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Aging an Ancient Maya Population from Actuncan, Belize using Dental X-Rays

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By

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Abstract

My goal is to determine an accurate age at death estimation of an ancient Maya population from the archaeological site of Actuncan, Belize. This was done by measuring the lengths of the coronal pulp cavities in the individuals' teeth. I used X-Ray images to measure the coronal pulp cavities of the teeth and estimated age using multiple regression formulae for premolars, molars, and incisors to make age estimations. The formulae came from two studies, Ikeda et al. (1985) and Drusini (2008), that form the basis of my research. Ikeda et al. (1985) was the first to use this aging method, while Drusini's (2008) research closely follows the Ikeda et al. (1985) method using a new population. The measurements on the Actuncan population were measured by myself, a peer Eden Irwin, and Dr. Carolyn Freiwald. The results were inconsistent, some that clearly were errors. These inconsistencies are largely due to lack of a tooth-wear scale to base the teeth on, third-party X-Rays, formulae from multiple articles, and measuring program sensitivity. Overall, the age estimations fell within the 20 - 50 year age range. This research is important because age-at-death is the basis for understanding ancient health and longevity in archaeological populations and for use in forensic applications.

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Chapter I: Introduction

It is very difficult to estimate age using forensic and archaeological methods. The first step to making an age estimate is to look at the individual's dental development. This means looking to see which teeth have developed and in what order they have erupted. When growth is complete, wear on their teeth and bones can provide an idea of an individual's age, but this is not a precise method of aging. Being able to determine an individual's age-at-death is crucial because it can help to identify missing persons and age archaeological populations. Learning this information gives us key insights about people's lived experiences such as their health, longevity, or natural disasters and diseases occurring at the time. This thesis applies an aging method to an archaeological sample using X-Ray technology on teeth. Teeth can be used as reliable biomarkers because they mature at predictable rates. X-Ray technology is the ideal method of imaging because it is non-invasive and cost-effective. This process has proven to be difficult, but it has provided new age estimates for an archaeological population.

Actuncan, Belize is a city located in the Upper Belize River Valley that was inhabited by the Maya people from 1000 BC - AD 900 (Figure 1). The Actuncan Archaeological Project discovered burials at the site from 2001-2019, but little is known about how long people lived or how healthy they were despite more than two decades of research on peoples' origins, diet, and burial patterns at the site. Forthcoming burial information from Carolyn Freiwald (forthcoming) reports that nine individuals were buried in graves at the site during the Preclassic (1000 BC-AD 250) and Early Classic (AD 250-600) periods…Late Classic period (AD 600-900) burials include at least eighteen individuals interred in occupied and unoccupied households.

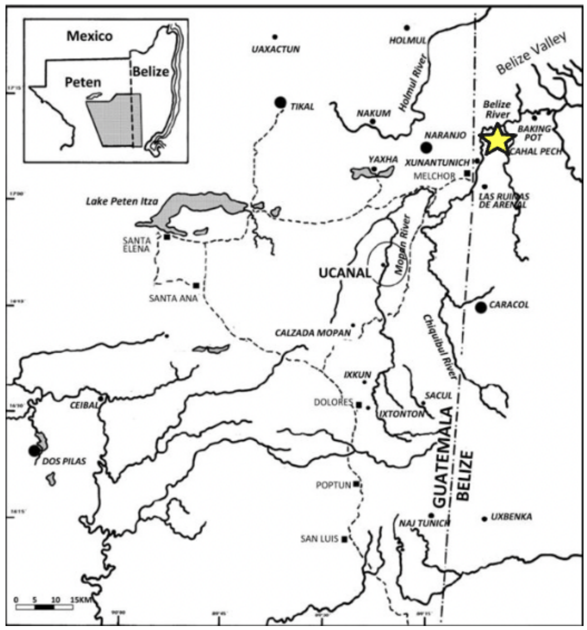

Figure 1 - Map of Archaeological Site in Actuncan, Belize.

This thesis focuses on using teeth as ways to make age estimations. There are two main imaging methods that can be used: X-Rays and CT-Scans. While both options are non-invasive, X-Rays are significantly easier to use and more efficient compared to CT-Scans that are precise but have specific software requirements. We took X-Rays of each of the teeth in the sample and measured certain components to make age estimations.

This thesis is composed of four main sections: the background, materials and methods, results, and discussion and conclusion. The background talks about the city of Actuncan, the sample population, the basics of tooth eruption, and the articles I used to base my research upon. The materials and methods section gives the sample along with information regarding how I measured the components of the teeth. The results section shows my results along with the results my peer received. The discussion and conclusion section compares my results to Freiwald's original osteological age estimates. This section also highlights the problems I faced and the things I would prefer to do differently in future research. In this thesis, I showed how this process works as well as the problems for a first-time user. We found the results to be inconsistent, but this method has potential. This type of research is essential because we will never learn more about past health and demography if we cannot find an accurate way to estimate age. We have to be able to compare ourselves to our ancestors, and we can't model ourselves if we are incapable of aging these people.

Chapter II: Background

This section describes the background for the project, including details about the sample population. This includes where they lived, how they were buried, a list of which teeth I examined, and isotopic analyses. I have also included figures that show which burials the teeth have come from, as well as information about general human tooth growth and development. Along with this information, I will also include summaries of the research studies I have used to base my research upon. This will entail the significance of each paper as well as their methods of study.

We want to know more about the ancient city of Actuncan, Belize, and background includes studies on how they lived and their diets. We used X-Ray and CT scan technology to examine their teeth in hopes of receiving information that will give us accurate age estimation data via measurement of various components of the teeth.

Actuncan was first settled during the Middle Preclassic period (1000 - 400 BC) and grew to a ceremonial and political center in the Late Preclassic period (400 BC - AD 250) (Fulton, 269). The city rose to political prominence during the Early Classic Period (AD 250 - 600). It was incorporated into another polity by the Late Classic period, later regaining its independence until it was eventually abandoned after AD 900.

The city of Actuncan is located in the upper portion of the Belize River Valley, neighboring the modern city San Jose de Succotz and the Guatemalan border. The site rests on an alluvial terrace west of the Mopan River, a tributary of the Belize River (Fulton, 268). The Mopan River is an important geographical feature, as it provided constant resources such as freshwater and dietary supplements, as well as transportation along the waterway of the Caribbean coast. Archaeologists believe that these neighborhoods played very important roles in the diversity and function of cities due to the social ties created between community members (Fulton, 267). These ties are likely the reason that the people of Actuncan made the conscious decision to not vacate their community when they began to face hardships.

Studying the human remains of the people buried at Actuncan can tell us a lot more about their lifestyles, health, diets, and how long they lived. Excavations during the years of 2001 and 2013 identified over eighteen burials at Actuncan. These excavations resulted in twenty-six, if not more, individuals within the age range of perinatal to middle-aged adult (Freiwald, Mixter, Billistrand, 95). The population found in the burial includes "...one perinate, two children, one juvenile, one adolescent, four probable adult males, one female, and four adults. Age estimates range from 1-3 years to >30 years based on dental development and attrition, with patterns that vary from unworn occlusal surfaces to complete crown loss" (Freiwald, Mixter, Billistrand, 97).

Dental analyses were performed on over two hundred teeth from individuals from this burial, showing evidence of hypoplastic activity on the teeth (Freiwald, Mixter, Billistrand, 97). Hypoplasias are defined as absent or thin enamel that causes a defect in the tooth. In fact, this analysis showed that the majority of the individuals have hypoplasias, and that seven individuals showed signs of multiple stress incidents (Freiwald, Mixter, Billistrand, 97). In fact, twenty-six percent of the sample (fifty-six teeth) have hypoplasias while eight percent (seventeen teeth) are characterized by having two or more defects in the enamel (Freiwald, Mixter, Billistrand, 97). Childhood health can impact longevity, so it may relate to age-at-death in the Actuncan population.

