost annulation can be used to estimate the season-of-death to a six-month timeframe. SOD is determined based on the opacity or translucency of the outer-most annulation. If the outer-most annulation is opaque, it is suggested that the season-of-death occurred during fall or winter months. On the other hand, if the outer-most annulation is translucent, it is suggested that the season-of-death occurred during the spring or summer months. This can inform researchers of an approximate SOD which can be included in case reports to cross-validate with missing person's reports. Therefore, cementochronology holds the potential to aid in understanding mortuary practices and mortality incidences by providing the season and age in which an individual died.

According to Ralston (2016) and Meckel (2016), cementochronology can be used to estimate SOD in faunal teeth much better than in human teeth since animals have shorter life spans. This reduces the potential for crowding of the annulations and permits more accurate counts. Crowding reduces the visibility and consistency of the deposition of annulations which consequently affects the estimation of SOD (Meckel, 2016; Ralston, 2016). Wedel (2007) demonstrated cementochronology had an accuracy rate of 99% when estimating SOD in human teeth. However, more recent research has suggested there is little reliability in using cementochronology for estimating SOD because the outer band is not reliably identified by observers due to lack of standardization of methodology (Meckel, 2016; Ralston, 2016). Again, it is important to recognize that the underlying factors associated with the deposition and opacity of annulations is not fully understood. The underlying biochemical factors associated with annulation deposition will aid in more accurately estimating SOD.

III. MATERIALS AND METHODS

A. OPID SAMPLE

Along the United States-Mexico border migrants attempt to enter the United States to seek a better life. Many of these migrants are of Latin American descent typically fleeing conflict and lack of necessary resources in Mexico and Central America. The undocumented migrants typically pass through the desolate areas where they are less likely to be apprehended by U.S. border patrol agents. In these areas, they have little or no access to resources such fresh water and shade resulting in a high volume of deaths (De León, 2015:29-34). Most of the deaths in Texas occur in Brooks County (Martinez et al. 2013). Because of jurisdictional procedures, high volume of deaths, and the lack of resources, the local authorities in Brooks County buried the undocumented migrants and did not take proper measures to assist in identifications (Personal Communication with Dr. Spradley, 2017-2018). Individuals suspected to be undocumented migrants have been buried with little to no analysis done to aid in their identification. Initially, exhumations of the unidentified migrants were completed by University of Indianapolis and Baylor University faculty, graduate students, and undergraduates (Anderson and Spradley, 2016). Since 2014, exhumations have been conducted by students and faculty from the University of Indianapolis and Texas State University during the months of January and May.

When forensic anthropology teams such as Texas State University and University of Indianapolis arrive to exhume the remains, a great deal of preliminary efforts are made to locate the remains. This is typically done based on memory and “hear-say” by the cemetery keepers. This process requires the recovery teams to do extensive informant interviews, ground surveying, and exploratory ground trenching to identify potential burials associated with unidentified migrants in these cemeteries. The use of associated cultural materials and deposition of the unidentified remains within the cemetery are used to support the differentiation between pauper and migrant burials. The skeletal and associated cultural materials are transferred from the recovery site to Texas State University for further analysis (Anderson and Spradley, 2016).

The goals of OpID is the recovery, analysis, and repatriation of unidentified migrants exhumed from Brooks County, Texas (Anderson and Spradley, 2016). The recovered undocumented migrants are taken to the Forensic Anthropology Center at Texas State (FACTS) for processing and analysis (Anderson and Spradley, 2016). Once the OpID cases are recovered, each skeleton is inventoried, measured, examined, and photographed using standardized methods and tools. Dental photographs, radiographs, and reports are taken by a forensic odontologist. Cranial and post-cranial measurements are taken using standardized methods as well. Sex, geographic origin, age, stature, trauma, and pathological conditions are estimated to construct a biological profile. The clothing is cleaned, dried, photographed, and inventoried. Skeletal elements are inventoried and processed using a kettle system, rinsed, and laid out. Since 2016, every set of remains have received age-at-death estimates based on the scoring of cranial sutures, auricular area, and pubic symphysis which were then reported using ADBOU to get a point age estimate. Samples are taken for DNA (metatarsals and femora) and histological analysis (6^{th} or mid-rib).

Pertinent case information is uploaded to NamUs with the goal of matching it to a missing person report (Anderson and Spradley, 2016). A NamUs report is required for

DNA submission. Therefore, an age estimate is essential for these reports to be submitted. Family members typically submit DNA through a non-government organization (NGO) to Bode Cellmark Forensics. DNA results are added to the Combine DNA Index System (CODIS) which can be matched to the University of North Texas DNA samples from the skeletal material that are sampled by FACTS (Anderson and Spradley, 2016). There are several barriers involved in the identification of unidentified migrants. Because there is not always a reference DNA sample from a family member of the decedent, DNA is not always an option for immediate positive identification (Anderson and Spradley, 2016). Non-U.S. nationals have an incredibly challenging time gaining access to lab services to submit DNA samples. Many databases that are used by legal authorities such as CODIS do not allow non-citizens to submit DNA samples to their facility and are only accessible by the Federal Bureau of Investigation (FBI) and a few other labs (Personal Communication with Dr. Spradley, March 2018). CODIS doesn't allow foreign nationals to submit DNA samples unless a match is already hypothesized (Personal Communication with Dr. Spradley, March 2018). Undocumented migrants and their families are not likely to go to local authorities, consulates, and embassies for fear of drawing attention to themselves and their undocumented status. In many cases, family members don't know that they can provide DNA.

i. DATA COLLECTION

Kaur et al. (2015) points out that many researchers have used incisors, canines, premolars, and molars for DCIA; however, Renz and Radlanski (2006) suggest their studies indicated that premolars were more reliable in age-at-death estimation. Kaur et al. (2015) also indicates there is no unanimity in a method used for sectioning of the teeth;

however, the overall literature suggests longitudinal or cross-sections are preferred (Naji et al., 2014; Colard et al.,2015). In their 2004 study, Wittwer-Backofen et al. used maxillary and mandibular incisors, canines, and premolars. Some of the teeth in their sample had superimposed cementum lines which excluded them from the study as accurate counts could not be measured. In many cases these were premolars (Wittwer-Backofen et al. 2004). By selecting single rooted teeth without any taphonomic or pathological alteration, the concern of poor annulation visibility should be reduced.

The sample for this research was obtained from the OpID cases, which consist of 279 individuals recovered from along the United States-Mexico border. The sample set for this research consisted of 18 females and 30 males. One single rooted tooth was extracted from a total of 48 individuals. Prior to sectioning, the teeth had all metrics, morphology, radiographs, photos, and casting completed. Thirty of the individuals included in this study had rib histological aging analysis completed. Only teeth that were still present in the alveolar bone of the maxilla or mandible were selected to avoid teeth that may have suffered from soil diagenesis (Wittwer-Backofen et al., 2012). Parameters that have been established for this sample include teeth with no evidence of periodontal disease, carious lesions, dental modifications (surgical or cosmetic), and limited diagenetic degeneration. If diagenetic alterations are macroscopically observed in any of the teeth sampled, they were omitted as advised by Hillier and Bell (2007). The teeth were selected because of their relatively good preservation and absence of macroscopic pathology such as caries and modifications.

Twenty-three teeth that were included in this sample set were previously sampled by Dr. Eric Bartelink at California State University for isotopic analysis. Dr. Bartelink provided these teeth following his analysis, so they could be used for this study. The other 25 teeth were intact following extraction from the crania. The crowns were removed for 33 of the 48 teeth to be used for additional isotopic analysis. The remaining 15 teeth had been previously sampled for isotopic analysis and the roots had microfractures that required the crown to remain intact and embedded with the root to provide structural support during sectioning.

ii. COMPARATIVE AGE DATA

Cementum age estimates were compared to age estimates obtained through rib histology and ADBOU. The rib histology age estimates are derived from published case reports whereas the ADBOU age estimates were established by the author scoring the cranial sutures, pubic symphyses, and auricular surfaces for all 48 cases.

Rib Histology Age Estimates

The ribs of select OpID cases have been sampled and aged by Sophia Mavroudas since 2014. Rib histological age-at-death estimates were previously compared to morphological methods such as dental development, pubic symphysis, auricular surface, skeletal fusion, and ADBOU in 19 OpID cases (Mavroudas et al., 2015). The midshaft of the 6^{th} rib was sampled from each individual. The age estimate of each method was compared to the actual age of positively identified individuals to examine the accuracy of the results. Patterns of overlap was considered for point age estimates from each method (Mavroudas et al., 2015). Mavroudas et al. (2015) found that skeletal fusion did not correspond to actual age and that rib histological age-at-death estimates provided more accurate and reliable age ranges in the positively identified individuals when compared to actual age and the other aging methods (Mavroudas et al., 2015).

For the current study, the point age estimates and ranges for rib histological analysis were derived from published case reports for 30 of the cases included in this sample. The point age estimates were used to assess the correlation with cementum and ADBOU age estimates. Rib thin sections were sampled from 5^{th}-7^{th} ribs at the midshaft and were processed according to standardized histological protocol. Age estimates for individuals with ADBOU age estimates over 35 years were calculated based on the Cho et al. (2002) method. Individuals that had ADBOU age estimates that were 35 years and younger had rib histological age estimates that were calculated based on the Stout and Paine (1992) method.

ADBOU Age Estimates

As of 2016, each OpID set of remains analyzed at Texas State received a macroscopic age-at-death estimate using ADBOU. ADBOU is based on the scoring of five features of the pubic symphysis, five segments of cranial sutures, and nine features from the iliac auricular surface. The left and right auricular surfaces and pubic symphyses can be scored independently, allowing for intermediate phases to be assigned if necessary (Boldsen et al., 2002). This information is used to calculate a maximum likelihood point age estimate which will be used in this research for each set of remains included in the sample.

The development of ADBOU sought to make morphological aging on adult skeletal remains unbiased with the use of transitional analysis (Boel, Boldsen, and Melsen, 2007). ADBOU is based on Bayesian transitional analysis using a known-age reference collection to perform conditional probability equations (Boldsen et al., 2002). These probabilities show the likelihood that an individual fits into a particular age group.

These condition probabilities are combined with the Bayes theorem using a prior age-at-death distribution to yield posterior probabilities that the unknown individual belongs to the predicted age group. ADBOU provides a point age estimate as well as a 90-99% age range (Boldsen et al., 2002). According to Bethard (2005), secular differences in aging between the test sample and reference population in ADBOU may result in errors. Selected priors for ADBOU are based on an archaeolgoical and forensic sample which do not always accurately represent the test population such as the unidentified migrant population. Archaeological priors are based on a 17th century Danish rural parish records. Forensic priors are based on 1998 United States homicides, which are more representative of a younger population as forensic cases typically involve young individuals. For the OpID cases, forensic (context) and unknown (ancestry) priors are selected in the ADBOU software program. Ideally, ADBOU age estimates could be revised using a migrant mortality model which could be provided by migrant death statistics from the Pima County Office of the Medical Examiner (PCOME) or Border Patrol apprehenison statistics. Since there are more unidentified migrant remains being discovered in the United States, it could be extremely beneficial to add in this migrant mortality model into the ADBOU software to provide more accurate age estimates for this population.

