THE UTILITY OF DENTAL CEMENTUM INCREMENT ANALYSIS FOR ESTIMATING SEASON-OF-DEATH IN NATURALLY DECOMPOSED SKELETONS

by

Lauren A. Meckel, B.S.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Arts
with a Major Anthropology
August 2016

Committee Members:

Daniel J. Wescott, Chair

M. Katherine Spradley

Sophia Mavroudas

COPYRIGHT

by

Lauren A. Meckel

2016

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Fair Use

This work is protected by the Copyright Laws of the United States (Public Law 94-553, section 107). Consistent with fair use as defined in the Copyright Laws, brief quotations from this material are allowed with proper acknowledgment. Use of this material for financial gain without the author's express written permission is not allowed.

Duplication Permission

As the copyright holder of this work I, Lauren A. Meckel, refuse permission to copy in excess of the "Fair Use" exemption without my written permission.

DEDICATION

This thesis is dedicated to the gracious donors of the Texas State University Donated Skeletal Collection and their families.

ACKNOWLEDGEMENTS

I am deeply moved by the support I have received from my colleagues, family, and friends while developing and completing this thesis.

Danny Wescott, thank you for believing in me and giving me a space to explore my creativity in science. Your consistent encouragement throughout this process has strengthened my confidence as a researcher and I will value your mentorship and our friendship for the rest of my life.

Sophia Mavroudas, my favorite bone biology nerd. Histology is one of my greatest passions because of you. I cannot thank you enough for all you have done for me. You are a brilliant wizard.

Christian Wallace, I owe you many nights of sanity. Thank you for choosing to dance with me- you are the perfect partner.

Mom, Dad, Melanie, and Amanda, thank you for supporting my path and occasionally filling in some pot holes. I love you all so much.

To my cohort, I feel so fortunate to have navigated this experience with you. You are all so talented, intelligent, and hilarious. I am honored to be your friend and colleague.

I feel extreme gratitude toward Dr. Kate Spradley and Dr. Michelle Hamilton for their instrumental role in the success of my graduate career, for inspiring me to pursue forensic anthropology as an eager undergraduate, and for minimizing the pain of graduate school with tasteful (sometimes) humor.

This study was possible thanks to the generous enthusiasm of Dr. Grady Early, The Grady Early Fellowship, and the Graduate College at Texas State University.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATIONS

PMI	**Postmortem Interval**
DCIA	**Dental Cementum Increment Analysis**
TXSTDSC	**Texas State University Donated Skeletal Collection**

ABSTRACT

Determining the season-of-death (i.e. spring/summer or fall/winter) from human remains has significant implications for forensic anthropological and bioarchaeological investigations. In forensic anthropology, determining the season-of-death may greatly increase the accuracy of estimating the postmortem interval (PMI) in human remains. In bioarchaeology, knowing the season-of-death may contribute to the understanding of mortuary patterns, mortality periods, and identify changes in human behavior over time.

Dental cementum increment analysis (DCIA), also known as cementochronology, is the microscopic examination of the alternating mineralized layers in dental cementum, the part of the tooth root that anchors the tooth to the bone. These layers are laid down incrementally twice a year and resemble the cross-section of a tree. Theoretically, under the microscope one bright ring represents the growth season (spring/summer) and one opaque ring represents the dormant season (fall/winter). Based on this theory, Wedel (2007) used DCIA to estimate the season-of-death with 99% accuracy in a sample of teeth extracted from living individuals. If Wedel (2007) is validated and the season-of-death can accurately be estimated in a sample of known individuals who decomposed in a natural environment, then this method will greatly improve the estimation of PMI in forensic contexts.

The following study used DCIA to validate Wedel (2007) to determine if band translucency can effectively estimate season-of-death in human remains using a known

date-of-death skeletal collection of individuals that have undergone natural decomposition processes similar to those examined by forensic anthropologists and bioarchaeologists. Additionally, causal factors for the appearance of cementum annulations in human teeth were investigated.

The results of two separate observations of 24 individuals show that there is not a strong relationship between the translucency of the band and the season-of-death as would be expected using methods outlined by Wedel (2007). In the first observation, no difference was found between the band translucency of individuals who died in the spring/summer and fall/winter, and bands were identified correctly in only 60% of the sample. In the second observation, individuals who died in the spring/summer were more likely to exhibit an opaque band, while those who died in the fall/winter were more likely to exhibit a bright band. Correctly correlating the bands to the actual season-of-death in the second observation only occurred in 18% of the sample. Intraobserver error tests determined that estimating the season-of-death based on the translucency of the outer cementum increment yielded results only slightly greater than those achieved by chance. That is, if one were to simply guess the outer band, the results would be about the same.

To investigate the impact of diet on cementum deposition and to ensure the method of preparation was valid, DCIA was tested on a sample of 9 wild deer and 2 domestic dogs. These results indicate there is a correlation between season and cementum deposition, but the extent of this relationship is poorly understood. However, it is unlikely diet plays as significant a role as implied by Lieberman (1994), since domestic mammals lack seasonal variation in diet. Stress factors related to cause-of-death were ruled out as possible confounding variables in this study, but periodontal disease and cementum diagenesis remain questionable limitations. It is likely that the age of the individual is the biggest influence on the clarity of the outer band, and; therefore, the ability of the investigator to estimate the season-of-death.

At this time DCIA is not recommended for use in forensic practice on individuals over fifty years of age; however, with a greater understanding of the biological basis for cementum deposition and the effects of degeneration on the outer band, the method described by Wedel (2007) shows promise for its utility in young humans and wild or domestic non-human mammals.

I. INTRODUCTION

Determining the season-of-death (i.e. spring/summer or fall/winter) from human remains has significant implications for forensic anthropological and bioarchaeological investigations. In forensic anthropology, determining the season-of-death may greatly increase the accuracy of estimating PMI in human remains, especially since there appears to be seasonal variation in accumulated degree days, insect biodiversity and activity, microbial biodiversity, and the amount of carbon and nitrogen released into the soil (Carter et al., 2007; Tomberlin et al., 2011; Bates, 2014; Aitkenhead-Peterson et al., 2015; Cobaugh et al., 2015; Bates and Wescott, 2016), all of which affect the rate of decomposition. In bioarchaeology, knowing the season-of-death may contribute to the understanding of mortuary patterns, mortality periods, and may identify changes in human behavior over time (Ubelaker and Willey, 1978; Wall-Scheffler, 2007; Broucker et al., 2015).

Dental cementum increment analysis (DCIA), also known as cementochronology, is the microscopic examination of alternating opaque and bright cementum increments or bands in teeth (described below. In the past this method has been shown to accurately reflect season of extraction in both marine and land mammals (Beasley et al., 1992; Lieberman and Meadow, 1992; Lubinski, 2001). More recently DCIA has been proposed as a valid method for estimating season-of-death in human remains. Wedel (2007) found DCIA to estimate season-of-extraction with a 99% accuracy in a sample of 92 extracted teeth from living individuals ranging from 15 to 90 years of age. However, in a more recent study, Ralston (2016) observed a low correlation between band translucency and season-of-death when investigating the utility of DCIA in a sample of 143 teeth with known dates of extraction from living individuals and medical cadavers. Ralston (2016) argues that there is low reliability in DCIA due to subjectivity in identifying the outer band as well as lack of standardized methods.

The main purpose of this study is to test the validity of Wedel's (2007) study of estimating season-of-death using DCIA in single-rooted teeth from individuals with known dates of death that have undergone natural decomposition processes in an outdoor environment. Since Wedel (2007) used teeth extracted from living humans and DCIA has never been validated on a sample of individuals with known dates-of-death who decomposed in an outdoor environment, it is unclear whether factors related to the decomposition process may alter the results of such analysis. If successful, the application of this method to a known skeletal collection that has undergone natural decomposition and taphonomic processes will bolster confidence in forensic and bioarchaeological practice.

In addition to testing the validity of DCIA for estimating season-of-death, the intention of this study is to expand the understanding of using DCIA by testing if the thickness of the outer band can reflect early or late periods in the season-of-death, which was hypothesized by Wedel (2007), and whether abiotic causal factors for seasonal effects of cementum translucency in humans can be determined (e.g., hours of sunlight and average daily temperature). This study will also explore possible causal factors for alternating cementum bands in humans. Previous research on causal factors in non-human mammals suggests that seasonal bands are the result of changes in cementum orientation and composition due to seasonal variation in diet (Lieberman, 1994).

However, this hypothesis does not adequately explain why cementum bands occur in modern humans lacking seasonal diets.

Since human teeth are commonly recovered and tend to remain well preserved over time, understanding the utility of DCIA can contribute to the analysis of forensic and bioarchaeological remains in many circumstances (Wittwer-Backofen et al., 2004; Meinl et al., 2008; Guatelli-Steinberg and Huffman, 2011; Gocha and Schutkowski, 2012). The broader impact of this study is that it will provide possible causal factors for seasonal effects in dental cementum among humans, which is poorly understood and widely debated, but has both forensic anthropological and bioarchaeological applications (Bosshardt and Schroeder, 1996; Grosskopf and McGlynn, 2011; Broucker et al., 2015). Furthermore, if a significant relationship is discovered between cementum band translucency and season-of-death, this research will contribute to the development of methods for accurately estimating PMI in medicolegal death investigations, especially since standardized methods for preparing and analyzing dental cementum do not exist at this time.

Hypothesis Flowchart

This project is a validation of Wedel (2007) but altered to analyze skeletons that have undergone natural decomposition. The study will test DCIA to determine if outer band translucency (i.e., bright or opaque) can effectively estimate season-of-death in human remains using a known date-of-death skeletal collection with natural taphonomic modifications. If differences are found between the translucency of spring/summer bands and fall/winter bands, the study will be expanded to explore: 1) whether band thickness (i.e., percent band completion) corresponds to early or late periods in the season, 2) possible causal factors (e.g., number of sun hours and temperature) for band color in humans, and 3) if there are possible regional effects in seasonal shifts. If differences are not found between the translucencies of the bands based on season-of-death, then this study will explore the possible reasons for the discrepancy.

This study was set up as a hypothesis flowchart (Figure 1.1). That is, the hypotheses tested will depend on the outcome of the Null 1 test. For example, if Null 1 is rejected then Null 2 and Null 3 will be tested. On the other hand, if Null 1 is not rejected then Null 4, Null 5, and Null 6 will be examined. The null hypotheses are as follows:

1) There is no statistically significant difference in outer band translucency of dental cementum in single-rooted human teeth between individuals that died during the spring/summer (April through September) and those that died during the fall/winter (October through March),

2) Percent of outer band width will not correspond to early (thin) or late (thick) period in the season,

3) Dental cementum translucency does not significantly correlate with seasonal environmental factors such as sunlight hours and temperature,

4) There is no difference in outer band translucency of dental cementum between the teeth of non-human mammals that died during the spring/summer and those that died during the fall/winter,

5) Nutrition or stress related factors, such as long-term illness, will not affect the determination of the cementum outer band translucency.