The burial population was uncovered in a variety of ways. Individuals were mostly buried in an extended body position and southern orientation, but other body positions such as seated were also reported. This could indicate status, although more data would be needed (whether it be biological, taphonomic, or contextual) in order to understand the true meaning behind each burial position (Freiwald, Mixter, Billistrand, 104). In Actuncan, the seated position is theorized to denote significance and importance of that individual, and it is also noticeably different from the standard burial practices associated with this site in the Late Classic period. This suggests that they are representative of an individual of significance (Freiwald, Mixter, Billistrand, 105). Components such as choice of burial location and body positioning can represent a certain lineage or claim of land in Actuncan (Freiwald, Mixter, Billistrand, 105). This information suggests that we can learn a lot about an individual based on where and how they were positioned in their burial when excavated.

Other research on the Actuncan population includes a study of diet and migration, both of which may relate to health and longevity. Isotopic ratios of different elements correspond to geographical location and others to the plants grown there. Both of these factors can influence health and how long people live.

Enamel begins growing while in utero and continues developing until the person is around twelve years old. Sampling enamel gives researchers an idea of nutrient intake during the early childhood years (Micklin, 22). Examining the development of the enamel also aids researchers in estimating the ages of the burial population.

In addition to this, two elements that are very important are strontium and oxygen. The ratio of strontium isotopes in the human body are important, especially those in the bones and dental enamel. It can indicate where the individual lived during their childhood and prior to death (Tiesler, 108). This information can provide confirmation of their area of residence. The element strontium is found within the landscape, and is therefore ingested within the food people eat. This would correspond to a linkage in dietary input and location. In addition, plants take up strontium due to rocks releasing it. Humans and animals ingest these plants, which then gives them corresponding strontium isotope values to the region they reside (Micklin, 23).

Oxygen is another important isotope; it can be ingested through drinking water and within food. It is found primarily in the two elemental forms of ^{18}O and ^{16}O. Oxygen isotope analysis is useful for gaining insight on migration patterns (Tiesler, 109). Examining the isotopes of these elements would give much needed insight into the diets of the population because it would show the different sources of water available to the population, whether it is ground water, ocean water, fresh water, etc.

Depending on the type of photosynthesis mechanism a plant uses, that plant will have different oxygen isotope concentrations. For example, a study found that some individuals ingested C4 plant isotopes (maize) while the majority of others had C3 plant isotopes (Micklin, 30). This statement proves that different levels of oxygen isotopes are consumed within plant sources, and it also gives researchers a better idea of a population's diet. This study found that those in the Belize River Valley region had other primary food sources other than maize compared to those in neighboring societies.

Teeth age in multiple different stages. Teeth begin as early as developing within the fetus. It is important that the mother's diet consists of sufficient levels of important components such as

Calcium, Phosphorus, Vitamin C, and Vitamin D to ensure proper tooth development. There are four main stages of tooth development, the first of which occurs at 6 weeks with the basic formation of tooth substance. The next stage consists of the formation of hard protective tissue around the tooth, at around 3 - 4 months. When the child is born, the next stage occurs when the tooth breaks the skin and erupts through the gums (John's Hopkins Medicine).

Primary (baby) teeth usually begin to erupt within six to twelve months, while the majority of these teeth will be completely in by the time the child is a year and a half. Generally, this process begins with the eruption of the lower central incisor, followed by the second central incisor. Next, the four upper incisors come in, the first four molars, and then the bottom two lateral incisors. After this, the cuspids appear, and then the last of the primary teeth (four second molars) erupt. Generally, the guideline is that the lower jaw teeth will erupt first, and then the corresponding upper dentition will erupt between one and two months later.

It is normal for children to start losing their primary teeth around age six, which are the central incisors. After this, the first permanent molars will erupt. Children continue to lose their deciduous teeth until around the age of twelve as the permanent set comes in. There are a total of thirty-two permanent teeth when the process is completed, with individual variation (i.e., extra or missing teeth) (Table 1).

Table 1 - Human Tooth Growth and Development Table. This chart shows the relative ages of tooth development, along with the different types of teeth.

Age Development Chart	
Perinatal (0)	
Infant (0-2)	Central Incisors (bottom) (6-10 months)
	Central Incisors (upper) (8-16 months)
	Four Lateral Incisors (13-19 months)
	Four Canines (16-23 months)
	Four Second Molars (25-33 months)
Early Childhood (3-6 years)	First Molars (6-7 years)
	Central Incisors (6-8 years)
Middle Childhood (7-10 years)	Lateral Incisors (7-8 years)
	Canine Teeth and Premolars (9-13 years)
Late Childhood (11-13 years)	Second Molars (11-13 years)
Adolescence (12-18 years)	
Young Adult (19+ years)	Wisdom Teeth (17-25 years)

There are four main parts of the tooth, which are the enamel, dentin, pulp, and root. The enamel is the outermost layer of the tooth, which protects the tooth from wear and tear. The dentin is the inner layer that makes up the majority of the tooth. The pulp is the soft tissue layer that contains the nerve, which supplies blood, and the capability to form dentin. Lastly, the root is what anchors the tooth to the jaw. First, the crown of the tooth forms from the enamel and dentin. Once the crown is complete, the root begins to form. At this point, the tooth will move toward the oral cavity, ensuring that it will erupt in the correct position (Colgate) (Figure 2).

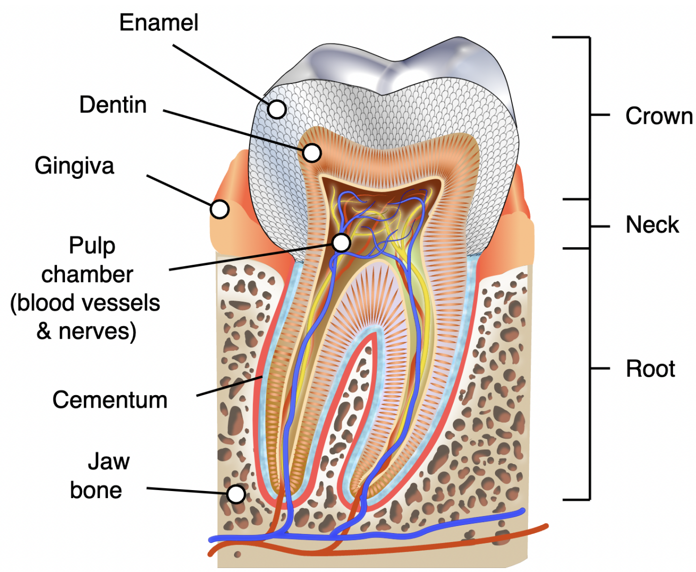

Figure 2 - General anatomy of a tooth. Figure 1 shows the anatomy of a molar at a vertical cross-section. (K. D. Schroeder)

As age increases and the tooth initial development is complete, there are a number of changes that occur in the dental pulp. These changes include decreased cell content, more fibrous appearance, and an overall decrease in volume of tooth. The surviving fibroblasts account for cell death by producing type 1 collagen fibers as opposed to type 2, so that a fibrous matrix is created. Fibroblasts are cells in connective tissue that produce collagen and other elastic fibers.

Type 1 collagen fibers are used for structural support and are tightly packed whereas type 2 fibers are associated with elasticity. Secondary dentin begins to form as the adult ages. In addition, any kind of stress to the tooth (injuries, cavities, etc.) can cause a tertiary dentin to form, each reducing the size of the pulp cavity. These are key factors, specifically the coronal pulp cavity size, in making age estimations.