In many instances, the OpID cases do not have sufficient skeletal material to complete macroscopic age-at-death estimates because the ossa coxae and cranium are fragmented or absent. Poor bone preservation has convoluted the macroscopic age-at-death estimates in many of the OpID cases. Furthermore, the macroscopic age-at-death estimates may be unreliable on the unidentified migrant population as the methods used for ADBOU have not been previously validated on these populations. Several of the cases that were included in this study did not have ADBOU age-at-death estimates completed. Because ADBOU has an interobserver bias and to assure that all 48 cases had ADBOU estimates, the author performed ADBOU assessments with a 95% probability on all the cases included in the sample. Cases included in this sample had to have cranial sutures or at least one os coxae that could be scored for ADBOU. In most cases, both cranial sutures and left and right ossa coxae were able to be scored.

B. CEMENTOCHRONOLOGY PREPARATION

Selection of tooth types was based on the recommendations of previous researchers such as Wittwer-Backofen et al. (2004), Alghonamy et al. (2015), Colard et al. (2015), Renz and Radlanski (2006), and Gocha and Schutkowski (2013). The sample included maxillary and mandibular single rooted teeth. Thirty-eight of the teeth were maxillary premolars, nine of the teeth were mandibular premolars, and one tooth was a maxillary canine (refer to Table 3.1). Of the 38 maxillary premolars, 25 were first premolars and 13 were second premolars. Of the nine mandibular premolars, three were first premolars and six were second premolars (refer to Table 3.1).

The teeth were processed according to the methods discussed in Naji et al. (2014), Colard et al. (2015), and Wittwer-Backofen et al. (2004) which are considered the optimal procedures for accurate and reliable measurements and counts of annulations. For teeth that were previously sampled for isotopic analysis, the tooth was embedded in a mixture of Buehler® EpoThin™2 resin and hardener prior to sectioning to the middle third of the root. If the crown was able to be removed without destroying the root, it was sectioned off using a Dremel saw with a fitted diamond wafer blade so the crown could be used in future research. The root was then embedded and sectioned at 1 mm sections using a Low Speed Saw Buehler IsoMet® 1000 Precision Saw fitted with a diamond-coated blade at 100 RPM.

Table 3.1 Sex, Tooth Number and Type, and Age of Root Completion

OpID #	Sex	Tooth #	Tooth Type	Age of Root Completion
365	F	12	Left Maxillary Pm1	12.5
368	M	29	Right Mandibular Pm2	13.5
379	F	12	Left Maxillary Pm1	12.5
381	M	12	Left Maxillary Pm1	13.5
383	M	13	Left Maxillary Pm2	13.5
384	F	11	Left Maxillary C	14.5
389	F	20	Left Mandibular PM2	13.5
390	M	12	Left Maxillary Pm1	13.5
391-A	M	12	Left Maxillary Pm1	13.5
392	F	12	Left Maxillary Pm1	12.5
395	M	4	Right Maxillary Pm2	12.5
397	M	4	Right Maxillary Pm2	12.5
398	M	12	Left Maxillary Pm1	13.5
400	M	12	Left Maxillary Pm1	13.5
401-C	F	13	Left Maxillary Pm2	13.5
406	F	13	Left Maxillary Pm2	13.5
409	M	28	Right Mandibular Pm1	13.5
415	M	29	Right Mandibular Pm2	13.5
416	F	13	Right Maxillary Pm2	13.5
417	F	12	Left Maxillary Pm1	12.5
421	M	12	Left Maxillary Pm1	13.5
422	M	12	Left Maxillary Pm1	13.5
423	M	12	Left Maxillary Pm1	13.5
426	M	5	Right Maxillary Pm1	12.5
427	M	12	Left Maxillary Pm1	13.5
430	M	4	Right Maxillary Pm2	12.5
440	M	4	Right Maxillary Pm2	12.5
446	F	4	Right Maxillary Pm2	13.5
448	F	5	Right Maxillary Pm1	12.5
451	M	12	Left Maxillary Pm1	13.5
464	F	20	Left Maxillary Pm2	13.5
467	M	5	Right Maxillary Pm1	12.5
469	M	5	Right Maxillary Pm1	12.5
470	M	21	Left Maxillary Pm2	13.5
471	M	12	Left Maxillary Pm1	13.5
477	F	29	Right Maxillary Pm2	12.5
482	M	12	Left Maxillary Pm1	13.5
485	F	5	Right Maxillary Pm1	12.5
490	F	12	Left Maxillary Pm1	12.5
491	M	4	Right Maxillary Pm2	12.5
503	M	5	Right Maxillary Pm1	12.5
504	M	20	Left Maxillary Pm2	13.5
505	M	13	Right Maxillary Pm2	13.5
506	F	13	Right Maxillary Pm2	13.5
508	F	12	Left Maxillary Pm1	12.5
514	F	21	Left Maxillary Pm2	12.5
608	M	5	Right Maxillary Pm1	12.5
611	M	4	Right Maxillary Pm2	12.5

For teeth that were not previously used for isotopic analysis, the roots were marked at the middle third. Once marked, the crown was removed using a Dremel saw with a fitted diamond wafer blade. Next, the root was sectioned to the middle third of the root using a Buehler precision saw. Once this step was complete, the root was Crystal Bonded to a slide and sectioned directly on the slide using the Buehler precison saw to 1 mm thickness. A total of four to five cuts was made to each tooth. The first slice removed the crown. The second cut made the acellular region of the root (the middle third) visible. Two to three 1 mm transverse cross-sections were taken from the middle third of the root using a Low Speed Saw Buehler IsoMet® 1000 Precision Saw fitted with a diamond-coated blade at 100 RPM. Isocut Fluid was used in the water basin of the Low Speed Saw Buehler IsoMet® 1000 Precision Saw to lubricate and cool down the wafering blade while sectioning.

Two to three sections from each tooth were taken to account for any structural, taphonomic, diagenetic, or pathological discrepancies in the layers of the tooth. Embedded sections (from previously sampled teeth) and Crystal Bonded sections were ground and polished using a Buehler® Metaserv® 3000 Diamond Grinding (15 micron, 8" disc) and Polishing pad at 100 RPM. The embedded sections were ground between the grinding pad and a slide fitted with BuehlerMet2 240 grit abrasive paper. The Crystal Bonded sections were ground directly on the slide in which it was mounted, between the grinding pad and a metal slide holder. Thickness of all tooth sections were measured consistently throughout the grinding process using a Lgsixe digital micrometer (0.004064 mm accuracy) until thickness measured to 70-100 µm. For Crystal Bonded sections, the slide thickness and Crystal Bond surrounding the tooth section was measured so that tooth thickness was known. The same procedure was used for polishing of all tooth sections with the use of MasterPrep Polishing Suspension, MetaDi Fluid, and 3 micron Diamond Polishing Compound. Once the section was ground and polished to necessary thickness, the section and cover slide was mounted to a slide using Protocol® SecureMount™ mounting medium. Each slide was etched with the OpID case number and tooth number which was covered with painters tape and coded by Sophia Mavroudas to eliminate any bias during the analysis process. Two slides were analyzed for each individual to assess topographic variation of each tooth.

C. AGE-AT-DEATH METHODS

Each slide was viewed under a bright field and polarized light using an Olympus CX41 microscope with a mounted Olympus DP27 camera. Each coded slide was assigned a quality value based on the visibility of the dental cementum annulations. These qualitative values were "good", "fair," and "poor". This information will allow for examination in the future of accuracy of age-at-death and season-of-death based on quality of dental cementum annulations. Images of regions of interests (ROI) were composited at 40x magnification and viewed using Cellsens software to count cementum annulation pairs to assess age-at-death. Two to five regions of interest images were composited for each slide with varied settings for the focus, aperture, and light exposure, which allowed for the best analysis of each ROI without losing the quality of the annulations by stitching and viewing an entire cross-section. Counting annulations from multiple regions of interest assured that all observable areas were examined and to account for possible bifurcation or superimposition of annulations. Each slide was examined for three observations, at least two weeks apart to perform live counts and photo counts. Live counts were performed on the best ROI. This process allowed for the assessment of counts from live acquisition and composited images to test the variation of consistency between the two practices. Additionally, counts from live acquisition and region of interest photos will allow for determination of the best method for counting dental cementum annulations once more positive identifications are made.

Counts for age-at-death estimation for each individual were done live under the microscope on each of the two slides for the three separate observations. Additionally, counts to estimate age-at-death were done from five region of interest photos for each slide associated with each individual. In other words, each individual had six counts performed based on live acquisition and 30 counts based on region of interest photos. Since each individual had two slides that were observed at two to five regions, on three separate observations, each tooth had slight variation in the number of annulations counted. To select the number of annulations counted for each individual, two methods were employed. The maximum number of annulations observed from the two slides for each individual was used to estimate the “max count” age estimate. The second method used was to average the number of annulations observed between all observations made from the two slides for each individual to provide an “average count” age estimate. The benefit of the max count method is that it accounts for the maximum number of annulations found in the tooth section. However, it is also possible that bifurcation of the annulations occurred in the region examined and therefore provides an inaccurate estimate. To cover all possibilities, the average count method is used to weed out the outlier counts that may be the result of bifurcating or unobservable annulations (whether it be due to lack of focus, superimposition of the annulation, or related cause). The average count method is utilized by other authors to account for the outliers (Colard et al., 2015; Naji et al., 2014). Average and max count methods are employed here to examine the best practice for counting annulations and to test the overall consistency between the two methods.

Age-at-death estimations were calculated by the following formula for both max and average counts: Estimated Age = Total number of cementum annulation pairs + Age of root completion of that tooth. Root completion is based on the descriptions laid out in the London Atlas by AlQahtani (2012). Tooth number, sex, and age of root completion for each case is listed in Table 3.1. The error range associated with DCIA for age-at-death estimates is 0.8 years for young adults and 2-3 years for adults at 2 standard deviations based on the results from a modern large known-age sample from Germany (Wittwer-Backofen et al., 2012). The OpID cases are associated with unidentified individuals, therefore a ±3 year age range was used for the cementum age ranges.