6) Age of the individuals used in the sample effect the ability to accurately identify the outer band.

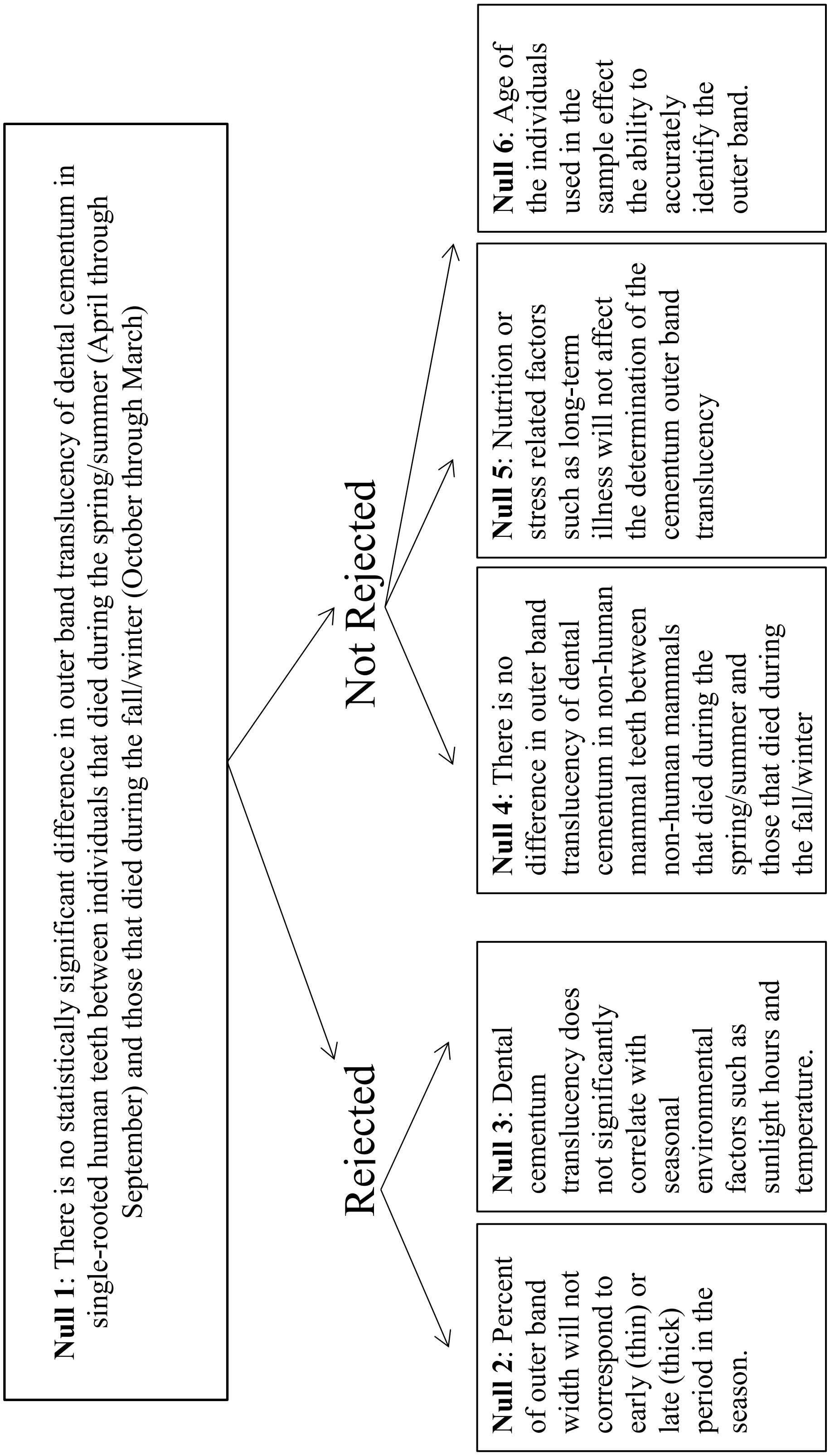

Figure 1.1. Hypothesis Flowchart

Cementum Composition and Optical Orientation

Under transmitted polarized light microscopy, dental cementum is identified by appositionally alternating bright and opaque bands, similar to rings in the cross-section of a tree (Wedel, 2007). These circumferential bands begin at the dentin cementum junction and extend to the outermost edge of the tooth. For years archaeologists and biological anthropologists have been analyzing band translucency data from non-human mammals, specifically ungulates, to estimate age-at-death and season-of-death (Mitchell 1963; Mitchell, 1967; Lieberman and Meadow, 1992; Lubinski and O'Brien, 2001; Stutz, 2002; Lieberman, 2004; Hillson, 2005).In humans cementochronology has the potential to estimate age-at-death (Stott et al., 1982; Charles et al., 1986; Condon et al., 1986; Miller et al., 1988; Solheim, 1990; Kagerer and Grupe, 2001; Wittwer-Backofen, 2004; Schug et al., 2012; Steinberg and Huffman, 2012; Gauthier and Schutkowski, 2013; Bertrand et al., 2014; Naji et al., 2014; Colard, 2015). This is possible because of the consistent biannual deposition of cementum where a single calendar year is represented by a pair of bands (one bright and one opqaue). Because the mineralized tissue is typically not resorbed, an imprint of the lifecycle of the mammal is embedded in the root of the tooth (Kagerer and Grupe, 2001; Naji et al., 2014; Colard et al., 2015).

Dental cementum is the multilayered hydroxyapatite rich tissue made up of 70% mineral, 21% collagen, and 1% extra organic material, that surrounds the dentin and anchors the tooth root to the alveolar bone via the periodontal ligament (Bosshardt and Selvig, 1997; Naji et al., 2004) (Figure 1.2). Cementum is also a complex milieu of cellular or acellular material and extrinsic collagenous periodontal fibers called Sharpey's fibers which are embedded in the calcified tissue matrix. These fibers are created by fibroblasts (building cells) in the periodontal membrane between the cementum and the alveolar bone and link the membrane to the cementum layer. In early development, Sharpey's fibers are characterized by a collagenous uncalcified core surrounded by a field of dense electrons. These fibers become mineralized as cementoblasts deposit hydroxyapatite into the cementum composition (Lieberman, 1994).

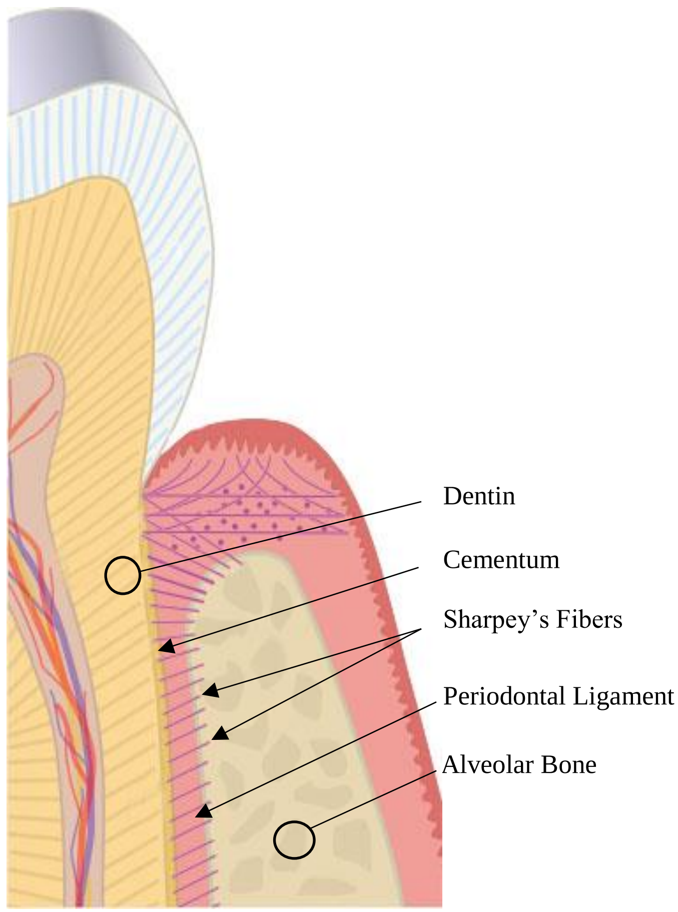

Figure 1.2. Anatomy of a Tooth (modified from Wikimedia Commons)

The extent of Sharpey's fiber mineralization depends on the tissue maturity and its location of attachment to the tooth. Fibers located at the middle third of the root are embedded acellular extrinsic fiber cementum, a secondary cementum lacking cementocytes (Bosshardt and Selvig, 1997). In acellular cementum, new tissue is deposited slowly and in a well-organized manner, similar to the mature organized deposition of lamellar bone. Sharpey's fibers are radially oriented in relation to the edge of the tooth and there appears to be a homogenous distribution of fiber mineralization in the mature stages of development (when the material is surrounded by new tissue) (Cool et al., 2002; Yamamoto et al., 2016). Incremental lines, visible as alternating layers of bright and opaque bands spanning the depths of the cementum, are clearer in this type of acellular cementum that lacks the chaos of the alternate matrix, cellular cementum (Bosshardt and Schroeder, 1996; Broucker et al., 2015). Additionally, the optical birefringence of these bands is believed to be dependent on the orientation of the Sharpey's fibers, which is hypothesized to be related to diet (Lieberman, 1994). For example, during mastication, strain in chewing is dependent on meal consistency which alters the angle at which collagenous Sharpey's fibers lay down. In the winter, food is typically tough and lacks nutrients. To make up for nutritional deficiencies non-human mammals must consume more tough meals; thus, excessive strain is placed on the periodontal ligament subsequently altering the orientation of the Sharpey's fibers. Additionally, strain placed on existing fibers can influence the rate of cementum apposition when these fibers are destroyed and new fibers must be laid down.

Cellular intrinsic fiber cementum, or cellular cementum, is composed of the same calcified material as acellular cementum but retains cementocytes housed in lacunae with associated canaliculi. This type of primary cementum is found at the apex of the root and in the furcation of multi-rooted teeth. It is laid down quickly, resulting in uncalcified Sharpey's fibers that may exhibit radial or parallel orientations. Incremental lines are difficult to distinguish in cellular regions of the root, thus, careful consideration must be taken when determining where thin sections are cut for DCIA analysis (Lieberman, 1994, Yamamoto et al., 2016).