Six studies analyze the coronal pulp cavities in human adult teeth to determine the person's age, including Drusini (2008), Ge et. al (2016), Ikeda et al. (1985), Karkhanis et al. (2013), Koranne et al. (2017), and Sasaki and Kondo (2013). These studies are important because they provided multiple methods of providing age estimations via teeth using different populations. This is important because it proved that the type of population did not affect the accuracy of the research. The studies also used a variety of different imaging methods. Two studies used intraoral periapical radiographs, and other sources such as microcomputed tomography scans, CBCT images (quantitative radiology), panoramic radiography, and regression analysis were used by other researchers. The teeth that these studies focused on using were the premolars and a variety of molars excluding the wisdom teeth. Most of the studies used premolars and the first mandibular molar, while some used only the first and second molars. Despite different methods, they are important to mention because they each concluded that the method of using teeth for age estimation works. These studies provided the groundwork for my research, as I was able to decide which methods from these studies to impart on my own work.

The study by Ikeda et al. (1985) is the first to describe the method in which I am using for determining the age at death of my population. This study is also the basis of research in which the Drusini (2008) study was modeled on. In summary, the study's population consisted of one hundred and sixteen human teeth, fifty-three incisors and sixty-three molars. To analyze these teeth, X-ray images were taken. Once the X-rays were received, the researchers calculated the tooth-coronal indexes by measuring the coronal pulp cavities and coronas of the teeth. The formula that they used to find the tooth-coronal index is shown below (Figure 3).

$$\text{tooth-coronal index} = \frac{\text{length of coronal pulp cavity} \times 100}{\text{length of the crown}}$$

Figure 3 - Formula for Finding the Tooth-Coronal Index using the lengths of the coronal pulp cavities and crowns. (Ikeda et al., 1985)

Furthermore, the Ikeda et al. (1985) study found that there was a correlation between the tooth-coronal index and age. As age increased, the tooth-coronal index decreased. To make age estimations, the researchers formed two equations specific for both incisors and molars. For incisors, they used the formula Y = -1.41X + 108.22 (± 6.06 years). For molars, they used the formula Y = -2.09X + 104.32 (± 5.73 years). In these equations, the X indicates the tooth-coronal index and the Y is the estimated age of the individual (Ikeda et al. 1985).

A follow-up study by Drusini (2008) consisted of sampling 846 human teeth from 433 individuals of known age and sex through the use of panoramic radiography. This study involved a combination of ideas from many other studies, including Bodecker (1925) and Ikeda et al. (1985). The research performed by Bodecker (1925) revealed that the positioning of the secondary dentine in a tooth can correlate to age. After the tooth crown is fully developed, the secondary dentine will begin to form. Dentine constantly forms throughout our lives, and the secondary dentine is set via odontoblasts in the pulp chamber. Odontoblasts are characterized as the cells in the pulp chamber that produce dentin. As the secondary dentine is continuously added onto the primary dentine, this negatively affects the size of the pulp cavity. Essentially, as we age, the amount of secondary dentine that forms will increase. This increase negatively correlates with the coronal pulp cavity, meaning that the coronal pulp cavity is intended to decrease as humans age. With this idea in mind, the researchers studied the lengths of the crowns (in mm), known as coronal length (CL) and the length (in mm) of the coronal pulp cavity length (CPCL). The tooth coronal index (TCI) was computed based on the research of the study by Ikeda et al (1985). The amount of secondary dentine formation is different depending on the type of tooth being analyzed. Because of the varying tooth types, the secondary dentine will be in higher concentrations in different areas of the teeth. For example, in molars, the floor of the pulp chamber has a higher dentine composition when compared to the lateral and occlusal walls (Drusini 2008). This indicates that there will be differences in this method of aging teeth, as there are significant possibilities of variation. Along with this, it also has to be considered that if the individual has undergone trauma, they may have developed an irregular dentine level. These changes in the structure of dentine in the tooth can be from nonconsistent odontoblasts. Nonconsistent odontoblast populations indicate them dying due to things like harmful or noxious stimuli, temperature conditions, or blunt trauma (Drusini 2008). The cells that survive will continue to produce dentin while some are susceptible to trauma, resulting in an irregular layer of dentin.

This research sample consisted of 425 premolars and 421 molars from 433 Caucasian individuals. This population consisted of 213 males and 220 females, all of known sex and age. The ages of the individuals within this population ranged anywhere from nine to seventy-six years. Panoramic X-ray technology was used, which is when the X-ray device floats around the individual to receive the photograph. There are many advantages to using the Panoramic X-ray method, and that is because the teeth can remain intact and the process is efficient in terms of time. Another advantage to using this method is that all of the teeth (both mandibular and maxillary) can be viewed at once. With this, any varieties in the teeth (damage, missing teeth, interrupted growth, etc.) can be visualized as well. The areas measured in the molars and premolars used in this research are shown in Figure 4.

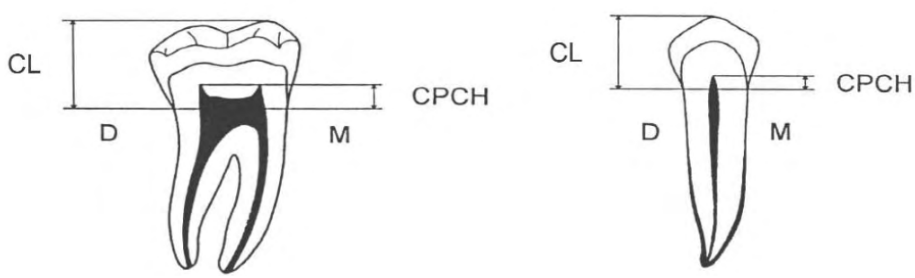

Figure 4 - Differences in the areas of the coronal pulp cavity in premolars and molars. This is what this study used as a basis for measurement. (Drusini, 2008: page 239, Figure 1)

As an individual faces more trauma and/or ages, the amount of coronal pulp cavity remaining for analysis decreases. To begin the age analyses, the researchers began with finding the tooth-coronal index. The formula that they used is below (Figure 5).

$$\text{tooth-crown index} = \frac{\text{enamel area} + \text{area of coronal pulp cavity} \times 100}{\text{area of coronal dentine}}$$

Figure 5 - Formula for Finding the Tooth-Crown Index using the areas of the enamel, coronal pulp cavity, and coronal dentine (Drusini, 2008)

After measuring tooth-crown indexes, the researchers moved on to finding the tooth-coronal index. This formula was inspired by the study done by Ikeda et al. (1985). Once the tooth-coronal indexes were found for this population, the researchers followed the work of Ikeda et al. (1985) and created regression equations for premolars and molars that would estimate the ages of the individuals. The equations are divided into combined samples, males, and females. For premolars, the equation for the combined sample is Y = 77.617 - 1.4636X. The male-specific equation is Y = 79.679 - 1.5356X. The female-specific equation is Y = 75.523 - 1.3896X. For molars, the combined sample equation used is Y = 76.073 - 1.4576X, the male equation is Y = 77.747 - 1.5066X, and the female equation is Y = 73.846 - 1.3906X (Drusini 2008). Like the equations used in the Ikeda et al. (1985) study, the X is indicative of the tooth-coronal index, and the Y is the age estimate.

The study found that the results showed a significant correspondence with the length of the coronal pulp cavity with chronological age. The research ultimately resulted in finding a ± 5 year accuracy in determining the ages of the male individuals. A very interesting aspect of this study is that the sexes of the individuals were taken into account. This study proved that there were no overarching correlations between age determination and sex, resulting in the idea that the use of sex-specific equations were not necessary. Overall, this study shows that the formulae work on multiple populations and additional teeth may be used. It also heavily supported the findings of the Ikeda et al. (1985) study. It used an entirely different population and slightly different methods that further promoted the technique of measuring coronal pulp cavities to find the tooth-coronal indexes, which can result in accurate age estimations.