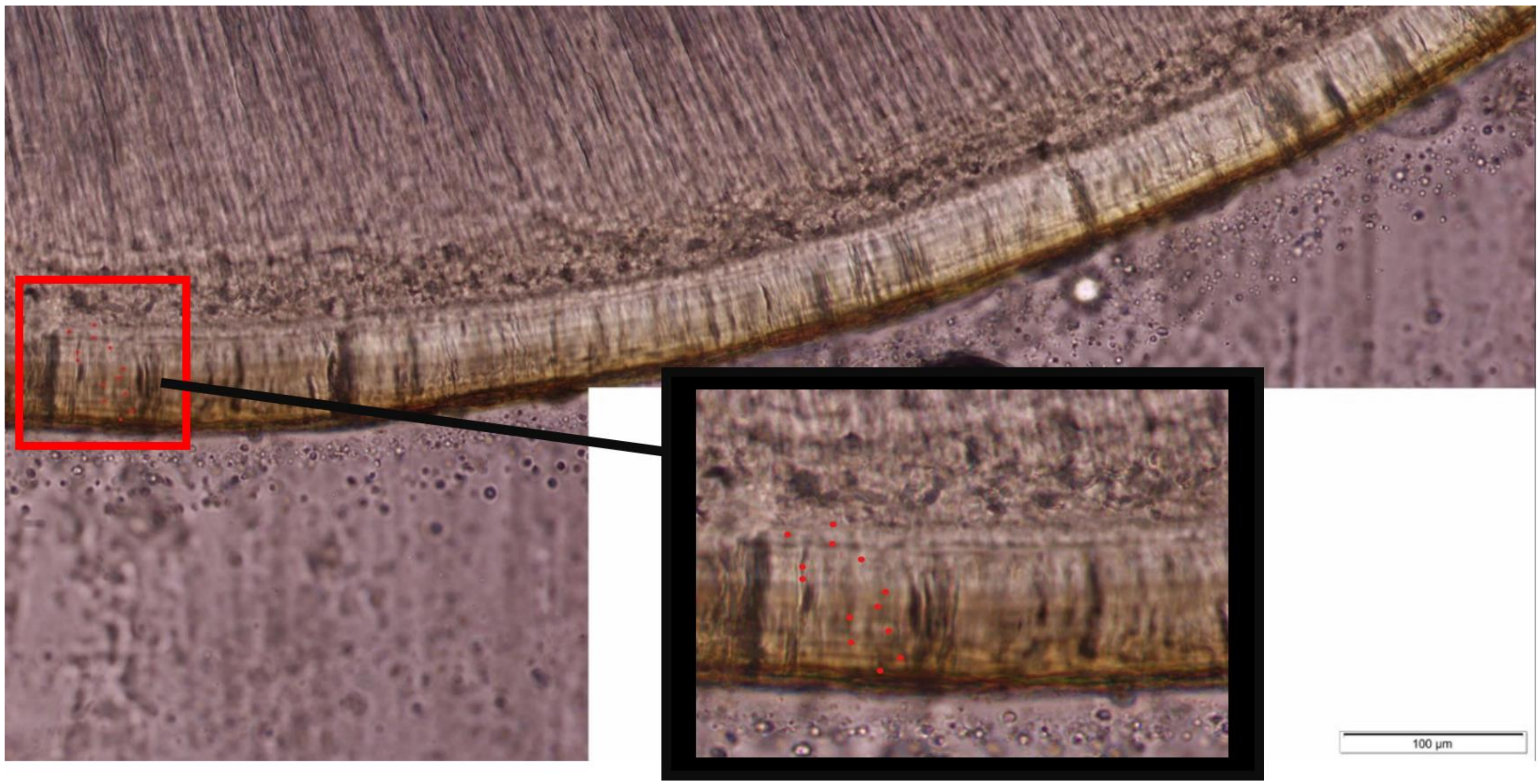

Figure 3.1 Dental Cementum Counts for Case OpID 0422. Thirteen Annulations Can Be Counted (marked by red dots). This is the region of interest photo of tooth #12 (left maxillary first premolar), in which root completion occurs around 13.5 years in males. This provides a cementochronology age estimate of 26.5

i. STATISITCAL ANALYSIS

Test of Difference in Means: Photo Vs. Live Acquisition

To examine the difference between the two counting methods (i.e. photo vs. live acquisition), a Wilcoxon rank signed test was performed in Excel using the XLSTAT add-on. Given the limited number of paired observation sets for each individual, a non-parametric test is recommended. A two-tailed Wilcoxon ranked signed test was performed for each individual to test the null hypothesis that the median difference between photo and live counts is 0 (i.e. both samples follow the same distribution). Since the exact p-values cannot be generated with tied values, the Hollander and Wolfe (1999) method was selected in XLSTAT to remove the tied observations during the statistical analysis[1].

To examine the average differences between paired counts by individual, count cohorts were created to show how many individuals had average individual count differences[2] of less than1, between 1 and 1.99, between 2 and 2.99, and 3 or greater. For example, individuals having an average count difference of less than one were tabulated and presented in the "<1" row (See Table 4.2). In addition, absolute differences for paired observations across all individuals were calculated. These values were similarly grouped into "difference" bins of 0,1,2, and 3 or greater. For example, all paired observations with

[1] It's important to note using this method can potentially lead to a significant ($p<0.05$) result in a scenario where a large proportion of values are tied. As a result, the analysis could lead to a rejection of the null hypothesis, when in fact most of the samples are equal (i.e. tied values). Tied values for individuals with significant results (Wilcoxon rank signed test) in this paper, however, are minimal. Out of the 12 significant outcomes, six have 0 ties, four have 1 tie, and two have 2 ties.

[2] Average individual count difference was calculated by taking the mean of the absolute differences between each paired sample for each individual. This value measures consistency between each paired observation (i.e. photo vs. live counts) for each individual.

a difference of 1 were tabulated and presented in the “1” count difference row (see Table 4.3).

Test of Correlation for Age-at-Death Methods

The age estimates for each method was tested for normality using the Shapiro-Wilk test in R (R Core Team, 2018) using the Rstudio GUI (https://www.rstudio.com/). Based on the Shapiro-Wilk test results, a non-parametric analysis was used (Kendall’s Tau Coefficient method (τ)), to test the correlations among cementochronology max count, cementochronology average count, rib histology, and ADBOU. A Pearson’s correlation analysis was also used to test rib histology and ADBOU, since they had normally ditributed data ($p < 0.05$) based on the results of the Shapiro-Wilk test. To provide a visual represetation of the correlations for cementochronology max count, cementochronology average count, rib histology, and ADBOU, a scatterplot matrix was performed in Rstudio. Additionally, a line graph was generated to provide a visual representation of the relationship of the age esitmates for each case.

D. SEASON-OF-DEATH METHODS

A subsample of nine individuals that were in early stages of decomposition at time of discovery and were well documented (e.g. photographs and reports) were included in the sample for SOD. The PMI estimates for these individuals were compared to the translucency or opacity of the outermost band to estimate SOD. Only individuals that were in early stages of decomposition at time of discovery or exhumation were used for this purpose of the study. The PMI estimate is more accurate during these decomposition stages. These observations may help establish whether cementochronology can be used to estimate SOD in this population, particularly with skeletonized remains. Total body score (TBS) was estimated based on photos of nine individuals from the OpID cases. These photos were taken at the location in which the bodies were first discovered by local authorities. Scoring of TBS is based on the Megyesi et al. (2005) descriptions with modifications recommended by Moffatt et al. (2016) where 3 points are deducted from the total score. Accumulated degree day (ADD) was determined based on Moffatt's calculations. ADD was reached by summing the average temperature for each day since date of discovery back to possible date of death (or exposure to the elements) until expected ADD was obtained. Average temperature for each day was obtained from http://mesowest.utah.edu. The weather stations selected for each case was either Falfurrias or Kingsville as they are the stations in closest proximity to where the bodies were found. Three observations at least two weeks apart were made for SOD based on two to five ROI images composited. SOD was also assessed based on live observation on three occasions at least two weeks apart for each slide. The purpose of viewing the slides live under the microscope for SOD is to allow the researcher to adjust region of interest, light and polarization, and focus.

Table 3.2 Circumstances in Which SOD Subsample Cases Were Found

Case Number	Sun/Shade	Skeletonized	Discovered by	Scavenged
384	Mostly Shaded	None Visible	Brooks Co Deputies	None Visible
390	Shaded	None Visible	Rancher	None Visible
391-A	Shaded	Eye Orbits	Texas Border Volunteers	None Visible
395	Sun	Maxillary region	Ranch Hand	None Visible
397	Sun	None	Ranch Hand	None Visible
400	Shaded	None Visible	Ranch Hand	None Visible
423	Sun	None Visible	Border Patrol	None Visible
446	Unknown	Frontal, Maxillary	Border Patrol	None Visible
451	Sun	None Visible	Passerby	None Visible

i. STATISTICS ANALYSIS

Test of Association for Expected SOD Vs. Observed SOD

A Fisher's Exact tests was used to determine if there are nonrandom associations between expected season-of-death and observed season-of-death for the OpID cases included in the SOD study. There were only nine individuals that could be included in this sample set, as they were the only individuals that were in early stages of decomposition at time of discovery. The small sample size is accounted for statistically by performing a Fisher's Exact test. The expected band color is based on the known SOD from the ADD calculations estimating PMI. For example, if the ADD calculations estimate that an individual died in the month of February, the expected outermost annulation is dark. The observed outermost band is based on the estimated SOD from the thin tooth sections.

IV. RESULTS

A. AGE-AT-DEATH

i. TEST OF DIFFERENCE IN MEANS: PHOTOS VS. LIVE ACQUISITION

To examine the difference between the two counting methods (i.e. photo vs. live acquisition), a Wilcoxon rank signed test was calculated in Excel using the XLSTAT add-on. The results of the Wilcoxon rank signed test indicated there is not a significant difference between the counts based on live acquisition and region of interest photos for the majority of the sample set (see Table 4.1 for statistical model outputs). However, a few OpID cases had significant differences between photo and live counts, which are denoted by an asterisk (*) in Table 4.1. This may be due to differences in the visibility of annulations for photos and live observations. It is recommended by Naji et al. (2014) and Colard et al. (2015) to use region of interest photos for estimating age-at-death to assure consistent counts throughout separate observations.