Age-at-death Estimation

To estimate age-at-death in mammals, investigators count the number of band sets in the tooth, usually by choosing a single band and counting like bands through the depth of the cementum (Wittwer-Backofen, 2004; Kagerer and Grupe, 2001). This number is then added to the age of eruption for that tooth. These studies have been very successful in the remains of non-human mammals; however, there are conflicting opinions surrounding the utility of this method for human skeletons. Some researchers claim that low accuracy and high inter- and intra-observer error in analysis have limited the use of this method (Jankauskas et al, 2001; Renz and Radlanski, 2006; Roksandic et al., 2009; Huffman and Antoine, 2010; Ralston, 2016), while others have found a significant correlation between age-at-death and cementum banding greater than many results obtained by gross morphological aging methods (Stott et al., 1982; Charles et al., 1986; Condon et al., 1986; Miller et al., 1988; Solheim, 1990; Kagerer and Grupe, 2001; Wittwer-Backofen, 2004; Blondiaux et al. 2006; ; Schug et al., 2012; Steinberg and Huffman, 2012; Gauthier and Schutkowski, 2013; Bertrand et al., 2014; Colard, 2015). The lack of standardized preparation methods for DCIA no doubt contributes to the rates of low accuracy in age-at-death estimation obtained by some researchers.

Season-of-death Estimation

Season-of-death in mammals is estimated by examining the outer band of cementum that attaches directly to the periodontal ligament in life (Lubinski and O'Brien, 2001; Stutz, 2001). Mammals that died during the spring/summer seasons (April-

September) display a bright outer band, while those that died during the fall/winter seasons (October-March) show an opaque band. This method has been performed successfully in archaeological contexts to examine use of mammal resources, infer hunting strategies, identify changes in human behavior, and explore climatic changes over time (Lieberman and Meadow, 1992; Lieberman, 1994; Klevezal and Shishlina, 2001; Lubinski and O'Brien, 2001; Wall-Scheffler, 2007). In modern humans, this method is most useful for estimating PMI in forensic death investigations wherein a forensic anthropologist may be able to inform law enforcement of the season an individual died (Wedel, 2007). Unfortunately, there are very few studies that test the applicability of this method to a known date-of-death sample and none that test the method on a sample of known individuals that have decomposed in an outdoor environment. Previous work by Wedel (2007) has only examined the date-of-tooth extraction in living people and Ralston (2016) estimated season-of-death in donated medical cadavers and season-of-extraction in living individuals.

The Perplexity of DCIA

Although the composition of cementum banding and its patterns have been extensively studied in humans and non-human mammals, the reasons for seasonal incremental patterns remain largely misunderstood. Lieberman (1994) observed that cementum banding in goats (N = 6) is primarily determined by the amount of mineralization in the cementum and the orientation of Sharpey's fibers. The author argued that these factors are related to seasonal changes in diet and may be a result of nutritional intake, hormonal cycles, and/or biomechanical forces during mastication.

However, most modern humans and domestic non-human mammals living in the United States do not have strict seasonal diets like those of wild mammals (Cool et al., 2002; Ralston, 2016). Furthermore, the diet of humans and domestic non-human mammals is relatively consistent throughout the year. Therefore, if there is a significant seasonal effect in human cementum banding, diet may not be the main cause for the differential orientation of the Sharpey's fibers. In fact, Saxon and Higham (1969) found the same positive relationship between seasonality and cementum banding in their domestic non-human mammal sample as would be expected in wild mammals when the experimental sample was provided with a sufficient winter food source. The authors suggest this relationship is primarily based on an innate metabolic cycle in non-human mammals that may involve a reduction in nutritional demands during the dormant months. This is contrary to Lieberman (1994) who found no difference in the orientation of Sharpey's fibers in non-human mammals that consumed the same diet all year round.

To gain a better understanding of the composition of cementum and, as a result, the habits of its orientation, Cool and colleagues (2002) used scanning electron microscopy (SEM) to determine if there is a relationship between birefringence in cementum bands and mineral content. Congruent with Lieberman (2004), the authors suggest mineral orientation and/or size may play a greater role in cementum translucency than collagen orientation since birefringence only changes when the mineral component is manipulated.

Summary

DCIA has been used successfully to estimate age and season-of-death in non-human mammals. However, its cogency for estimating season-of-death in human teeth is limited. Though Wedel (2007) argued that there is a strong correlation between outer band translucency in human teeth and the season-of-death, more recent studies by Ralston (2016) found contradictory results.

This study will further investigate the relationship between season-of-death and dental cementum increments in modern humans from a known date of death skeletal collection. The goal of this research is to test the validity and reliability of the research conducted by Wedel (2007) and to contribute to the body of knowledge regarding the utility of DCIA in a forensic context.

II. MATERIALS AND METHODS

Thirty-one single-rooted teeth from individuals of the Texas State University Donated Skeletal Collection (TXSTDSC) were obtained for DCIA. Approval for destructive analysis was granted by the Forensic Anthropology Center at Texas State (FACTS) Board. Individuals from this collection decomposed in an outdoor environment at the Forensic Anthropology Research Facility (FARF) at Freeman Ranch in San Marcos, Texas and were not injected with embalming fluids or any other preservation modifications. Sampling was limited to single rooted teeth to ensure only the analysis of acellular cementum was analyzed as cellular cementum in the furcation of a double rooted tooth can be difficult to read (Lieberman 1994, Wittwer-Backofen et al. 2004). Due to sampling restrictions and to control for age, samples were only taken from individuals over the age of fifty years. Therefore, the average age of the entire sample was 67 years. Once the samples were collected the teeth were separated into a "learning" sample and a "validation" sample. For consistency only single rooted teeth were analyzed. All methodology was based on Wedel (2007) and personal experience training for one week with Vicki Wedel in her histology lab at Western University of Health Sciences.

Learning Sample

The learning sample was comprised of six teeth, three from individuals that died in the fall/winter (October-March) and three from individuals that died in the spring/summer (April-September). Each season was defined by months outlined in Wedel (2007). The average age for this sample is 63.6 years old (Table 2.1). The learning sample was of known season-of-death to the observer and was used to develop the method, examine initial intra-observer error, and insure that band translucency can be accurately determined with the available equipment. Ralston (2016) verified that there is no difference between geographic origin of the individual and season to band correlation. Therefore, geographic origin was not considered a limiting factor in band identification.

2.1. Texas State University Donated Skeletal Collection Learning Sample

ID	Tooth Type	Month of Death	Age	Residence at Death
T1	Incisor	August	54	Texas
T2	Incisor	June	56	Texas
T3	Incisor	June	86	Texas
T4	Incisor	February	55	Texas
T5	Incisor	January	63	Texas
T6	Canine	January	68	Texas

Validation Sample

The validation sample was comprised of teeth from 24 individuals with an average age of 67.8 years old (Table 2.2). Initially, teeth were to be taken from two individuals who died in each month of the year; however, because of the limited availability of teeth from individuals over the age of 50 years, samples were taken from any individual over the age of 50 years with single rooted teeth until 24 samples were obtained. This resulted in a validation sample of seven summer/spring deaths and 17 winter/fall deaths. In order to be blind to the information regarding this sample until after the band translucency data was collected, Dr. Daniel J. Wescott randomly assigned each tooth a unique number using an online random number generator.

Two observations were conducted on the validation sample at least two weeks apart. A Fisher's Exact test was conducted to determine if there were any differences in the translucency of bands identified as spring/summer and those identified as fall/winter in each observation. To examine rates of intra-observer error, Cohen's Kappa was used to determine if there were significant differences between each observation and how much of the agreement was likely due to chance (i.e. intraobserver error).

After the Null 1 hypothesis was tested, permission was granted by FACTS to sample one 22 year old individual. This tooth was analyzed to examine the possible effect of age on the identification of the outer band (Null 6). Due to sampling restrictions, only one sample from an individual under the age of 50 was collected.

2.2. Texas State University Donated Skeletal Collection Validation Sample

ID	Tooth Type	Month of Death	Age	Residence at Death
35	Premolar	August	72	Texas
74	Premolar	August	63	Oklahoma
48	Premolar	October	50	Texas
68	Premolar	October	57	Texas
86	Premolar	October	67	Texas
31	Premolar	October	60	Pennsylvania
42	Premolar	November	65	Texas
7	Premolar	November	60	Texas
91	Premolar	November	68	Texas
20	Premolar	February	84	Texas
54	Premolar	February	89	Texas
45	Canine	February	58	Texas
34	Premolar	February	53	Texas
40	Premolar	March	91	Texas
83	Premolar	March	60	Texas
77	Premolar	March	67	Texas
25	Premolar	May	53	Texas
36	Premolar	April	62	Nevada
3	Premolar	October	61	Texas
63	Premolar	October	63	Oklahoma
98	Premolar	October	58	Texas
41	Premolar	June	87	Texas
29	Premolar	July	88	Texas
33	Premolar	July	91	Texas
-	Premolar	September	22	Oklahoma

Non-Human Sample

It is widely recognized that seasonal increments are identifiable in wild animal species (Laws, 1952; Saxon and Higham, 1969; Spiess, 1976; Bourque et al., 1978; Stallibrass, 1982; Charles et al., 1986, Condon et al., 1986; Gordon, 1988; Pike-Tay, 1991; Beasley et al., 1992; Lieberman and Meadow, 1992; Lieberman, 1994). To validate the sample preparation method used for the human teeth (described below) and to conduct an initial investigation for the primary cause of cementum banding, a sample of 11 wild deer and two domestic dogs with known season-of-death were prepared (Table 2.3). If the season-of-death could accurately be estimated from non-human mammals then the method of preparation for the human sample will be considered valid. Additionally, confirmation of the utility of DCIA for domestic animals may call into question the relationship between diet and cementum band birefringence. In this sample of non-human teeth the season-of-death was unknown to the observer until after a blind estimation was performed.

Tooth Preparation

Preparation methods are a major point of contention for researchers conducting DCIA and are likely the cause of much disagreement on the efficacy of the method (Kagerer and Grupe, 2001; Renz and Radlanski, 2006; Ralston, 2016). In order to stay true to the validation study, few alterations were made from Wedel's (2007) preparation and data collection methodology, but the teeth were prepared in a manner best suited for preservation.

2.3. Texas State University Non-Human Sample

Non-human Mammal	ID	Tooth Type	Month of Death
Deer	4	Incisor	October
Deer	6	Incisor	October
Deer	7	Molar	October
Deer	8	Incisor	October
Deer	9A	Incisor	October
Deer	9B	Incisor	October
Deer	10	Molar	October
Deer	J	Molar	April
Deer	D1	Molar	January
Deer	F1	Incisor	November
Deer	F2	Incisor	November
Sheepdog	S1	Incisor	November
Rottweiler	R1	Incisor	April

First, photographs were taken of the teeth to document the condition of the sample prior to sectioning. Any deformity of the tooth due to dental disease, medical procedure, or handling was recorded. This would allow for further investigation in any case where cementum annulations are obstructed or unobservable; however, none of the teeth exhibited any pathology that would require exclusion of the tooth from the sample (Wittwer-Backofen 2004).