Another study aiming to test the reliability in age estimation via measuring the coronal pulp cavity index method is the research led by Karkhanis et al. (2013). This study's aim was to use the methods used in the Drusini (2008) research on a different population. So, the researchers sampled 450 individuals from a Western Australian population. This sample consisted of 220 females and 230 males. They used left and right mandibular premolars, as well as first and second molars. Again, this study followed the Drusini method and used orthopantomograms to measure each of the individuals' teeth. Once the panoramic X-Rays were received, they were imported into the ImageJ imaging software to collect data. It was here that they measured the crown heights, which was a vertical length beginning at the cervical margin and up to the highest cusp (Karkhanis et al. 2013). Next, the coronal pulp cavity was measured, and the tooth-coronal index was calculated once the information prior was received.

This data was compiled using bilateral asymmetry, linear correlation, and simple linear regression models. The most telling component is the linear correlation, as the study found that there is again a negative correlation between chronological age and TCI. These results support the studies of Ikeda et al (1985) and Drusini (2008). This research found that the most accurate age estimations came from the right first molar, with a standard error of ± 8.271 years and the left first molar which had a standard error of ± 8.337 years. They also found the standard errors for sex-specific teeth, whereas the standard error for females ranged from ± 8.062 years (left second molar) to ± 10.344 years (left first premolar). The standard error for males ranged from ± 8.212 years (right first molar) and ± 8.245 years (combined TCI scores of left and right first molars). Overall, this study was congruent with the Drusini (2008) research because it found that the best tooth to use for the most accurate age estimations was the right first molar. This was the first study to be completed on a Western Australian population, showing that this method can be applied across any community despite location. This research also agreed that the TCI method of age estimation is suitable for biological and forensic applications.

The study led by Ge et al. (2016) made an effort to evaluate which types of teeth demonstrated the strongest relationship between pulp cavity volume and age as well as explore if the addition of teeth in the same stage of dentition would affect the accuracy of age estimation (Ge et al. 2016). The sample population in this research consisted of 240 subjects with confirmed age via the Peking University Hospital of Stomatology in Beijing, China. In contrast to the prior studies, the method of research consisted of using cone beam computed tomography (CBCT) images. This study used thirteen tooth types, which consisted of both single and multi-rooted teeth. The single root category is comprised of maxillary central and lateral incisors, maxillary canines, maxillary second premolars, mandibular central and lateral incisors, mandibular canines, and mandibular first and second premolars. The multi-rooted tooth category consists of both maxillary and mandibular first and second molars (Ge et al. 2016). When choosing which teeth to perform analyses on, teeth that passed all of the categories of the tooth-wear index (TWI) were carefully selected.

For single-rooted teeth, the volume of the tooth pulp cavity was calculated. To avoid the influence of the root systems in the multi-rooted teeth, the researchers implemented a cut plane on the pulp chamber floor. They then cut off the roots to calculate the volumes of the tooth pulp chambers (Ge et al. 2016). This study used the computer software ITK-SNAP 2.4 in order to gain the volume measurements of the pulp cavities and chambers. To test the accuracy of their findings, the study used descriptive statistics of the pulp cavity and chamber volumes for each of the thirteen tooth types. Paired t tests were used to determine the inter- and intra-observer variabilities, which states that a p value that is equal to or less than 0.05 would be considered significant (Ge et al. 2016). Logarithmic regression analysis was another statistical method used to establish correlations between age and pulp cavity/chamber volumes which gave rise to the coefficient of determination. The research used the coefficient of determination (R^2) from the regression analyses to calculate the relationship between chronological age and pulp cavity and pulp chamber volumes (Ge et al. 2016). Once the R^2 value was obtained, the researchers were able to calculate the standard errors of the analyses, which showed the level of accuracy in the study. The analyses showed that twelve out of the thirteen tooth types were statistically significant for both genders. The one exclusion is the mandibular first molars, with the only p value that was greater than 0.05. The multiple linear regression models suggested high R^2 values and small standard error values for both males and females. Along with this, the research deemed that there were no significant differences found for inter- and intra-observer error (Ge et al. 2016). This study theorizes that maxillary second molars are the best tooth type to use for accurate age estimation, while maxillary canines are the worst. There are many reasons thought to support this. For example, the main functions of molars vs. canines, the amount of dentition apposition in the teeth, and the anatomy of the pulp chambers in booth tooth types are taken into consideration.

This research study is very helpful, as it sampled a different population and found that using multiple types of teeth can potentially improve the accuracy of age estimation compared to only using one type of tooth (Ge et al. 2016). This study is also different in the fact that the researchers chose to use micro-CT and CBCT images rather than panoramic X-rays. They chose this route due to the belief that micro-CT and CBCT images provide more precise visualizations of the teeth. This study also contradicts the Drusini study in the fact that it recommends the use of sex-specific equations due to the gender differences in the pulp cavities and chambers. The study decided that this method of estimation was reasonably accurate for determining the age of individuals.

The study performed by Koranne et al. (2017) aimed to study the accuracy of the TCI method of age estimation. The sample consisted of mandibular second premolars and first molars from an adult Indian population. These specific teeth were selected for research for a variety of reasons including the radiographic resolution differences, morphological attributes, dimensions available for measurements, and a higher likelihood of these teeth being more intact compared to others. Mandibular teeth have a higher resolution compared to maxillary ones due to their locations in the mouth. The third molars have been known to differ substantially in morphology and position, making the second molars the ideal candidates for sampling. The mandibular second molar also has a much smaller size, making the measuring process significantly easier. When compared to the first molar, the second molar has larger attributes (crown, coronal pulp chamber, roots). This concludes that the second molar has the most defined, yet smaller and most consistent components. These necessities make the second molar the best contender when selecting an ideal tooth for research.

The method of imaging used was intraoral periapical radiographs. The study states that each of the individuals involved belonged to the age group between twenty and sixty years (Koranne et al. 2017). The research began by measuring the cervical lines, crown heights/lengths, and pulp heights of 400 individuals' premolars and molars. From these measurements, they were able to compute the tooth coronal indexes. Next, the study used SPSS (Statistical Package for Social Sciences) and Pearson's correlation coefficient to determine the correlation overlapping between TCI and the ages of the individuals as well as to find the interobserver error. Paired t-tests were used to find the intraobserver reliability (Koranne et al. 2017). The statistical analyses revealed that the interobserver reliability were relatively high for CPCH, CL, and TCI measurements. The intraobserver reliability showed the same. The researchers derived regression analysis equations for each of the samples, which were $Y = 78.63 - 0.9666*X$ (combined sample), $Y = 69.72 - 0.7425*X$ (male sample), and $Y = 78.15 - 0.9779*X$ (female sample) (Koranne et al. 2017). In each of these equations, the Y represents the age in years while the X represents the TCI. This research supports those mentioned prior, in that it agreed that there is a negative correlation between TCI and age in years. This study is important because the researchers created their own regression formulae specific for the Indian adult population. It is different because it used intraoral periapical radiographs as an imaging method rather than orthopantomographs. It also used a different tooth to focus on compared to the other studies. Contradicting the works of Ge et al. (2016), this study supports the research completed by Ikeda et al. (1985), Drusini (2008), and Karkhanis et al. (2013) because it agrees that sex does not play a role in aging the individuals via the tooth coronal index measuring method. These researchers concluded that TCI was a precise, noninvasive, quick, and inexpensive method for age estimation, with an accuracy of ± 9 months.