While the Wilcoxon rank signed test is useful to determine potential differences in medians between the two counting methods, the statistical test has limited ability to detect variation/differences for each paired observation. For example, OpID case 379, which has a non-significant p-value (Table 4.1), has one of the highest average individual count difference (see footnote 1 above for a description of this statistic) among all individuals (Appendix F). To account for variation in counts for each paired observation per individual, count cohorts were created to show how many individuals in the sample set had average individual count differences of less than 1, between 1 and 1.99, between 2 and 2.99, and 3 or greater. Differences (absolute value) in counts for all paired observations were also calculated. The count cohorts indicate that much of the sample set (i.e. all individuals) have similar counts for photos and live observations. It can be seen in Table 4.3 that 90% of all paired observations have count differences of 2 or less. The mean difference for all paired observations is 1.2. Similarly, Table 4.2 indicates 88% of individuals have average individual count differences of less than 2. In other words, 88% of individuals did not exceed an average difference of 2 annulation counts. As indicated above, absolute differences were used for the calculations described above. It should be noted the actual mean difference (i.e. not using absolute differences) between all paired observations is -0.43 (Table 4.4), indicating similar values for both the photo and live count methods, with live counts tending to be slightly higher on average[3]. It is expected to have higher counts for live acquisition because as the microscope settings are adjusted, it is possible that there would appear to be more annulations as light transmits through the tooth section. However, it is recommended throughout the literature to use photos for cementum annulation counts for age-at-death estimation.

[3] Note that differences were calculated by the following formula: Difference = (Photo) – (Live). Thus, a negative value indicates a higher count for the live count method.

Table 4.1 Results for Wilcoxon Rank Signed Test. Testing the Difference in the Medians Between Live Acquisition and ROI Photo Count Methods to Estimate Age-at-death

Individual Case	Wilcoxon Test Statistic (V)	*p*-Value
365	4	0.705
368	10.5	1
379	6.5	0.399
381	5	1
383	1.5	0.194
384	0	0.039*
389	2	0.564
390	6	0.655
391-A	4.5	0.396
392	9	0.746
395	10	0.063
397	0	0.157
398	2	0.564
400	15	0.335
401-C	10.5	1
406	10.5	0.396
409	0	0.102
415	6	0.102
416	0	0.024*
417	14	0.414
421	0	0.157
422	0	0.102
423	2.5	0.092
426	4	0.564
427	7.5	0.317
430	2	0.564
440	0	0.026*
446	0	0.083
448	12	0.180
451	0	0.038*
464	0	0.180
467	15	0.025*
469	0	0.157
470	0	0.059
471	0	0.038*
477	0	0.026*
482	0	0.024*
485	10	0.046*
490	21	0.026*
491	6	0.083
503	0	0.027*
504	10.5	1
505	0	0.059
506	18	0.096
508	6	0.102
514	1.5	0.414
608	6	0.083
611	0	0.046*

* Denotes Values that are Significant

Table 4.2 Average Differences in Cementochronology Counts (Photo vs. Live) by Each Individual

Average Individual Count Differences	# Individuals	% Average Differences	Cumulative % Differences
<1	21	44	44
1-1.99	21	44	88
2-2.99	3	06	94
≥3	3	06	1[1]

1 The largest average count difference for each individual is 5, therefore 100% of the count differences are 5 or less.

Table 4.3 Differences in Cementochronology Counts (Photo vs. Live) for All Observations

Count Differences All Samples	N Differences	% Differences	Cumulative % Differences
0	78	27	27
1	123	43	70
2	58	20	90
≥3	29	10	1[1]

1 The largest count difference for all samples is 6, therefore 100% of the count differences are 6 or less.

Table 4.4 Mean Differences Among All Paired Observations

Mean of Absolute Differences	Mean of Actual Differences
1.2049	-0.4340

ii. TEST OF CORRELATION FOR AGE-AT-DEATH METHODS

Shapiro-Wilk tests were performed in R to test the normality for each of the method's data sets for age-at-death estimates (Table 4.5). The results of these tests suggest that the cementum max count and average count data were not normally distributed, whereas the rib histology and ADBOU data followed a normal distribution (based on $p > 0.05$). Because the data generally followed a non-normal distribution, with some exceptions, the main statistical analysis used the non-parametric test, Kendall's Tau, to calculate Kendall's Tau rank correlation coefficients. A parametric test was also performed to test the correlation between rib histology and ADBOU using Pearson's correlation (see Table 4.6 for results), since the data with these two methods followed a normal distribution. Kendall's Tau coefficients, Pearson's correlations, and scatterplot matrices for all the methods were calculated and produced in R within the Rstudio GUI. Scatterplot matrices, including Kendall's Tau coefficients and *p*-values, can be referenced in Figure 4.1 and Table 4.7. It should be noted that the correlation coefficients calculated for the rib histology method are limited to 30 rib samples that had age estimates. In contrast, the cementochronology (both average and max counts) and ADBOU age estimates had 48 samples.

Based on the results shown in the scatterplot matrices below, all the correlations among the aging methods have a statistically significant positive correlations ($p < 0.05$; see Table 4.7). However, some of these correlations are stronger than the others. It can be seen in Figure 4.1 and Table 4.7, specifically, that the cementochronology average and max count methods had a τ value of 0.87, which indicates a moderately strong correlation. Cementochronology average and max counts to rib histology analyses had τ values of approximately 0.41-0.44, which indicates a generally moderate correlation. The cementochronology (average and max counts) to ADBOU analysis had τ values of approximately 0.21-0.24, which indicates weaker correlations. The rib histology and ADBOU analysis produced a τ value of 0.42, which indicates a generally moderate correlation. Because the rib histology and ADBOU age estimates were determined to be normally distributed ($p > 0.05$), a parametric test was also performed (Pearson's correlation). The results of the Pearson's correlation test produced an R-value of approximately 0.71, with a *p*-value less than 0.0001. These results indicate a statistical significance and a generally moderate to strong positive correlation. After visual inspection of the scatterplot matrix (Figure 4.1), the fitted lines for cementochronology max and average counts and rib histology generally show more overlap with the 1:1 line, consistent with the τ values presented in Table 4.7. This indicates a more closely related age estimate, whereas the correlations based on cementochronology or rib histology to ADBOU are less positively correlated. It's important to note the cementochronology max and average age estimate scatterplot shows the strongest correlation (see overlap with 1:1 line) and tightest fit around the regression (fit) line.

Table 4.5 Shapiro-Wilk Normality Test Results

Method	**W Statistic**	***p*-Value**
Average Cementochronology	0.93727	0.01276*
Max Cementochronology	0.91162	0.00153*
Rib Histo	0.93456	0.06497
ADBOU	0.96436	0.15140
*Significant based on $\alpha = 0.05$		

Table 4.6 Pearson's Correlation Results for Rib Histology and ADBOU

Correlation Coefficient	***p*-Value**
R=0.71219	P<0.0001
*Significant based on α=.05	

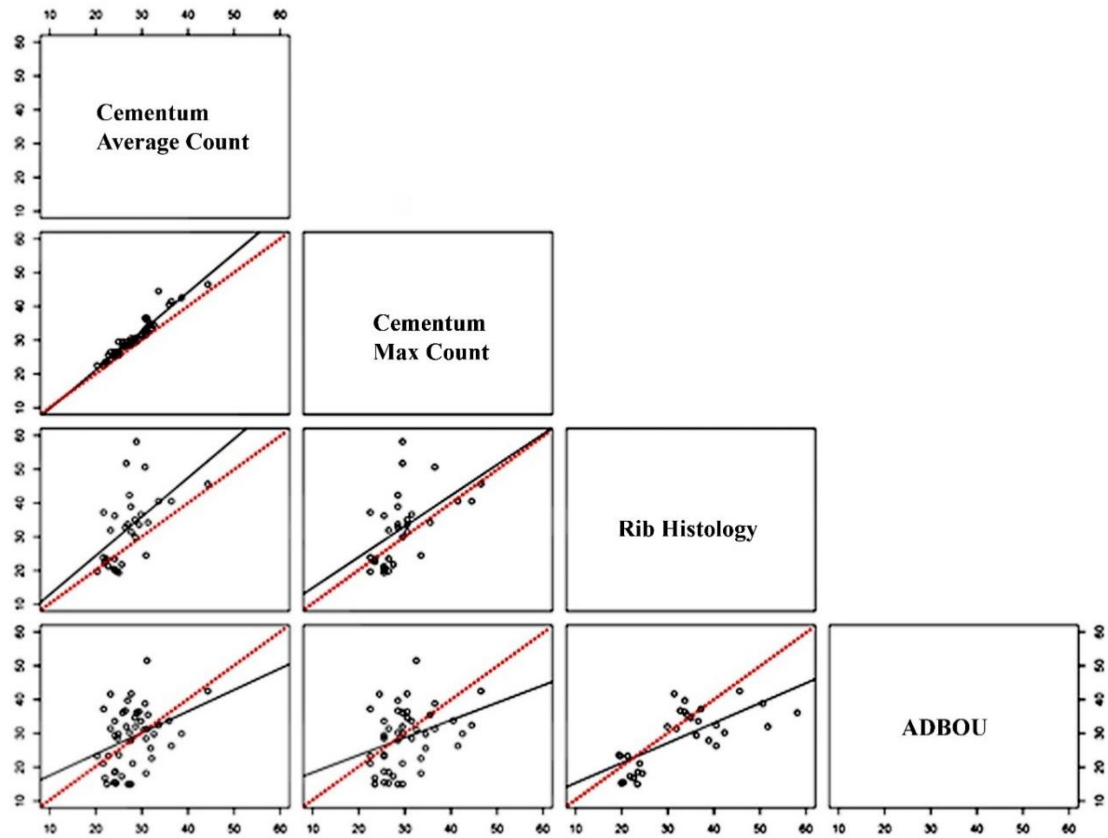

Figure 4.1 Scatterplot Matrix with Fitted Line (Solid) and 1:1 Line (Dashed). This Figure Shows the Correlation Between Each Age-at-death Estimation

Table 4.7 *p*-values (Lower Triangle) and Kendall's Tau Coefficient Values (τ) (Upper Triangle) Associated with the Method Correlations

	Average Cemento.	Max Cemento.	Rib Histo	ADBOU
Average Cemento.	--------------	0.8702	0.4124	0.2063
Max Cemento.	<0.0001	----------------	0.4481	0.2396
Rib Histo	0.0014	0.0007	-----------------	0.4198
ADBOU	0.0399	0.0197	0.0012	-------------------

iii. AGE-AT-DEATH ESTIMATES FOR IDENTIFIED INDIVIDUALS

There have been two positive identifications included in this sample set. OpID case 0608 was identified as a 37-year-old male. The point age estimate provided by cementochronology (31.1 years- average count; 36.5 years- max count) was closer than the rib histology (42.6 years) and ADBOU estimates (31.3 years). The age ranges used for each method is as follows: cementum average count- ±3 years, cementum max count- ±3 years, rib histology- ±16.02 years, and ADBOU ±7.4 years. It can be seen in Figure 4.2 that the age ranges for cementum max count, rib histology, and ADBOU include the known age. The cementum average count age range does not include the known age of case 0608. It is possible that the average count method weeded out "outliers" that were not in fact outliers, therefore underestimating the age.