Next, the crown of each tooth was removed just below the cervix using a Dremel® tool with a diamond blade wheel while the tooth was secured with a vice by the root. The vice allowed for a secure cut that preserved the integrity of the crown in most cases. Though precautions were taken, if the crown exhibited fractures prior to the cut it was likely to splinter into pieces. The crown was returned to the collection once it was removed.

To embed the teeth, each root was positioned on its side in the middle of a 1 oz. plastic cup with the cut end of the root as close to the side of the cup as possible without making contact. Then, a small paper label with the sample number was positioned behind the apex of the root to ensure proper identification throughout preparation. A mixture of two parts Buehler® EpoThin™ 2 Fast Cure Epoxy Resin 20-3440-032 with one part EpoThin™ 2 Epoxy Hardener 20-3442-064 was combined by stirring slowly in a continuous circular motion for two minutes. The root was then embedded in the resin/hardener mixture by pouring it over the root until completely covered. The samples were left to harden for at least 24 hours.

Maat et al. (2006) suggests optimizing the visibility of the cementum to create a clear contrast between opaque and translucent annulation lines by cutting the root perpendicular to the long axis. Following Maat et al. (2006), if the root curved in any particular direction a line was drawn with a permanent marker parallel to the exterior of the tooth. The first transverse cut was then made perpendicular to the marked line to reach the middle third of the root using a Buehler IsoMet® 1000 Precision Saw.
Next, three more sections were cut to a thickness of 1 millimeter (mm). Multiple sections were cut in case of error in preparation or to compare taphonomic distortion. One section from each sample was then mounted to a glass slide using Buehler® Crystalbond Mounting Wax. Initially, grinding was done at 150 rpm using a metal slide holder on a MetaServ®3000 Variable Speed Grinder-Polisher. However, it was determined that it was easier to control the thickness and prevent destruction of the section by manually rubbing the sample into the grinding plate instead of relying on the rotation of the plate to thin the section. The slide was pressed into the grinding plate using the metal slide holder and

moved in a forward and backward motion for about 20 seconds before checking the visibility of the cementum under the microscope. This was done two to three times depending on the tooth and certain regions were targeted if they appeared thinner than surrounding areas. Each sample was polished using the same method but with a polishing cloth, Buehler MetaDi® Fluid, and 0.05 Micron MasterPrep® Polishing Suspension. The sample appeared thin enough when annulations throughout the cementum could be discerned with ease in polarized light at 20x magnification under the microscope. At this time, the slide was cleaned and preparation concluded with the application of a glass coverslip adhered to the slide using Protocol® SecureMount™ Mounting Medium.

Outer Band Examination

Dental cementum was examined under transmitted polarized light using an Olympus CX41 microscope with 20X objective and digital photographs of the slides were taken using a mounted Infinity1-3C camera. The color of the outer band was scored as opaque or translucent by visual examination through the microscope lens. It was recommended by Vicki Wedel (personal communication) to use the microscope to reliably examine the outer band by taking advantage of the polarizing light and the manual focus. According to Wedel, the outer band should be distinguishable by a change in morphology (width and density) when the focus is manipulated.

In order to identify the outer band, I first examined the entire cross-section for a clear area of analysis. Sections of the cementum considered clear were not obstructed by taphonomic disturbances, blurred, obliterated, or separated from the dentin. Next, I focused on this area using manipulation of the focus and polarizing light to bring out the features of the cementum. Each clear area of the cementum was considered before making a final decision on the translucency of the outer band. This was done for each tooth in the sample.

Outer Band Examination of the Learning Sample

The learning sample was examined to determine the visible qualities of a spring/summer band and a fall/winter band. The qualities of a spring/summer band were seemingly simple to identify. In the analysis of summer deaths T1, T2, and T3, a translucent bright band was observed along the outer edge of the cementum (Figure 2.1). This translucent band was preceded by an opaque band and was more vibrant along the outer edge of the tooth than most of the inner cementum bands. Based on the observations of the three spring/summer learning samples, a spring/summer band should cover the majority of the outer edge of the tooth, be brighter than the other bands in the cementum, and follow an opaque band that is preceded by another translucent band.

The fall/winter bands, T5 and T6 did not exhibit the same clear banding distinction as the spring/summer bands. Instead of observing an obvious opaque band surrounding the cementum, areas of reduced vibrancy were observed. Some areas of the tooth appeared more opaque than others and some appeared bright but were not considered as bright as those in the spring/summer sample (Figure 2.2). Therefore, fall/winter teeth were categorized as those samples whose outer cementum exhibited a faded appearance of the cementum banding, or a dark band. This is in opposition to the spring/summer test samples that exhibit high vibrancy, described as bright banding, throughout most of the outer edge. Training sample tooth T4 could not be identified as either color band due to the lack of clarity in the cementum (Table 2.4). The qualitative distinctions between the fall/winter test sample and the spring/summer test sample were used as a basis for estimating season-of-death in the validation sample.

Table 2.4. Learning Sample: Date of Death Information and Band Identification

Code	Actual DOD	Actual SOD	Band Observed
T1	8/11/2010	Summer	Bright*
T2	6/6/2012	Summer	Bright*
T3	6/5/2013	Summer	Bright*
T4	2/13/2013	Winter	Undetermined
T5	1/27/2012	Winter	Opaque*
T6	1/24/2013	Winter	Opaque*

***Indicates Outer Band Matched Season-of-death**

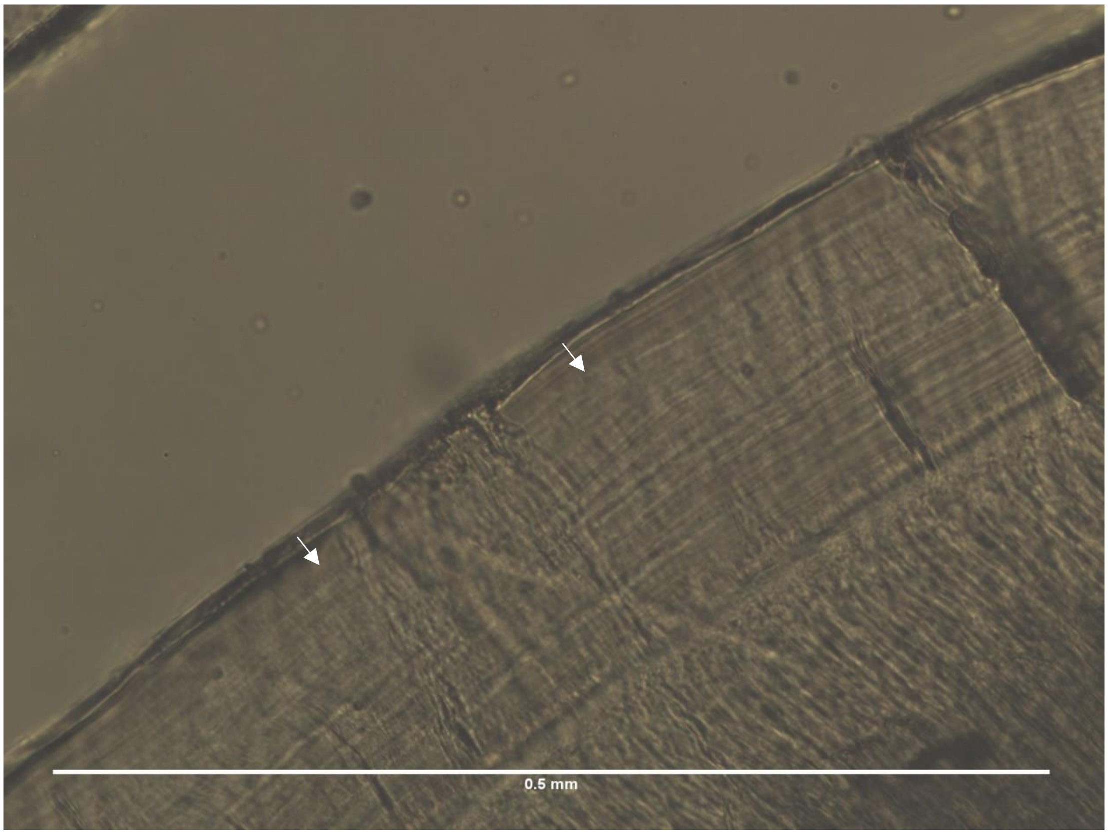

Figure 2.1. Bright Banding in a Summer Tooth, T1 (white arrows) (20x)

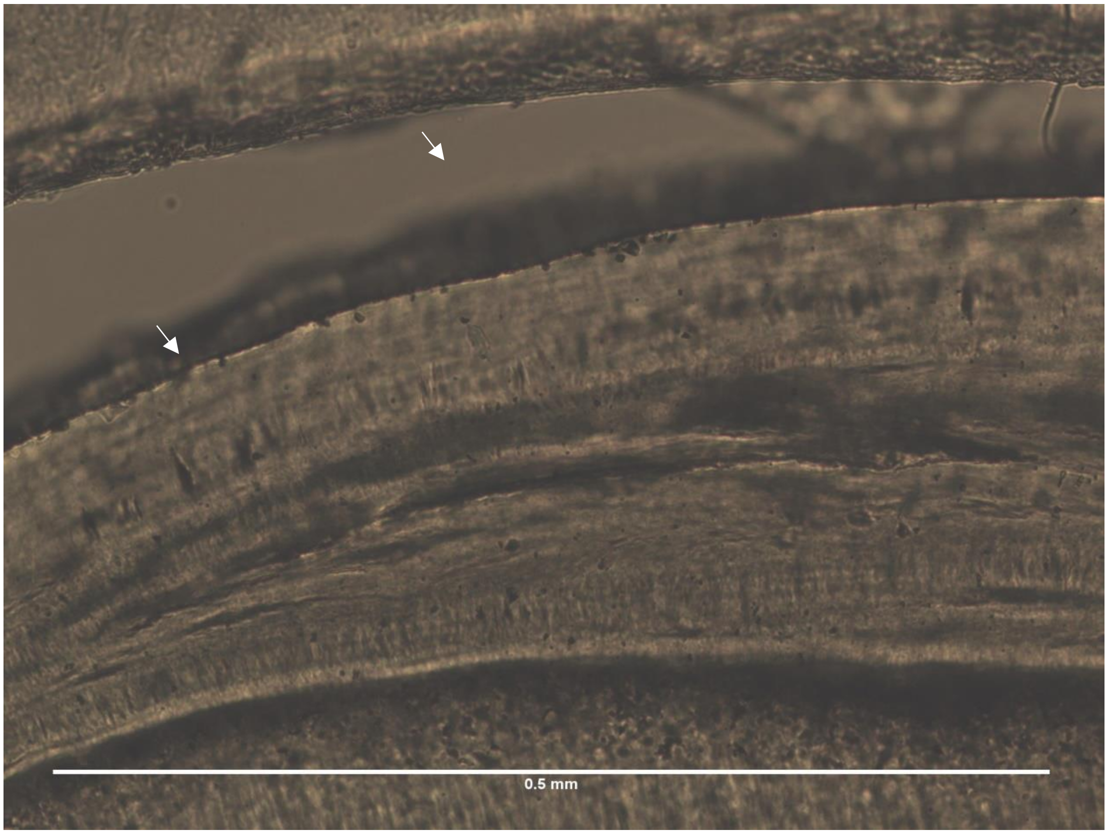

Figure 2.2. Dark banding in a Fall/Winter Tooth, 6 (white arrows) (20x)

Stress Related Factors

A test of the Null 5 hypothesis was conducted to determine if there were any consistent medical variables within the sample that may account for the lack of clarity in cementum and, as a result, the inability to identify the translucency of the outer band. I examined the information on file for each donor searching for patterns of long term cancer, treatment, or any significant illness or medical intervention in the medical history of the sample that may have had an influence on the development of the outer band.