The study led by Sasaki and Kondo (2013) used a Bayesian approach to estimate the ages of individuals in a Japanese population by examining the pulp volume ratio from lower-canine teeth. They calculated the probability density distributions of the estimated ages using the Bayes' theorem (Sasaki and Kondo 2013). The Bayes' theorem states that the probability of being a certain age *A* conditional on the age indicator being a certain value (or state) *I* is dependent on the age distribution, but the probability of being *I* is conditional on being *A* (Sasaki and Kondo 2013). In this study, the pulp volume ratio is represented by *I* (age indicator) and age range is represented by *A* (Sasaki and Kondo (2013). The researchers opted to strictly use mandibular canines in this study, due to the fact that canines are not a popular tooth selected for the age estimation processes. The canines selected depended on the caries or damages to the teeth, and the canine in the best condition was used in the sample. The sample consisted of 363 individuals' canines. The study used microfocal CT scans to visualize the volumes of the pulp cavities in the teeth (Sasaki and Kondo 2013). Since canines are morphologically very different from the typical teeth used in age estimation research, the study concluded new methods of measuring the volumes of the pulp cavities. First, they decided on a longitudinal axis which bisects the major part of the root. From there, a horizontal plane was placed to segment the tooth into coronal and root portions. The horizontal plane was placed at the height of the tallest apical point of the cementoenamel junction (Sasaki and Kondo 2013). Since the coronal portions of the teeth are more susceptible to traumas and wear, only the root portions were used for further analyses in an attempt to eliminate bias. Next, the pulp cavity volumes were measured by counting voxels and imputed into the PVRrt ratio. The PVRrt is the volume of the pulp cavity divided by the volume of the root (Sasaki and Kondo 2013). This gave the researchers the volumes of the pulp cavities within the root portions of the teeth. The PVRrt values were then used in the Bayes' theorem.

In general, the study found a negative correlation between age and PVRrt values. The study could not find any advantages to using this method over the TCI method, but it did provide a lot of insights on aging females. The male sample received an error of up to ten years, making it unsuitable for precise estimations (Sasaki and Kondo 2013). On the other hand, females typically have a longer lifespan than males. This means that most of the elderly population is female. Once an individual reaches fifty years of age, the compositions of their teeth are less likely to deviate. This is an advantage of using the PVRrt method for aging in a female population because it has the capability of being much more precise compared to a male population. This study is important because it used different teeth than the studies prior. It provided more information to the forensic and archaeological communities on age estimation by using a new method and a tooth with different anatomical structure. This study deemed PVRrt as a useful method for age indication in a female population. It also decided that the PVRrt method does not have a higher accuracy compared to other methods such as the TCI method.

Chapter III: Materials & Methods

To estimate ages for the burial population of Actuncan, Belize, we used two imaging techniques: X-Ray and CT-Scan technologies. X-Rays were performed at the University of Wisconsin-Madison School of Veterinary Medicine under the supervision of Jason Soukup, DVM by Kate Kudrna in October 2022. CT scans are in process at the UW-Madison School of Medicine.

X-Ray imaging works by heating a pair of electrodes (cathode and anode). When the cathode is heated, it scatters electrons. The anode pulls the electrons across the tube and generates energy. The electrons collide with Tungsten molecules in the anode and knock loose an electron in a lower orbital. An electron in a higher orbital then falls down to replace the missing electron, releasing energy in the form of a photon. The atoms in our bodies absorb photons at different levels. Larger atoms have a greater capability of absorption rather than smaller ones. Our bones are made of Calcium (a very large atom), so our bones absorb photons well and reflect white on X-Ray images. This is in contrast to lung tissue that is composed of many small ions, which reflects gray on X-Ray images.

Computerized tomography (CT) scans use a combination of X-Ray images that are taken from multiple angles. These images are put into a software that creates cross-sectional images of the part of the body you are examining. The ability to be able to observe the subject from multiple angles allows for CT scans to be much more precise than X-Rays. Due to not having a software suitable to process the CT scans, we opted to focus on X-Rays for tooth analysis.

The sample consists of twenty-four human teeth from the Actuncan, Belize population. The teeth are a mixture of five incisors, three canines, five premolars, ten molars, and an unidentified tooth. Molars were selected for X-Ray imaging and incisors for CT scans. Samples were selected at the UW-Madison Zoological Museum by Carolyn Freiwald and Sarah Traynor, preferentially choosing single rooted teeth for CT scans and molars for X-Rays where possible (Table 2).

Table 2 - Actuncan, Belize burial sample. This chart indicates the burials and individuals, along with sex, age, and the tooth we used. The teeth are also identified using the ADA (American Dental Association) naming system. Ages were estimated by Freiwald.

Burial and Individual	Sex	Age	Research ID	Tooth ID	ADA ID
Op. 18I		none	A9	LLP4	21
Bone 4 Op. 18Z		none	A10	ULI2	10
Op. 50C		none	A6	LRM2	31
Op. 50C		none	A7	LRM2	31
Op. 5A		none	A3	ULC	11
Op. 5A		none	A4	URM2	2
Op. 5A		none	A5	ULC	11
Op. 56A		none	A2	LLP4	
Op. 56A		none	A1	LRM1	30
Op. 1D		none	A8	ULC	11
Op. 1C		none	A14	LLM3	32
Op. 1C		none	A13	URP3	
Op. 1C		none	A12	LLM2	18
Op. 50C		none	A11	LI	
Op. 12	F	Ao	A17	ULP4	
Op. 12	M	Mad	A20	LLM3	17
Op. 12	F	Y-Mad	A16	URM2	2
Op. 12	M	Mad	A21		
Op. 12	M	Oad	A25	LLI1	24
Op. 18HS		none	A24	M	

Op. 18H3		none	A26	LRP4	
Op. 18B		none	A27	LI	
Op. H5		none	A22	LLM2	18
Op. 24		none	A23	URI2	7
Op. G6, H7, I6		Yad-Mad	?		

The categories used to estimate age in the original research are very broad, following Buikstra and Ubelaker (1994: 9):

F = Fetal (<birth)
I = Infants (b - 3 years)
C = Children (3 - 12 years)
AO = Adolescents (12 - 20 years)
YAd = Young Adults (20 - 35 years)
MAd = Middle Adults (35 - 50 years)
OAd = Old Adults (50+ years)

Once X-Rays came back, I used the computer program InkScape to measure the lengths of the coronal pulp cavities and coronas of each tooth. The coronal pulp cavity is measured vertically from the cervical line of the tooth to the pulp horn with the highest peak (Doni, Patil, Agrawal, et. al). The corona is the length of the coronal pulp cavity to the top of the tooth (Figure 3). Using the measurements I found, I divided the length of the coronal pulp cavity by the length of the corona and multiplied this value by 100 to get the tooth-coronal index. Once I received the tooth-coronal index, I used the following regression formulae from the Ikeda et al. (1985) study for incisors ($Y = -1.41X + 108.22 \pm 6.066$) and molars ($Y = -2.09X + 104.34 \pm 5.73$). The “x” value represents the TCI to find an age estimate of the individual.

The regression formulae used were created from a Japanese population on a new White Italian population (both of which are of known ages) and archaeological samples (which are of unknown ages) (Ikeda et al. 1985). It was found that the age estimates for the known age populations were accurate, indicating that the age regression formulas are not required to be specific to that population. With this information, we know that the formulae have the potential to be accurate in aging our unknown population (Figure 6).