OpID case 0383 was identified as a 23-year-old male. The point age estimate provided by cementochronology (29.8 years- average count; 31.5 years- max count) was closer to the known age than the rib histology (36.6 years) and ADBOU estimates (33.6 years). The age ranges used for each method is as follows: cementum average count- ±3 years, cementum max count- ±3 years, rib histology- ±16.02 years, and ADBOU ±5.7 years. It can be seen in Figure 4.2 that the age ranges for cementum max count, cementum average count, and ADBOU do not include the known age. The only age range that does include the known age of case 0383 is rib histology. Case 0383 was overaged by all of the aging methods, suggesting that the skeletal morphology and microstructure makes this case an outlier.

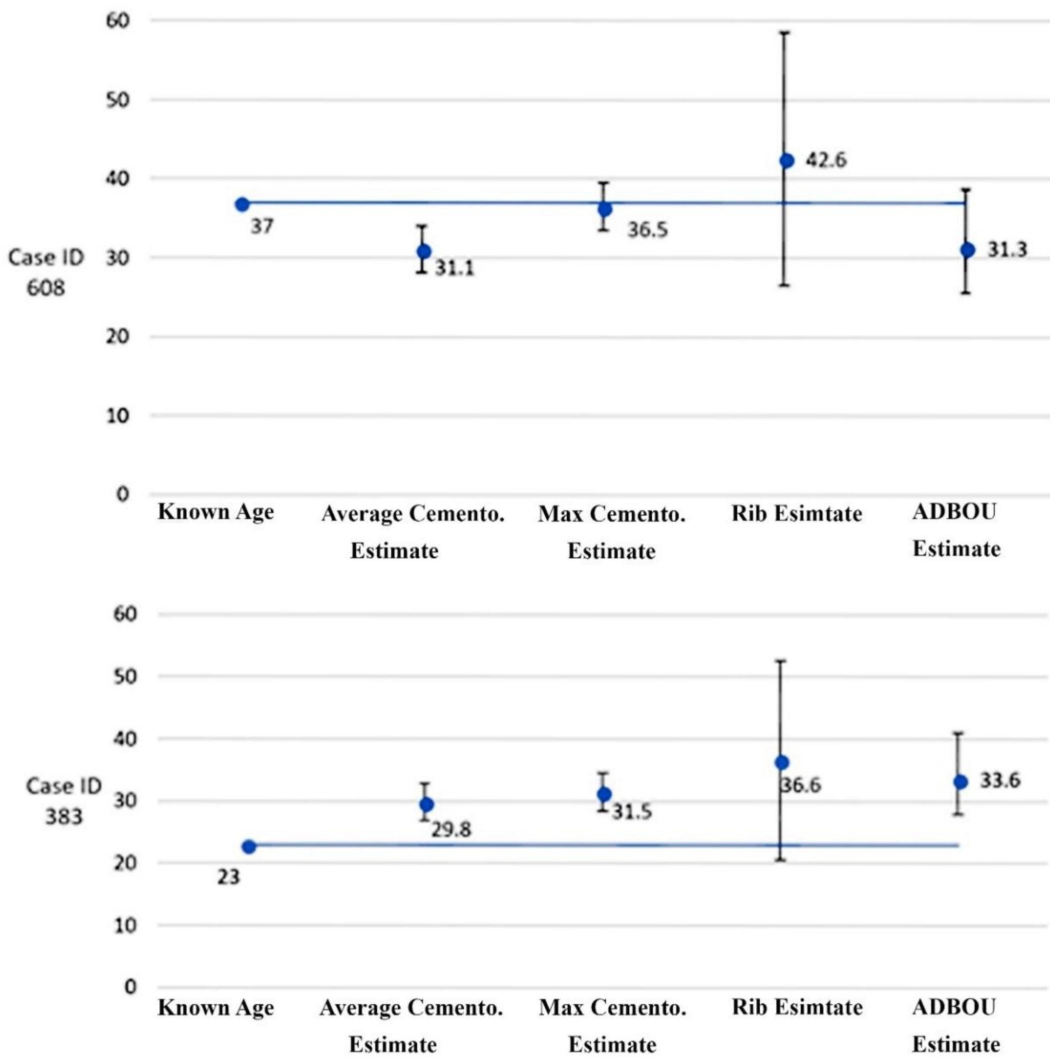

Figure 4.2 Line Graph with Error Ranges Indicating Fitted Age Estimates to Known Age for Positive Identifications

B. SEASON-OF-DEATH

i. <u>TEST OF ASSOCIATION FOR EXPECTED SOD AND OBSERVED SOD</u>

To test the relationship between the expected and observed observations for estimating season-of-death, a Fisher's Exact test was performed in JMP Pro 13. Because the sample size for season-of-death includes only nine individuals, a Fisher's Exact test was performed to account for the small sample size. The Fisher's Exact test was used to test the null hypothesis that the observed outermost bands are not significantly different than the expected outermost bands. Based on a *p*-value of 0.5238, the null hypothesis was not rejected (Figure 4.3), indicating no significant difference between the expected SOD and observed SOD estimates. However, the results of the Fisher's Exact test may be misleading, as only 6 of the 9 observed SOD matched the expected SOD (Table 4.8), which is not much better than to occur by chance alone. This mismatch demonstrates that the Fisher's Exact test has limited ability to detect differences between the expected and observed SOD for each individual OpID case number (i.e. differences in the paired color band comparisons for expected and observed). It's also important to note that due to the small sample size used in this assessment (n=9), the results may not be representative of the potential accuracy of cementochronology to estimate SOD.

Table 4.8 Expected and Observed Season-of-Death Estimates. Asterisk (*) Indicate the Expected SOD Matches the Observed SOD

OpID #	TBS (Low/ High)	ADD (Low/High)	Most Likely Month of Death	Expected Band Color	Expected SOD	Observed Band Color	Observed SOD
384	10/14	103/174	August	Bright	Spring/Summer	Dark	Fall/Winter
390	10/12	103/132	June	Bright	Spring/Summer	Dark	Fall/Winter
391-A	13/16	151/234	June	Bright	Spring/Summer	Bright	Spring/Summer*
395	12/16	132/234	May	Bright	Spring/Summer	Bright	Spring/Summer*
397	1/3	50/55	October	Dark	Fall/Winter	Dark	Fall/Winter*
400	10/13	103/151	June	Bright	Spring/Summer	Bright	Spring/Summer*
423	12/15	132/201	October	Dark	Fall/Winter	Dark	Fall/Winter*
446	14/17	174/274	November	Dark	Fall/Winter	Bright	Spring/Summer
451	1/7	50/75	February	Dark	Fall/Winter	Dark	Fall/Winter*

Contingency Analysis of Expected Band Color by Observed Band Color
Contingency Table
Observed Band Color By Expected Band Color

Count Total % Col %	Bright (0)	Dark (1)	Total
Bright (0)	3 33.33 60.00	1 11.11 25.00	4 44.44
Dark (1)	2 22.22 40.00	3 33.33 75.00	5 55.56
Total	5 55.56	4 44.44	9

Tests

Fisher's Exact Test	Prob	Alternative Hypothesis
Left	0.9603	Prob(Expected Band Color=1) is greater for Observed Band Color=0 than 1
Right	0.3571	Prob(Expected Band Color=1) is greater for Observed Band Color=1 than 0
2-Tail	0.5238	Prob(Expected Band Color=1) is different across Observed Band Color

Fisher's Exact Test

Table Probability (P)	Two-sided Prob ≤ P
0.31746	0.5238

Figure 4.3 Fisher's Exact Test for Observed Band Color and Expected Band Color

ii. SEASON-OF-DEATH ESTIMATES FOR IDENTIFIED INDIVIDUALS

Case 0608 estimated season-of-death is Winter based on the dark outermost annulation. However, the remains were recovered completely skeletonized on July 31, 2012 and the date when case 0608 was last seen alive is unknown. Therefore, there is no accurate known season-of-death. Live observation of case 0608 indicated a dark outermost band for all six observations (three separate observations from two slides). Photo observation of case 0608 also indicated that the outermost band was dark for all six observations. It is possible that once the repatriation occurs, a known date that case 0608 was last seen alive will be established.

The known season-of-death for case 0383 was Summer and the estimated season-of-death from cementochronology was also Summer. The last date known alive for case 0383 was September 8, 2012. Live observation of case 0383 indicated a bright outermost band for all but one of the six observations (three separate observations from two slides). Photo observation of case 0383 indicated that the outermost band was dark for three of the six observations. These results indicated that the live observation was the best method for estimating season-of-death.

V. DISCUSSION

A. AGE-AT-DEATH

Age-at-death was assessed for intraobserver consistency by using a Wilcoxen rank signed test. Additionally, the correlation of all the aging methods were tested using a scatterplot matrix. The scatterplot matrices indicate that there is a weak to moderately strong positive correlation among all four aging methods. Cementochronology max count and average count age estimates are more closely correlated with rib histology estimates than with the ADBOU age estimates. As previously mentioned, prior research conducted by Mavroudas et al. (2015) indicated that for positively identified individuals, the rib histology age estimates were more accurate than the ADBOU age estimates. Because the cementochronology (max and average) method showed a higher correlation with rib histology, this suggests that cementochronology is likely a more accurate method relative to ADBOU for use on the OpID cases. However, it is best to use multiple methods to estimate age when possible.

As seen in Figure 4.1, the relationship between rib and max/average cementochronology has a few extreme outliers. This is likely due to the poor visibility or crowding of the annulations in these teeth. It is also possible that a couple of these individuals had poor visibility of the rib thin section. In several of the OpID cases, there were moderate to high levels of resorption bays in the rib sections and scalloping of the cementum. These traits are indicative of biodeterioration of the rib and tooth samples, either from soil or decomposition diagenetic alteration.

Many of the teeth included in this sample were premolars. As discussed by Wittwer-Backofen et al. (2012), premolars typically have superimposed annulations.

Therefore, it is possible that some of the teeth in this sample had lower age estimates than the actual age due to superimposed annulations. Since the OpID cases are open forensic cases, it would be best to have a broader age range so as not to exclude possible identification matches. For this reason, a broader age range than ±2-3 years should be used in case reports that utilize these cementum age estimates. Recent work completed by Lanteri et al. (2018), indicates that a probabilistic method provides a way to estimate a more representative age range based on the available data by incorporating count errors and dental development variation. Application of a probabilistic method in estimating age ranges based on cementochronology was used for reconstructing paleodemography in archaeological sites. The use of a probabilistic method can be used in future studies to tailor appropriate age ranges to each sample used for cementochronology. Additionally, it would be useful for a known standard deviation for dental eruption to be established for the samples referenced in the London Atlas, so they can then be used in the formulae provided by Lanteri et al. (2018). The cementochronology age estimates with error ranges (±2-3 years) and tooth root formation with error ranges (to be established by the London Atlas web interface) can be used to calculate more representative age ranges for the OpID cases.