A Single Sample Under 50 Years of Age

While using dental cementum to estimate age-at-death, Aggarwal and colleagues (2002) experienced a lower correlation between age and number of cementum annulations in a portion of the sample over the age of 60.. Similar studies (Condon et al., 1986; Lipinsic et al., 1986; Solheim, 1990; Stein and Corocan, 1994; Kvaal et al., 1995; Klevezal and Shishlina, 2001; Pilloud, 2004; Wittwer-Backofen et al., 2004; Gauthier and Schutkowski, 2013) obtained comparable results in individuals over the age of 50 years old. Considering the original sample from the Texas State University Donated Skeletal Collection is composed of 24 individuals over the age of 50 years old, the possibility remains that age is the primary limiting factor when estimating season-of-death in human teeth.

In a final attempt to explore the limitations involved when estimating season-of-death in the human sample the Null 6 hypothesis was tested on a left third premolar from a twenty-two year old male. Though the sample size is one, the results of this test may provide insight regarding the impact of age on dental cementum annulation clarity and development.

III. RESULTS

Null 1 Hypothesis: Human Sample

The results of the first round of validation observations resulted in 12 out of 20, or 60%, of the bands identified correctly according to the actual season-of-death and expected band translucency (Table 3.1). In this observation, four teeth were excluded because I was unable to make a determination due to obscure cementum bands, bringing the sample size down to 20 teeth. A Fisher's Exact Test demonstrated there is no significant difference between bands identified in the spring/summer and those identified in the fall/winter ($p = .362$; $p > .05$) (Table 3.2). This indicates that incorrect classifications are not specific to one season (Table 3.3).

The second round of observations resulted in the correct identification of 4 out of 22 bands, or 18%, according to the actual season-of-death. Two of the same teeth excluded in the first observation (86 and 83) were excluded from the second observation bringing the sample size down to 22 teeth. In this observation, only 36.4% of the fall/winter deaths were recorded as a dark band and only 9.1% of spring/summer deaths were positively correlated as a bright band according to season-of-death (Table 3.4). However, the results of the Fisher's Exact test show a significant difference in band translucency between the seasons ($p = .024$; Fisher's Exact Test) (Table 3.5).

Intraobserver error between the two observations was calculated using the Cohen's Kappa test for differences between two independent observations (Table 3.6). The results of the Cohen's Kappa indicate that only 17% agreement in band translucency can be explained beyond chance (Table 3.7). However, of the 24 teeth observed only 50% were scored the same in both observations. In 50% the band translucency was reversed. Table 3.8 shows the band identification in both observations for each tooth in the spring/summer and fall/winter months.

Table 3.1. Observation 1: Date of Death Information and Band Identification

ID	Actual DOD	Actual SOD	Band Color
35	8/27/2012	Summer	Opaque
74	8/29/2012	Summer	Bright*
48	10/3/2012	Fall	Bright
68	10/23/2012	Fall	Bright
86	10/29/2012	Fall	X
31	10/25/2012	Fall	X
42	11/25/2012	Fall	Opaque*
7	11/18/2012	Fall	Bright
91	11/26/2012	Fall	Opaque*
20	2/8/2013	Winter	Bright
54	2/9/2013	Winter	Opaque*
45	2/19/2013	Winter	Opaque*
34	2/24/2013	Winter	X
40	3/26/2013	Winter	Opaque*
83	3/15/2013	Winter	X
77	3/28/2013	Winter	Bright
25	5/5/2013	Spring	Bright*
36	4/4/2013	Spring	Opaque
3	10/27/2013	Fall	Opaque*
63	10/13/2013	Fall	Opaque*
98	10/27/2013	Fall	Opaque*
41	6/26/2013	Summer	Opaque
29	7/11/2013	Summer	Bright*
33	7/15/2010	Summer	Bright*

***Indicates Outer Band Matched Season-of-death**
X- Excluded from Sample

Table 3.2. Observation 1: Fisher's Exact Test

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Fisher's Exact Test				**0.362**	**0.205**
N of Valid Cases	20				

Table 3.3. Observation 1: Crosstabulation of Fisher's Exact Test

			Band Translucency		Total
			Winter/Fall	Spring/Summer	
Season-of-death	Fall/Winter	Count	8	4	12
		% within Season-of-death	66.70%	33.30%	100.00%
		% within Band Translucency	72.70%	44.40%	60.00%
		% of Total	40.00%	20.00%	60.00%
	Spring/Summer	Count	3	5	8
		% within Season-of-death	37.50%	62.50%	100.00%
		% within Band Translucency	27.30%	55.60%	40.00%
		% of Total	15.00%	25.00%	40.00%
Total		Count	11	9	20
		% within Season-of-death	55.00%	45.00%	100.00%
		% within Band Translucency	100.00%	100.00%	100.00%
		% of Total	55.00%	45.00%	100.00%

Table 3.4. Observation 2: Date of Death Information and Band Identification

ID	Actual DOD	Actual SOD	Band Color
35	8/27/2012	Summer	Opaque
74	8/29/2012	Summer	Opaque
48	10/3/2012	Fall	Bright
68	10/23/2012	Fall	Bright
86	10/29/2012	Fall	X
31	10/25/2012	Fall	Bright
42	11/25/2012	Fall	Bright
7	11/18/2012	Fall	Opaque*
91	11/26/2012	Fall	Opaque*
20	2/8/2013	Winter	Bright
54	2/9/2013	Winter	Opaque*
45	2/19/2013	Winter	Bright
34	2/24/2013	Winter	Bright
40	3/26/2013	Winter	Bright
83	3/15/2013	Winter	X
77	3/28/2013	Winter	Bright
25	5/5/2013	Spring	Opaque
36	4/4/2013	Spring	Opaque
3	10/27/2013	Fall	Bright
63	10/13/2013	Fall	Bright
98	10/27/2013	Fall	Opaque*
41	6/26/2013	Summer	Opaque
29	7/11/2013	Summer	Opaque
33	7/15/2010	Summer	Opaque

***Indicates Outer Band Matched Season-of-death**
X- Excluded from Sample

Table 3.5. Observation 2: Fisher's Exact Test

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Fisher's Exact Test				**0.024**	**0.012**
N of Valid Cases	22				

Table 3.6. Intraobserver Error Crosstabulation

			Ob1			Total
			Dark	Bright	NoID	
Ob2	Opaque	Count	6	5	0	11
		Expected Count	5	4.1	1.8	11
	Bright	Count	5	4	2	11
		Expected Count	5	4.1	1.8	11
	NoID 3	Count	0	0	2	2
		Expected Count	0.9	0.8	0.3	2
Total		Count	11	9	4	24
		Expected Count	11	9	4	24

Table 3.7. Results of the Cohen's Kappa Test

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.172	.182	1.105	.269
N of Valid Cases		24			

Table 3.8. Band Identification between Observations

ID	Actual SOD	Observation 1	Observation 2	Agreement
35	Summer	Opaque	Opaque	Yes
74	Summer	Bright*	Opaque	No
48	Fall	Bright	Bright	Yes
68	Fall	Bright	Bright	Yes
86	Fall	X	X	Yes
31	Fall	X	Bright	No
42	Fall	Opaque*	Bright	No
7	Fall	Bright	Opaque*	No
91	Fall	Opaque*	Opaque*	Yes
20	Winter	Bright	Bright	Yes
54	Winter	Opaque*	Opaque*	Yes
45	Winter	Opaque*	Bright	No
34	Winter	X	Bright	No
40	Winter	Opaque*	Bright	No
83	Winter	X	X	Yes
77	Winter	Bright	Bright	Yes
25	Spring	Bright*	Opaque	No
36	Spring	Opaque	Opaque	Yes
3	Fall	Opaque*	Bright	No
63	Fall	Opaque*	Bright	No
98	Fall	Opaque*	Opaque*	Yes
41	Summer	Opaque	Opaque	Yes
29	Summer	Bright*	Opaque	No
33	Summer	Bright*	Opaque	No

***Indicates Outer Band Matched Season-of-death**
X- Excluded from Sample

The low accuracy in cementum band translucency identification when compared to actual season-of-death indicates there are many limitations for using DCIA for estimation of PMI. The results of the Fisher's Exact test show a failure to reject the Null 1 hypothesis in the first observation (no differences between band translucency in the spring/summer and fall/winter), and reject the Null 1 in the second observation (a significant difference between band translucency in the spring/summer and fall/winter). Although the Null 1 was rejected, the pattern of observed versus expected in the second observation was the exact opposite of what would be predicted based on the study by Wedel (2007). That is, in the second observation, cementum from individuals that died in the spring/summer displayed significantly more opaque outer bands (Figure 3.1), while individuals that died in the fall/winter displayed significantly more bright outer bands (Figure 3.2) (Table 3.9). This is contrary to Wedel (2007) who observed opaque bands in almost all the teeth of individuals that died in the fall/winter, and bright bands in the teeth of individuals that died in the spring/summer.

The results achieved in this study are not consistent with 99% accuracy obtained by Wedel (2007); however, they are similar to those obtained by Rolston (2016) who obtained accuracy between 61.54% and 71.15% when investigating the utility of DCIA on a sample of 143 teeth with known dates of extraction.

Null 4 Hypothesis: Non-human Sample

A test of the Null 4 hypothesis showed there is a difference in the translucency of cementum banding between the spring/summer and fall/winter seasons in the teeth of non-human mammals. Of the 11 wild deer, two were excluded due to distortion and lack of clarity in the cementum. In the remaining sample of nine deer and two dogs the outer cementum band was correctly identified 100% of the time. In each observation a bright band was observed in the non-human mammals that died in the spring/summer and an opaque band was observed in the non-human mammals that died in the fall/winter. Table 3.10 shows the results for the non-human teeth.