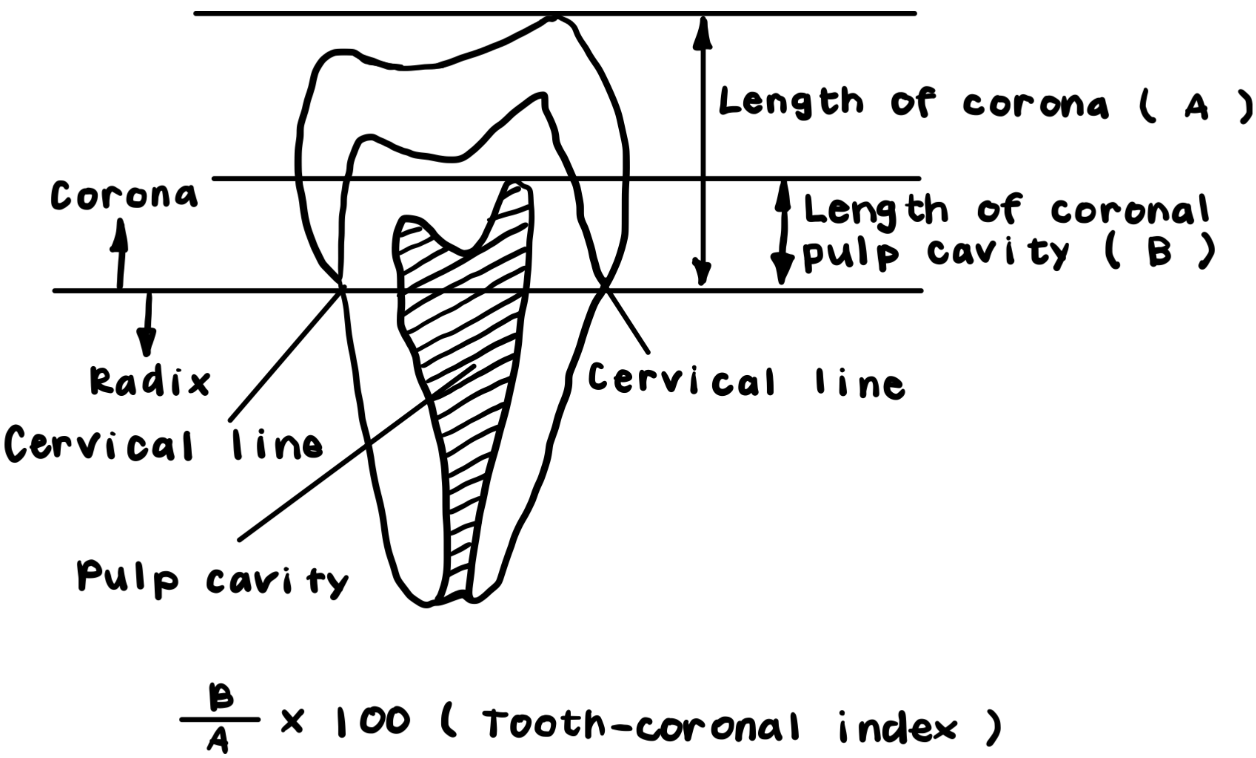

Figure 6 - Diagram showing the areas of measurement used for molar teeth. Length of Corona (A) and Length of Coronal Pulp Cavity (B) are both used to calculate the tooth-coronal index. (Ikeda et al. 1985: page 248, Figure 3)

In addition to using the Ikeda et al. (1985) method, I also used the Drusini (2008) method. The Drusini (2008) method provided both molar (Y = 76.073 - 1.4576X) and premolar (Y = 77.617 - 1.4636X) formulae. I used the same process as I did with the Ikeda et al. (1985) method by measuring the lengths of the corona and coronal pulp cavities. The difference in the methodologies of the Drusini (2008) and the Ikeda et al. (1985) research is that the Drusini (2008) focused on molars and premolars rather than molars and incisors.

This method of age estimation has not been widely researched, which is why I chose to base my research on it. I find it fascinating that something as small as our teeth can be used as an accurate tool for aging. This method can be used for both living and skeletal samples, making it uniform no matter the circumstances of the population.

Chapter IV: Results

My sample consisted of twenty-four teeth - ten molars, five premolars, three canines, five incisors, and one unidentified tooth. I was only able to estimate the age of individuals with molars, premolars, and incisors based on published formulae (Drusini 2008; Ikeda et al. 1985), resulting in age estimations for nineteen teeth. The Ikeda et al. (1985) study sample consisted of molars and incisors, while the Drusini (2008) sample consisted of premolars and molars. With both studies having different formulae associated with tooth-type, I focused my research on molars, premolars, and incisors.

Using the Ikeda et al. (1985) method, I measured the lengths of the coronas (crowns) and pulp cavities of each of my molars and incisors (Figures 7, 8).

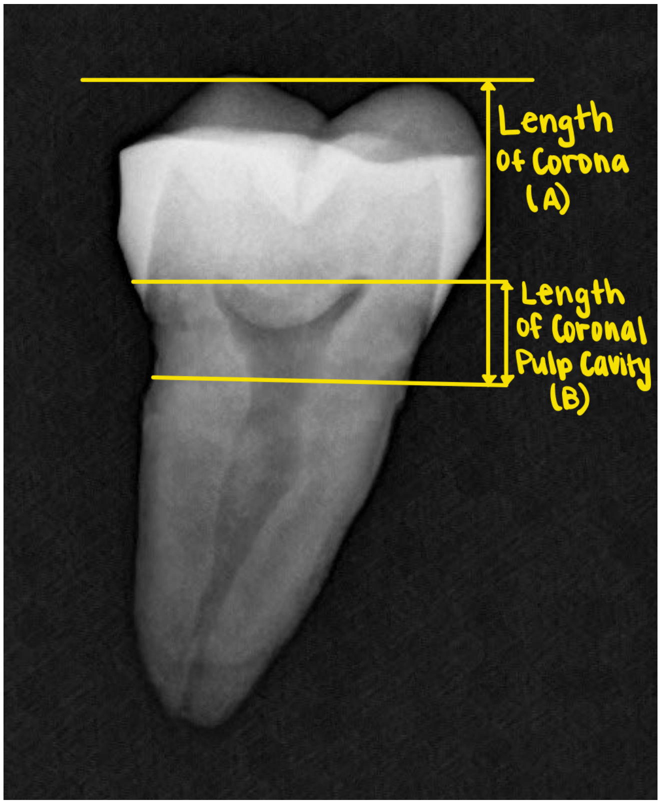

Figure 7 - My measurement method for molars. This is from individual B1, depicted as A12 - LLM2. This indicates that this is a mandibular second molar (ADA ID: 18).

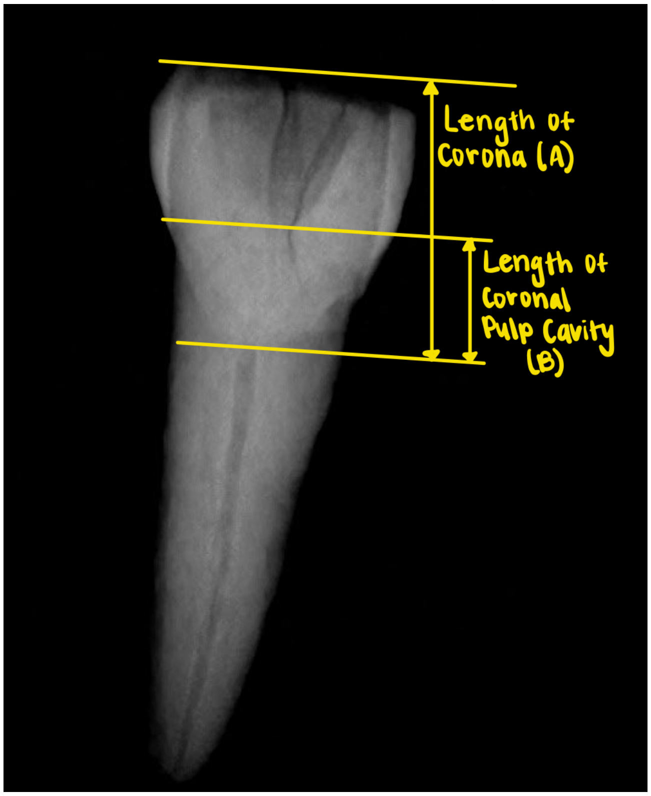

Figure 8 - My measurement method for incisors. This tooth is from Bone 4 Bag 54, depicted as A10 - ULI2. This indicates that this tooth is a maxillary second incisor (ADA ID: 10).