In this sample, region of interest photo counts for age-at-death estimation provided more accurate observations. However, it should be noted this accuracy is only based on two positively identified individuals from this sample set. It has been recommended by previous researchers that images be used to estimate age-at-death (Naji et al., 2014; Colard et al., 2015, Wedel, 2009). The preliminary results from this study support this recommendation. Since only two individuals have been positively identified, the age estimations based on photo and live counting methods can only be compared to these two individual's known age. As more data (i.e. positive identifications) on known ages becomes available, further evaluation can help determine which method produces more accurate counts. In the case of the two individuals positively identified from this sample set, case 0383 was more accurate based on averaging the photo counts, while the max cementochronology age estimate was more accurate in case 0608. It is recommended based on the results of this study to expand the error range for cementum age estimates. Using a ±2-3-year age range for cementum age ranges is likely to exclude possible identification matches due to the narrow age range it produces. As more identifications are made from this sample, it will be possible to determine the best age range to use.

B. SEASON-OF-DEATH

Regarding the Fisher's Exact test results, even though there was not a significant difference between the known (expected) SOD and the observed (estimated) SOD based on analysis of the outermost band color, the overall accuracy of these data suggests only a 66% (6 correct out of 9 total) success rate. This is just slightly better than correctly identifying SOD based on chance. This accuracy rate is significantly lower than the 99% success rate suggested by Wedel (2007). However, these results are consistent with those found by Ralston (2016) and Meckel (2016). This suggests that cementochronology holds potential to be used to accurately estimate SOD in some cases; however, it warrants further investigation with a larger known migrant and Latin American sample sizes. It is recommended to examine the outermost band for SOD under live acquisition, so the microscope settings and areas of interest can be adjusted as necessary. Due to the small sample size in this study, it is recommended to make additional comparisons once more OpID positive identifications are made. This will aid in the validation of utilizing cementochronology for estimating SOD in an unidentified migrant population.

C. POSITIVE IDENTIFICATIONS

In both identified cases, the cementochronology point age estimates were closer to the known age. The age estimate from average count was closer to the known age of case 0383 and the age estimate from max count was closer to the known age of case 0608. This complicates the determination of which method is best to use for cementochronology counts. However, it was shown in the Scatterplot matrix in Figure 4.1, that the average and max count estimates were strongly correlated. Once more positive identifications are made, perhaps either max or average counts can be established as superior.

The estimated SOD for case 0383 matched the known SOD based on cementochronology. Case 0608 does not have a known last date seen alive, so it cannot be determined if the estimated season-of-death is accurate for this individual. However, once repatriation occurs, it is possible that a known date of last seen alive will be established. The remains of case 0608 were completely skeletonized which suggests that they were exposed to the environment for an extended period. This indicates that it is possible that this individual died late in the “winter” SOD timeframe (approximately March) or early in the “summer” SOD timeframe (approximately April-June). Even though two positive identifications are not enough to validate cementochronology for age-at-death or SOD, it is suggestive that dental histology is a useful age estimator in the OpID unidentified migrants.

VI. CONCLUSIONS

Learning the protocol for dental histology has a steep learning curve initially. It is recommended to use a training sample with a known age, prior to applying age-at-death and season-of-death estimates to unidentified individuals. It is also recommended that labs intending to perform dental histological analysis should invest in equipment that is suitable for sectioning teeth. In other words, it is best to have a precision saw that allows the user to section the teeth at 70-100 μm so that grinding is not required, and only minimal polishing can remove the striations created by the sectioning saw. Additionally, it is essential for the microscope to have a mounted camera with slide image processing software. Annulations are visible with a 20x objective, but the quality of the annulations are significantly improved with a 40x objective. Once a researcher knows how to process histological samples, the learning curve is minimized. If the researcher uses a known age sample to train, age estimates are more accurate as the observer can compare estimates to known ages.

In many of the cases, teeth have already been sampled for isotopic analysis. As long as the root is preserved for cementochronology, an age estimate can be produced. Based on the results from this study, it can be concluded that cementochronology serves as a valid age-at-death estimation technique for the OpID cases. Interobserver error was not performed in this study, however intraobserver error indicated that the count differences (the age cohorts) show that 90% of the sample had annulation pair counts of two or less throughout all observations (refer to Table 4.3). In other words, the number of annulations counted for each individual did not exceed two annulations. This indicates a significant degree of consistency for age-at-death counts from cementum annulations. In the future, it would be beneficial to have two additional observers perform age-at-death estimates from the same photos used in this study to test the interobserver error.

As has been shown by this and previous research, ADBOU is a less reliable method to estimate actual age of the OpID cases. Cementochronology provided age estimates that were moderately correlated with those produced by rib age estimates, suggesting cementochronology can provide age estimations consistent with rib histology. Therefore, it can be concluded that cementochronology is a suitable aging technique in cases where ribs and ossa coxae are not present or in conjunction with these methods. There are no other aging techniques that are capable of providing a chronological age estimate. Other aging methods are based on epiphyseal union and degeneration of joint surfaces, which occur at different ages for some individuals. Furthermore, teeth preserve much more consistently than do ribs and other elements used for common aging techniques. The application of cementochronology for an unidentified migrant population is a valid method for estimating age-at-death. Using a multifactorial approach to estimating age-at-death in the OpID cases is crucial for aiding in positive identifications, as it allows for potential matches to be narrowed down. Using a combination of ADBOU, rib histology, and cementochronology will provide an accurate age range for the unidentified OpID cases. ADBOU, rib histology, and cementochronology will produce a narrower age estimate than ADBOU or rib histology do on their own; however, the age estimates will be broader that with just cementochronology. The use of cementochronology will be a suitable aging technique in more cases simply due to their better preservation.

Some of the teeth included in this sample may have inaccurate age estimates due to the taphonomic alterations or bifurcation/superimposition of annulations in the tooth sections. If any of the teeth in this sample had superimposed cementum lines, it is likely the individual was underaged. With additional studies on the underlying biochemical factors associated with the deposition and opacity of dental cementum annulations will increase our understanding of how to apply them more accurately to estimation of age-at-death and season-of-death. In previous studies, teeth chosen for cementochronology have been extremely selective causing doubt in the applicability of the method for less ideal tooth samples. It is important to conduct more studies on teeth that have been exposed to diagenetic alterations, periodontal disease, and dental modifications (e.g. fillings).

Future recommendations for the application of cementochronology for the OpID cases include sampling of a single rooted tooth that can be used for DNA, isotopes, and dental histology; training with the slides from the Texas State Skeletal Donation Collection samples and identified OpID cases that have known season-of-death and age-at-death; and finally training of standardized histological procedures including those established by Naji et al. (2014), Colard et al. (2015), with modifications established in this research to accommodate the equipment available at the FACTS facilities for cementochronology. Once these steps are followed, the learning curve associated with cementochronology is minimal. Additionally, the FACTS facilities already have the appropriate histology equipment and the costs associated with cementochronology are reduced to consumables already used for skeletal histology. An itemized budget for the consumable items has been established through this research and can be requested at any time to aid in the planning of future tooth sampling for the purpose of cementochronology. It is recommended to use a composited image of the regions of interests for age-at-death and live acquisition for estimating season-of-death. Additionally, it is best practice to composite a full cross-section of the tooth section for replication of the regions of interests selected as well as to have a permanent documentation of the tooth. These steps will preserve as much information for future studies as well as replicability of the results.

APPENDIX SECTION

Title **Page**

APPENDICES

Appendix A. Sample Demographic Information and Compiled Aged Estimates (ADBOU age ranges are provided in Appendix B).

OpID #	Sex	Code for Sex Certainty	Max DCIA	Average DCIA	Rib Histo	ADBOU	Known Age	Estimated SOD	Known SOD
365	F	1	22.5	20.3	19.7	23.3			
368	M	3	28.5	26.9	33.7	39.7			
379	F	1	41.5	36.4	40.5	26.3			
381	M	1	30.5	28.5	35	34.6			
383	M	3	31.5	29.8	36.6	33.6	23	Summer	Summer
384	F	3	44.5	33.6	40.5	32.4			
389	F	1	28.5	27.3	42.3	30.2			
390	M	1	33.5	30.9	24.5	18.2			
391-A	M	1	29.5	28.8	58.1	36.1			
392	F	3	27.5	25.6	21.8	17.3			
395	M	1	25.5	22.7	21.3	23.3			
397	M	3	28.5	27.6	38.9	27.9			
398	M	1	29.5	26.6	51.7	32			
400	M	1	25.5	24.1	36.2	29.4			
401-C	F	3	26.5	24.4	19.9	15.3			
406	F	3	30.5	27.7	31.4	41.7			
409	M	3	42.5	38.6		29.9			
415	M	3	34.5	32.6		29.8			
416	F	1	35.5	31.3	34.2	35.5			
417	F	1	22.5	21.7	37.2	37.2			
421	M	3	25.5	25	19.4	23.6			
422	M	1	26.5	23.2	31.9	31.4			
423	M	1	36.5	30.7	50.6	38.8			
426	M	3	30.5	29.4	33.6	36.4			
427	M	1	25.5	24	20.4	15.5			
430	M	1	26.5	24.1	23.5	18.5			
440	M	1	32.5	30.4		31.1			
446	F	1	29.5	28.6	29.9	32			
448	F	3	22.5	21.6	23.9	21.1			
451	M	1	23.5	22.4	23.4	15			
464	F	3	34.5	31.9		25.6			
467	M	3	28.5	26.4	32.7	36.7			
469	M	3	31.5	30.9		28.5			
470	M	3	29.5	25.8		36.1			
471	M	1	29.5	24.9		29.8			
477	F	3	25.5	24.7		28.5			
482	M	1	25.5	24.2		18.7			
485	F	3	29.5	27.6		15			
490	F	3	28.5	27		15			
491	M	3	25.5	24.1		33.6			
503	M	1	40.5	35.9		33.7			
504	M	1	33.5	32.1		22.6			
505	M	1	32.5	31.1		51.5			
506	F	1	46.5	44.3	45.6	42.5			
508	F	1	23.5	22	22.6	16.8			
514	F	3	24.5	23.2		41.6			
608	M	3	36.5	31.1	42.6	31.3	37	Winter	Unknown
611	M	3	29.5	28		21.1			

Codes for Sex Certainty: 1 – Confirmed through DNA; 2 – Confirmed through soft tissue indicators (e.g. genetalia); 3 – Estimated skeletally