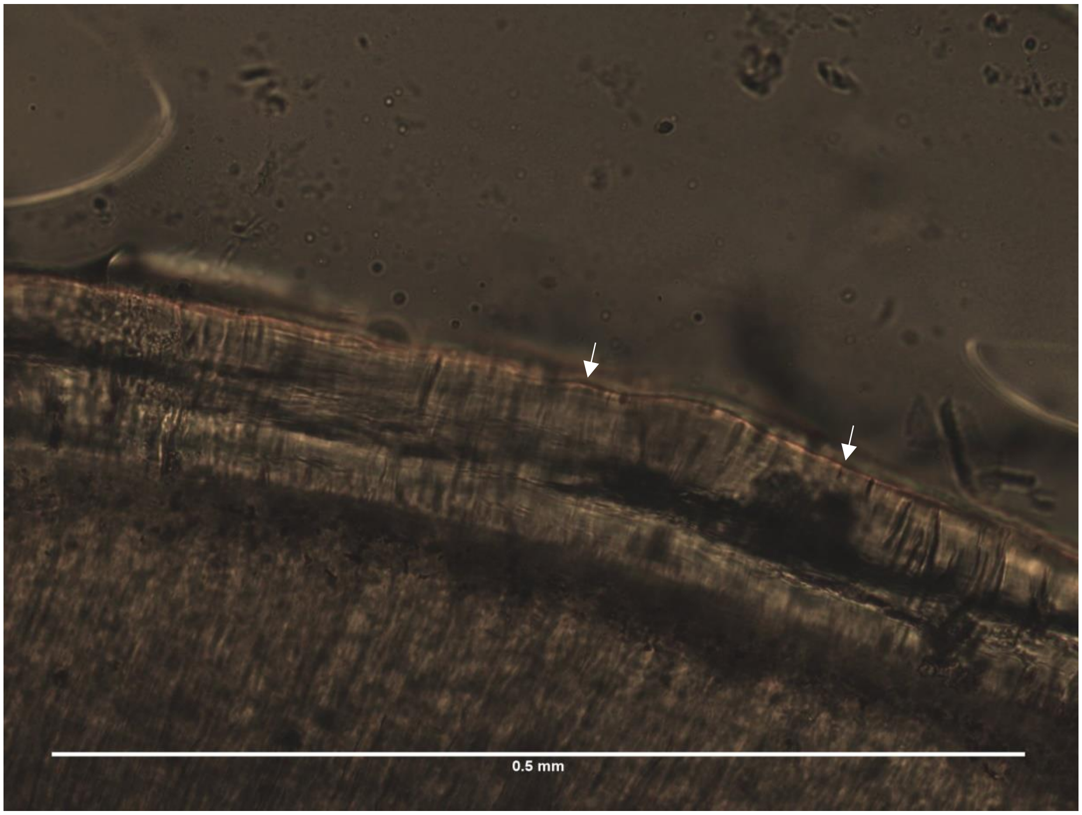

Figure 3.1. Dark banding (white arrows) around Spring Tooth, 36 (20x)

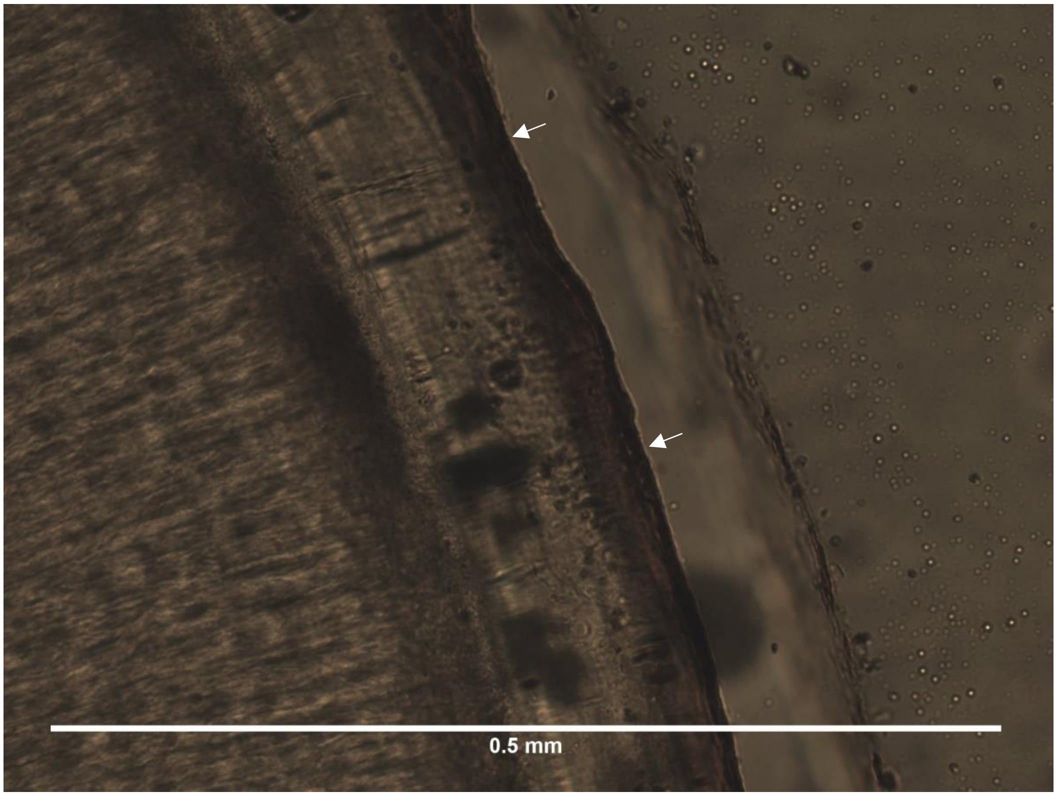

Figure 3.2. Bright banding (white arrows) around Fall Tooth, 3 (20x)

Table 3.9. Observation 2 Crosstabulation

			Band Translucency		Total
			Winter/Fall	Spring/Summer	
Season-of-death	Fall/Winter	Count	4	10	14
		% within Season-of-death	28.60%	71.40%	100.00%
		% within Band Translucency	36.40%	90.90%	63.60%
	Spring/Summer	Count	7	1	8
		% within Season-of-death	87.50%	12.50%	100.00%
		% within Band Translucency	63.60%	9.10%	36.40%
		% of Total	31.80%	4.50%	36.40%
Total		Count	11	11	22
		% within Season-of-death	50.00%	50.00%	100.00%
		% within Band Translucency	100.00%	100.00%	100.00%
		% of Total	50.00%	50.00%	100.00%

Table 3.10. Non-Human Sample

ID	Month of Death	Expected Band	Observed Band
4	October	Opaque	X
6	October	Opaque	Opaque
7	October	Opaque	Opaque
8	October	Opaque	Opaque
9A	October	Opaque	Opaque
9B	October	Opaque	Opaque
10	October	Opaque	Opaque
J	April	Bright	Bright
D1	January	Opaque	X
F1	November	Opaque	Opaque
F2	November	Opaque	Opaque
S1	November	Opaque	Opaque
R1	April	Bright	Bright

Null 5 Hypothesis: Stress-related Factors

Of the 20 individuals whose outer cementum band did not match up with the season-of-death in at least one of the two observations, 20% died as a result of a traumatic event (gunshot to the head, an unknown form of homicide, poisoning, and closed head injury from a fall), 10% died as a result of hemorrhage or pulmonary embolism, 25% died as a result of various forms of cancer, another 25% died as a result of organ failure or disease of the heart or liver, 15% died as a result of respiratory failure, and 5% suffered from debility and dementia. An examination of available data on file for each individual in the TXSTDSC sample showed there is no clear pattern between the misclassification of the season-of-death and indicators of stress; therefore, the Null 5 hypothesis could not be rejected.

Null 6 Hypothesis: A Single Sample Under 50 Years of Age

The cementum of the 22 year old individual displayed a small opaque band visible along the outside of the tooth. This line was not continuous and seemed to break up in some regions before appearing again. Of interest is the fact that this individual died on the 28^{th} of September, straddling the line between a summer death and a fall death. It is possible the opaque band was in the process of deposition before growth was halted at death as described by Wedel (2007) regarding transitory teeth present in her sample.

Table 3.12. Stress Related Factors

ID	Correct ID	Cause of Death
35	No	Acute gastrointestinal hemorrhage
74	Yes	Remote blunt trauma
48	No	Terminal melanoma
68	No	Lung cancer
86	No	Metastatic breast cancer
31	No	Pulmonary embolism due to deep vein thrombosis
42	Yes	Respiratory failure, pneumonia
7	Yes	Hypertensive cardiovascular disease
91	Yes	Hypertensive and atherosclerotic
20	No	Debility
54	Yes	Diabetes, dementia, chronic kidney failure, hypertension
45	Yes	Ischemic stroke, metastatic renal cell carcinoma
34	No	Respiratory failure; morbid obesity
40	Yes	Cardiac arrhythmia
83	No	Intraorbital gunshot wound to the head
77	No	Alcoholism, cirrhosis
25	Yes	Hepatic insufficiency
36	No	Homicide
3	Yes	Ethylene glycol toxicity
63	Yes	Respiratory failure
98	Yes	Contact gunshot wound of the head
41	No	Closed head injury
29	Yes	Cardiopulmonary failure
33	Yes	Arteriosclerotic heart disease

IV. DISCUSSION AND CONCLUSION

Null 1 Hypothesis: Human Sample

The purpose of this study was to evaluate the use of dental DCIA to estimate the season-of-death from human teeth that underwent natural decomposition processes. Previous research by Wedel (2007) showed that DCIA can be successfully used by forensic scientists to determine the season-of-death by determining the translucency of the outer band. However, more recent studies (Ralston, 2016) have demonstrated a weak correlation between the outer cementum band and season-of-death.

This study was set up as a hypothesis flowchart based on the rejection or acceptance of the Null 1 hypothesis. The Null 1 hypothesis is that there is no statistically significant difference in outer band translucency of dental cementum in single-rooted human teeth between individuals that died during the spring/summer and those that died during the fall/winter.

Contrary to Wedel (2007), the Null 1 was not rejected in the first observation. Although a significant difference was found between spring/summer and fall/winter bands in the second observation, the difference was opposite from what was expected. In the second observation, fall/winter deaths were more likely to be identified as a bright band instead of an opaque band while spring/summer deaths were more likely to be identified as an opaque band. This pattern explains the reason for a statistically significance difference in banding between seasons, though low accuracy was obtained based on the band translucency and season assignments outlined by Wedel (2007). The results of the Cohen's Kappa intraobserver error test indicate that much of the agreement between observations is likely related to chance.