For molars, I used the formula $Y = -2.09X + 104.32 \pm 5.73$, where the X represents the tooth coronal index and the Y represents the estimated age. For incisors, the equation I used was $Y = -1.41X + 108.22 \pm 6.066$. The age estimations I computed for each of the molars and incisors in my sample are listed in the chart (Table 3).

Table 3 - Age estimations based on the Ikeda et al. (1985) method. Those bolded were used in analysis.

Tooth	Length of Coronal Pulp Cavity (B)	Length of Corona (A)	Tooth-coronal Index Formula, B/A	Tooth-coronal Index (B/A)*100	Age
A1 LRM1	18.38	25.83	0.7115756872	71.15756872	-44.40
A2 LLP4	14.81	30.25	0.4895867769	48.95867769	
A3 ULC					
A4 URM2	11.92	35.81	0.332867914	33.2867914	34.75
A5 ULC					
A6 LRP4	13.32	44.87	0.2968575886	29.68575886	
A7 LRM2	12.1	31.34	0.3860880664	38.60880664	23.63
A8 ULC					
A9 LLP4	12.07	24.92	0.4843499197	48.43499197	
A10 ULI2	10.95	31.97	0.3425086018	34.25086018	59.93
A11 LI	8.64	22.51	0.3838294092	38.38294092	54.10
A12 LLM2	8.13	26.78	0.3035847647	30.35847647	40.87
A13 URP3	10.54	20.76	0.5077071291	50.77071291	
A14 LLM3	7.75	21	0.369047619	36.9047619	27.19
A15 unidentified					
A16 URM2	12.82	30.95	0.4142164782	41.42164782	17.75
A17 ULP4	11.15	35.99	0.3098082801	30.98082801	
A18 unidentified					
A19 unidentified					
A20 LLM3	12.33	26.28	0.4691780822	46.91780822	6.26
A21 unidentified					
A22 LLM2	8.87	25.37	0.349625542	34.9625542	31.25
A23 URI2	7.71	23.36	0.3300513699	33.00513699	61.68

A24 M	8.77	30.95	0.2833602585	28.33602585	45.10
A25 LL12					
A26 LRP4	12.77	27.74	0.4603460707	46.03460707	
A27 LI	6.98	27.71	0.2518946229	25.18946229	72.70

Drusini (2008) provided updated formulae for this study that consisted of premolars and molars. I applied this methodology to my sample. The measurement process of finding the lengths of the coronas and pulp cavities were based on the Ikeda et al. (1985) method (Figure 9).

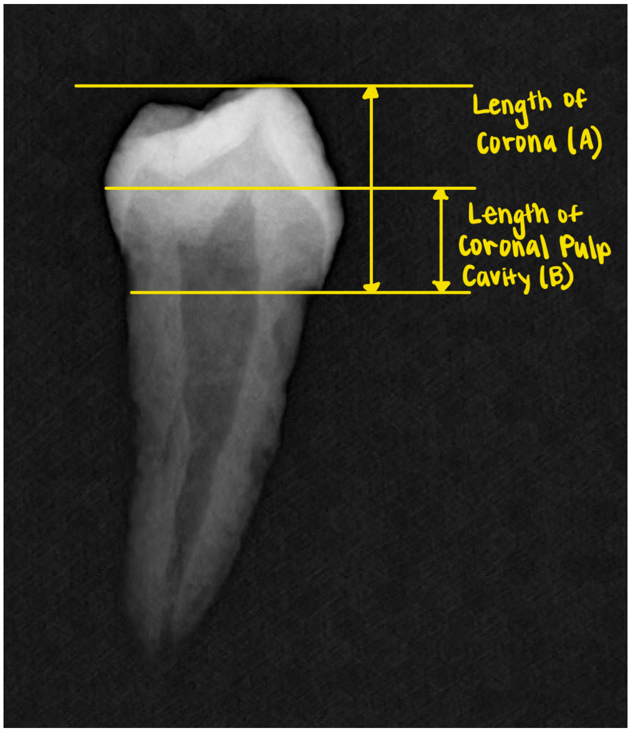

Figure 9 - My measurement method for premolars. This tooth is from an unknown individual, depicted as A26 - LRP4. This is a mandibular second premolar (ADA ID: 29)

My results were different following the Drusini (2008) methodology reflecting the different equations used in this study (Table 4). The molar equation formulated by the Drusini (2008) study is as follows: Y = 76.073 - 1.4576X where X represents the tooth coronal index and

Y represents the chronological age. The premolar equation is Y = 77.617 - 1.4636X where X and Y both represent the same values as mentioned in the molar equation.

Table 4 - Chronological age estimations using the Drusini (2008) method. Teeth in bold were used in analysis.

Tooth	Length of Coronal Pulp Cavity (B)	Length of Corona (A)	Tooth-coronal Index Formula, B/A	Tooth-coronal Index (B/A)*100	Age
A1 LRM1	18.38	25.83	0.7115756872	71.15756872	-27.52
A2 LLP4	14.81	30.25	0.4895867769	48.95867769	5.96
A3 ULC					
A4 URM2	11.92	35.81	0.332867914	33.2867914	27.55
A5 ULC					
A6 LRP4	13.32	44.87	0.2968575886	29.68575886	34.17
A7 LRM2	12.1	31.34	0.3860880664	38.60880664	19.80
A8 ULC					
A9 LLP4	12.07	24.92	0.4843499197	48.43499197	6.73
A10 ULI2	10.95	31.97	0.3425086018	34.25086018	
A11 LI	8.64	22.51	0.3838294092	38.38294092	
A12 LLM2	8.13	26.78	0.3035847647	30.35847647	31.82
A13 URP3	10.54	20.76	0.5077071291	50.77071291	3.31
A14 LLM3	7.75	21	0.369047619	36.9047619	22.28
A15 unidentified					
A16 URM2	12.82	30.95	0.4142164782	41.42164782	15.70
A17 ULP4	11.15	35.99	0.3098082801	30.98082801	32.27
A18 unidentified					
A19 unidentified					
A20 LLM3	12.33	26.28	0.4691780822	46.91780822	7.69
A21					

unidentified					
A22 LLM2	8.87	25.37	0.349625542	34.9625542	25.11
A23 URI2	7.71	23.36	0.3300513699	33.00513699	
A24 M	8.77	30.95	0.2833602585	28.33602585	34.77
A25 LL12					
A26 LRP4	12.77	27.74	0.4603460707	46.03460707	10.24
A27 LI	6.98	27.71	0.2518946229	25.18946229	

A peer, Eden Irwin, worked on a similar project and also calculated age for Actuncan individuals using the same methods for an interobserver comparison (Table 5).

Table 5 - Irwin's Age Estimations using the Ikeda et al. (1985) method.