Appendix B. ADBOU Summary

OpID Case	Sex Prior	Ancestry Prior	Contextual Prior	Point Age Estimate	Age Range
365	F	Unknown	Forensic	22.3	22.3-33
368	M	Unknown	Forensic	39.7	31.4-52.3
379	F	Unknown	Forensic	26.3	15.9-39.4
381	M	Unknown	Forensic	34.6	28.2-43.9
383	M	Unknown	Forensic	33.6	27.9-41
384	F	Unknown	Forensic	32.4	25.6-42.4
389	F	Unknown	Forensic	30.2	23.5-39.8
390	M	Unknown	Forensic	18.2	18.2-24.7
391-A	M	Unknown	Forensic	36.1	28.2-48.4
392	F	Unknown	Forensic	17.3	17.3-23.4
395	M	Unknown	Forensic	23.3	19.5-27.7
397	M	Unknown	Forensic	27.9	34.1-42.9
398	M	Unknown	Forensic	32.0	26.2-39.6
400	M	Unknown	Forensic	29.4	24.3-35.9
401-C	F	Unknown	Forensic	15.3	15.3-22.8
406	F	Unknown	Forensic	41.7	30.3-57.5
409	M	Unknown	Forensic	29.9	24.6-36.6
415	M	Unknown	Forensic	29.8	25.1-36.3
416	F	Unknown	Forensic	35.5	27.6-47
417	F	Unknown	Forensic	37.2	28.1-50.2
421	M	Unknown	Forensic	23.6	19-29.4
422	M	Unknown	Forensic	31.4	25.6-39.6
423	M	Unknown	Forensic	38.8	29.9-53
426	M	Unknown	Forensic	36.4	29.4-46.8
427	M	Unknown	Forensic	15.5	15.5-21
430	M	Unknown	Forensic	18.5	18.5-22.9
440	M	Unknown	Forensic	31.1	25-40
446	F	Unknown	Forensic	32.0	25-42.6
448	F	Unknown	Forensic	21.1	17.3-25.6
451	M	Unknown	Forensic	15.0	15.0-19.9
464	F	Unknown	Forensic	25.6	20.7-32
467	M	Unknown	Forensic	36.7	27.2-51.9
469	M	Unknown	Forensic	28.5	24.1-34
470	M	Unknown	Forensic	36.1	28.5-47.2
471	M	Unknown	Forensic	29.8	25.2-35.8
477	F	Unknown	Forensic	28.5	22.7-36.4
482	M	Unknown	Forensic	18.7	18.7-22.7
485	F	Unknown	Forensic	15.0	15.0-24.2
490	F	Unknown	Forensic	15.0	15.0-21
491	M	Unknown	Forensic	33.6	27-43.4
503	M	Unknown	Forensic	33.7	26.9-43.1
504	M	Unknown	Forensic	22.6	18.1-28.1
505	M	Unknown	Forensic	51.5	34.8-73.5
506	F	Unknown	Forensic	42.5	29.9-59.9
508	F	Unknown	Forensic	16.8	16.8-20.9
514	F	Unknown	Forensic	41.6	30.7-57.3
608	M	Unknown	Forensic	31.3	26.2-38.2
611	M	Unknown	Forensic	21.1	16.7-26.2

Appendix C. Rib Histology Age-at-death Estimates

OpID #	Sex	Point Esimate	Age Range
365	F	19.7	8-32
368	M	33.7	17.7-49
379	F	40.5	24-57
381	M	35	18.98-51.02
383	M	36.6	20.6-52.62
384	F	40.5	24.5-56.5
389	F	42.3	26.3-58.32
390	M	24.5	12-37
391-A	M	58.1	42.1-74.1
392	F	21.8	10-34
395	M	21.3	9-34
397	M	38.9	22.9-54.9
398	M	51.7	35.6-67.7
400	M	36.2	20-52
401-C	F	19.9	8-32
406	F	31.4	15.4-47.4
409	M		
415	M		
416	F	34.2	18-50
417	F	37.2	21-53
421	M	19.4	No age range provided
422	M	31.9	15.9-47.9
423	M	50.6	35-67
426	M	33.6	17.6-49.6
427	M	20.4	8-33
430	M	23.5	11-36
440	M		
446	F	29.9	13.88-45.9
448	F	23.9	12-36
451	M	23.4	11-36
464	F		
467	M	32.7	16.7-48.7
469	M		
470	M		
471	M		
477	F		
482	M		
485	F		
490	F		
491	M		
503	M		
504	M		
505	M		
506	F	45.6	29-72
508	F	22.6	10-25
514	F		
608	M	42.6	26.6-58.6
611	M		

Appendix D. Cementochronology Max Count Observations for Age-at-death with Age of Root Completion and Age Estimate

OpID Case	Max Count	Age of Root Completion	DCIA Age Estimate
365	10	12.5	22.5
368	15	13.5	28.5
379	29	12.5	41.5
381	17	13.5	30.5
383	18	13.5	31.5
384	30	14.5	44.5
389	15	13.5	28.5
390	20	13.5	33.5
391-A	16	13.5	29.5
392	15	12.5	27.5
395	13	12.5	25.5
397	16	12.5	28.5
398	16	13.5	29.5
400	12	13.5	25.5
401-C	13	13.5	26.5
406	17	13.5	30.5
409	29	13.5	42.5
415	21	13.5	34.5
416	22	13.5	35.5
417	10	12.5	22.5
421	12	13.5	25.5
422	13	13.5	26.5
423	23	13.5	36.5
426	18	12.5	30.5
427	12	13.5	25.5
430	14	12.5	26.5
440	20	12.5	32.5
446	16	13.5	29.5
448	10	12.5	22.5
451	10	13.5	23.5
464	21	13.5	34.5
467	16	12.5	28.5
469	19	12.5	31.5
470	16	13.5	29.5
471	16	13.5	29.5
477	13	12.5	25.5
482	12	13.5	25.5
485	17	12.5	29.5
490	16	12.5	28.5
491	13	12.5	25.5
503	28	12.5	40.5
504	20	13.5	33.5
505	19	13.5	32.5
506	33	13.5	46.5
508	11	12.5	23.5
514	12	12.5	24.5
608	24	12.5	36.5
611	17	12.5	29.5

Appendix E. Cementochronology Average Count Observations for Age-at-death with Age of Root Completion and Age Estimate

OpID Case	Average Count	Age of Root Completion	Age Estimate
365	7.8	12.5	20.3
368	13.4	13.5	26.9
379	23.9	12.5	36.4
381	15	13.5	28.5
383	16.3	13.5	29.8
384	19.1	14.5	33.6
389	13.8	13.5	27.3
390	17.4	13.5	30.9
391-A	15.3	13.5	28.8
392	13.1	12.5	25.6
395	10.2	12.5	22.7
397	15.1	12.5	27.6
398	13.1	13.5	26.6
400	10.6	13.5	24.1
401-C	10.9	13.5	24.4
406	14.2	13.5	27.7
409	25.1	13.5	38.6
415	19.1	13.5	32.6
416	17.8	13.5	31.3
417	9.2	12.5	21.7
421	11.5	13.5	25
422	9.7	13.5	23.2
423	17.2	13.5	30.7
426	16.9	12.5	29.4
427	10.5	13.5	24
430	11.6	12.5	24.1
440	17.9	12.5	30.4
446	15.1	13.5	28.6
448	9.1	12.5	21.6
451	8.9	13.5	22.4
464	18.4	13.5	31.9
467	13.9	12.5	26.4
469	18.4	12.5	30.9
470	12.3	13.5	25.8
471	11.4	13.5	24.9
477	12.2	12.5	24.7
482	10.7	13.5	24.2
485	15.1	12.5	27.6
490	14.5	12.5	27
491	11.6	12.5	24.1
503	23.4	12.5	35.9
504	18.6	13.5	32.1
505	17.6	13.5	31.1
506	30.8	13.5	44.3
508	9.5	12.5	22
514	10.7	12.5	23.2
608	18.6	12.5	31.1
611	15.5	12.5	28

Appendix F. Paired Observations Used for Photo vs Live Counts

Indiv.	Slide	Observation #	Photo Count	Live Count	Actual Difference	Absolute Difference	Indiv. Avg. Difference
365	22.1	1	9	8	1	1	0.8333
365	22.2	1	7	9	-2	2	
365	22.1	2	9	8	1	1	
365	22.2	2	7	8	-1	1	
365	22.1	3	8	8	0	0	
365	22.2	3	8	8	0	0	
368	20.1	1	15	14	1	1	1
368	20.2	1	13	14	-1	1	
368	20.1	2	15	14	1	1	
368	20.2	2	13	14	-1	1	
368	20.1	3	15	14	1	1	
368	20.2	3	13	14	-1	1	
379	18.1	1	19	23	-4	4	3.1667
379	18.2	1	19	16	3	3	
379	18.1	2	19	24	-5	5	
379	18.2	2	19	17	2	2	
379	18.1	3	19	22	-3	3	
379	18.2	3	19	17	2	2	
381	41.1	1	16	18	-2	2	1
381	41.2	1	17	17	0	0	
381	41.1	2	17	15	2	2	
381	41.2	2	17	17	0	0	
381	41.1	3	17	16	1	1	
381	41.2	3	16	17	-1	1	
383	10.1	1	13	14	-1	1	1
383	10.2	1	17	17	0	0	
383	10.1	2	13	15	-2	2	
383	10.2	2	17	16	1	1	
383	10.1	3	13	15	-2	2	
383	10.2	3	17	17	0	0	
384	43.1	1	17	20	-3	3	1.3333
384	43.2	1	17	18	-1	1	
384	43.1	2	18	20	-2	2	
384	43.2	2	17	18	-1	1	
384	43.1	3	19	20	-1	1	
384	43.2	3	18	18	0	0	
389	7.1	1	15	14	1	1	0.5
389	7.2	1	13	13	0	0	
389	7.1	2	13	14	-1	1	
389	7.2	2	13	13	0	0	
389	7.1	3	13	14	-1	1	
389	7.2	3	13	13	0	0	
390	30.1	1	14	16	-2	2	1.6667
390	30.2	1	20	18	2	2	
390	30.1	2	14	16	-2	2	
390	30.2	2	20	18	2	2	
390	30.1	3	14	16	-2	2	
390	30.2	3	18	18	0	0	
391-A	13.1	1	16	14	2	2	1.5
391-A	13.2	1	14	16	-2	2	
391-A	13.1	2	16	15	1	1	
391-A	13.2	2	14	16	-2	2	
391-A	13.1	3	15	15	0	0	
391-A	13.2	3	14	16	-2	2	