Overall, it seems DCIA is not a good estimate of season-of-death in a sample of naturally decomposed individuals; therefore, the Null 2 and Null 3 hypothesis were not tested and the Null 4 and Null 5 hypotheses were considered to parse out the limitations of this method.

The Null 4 hypothesis was tested to eliminate sample preparation or observer inexperience as limiting factors in band identification and to determine if diet can be ruled out as a primary causal factor for seasonal differences in cementum translucency. Additionally, the Null 5 hypothesis was tested to examine if nutrition or stress related factors such as long-term illness played a role in the determination of the cementum outer band translucency.

Null 4 Hypothesis: Non-human Sample

The Null 4 hypothesis is that there is no significance difference in outer band translucency of dental cementum in non-human mammal teeth between those mammals that died during the spring/summer and those that died during the fall/winter. This step was designed to determine if the method used to prepare the samples was detrimental to the integrity of the tooth and, as a result, could have prevented the possibility of observing a clear outer cementum band.

Two of the 11 deer teeth (18%) were excluded because of distortion and lack of clarity in the cementum banding. It is likely that the lack of visibility in tooth D1 is due to the fact that this tooth is a double-rooted molar. The double-rooted molar can create issues in analysis because of the abundance of cellular cementum in the apex of the root and in the furcation between the two roots, which only leaves a small section of organized acellular cementum (Lieberman, 1994; Pike-Tay and Cosgrove, 2002).

However, wild mammal samples 7, 10, and J are double-rooted teeth, each of which was identified accurately. Since there is so much variation between teeth it is possible that Tooth D1 was simply an outlier in the sample. Tooth 4, also eliminated from the sample, is an incisor with an unidentifiable outer band. In this particular tooth, the outer edge appears opaque in some regions but a clear bright line appears on the outer edge of the cementum in others.

The remaining nine teeth all exhibit clear cementum annulations and the outer band was identified correctly according to the season-of-death in 100% of the sample. Notable differences in the morphology of the non-human mammals cementum compared to the human cementum include not only the presence of clear bright and opaque bands, but many areas of the root still maintained an articulated portion of the periodontal ligament. The recognizable differences in tissue anatomy led to increased confidence when making a determination about the translucency of the outer band.

The ability to clearly distinguish cementum annulations in wild mammals provided confidence that error in the method of preparation and analysis of the human sample did not play a major role in the misidentification of the outer band translucency. Additionally, it appears observer experience did not impact the analysis of the human sample since band identification in the wild mammal sample was relatively simple.

In both the wild and domestic non-human sample the Null 4 hypothesis was rejected as a significant difference was found between the band translucency in non-human mammals that died in the spring/summer and those that died in the fall/winter. In both samples, non-human mammals that died in the spring/summer exhibited bright bands, while those that died in the Fall/Winter exhibited opaque bands.

The results from this analysis indicate three things. First, the associated seasons and outer band translucency in the non-human teeth agree with the results of Wedel (2007) indicating that, in a situation where season-of-death can be accurately estimated, spring/summer bands will likely be bright and fall/winter bands will likely be opaque. Second, variation in diet is likely not a major contributor to the seasonal deposition of cementum annulations. It is possible that ecology (e.g. temperature, exposure to ultraviolet light, humidity, altitude or pollution), natural metabolic changes influenced by fluctuating hormones (i.e. the parathyroid hormone), or any combination of these factors may play a more significant role than diet (Klevezaal and Kleinenberg, 1967; Mitchell, 1967; Morris, 1972; Grue, 1979; Lieberman, 1993; Kagerer and Grupe, 2001; Wittwer-Backofen et al., 2004; Aggarwal et al., 2008). Finally, it seems the success of the non-human sample compared to the human sample is likely due to cementum band crowding as a result of increased age and loss of available area within the greater cementum layer.

Null 5 Hypothesis: Stress-related Factors

The Null 5 hypothesis states that nutrition or stress related factors such as long-term illness will not affect the determination of the cementum outer band translucency. Patterns such as long term cancer treatment or any other significant illness were not observed; therefore, the results of this investigation do not support or deny any evidence for a medically related cause of misidentification of the outer band. However, prior to preparation, many of the teeth exhibited dental restorations, caries, and minor resorption of the alveolar bone. Although periodontal and other common dental diseases are not frequently a limitation when counting cementum annulations for estimating age-at-death (Jankauskas et al., 2001; Wittwer-Backofen, 2004; Aggarwal et al., 2008; Broucker et al., 2013; Bertrand et al., 2014), it is possible that the lack of functionality for dental cementum when the root is no longer attached to the alveolar bone curtails the normal rhythmic apposition of cementum annulations in the resorbed locations (Gocha and Schutkowski, 2012). Therefore, periodontal disease may be a limitation of season-of-death analysis in humans.

Null 6 Hypothesis: A Single Sample Under 50 Years of Age

This sample may indicate some promise for estimating season-of-death in individuals under the age of 50 years old, especially since many researchers have noted a pattern of inconsistent band identification in older individuals. Therefore, a sample of individuals under the age of 50 should be tested in order to better understand how age affects the rate of cementum deposition.

Further Consideration

<u>Region of Analysis</u>

In a study using DCIA to estimate age-at-death, Renz and Radlanski (2006) found high variation in cementum annulation counts depending on the region of the root being studied. Additionally, Ralston (2016) found differences in cementum clarity between adjacent cross sections of the same root. Both rounds of observation in the experimental sample demonstrated that dental cementum is highly variable between and within individuals. Some have wide, thick bands of cementum surrounding the root, while some are thin and barely visible under 20X magnification. Many of the samples wavered and were inconsistent in their cementum thickness and clarity. The sample sections can be easily ground and polished without breaking, or they can be fragile and flake away under stress. In many teeth there were only a few small areas in the circumference of the tooth that I felt comfortable enough to make an estimate on the band translucency.

A greater understanding of the appropriate region for DCIA may lead to more accurate determination of band translucency for estimating season-of-death; especially since Hillson (1996, 2005) described cementum deposition and stress along the periodontal ligament as occurring disproportionately in the root in response to mastication. For example, Pike-Tay and Cosgrove (2002) found that the distal portion of the root is more accurate for estimating season-of-death than the mesial surface in a sample of wallaby incisors.

Taphonomic Factors in DCIA

Taphonomic factors, such as chemical diagenesis of the cementum, may affect determination of the outer cementum band translucency. Since cementum exhibits some of the same properties as bone, it is possible that collagen leaching or growth of apatite crystal structures can cause disruption in the cementum banding, possibly even mimicking seasonal annulations. According to Stutz (2002), chemical diagenesis in archaeological dental cementum most frequently mimics the appearance of incremental growth (bright) layers.

Stutz (2002) suggests using a modified polarizing microscopy approach to determine if collagen leaching or apatite growth has occurred and affected the outer edge of the cementum. The modified approach involves using a wave (λ) plate designed to distinguish collagen fibers from apatite crystals in polarized microscopy. This plate allows the researcher to determine if the outer cementum increment was a true band created by natural biogenic effects or if collagen leaching caused the band to look like a growth layer under polarized light. Stutz (2002) tested the modified approach method on a Paleolithic deer specimen and described the false band as displaying variation in its birefringence during analysis, alternating between a dark and light appearance. The author determined that this behavior in the banding rendered the tooth an excellent candidate for testing the modified method of polarized microscopy and subsequently determined that the cementum had been taphonomically altered.

Similar descriptions of variable cementum translucency were recorded during analysis of the TXSTDSC sample. It is possible that the cementum has been altered by taphonomic changes, like collagen leaching or apatite growth, that would not have had the time or environmental pressure to develop in a sample of teeth extracted from living individuals, like those used by Wedel (2007). These observations indicate the need for a greater understanding of the effects of chemical diagenesis within the time span of forensic significance. Since the decomposition process of the TXSTDSC sample is more representative of the context of a forensic case than teeth extracted from living individuals, it is suggested that future studies of this sort include analyzing the cementum using polarized microscopy with a λ plate.

<u>Luminosity Testing</u>

Finally, future research examining the utility of DCIA should involve the examination of cementum using digital images for luminosity testing in image analysis programs and exploration of the orientation of Sharpey's fibers using a scanning electron microscope and mircoradiographic analysis. These methods are frequently used by archaeologists studying mortality patterns in faunal remains and may help to obtain a better understanding of the differences in human band translucency and improve methods to identify clandestine banding within crowded cementum (Lieberman and Meadow, 1992; Lieberman, 1994).

Conclusion

This study was intended to validate the utility of DCIA to estimate season-of-death in the teeth of naturally decomposed skeletons. The study was based on methods described by Wedel (2007) who estimated the season-of-death with 99% accuracy in a sample of teeth extracted from living individuals. If season-of-death can accurately be estimated in a sample of known individuals who decomposed in a natural environment, then this method will greatly improve the estimation of PMI in forensic contexts.

However, the recent study by Ralston (2016) reported low accuracy in correlating the outer cementum band with the known season-of-death. Ralston used methods described by Wedel (2007) to prepare a sample of 143 teeth for dental cementum increment analysis, yet only achieved between 61.54% and 71.15% success with significant interobserver error. Ralston's results are in congruence with those obtained in this study of 24 individuals from a known date of death skeletal collection where, at most, 60% of the band translucency correlated with the actual season-of-death. It was determined that estimating the season-of-death based on the translucency of the outer cementum increment yielded results only slightly greater than those achieved by chance. That is, if one were to simply guess the color of the outer band, the results would be about the same.

Season-of-death was successfully estimated in a sample of wild deer and domesticated dogs with 100% accuracy in one observation. The results of this analysis lend confidence to the idea that dental cementum increments may have some correlation with season in humans as they do in non-human mammals, but the context of this relationship is unknown.

The broader impact of this study is that possible causal factors for seasonal effects in the dental cementum of human teeth can potentially be ruled out by the results of this investigation. The biological significance of dental cementum increments is poorly understood in non-human mammals and even less so in humans since the prevailing hypothesis requires seasonal variation in diet, which humans, and other domesticated mammals, lack (Lieberman, 1994; Cool et al., 2002; Ralston, 2016). Although it is difficult to estimate the season-of-death in humans, as this study shows, human teeth and those of domestic dogs do exhibit cementum increments of alternating birefringence. The presence of these increments, and the potential ability to estimate season-of-death in the remains of domestic dogs, indicates an underlying cause with greater influence than that of diet. Therefore, further research of this kind should consider investigating the impact of diet as it relates to incremental banding in dental cementum.

Furthermore, this study determined that the ability to identify the outer cementum band was not limited by stress-related factors leading up to death of the individual. However, it is possible that the lack of functionality for dental cementum when the root is no longer attached to the alveolar bone may disrupt the normal apposition of cementum. As alveolar resorption tends to increase with age, it is possible there is a relationship between the clarity of the outer band and periodontal disease associated with degeneration.