Tooth	Length of Coronal Pulp Cavity (B)	Length of Corona (A)	Tooth-coronal Index Formula, B/A	Tooth-coronal Index (B/A)*100	Age
A1 LRM1	39	137	0.2846715328	28.46715328	44.82
A2 LLP4					
A3 ULC					
A4 URM2	38	164	0.2317073171	23.17073171	55.89
A5 ULC					
A6 LRP4					
A7 LRM2	43	142	0.3028169014	30.28169014	41.03
A8 ULC					
A9 LLP4					
A10 ULI2	68	161	0.4223602484	42.23602484	48.67
A11 LI	50	130	0.3846153846	38.46153846	53.99
A12 LLM2	30	107	0.2803738318	28.03738318	45.72
A13 URP3					
A14 LLM3	22	104	0.2115384615	21.15384615	60.12
A15 unidentified					
A16 URM2	45	140	0.3214285714	32.14285714	37.14
A17 ULP4					
A18 unidentified					
A19 unidentified					
A20 LLM3	35	130	0.2692307692	26.92307692	48.05
	58	200	0.29	29	43.71
A21 unidentified					
A22 LLM2	23	147	0.156462585	15.6462585	71.62

	21	136	0.1544117647	15.44117647	72.05
A23 URI2	31	111	0.2792792793	27.92792793	68.84
A24 M	36	122	0.2950819672	29.50819672	42.65
	26	111	0.2342342342	23.42342342	55.37
A25 LL12	39	158	0.246835443	24.6835443	73.42
A26 LRP4					
A27 LI	35	117	0.2991452991	29.91452991	66.04

I calculated the percent differences between the two sets of measurements to understand interobserver error (Figure 10), finding larger differences in molars than incisors using the Ikeda et al. (1985) method. The mean difference between the age estimations in molars is 35.68%, while the value for incisors is 11.35%. The overall combined percent difference is 31.07%. Despite using the same formulas, our results proved to be very different.

$$\% \text{ difference} = \frac{|A - B|}{\frac{(A+B)}{2}}$$

Figure 10 - The formula used to calculate interobserver differences in the measurements.

Chapter V: Discussion & Conclusion

The analyses from the X-Rays provided much more precise measurements than the original report (Freiwald, personal communication). The ages included in her research belonged to broad categories like adolescent, middle-aged adult, old-aged adult, etc., which are almost meaningless when trying to understand the health and longevity of a population (Buikstra, J. E., & Ubelaker, D., 1994). Since I used two studies with different sets of equations to age the individuals in my sample, the age estimations I received from each study were not consistent with eachother. Using the Ikeda et al. (1985) method, I received one child, one adolescent, four young adults, two middle-aged adults, four old adults, and one inconclusive result. This is different in comparison to my results using the Drusini (2008) method, which provided four children, two adolescents, seven young adults, zero middle-aged adults, zero old adults, and one inconclusive result. Receiving age estimations of young children is interesting, as we sampled the teeth of primarily adults and one adolescent.

My analyses proved to differ significantly not only from eachother but also from Freiwald's estimates in several examples. Freiwald had estimated that individual B7-1-A17 is an adolescent (12 - 20 years). I received an age estimation that individual B7-1-A17 is 32.27 years of age. According to my research, this individual would fall into the young adult (20 - 35 years) age category. This is significantly different given that the individual had incomplete bone fusion and likely was 12 - 14 years of age. A second difference was Individual 2 in Burial 7 (sample A20) who Freiwald aged in the middle-aged adult category (35 - 50 years). This contradicts my findings. In analyses of both of this individual's teeth, I estimated the age of B7-2-A20 to be 6.26 years and 7.68 years. Both of my measurements are fairly close to one another, but do not support Freiwald's findings.

These results provide us insight into ancient health. Seeming that the majority of my results fell into the age range of 20 - 50 years of age, we can conclude that the life expectancy for the Maya population in Actuncan, Belize is comparable to the United States' during the 1900s. This is a 2,000+ year difference, indicating that the Maya people were fairly healthy for their time. This kind of information can provide us the precursors to learn more about diseases, natural disasters, war, or food famines that potentially occurred during their time. This information is very important as it enables us to learn about our ancestors and it helps predict our future.

These results may be flawed due to the inexperience of the users, including the X-Ray technician, but may also reflect uneven secondary and tertiary dentin formation on teeth. For example, Freiwald notes that the 14-yr-old whose teeth had a much older age had dental modification in the form of notching. That type of trauma may have affected her tooth development.

I also used formulas from two separate articles. The Ikeda et al. (1985) is the primary article in this method of research. The Drusini (2008) study followed the methods of Ikeda et al. (1985) but used different tooth types. Despite the research of these articles working in tandem, they formed different equations for estimating age that produced different results.

For example, both the Ikeda et al. (1985) and the Drusini (2008) studies gave formulae for estimating chronological age based on molars that I used in my sample. Using Ikeda et al.

(1985), I estimated the age of individual B2 (sample A4) to be 34.75 years. Using the Drusini (2008) formula for the same individual, I estimated the age to be 27.55 years. These differences are not substantial, but they still have a considerable difference of ~seven years. Another complication of using two studies is that they did not provide formulae for the same tooth types. There were overlapping molar formulae. This allowed me to compare the studies with my own results. However, there was only a single formula for incisors and for premolars so I was not able to assess the consistency of my results.

An additional problem is the program, InkScape, that I used to measure the lengths of the coronas and pulp cavities of the teeth. This free program allowed me to upload the X-Ray images and measure the lengths of the tooth components. The images were subjected to conversions (.dcm to .jpg) in order to input them into the software so the X-Ray files were compatible with InkScape. The conversion process was time consuming with twenty-four tooth images. I searched to find conversion websites that had the capacity to convert multiple images at once. I found one (https://convertio.co), but could only convert ten images per day for free. I made sure to use Convertio for all of the images so that they would be uniform, but it took a few consecutive days to convert all of my X-Ray images into the correct formatting. Despite being as uniform as possible, the sizes of my images varied once inputted into InkScape. This required me to manually size each image to a consistent size, which resulted in a lot of additional effort.

I began to measure the data once the images were consistent. The InkScape program is very sensitive. Even though the measurements are proportional, the data was affected by the smallest of movements in the software. In addition, it was very difficult to find exact places to measure in some of the X-Rays. Some of the teeth had broken enamel, or coronal cavities were unclear in the images. This made it difficult to decide where exactly to place measurement lines.

Due to the sensitivity of the software and unclear measurement placement, the data may not be as accurate as it should be.

I included Eden Irwin's measurements (Table 14) for this reason. I calculated the percent differences between our measurements in an attempt to quantify intraobserver error. The percent difference between our measurements is a mean of 31.07%. This mean was computed from eleven teeth. This high percent difference between our measurements shows how hypersensitive the InkScape software was to work with.

Another issue is that most of the studies I mentioned in my background used a tooth wear scale to estimate age at death. This means that they observed each tooth in their sample, and scaled them based on deformities, trauma, enamel wear percentages, etc. If the tooth failed to fall in a certain range of the tooth wear scale, the tooth was not used in their research. Tooth wear and dental health have not yet been studied in the Actuncan burial population. Due to my population being from an ancient Maya civilization, many of my teeth had broken enamel, irregular tertiary dentine formations, and overall differences in their morphologies. The poor condition of some of the teeth may have affected my results. As I mentioned before, it was very difficult on some of the images to decide where the coronas and pulp cavities were in the teeth due to the irregularities in them. Since we did not use a wear scale, we know very little about the trauma and pathologies of the individuals that these teeth came from.

Understanding how long people lived at Actuncan and in the Maya region is important, and finding an age at death using X-Rays or CT scanning technology may be useful once we learn how to use it in this region. Once we have a better idea about age at death, we can explore how longevity was connected to diet or migration and connect health with peoples' life histories (Micklin 2015). We also can work more to see how childhood health affected a person's lifespan at the individuals, site, and regional level (Billistrand 2014). Based on the studies I mentioned in the background, I think that this method has potential to work under the right conditions. My results are inconsistent, but they are the first means of providing age estimations for these people. This small study at Actuncan is the first to use X-Rays on human lifespans in the Americas and provides a good place to start for future researchers

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