Appendix F (Continued). Paired Observations Used for Photo vs Live Counts

Indiv.	Slide	Observation #	Photo Count	Live Count	Actual Difference	Absolute Difference	Indiv. Avg. Difference
392	4.1	1	13	14	-1	1	1.6667
392	4.2	1	12	14	-2	2	
392	4.1	2	15	13	2	2	
392	4.2	2	12	14	-2	2	
392	4.1	3	15	13	2	2	
392	4.2	3	13	14	-1	1	
395	23.1	1	12	11	1	1	1
395	23.2	1	9	9	0	0	
395	23.1	2	12	12	0	0	
395	23.2	2	11	9	2	2	
395	23.1	3	12	11	1	1	
395	23.2	3	11	9	2	2	
397	45.1	1	16	16	0	0	0.3333
397	45.2	1	15	16	-1	1	
397	45.1	2	16	16	0	0	
397	45.2	2	15	16	-1	1	
397	45.1	3	16	16	0	0	
397	45.2	3	15	15	0	0	
398	29.1	1	12	13	-1	1	0.5
398	29.2	1	13	13	0	0	
398	29.1	2	12	13	-1	1	
398	29.2	2	13	12	1	1	
398	29.1	3	13	13	0	0	
398	29.2	3	13	13	0	0	
400	12.1	1	9	10	-1	1	1.5
400	12.2	1	12	10	2	2	
400	12.1	2	9	10	-1	1	
400	12.2	2	12	10	2	2	
400	12.1	3	9	10	-1	1	
400	12.2	3	12	10	2	2	
401-C	15.1	1	11	10	1	1	1
401-C	15.2	1	11	12	-1	1	
401-C	15.1	2	11	10	1	1	
401-C	15.2	2	11	12	-1	1	
401-C	15.1	3	11	10	1	1	
401-C	15.2	3	11	12	-1	1	
406	31.1	1	16	14	2	2	1.5
406	31.2	1	14	15	-1	1	
406	31.1	2	16	14	2	2	
406	31.2	2	16	14	2	2	
406	31.1	3	14	14	0	0	
406	31.2	3	13	15	-2	2	
409	40.1	1	25	25	0	0	0.6667
409	40.2	1	23	25	-2	2	
409	40.1	2	24	25	-1	1	
409	40.2	2	25	25	0	0	
409	40.1	3	24	25	-1	1	
409	40.2	3	25	25	0	0	

Appendix F (Continued). Paired Observations Used for Photo vs Live Counts

Indiv.	Slide	Observation #	Photo Count	Live Count	Actual Difference	Absolute Difference	Indiv. Avg. Difference
415	24.1	1	21	19	2	2	0.6667
415	24.2	1	18	18	0	0	
415	24.1	2	21	20	1	1	
415	24.2	2	18	18	0	0	
415	24.1	3	21	20	1	1	
415	24.2	3	18	18	0	0	
416	34.1	1	18	19	-1	1	2
416	34.2	1	15	18	-3	3	
416	34.1	2	18	19	-1	1	
416	34.2	2	15	18	-3	3	
416	34.1	3	18	19	-1	1	
416	34.2	3	15	18	-3	3	
417	19.1	1	10	9	1	1	1
417	19.2	1	9	10	-1	1	
417	19.1	2	10	9	1	1	
417	19.2	2	10	9	1	1	
417	19.1	3	10	9	1	1	
417	19.2	3	9	10	-1	1	
421	3.1	1	10	12	-2	2	0.6667
421	3.2	1	12	12	0	0	
421	3.1	2	10	12	-2	2	
421	3.2	2	12	12	0	0	
421	3.1	3	12	12	0	0	
421	3.2	3	12	12	0	0	
422	17.1	1	8	11	-3	3	1.1667
422	17.2	1	10	10	0	0	
422	17.1	2	8	10	-2	2	
422	17.2	2	10	10	0	0	
422	17.1	3	8	10	-2	2	
422	17.2	3	10	10	0	0	
423	37.1	1	11	14	-3	3	2.5
423	37.2	1	16	14	2	2	
423	37.1	2	11	14	-3	3	
423	37.2	2	16	18	-2	2	
423	37.1	3	11	15	-4	4	
423	37.2	3	17	18	-1	1	
426	32.1	1	18	19	-1	1	0.5
426	32.2	1	17	16	1	1	
426	32.1	2	18	18	0	0	
426	32.2	2	17	16	1	1	
426	32.1	3	18	18	0	0	
426	32.2	3	16	16	0	0	
427	16.1	1	10	9	1	1	0.6667
427	16.2	1	9	8	1	1	
427	16.1	2	10	10	0	0	
427	16.2	2	9	8	1	1	
427	16.1	3	9	10	-1	1	
427	16.2	3	9	9	0	0	

Appendix F (Continued). Paired Observations Used for Photo vs Live Counts

Indiv.	Slide	Observation #	Photo Count	Live Count	Actual Difference	Absolute Difference	Indiv. Avg. Difference
430	5.1	1	13	13	0	0	0.5
430	5.2	1	11	11	0	0	
430	5.1	2	13	12	1	1	
430	5.2	2	11	11	0	0	
430	5.1	3	12	13	-1	1	
430	5.2	3	10	11	-1	1	
440	42.1	1	20	24	-4	4	5
440	42.2	1	19	24	-5	5	
440	42.1	2	20	24	-4	4	
440	42.2	2	19	25	-6	6	
440	42.1	3	18	24	-6	6	
440	42.2	3	19	24	-5	5	
446	35.1	1	14	15	-1	1	0.5
446	35.2	1	15	15	0	0	
446	35.1	2	14	15	-1	1	
446	35.2	2	15	15	0	0	
446	35.1	3	14	15	-1	1	
446	35.2	3	15	15	0	0	
448	36.1	1	9	8	1	1	0.8333
448	36.2	1	10	9	1	1	
448	36.1	2	9	9	0	0	
448	36.2	2	10	9	1	1	
448	36.1	3	8	9	-1	1	
448	36.2	3	10	9	1	1	
451	11.1	1	8	9	-1	1	1.1667
451	11.2	1	9	11	-2	2	
451	11.1	2	8	9	-1	1	
451	11.2	2	9	11	-2	2	
451	11.1	3	9	9	0	0	
451	11.2	3	9	10	-1	1	
464	33.1	1	20	21	-1	1	0.5
464	33.2	1	20	20	0	0	
464	33.1	2	21	21	0	0	
464	33.2	2	20	20	0	0	
464	33.1	3	19	21	-2	2	
464	33.2	3	20	20	0	0	
467	14.1	1	14	13	1	1	0.8333
467	14.2	1	14	14	0	0	
467	14.1	2	14	13	1	1	
467	14.2	2	14	13	1	1	
467	14.1	3	14	13	1	1	
467	14.2	3	14	13	1	1	
469	48.1	1	18	19	-1	1	0.3333
469	48.2	1	18	18	0	0	
469	48.1	2	18	18	0	0	
469	48.2	2	18	18	0	0	
469	48.1	3	18	19	-1	1	
469	48.2	3	18	18	0	0	

Appendix F (Continued). Paired Observations Used for Photo vs Live Counts

Indiv.	Slide	Observation #	Photo Count	Live Count	Actual Difference	Absolute Difference	Indiv. Avg. Difference
470	46.1	1	11	12	-1	1	1
470	46.2	1	13	13	0	0	
470	46.1	2	12	12	0	0	
470	46.2	2	13	14	-1	1	
470	46.1	3	12	13	-1	1	
470	46.2	3	11	14	-3	3	
471	38.1	1	13	15	-2	2	2
471	38.2	1	13	15	-2	2	
471	38.1	2	12	15	-3	3	
471	38.2	2	12	14	-2	2	
471	38.1	3	12	15	-3	3	
471	38.2	3	12	12	0	0	
477	21.1	1	12	13	-1	1	1.8333
477	21.2	1	10	13	-3	3	
477	21.1	2	12	13	-1	1	
477	21.2	2	11	13	-2	2	
477	21.1	3	12	13	-1	1	
477	21.2	3	10	13	-3	3	
482	26.1	1	8	10	-2	2	1.5
482	26.2	1	10	11	-1	1	
482	26.1	2	9	11	-2	2	
482	26.2	2	10	11	-1	1	
482	26.1	3	9	11	-2	2	
482	26.2	3	10	11	-1	1	
485	6.1	1	16	15	1	1	0.6667
485	6.2	1	15	14	1	1	
485	6.1	2	15	15	0	0	
485	6.2	2	15	14	1	1	
485	6.1	3	15	15	0	0	
485	6.2	3	15	14	1	1	
490	27.1	1	15	14	1	1	1.8333
490	27.2	1	14	12	2	2	
490	27.1	2	15	14	1	1	
490	27.2	2	15	12	3	3	
490	27.1	3	15	14	1	1	
490	27.2	3	15	12	3	3	
491	28.1	1	12	11	1	1	0.5
491	28.2	1	12	12	0	0	
491	28.1	2	12	11	1	1	
491	28.2	2	12	12	0	0	
491	28.1	3	12	11	1	1	
491	28.2	3	12	12	0	0	
503	2.1	1	20	22	-2	2	3.5
503	2.2	1	18	23	-5	5	
503	2.1	2	20	23	-3	3	
503	2.2	2	18	23	-5	5	
503	2.1	3	20	22	-2	2	
503	2.2	3	19	23	-4	4	

Appendix F (Continued). Paired Observations Used for Photo vs Live Counts

Indiv.	Slide	Observation #	Photo Count	Live Count	Actual Difference	Absolute Difference	Indiv. Avg. Count Difference
504	39.1	1	17	18	-1	1	1
504	39.2	1	19	18	1	1	
504	39.1	2	17	18	-1	1	
504	39.2	2	19	18	1	1	
504	39.1	3	17	18	-1	1	
504	39.2	3	19	18	1	1	
505	8.1	1	18	18	0	0	1.1667
505	8.2	1	17	19	-2	2	
505	8.1	2	18	19	-1	1	
505	8.2	2	17	19	-2	2	
505	8.1	3	18	18	0	0	
505	8.2	3	17	19	-2	2	
506	44.1	1	32	31	1	1	1.1667
506	44.2	1	31	30	1	1	
506	44.1	2	32	30	2	2	
506	44.2	2	31	30	1	1	
506	44.1	3	31	32	-1	1	
506	44.2	3	30	29	1	1	
508	25.1	1	11	9	2	2	0.8333
508	25.2	1	10	10	0	0	
508	25.1	2	11	10	1	1	
508	25.2	2	10	10	0	0	
508	25.1	3	11	9	2	2	
508	25.2	3	10	10	0	0	
514	1.1	1	10	11	-1	1	0.6667
514	1.2	1	9	11	-2	2	
514	1.1	2	11	11	0	0	
514	1.2	2	11	11	0	0	
514	1.1	3	12	11	1	1	
514	1.2	3	11	11	0	0	
608	47.1	1	17	17	0	0	0.5
608	47.2	1	24	23	1	1	
608	47.1	2	18	17	1	1	
608	47.2	2	23	23	0	0	
608	47.1	3	18	17	1	1	
608	47.2	3	23	23	0	0	
611	9.1	1	16	16	0	0	0.6667
611	9.2	1	16	17	-1	1	
611	9.1	2	16	17	-1	1	
611	9.2	2	16	17	-1	1	
611	9.1	3	16	16	0	0	
611	9.2	3	16	17	-1	1	

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