On the contrary, the lack of clarity may simply be due to the crowding of annulations later in life. A greater understanding of the effect of age on the clarity of the outer cementum band may be gained by investigating a larger sample of known individuals with a wider age range.

The results of this study and the previous study by Ralston (2016) imply that the understanding and replicability of DCIA for estimating season-of-death is still within its infancy. Forensic anthropologists who utilize DCIA in forensic case work should interpret the results with caution, especially if the estimated age at death of the unknown individual is over 50 years.

LITERATURE CITED

Aaron JA. 2002. Polarizing microscopy identification of chemical diagenesis in archaeological cementum. J Archaeol Sci 29:1327-1347.

Aggarwal P, Saxena S, Bansal P. 2008. Incremental lines in root cementum of human teeth: An approach to age estimation using polarized microscopy. Indian J Dent Res. 19(4): 326-330.

Aitkenhead-Peterson Ja, Alexander MB, Bytheway JA, Carter DO, Wescott DJ. 2015. Applications of soil chemistry in forensic entomology. In Tomberlin JK, Benbow ME (eds.). Forensic entomology: international dimensions and frontiers. Boca Raton: CRC Press, pp. 283-296.

Bates LN. 2014. Comparison of decomposition rates between autopsied and non-autopsied human remains in Central Texas [MA thesis]. San Marcos (TX): Texas State University. 66 p.

Bates LN, Wescott DJ. 2016. Comparison of decomposition rates between autopsied human remains. Forensic Sci Int. 261:93-100.

Beasley MJ, Brown WAB, Legge AJ. 1992. Incremental banding in dental cementum: Methods of preparation for teeth from archaeological sites and for modern comparative specimens. Int J Osteoarchaeol 2:37-50.

Bertrand B, Schug GR, Polet C, Naji S, Colard T. 2014. Age-at-death estimation of pathological individuals: A complementary approach using teeth cementum annulations. Int J Paleopathol 132:1-8.

Blondiaux J, Gabart N, Alduc-Le Bahousse A, Niel C, Tyler E. 2006. Relevance of cement annulations to paleopathology. Paleopathol Newsl 135: 4-13.

Bourque BJ, Morris K, Spiess A. 1978. Determining the season-of-death of mammal teeth from archaeological sites: a new sectioning technique. Science 199:530-531.

Bosshardt DD and Schroeder HH. 1996. Cementogenesis reviewed: A comparison between human premolars and rodent molars. Anat Rec 245:267-292.

Bosshardt DD and Selvig KA. 1997. Dental cementum: The dynamic tissue covering the root. Periodontol 13(1):41-75.

Broucker A, Colard T, Penel G, Blondiaux J, Stephan N. 2015. The impact of periodontal disease on cementochronology age estimation. Int J Paleo DOI: 10. 1016.

Carter DO, Yellowlees D, Tibbett M. 2007. Cadaver decomposition in terrestrial ecosystems. Naturwissenschaften 94:12-24.

Charles DK, Condon K, Cheverud JM, Buikstra JE. 1986. Cementum annulation and age determination in *Homo sapiens*. I. Tooth variability and observer Error. Am J Phys Anthropol 71:311-320.

Cobaugh KL, Schaeffer SM, DeBruyn JM. 2015. Functional and structural succession of soil microbial communities below decomposing human cadavers. PlosOne 10(6): e0130201.

Colard T, Bertrand B, Naji S, Delannoy Y, Becart A. 2015. Toward the adoption of cementochronology in forensic context. Int J Leg Med. DOI 10.1007/s00414-015-1172-8.

Condon K, Charles DK, Cheverud JM, Buikstra JE. 1986. Cementum annulation and age determination in *Homo sapiens.* II. Estimates and accuracy. Am J Phys Anthropol 71: 321-330.

Cool SM, Forwood MR, Campbell P, Bennett MB. 2002. Comparisons between bone and cementum compositions and the possible basis for their layered appearances. Bone 30(2): 386-392.

Gauthier J, Schutkowski H. 2013. Assessing the application of tooth cementum annulation relative to macroscopic aging techniques in an archaeological sample. Homo 64(1): 42-57.

Gocha TP, Schutkowski H. 2012. Tooth cementum annulation for estimation of age-at-death in thermally altered remains. J Forensic Sci 58(SI):151-155.

Gordon BC. 1988. Of men and reindeer herds in French Magdalenian Prehistory. Oxford: BAR International Series 390.

Grosskopf B, McGlynn G. 2011. Age diagnosis based on incremental lines in dental cementum: A critical reflection. Anthropol Anz 68(3):275-289.

Grue H, Jenson B. 1979. Review of the formation of incremental lines in tooth cementum of terrestrial mammals. Dan Rev Game Biol 11:1-48.

Guatelli-Steinberg D, Huffman M. 2011. Histological features of dental hard tissues and their utility in forensic anthropology. In: Crowder C, Stout S, editors. Bone histology: An anthropological perspective. Boca Raton: CRC Press. p 91-105.

Jankauskas R, Barakauskas S, Bojarun R. 2001. Incremental lines of dental cementum in biological age estimation. Homo 52(1):59-71.

Kagerer P, Grupe G. 2001. Age-at-death diagnosis and determination of life-history parameters by incremental lines in human dental cementum as an identification aid. Forensic Sci Int 118:75-82.

Klevezaal GA, Kleinenberg SE. 1967. Age determination of mammals by layered structures in teeth and bones. U.S.S.R.: Academy of Sciences. Translated 1969 from Russian by the Translation Bureau, Foreign Languages Division, Department of State of Canada. Quebec: Fisheries Research Board of Canada.

Klevezal GA, Shishlina NI. 2001. Assessment of the death of season of ancient human and cementum layers. J Archaeol Sci 28:481-486.

Kvaal SI, Solheim T. 1995. Incremental lines in human dental cementum in relation to age. Eur J Oral Sci 103:225-230.

Laws RM. 1952. A new method of age determination for mammals. Nature 169:972-973.

Lieberman DE. 1994. The biological basis for seasonal increments in dental cementum and their application to archaeological research. J Archaeol Sci 21:525-539.

Lieberman D, Meadow RH. 1992. The biology of cementum increments (an archaeological perspective). Mammal Rev 22:57-77.

Lipinsic FE, Paunovich E, Houston DG, Robison SF. 1986. Correlation of age and incremental lines in the cementum of human teeth. J Forensic Sci 31:982-989.

Lubinski PM, O'Brien CJ. 2001. Observations on seasonality and mortality from a recent catastrophic death assemblage. J Archaeol Sci 28:833-842.

Maat JR, Gerretsen RRR, Aarents MJ. 2006. Improving the visibility of tooth cementum annulations by the cutting angle of microscopic sections. Forensic Sci Int 159:95-99.

Meinl A, Huber CD, Tangl S, Gruber Gm, Teschler-Nicola M, Watzek G. 2008. Comparison of the validity of three dental methods for the estimation of age at death. Forensic Sci Int 178: 96-105.

Mitchell B. 1967. Growth layers in dental cementum for determining the age of red deer. J Anim Ecol 36(2): 279-293.

Morris ML. 1972. A study of the inductive properties of the organic matrix of dentin and cementum. J Periodontol 43(1):10-16.

Naji S, Colard T, Blondiaux J, Bertrand B, d'Incau E, Bocquet-Appel J. 2014. Cementochronology, to cut or not to cut. Int J Paleopathol doi:10.1016/j.ijpp.2014.05.003.

Naylor JW, Miller WG, Stokes GN, Stott GG. Cementum annulation enhancement: A technique for age determination in man. Am J Phys Anthropol 68: 197-200.

Ralston CE. 2016. Dental cementum increment analysis and estimating season-of-death in humans [MS thesis]. Boston (MA): Boston University School of Medicine. 84 p.

Renz H and Radlanski RJ. 2006. Incremental lines in cementum of human teeth- A reliable age marker? HOMO 57:29-50.

Roksandic M, Dejana V, Schillaci MA, Voicu D. 2009. Technical note: Applicability of tooth cementum annulation to an archaeological population. Am J Phys Anthropol 140: 583-588.

Pike-Tay A and Cosgrove R. 2002. From reindeer to wallaby: Recovering patterns of seasonality, mobility, and prey selection in the Paleolithic Old World. J Archaeol Method Th 9(2): 101-146.

Pilloud S. 2004. Can there be age determination on the basis of the dental cementum also in older individuals as a significant context between histological and real age determination. Anthropol Anz 62(2):231-239.

Saxon A, Higham C. 1969. A new research method for economic historians. Am Antiqu 303-311.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH image to ImageJ: 25 years of image analysis. Nature Methods 9:671-675.

Schug GR, Brandt ET, Lukacs JR. 2012. Cementum annulations, age estimation, and demographic dynamics in Mid-Holocene foragers of North India. Homo 63:94-109.

Solheim T. 1990. Dental cementum apposition as an indicator of age. Scand J Dent Res 98:510-519.

Spiess AE. 1976. Determining season-of-death of archaeological fauna by analysis of teeth. Artic 29(1):53-55.

Stallibrass S. 1982. The use of cement layers for absolute ageing of mammalian teeth: A selection review of the literature, with suggestions for future research and alternative applications. In: Wilson B, Grigson C, Payne S, editors. Aging and sexing animal bones from archaeological sites. Oxford: BAR British Series 109:109-126.

Stein TJ and Corocan JF, 1994. Pararadicular cementum deposition as a criterion for age estimation in human beings. Oral Surg Oral Med Oral Pathol 77(3): 266-270.

Stott GG, Sis RF, Levy BM. 1982. Cementum annulation as an age criterion in forensic dentistry. J Dent Res 61(6):814-817.

Tomberlin JK, Mohr R, Benbow ME, Tarone AM, VanLaerhoven S. 2011. A roadmap for bridging basic and applied research in forensic entomology. Annu Rev Entomol 56:401-421.

Ubelaker DH and Willey P. 1978. Complexity in Arikara mortuary practice. Plains Anthropol 23(79):69-74.

Wedel VL.2007. Determination of season-of-death using dental cementum increment analysis. J Forensic Sci 52(6):1-4.

Wikimedia Commons. Anatomy of a Tooth. Modified from https://commons.wikimedia.org/wiki/File:Periodontium.svg

Wittwer-Backhofen U, Gampe J, Vaupel JW. 2004. Tooth cementum annulation for age estimation: Results from a large known-age validation study. Am J Phys Anthropol 123:119-129.

Yamamoto T, Hasegawa T, Yamamoto T, Hongo H, Amizuka N. 2016. Histology of dental cementum: Its structure, function, and development. Jpn Dent Sci Rev 52:63-74.