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RESEARCH PAPER

Estimating the Age- and Season-of-Death for Wild Equids: a Comparison of Techniques Utilising a Sample from the Late Neolithic Site of Bad Buchau-Dullenried, Germany

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In this paper, various techniques for determining the age- and season-of-death of wild horse specimens are systematically compared using a sample derived from the Neolithic site of Bad Buchau-Dullenried, Germany. Tooth eruption and wear, crown height, manual optical analysis of dental cementum, line histogram analysis of dental cementum, and a new automated digital technique for analysing dental cementum are employed. Each tooth was measured, thin-sectioned, digitally photographed under microscopy, and the cementum layers analysed. The goal is to determine which technique is the most efficient (in terms of time and resources) and accuracy (in terms of age- and season-of-death) for analysts to employ. The data demonstrate that there is substantial variation between the techniques for determining the age- and season-of-death of individuals, and even between teeth of the same specimen. The results demonstrate that the digital automated technique has an advantage over conventional cementum increment counting in terms of reduced subjectivity, efficiency and accuracy for the identification of season- and age-of-death.

Keywords: Age-at-Death; Seasonality; Equidae; Thin-Sectioning; Dental Cementum

Introduction

Four major techniques for estimating the age- (and by implication, season-) of-death of equids from zooarchaeological assemblages have been utilised: epiphyseal fusion, tooth eruption and wear (Schmid 1972; Silver 1969), cheek tooth crown height (Levine 1982), and cementum analysis (Burke 1995; Klevezal 1995; Klevezal & Kleinenberg 1967). The epiphyseal fusion technique has long been recognised as too coarse a technique to calculate age-at-death because the sequence for the epiphyseal fusion of bones does not provide a continuous record of growth once adulthood is achieved. In contrast, tooth eruption, wear and crown height have been widely recognised to be more accurate indicators of age-at-death. Teeth are grown and/or are worn down continuously through the organism's lifetime and are less subject to the various taphonomic forces that cause higher attrition among the younger age groups (Grant 1975; Hillson 2005; Lyman 1994; Munson 2000; Payne 1972). Various

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studies have demonstrated that these techniques are far less accurate than dental cementum analysis (Burke 1995; Burke & Castanet 1995; McKinley & Burke 2000). Unfortunately, cementum analysis is not utilised more often because it is time consuming, expensive, destructive, and requires a high degree of technological proficiency and observer training for accurate and consistent results.

In recent years, advances in digital and computer automation technology have enabled the introduction of a new automated digital approach to cementum analysis (Czermak et al. 2006, Klauenberg & Lagona 2007, Wedel 2007). The new automated digital counting approach obviates several problems inherent in cementum analysis. In particular, it provides a more systematic means for identifying and quantifying increments which leads to less variability in inter-observer differences. It allows fossil (palaeontological and zooarchaeological) specimens with poorly preserved dentition to be more systematically analysed despite their diagenetic deficiencies (i.e., structural damage from the burial environment). Furthermore, this new technique achieves superior results in a fraction of the time compared to conventional increment counting assessments of age-at-death, and with a higher degree of replication. Inaccurate and/ or subjective observations and measurements can be circumvented with the use of computer analysis and quality thin-section preparation.

In this paper, we systematically compare the results of four age-at-death and season-of-death techniques using dental data in order to ascertain which is the most efficient (in terms of time and resources) and accurate (in terms of age- and season-of-death) for analysts to employ. The dental remains of four wild horses from a German Neolithic deposit are used (Steppan 2004). The data are employed to illustrate that there is substantial variation between the various dental age- and season-at-death techniques. It also will demonstrate that cementum analysis has an advantage compared to the low-technology techniques (i.e., tooth eruption/wear and crown height), and that there is substantial variation between teeth of the same specimen even in cementum analysis.

Anatomy of horse cementum

An increment is a growth layer (or ring). It broadly refers to any layering in hard tissue (shell, bone, enamel, dentin, or cementum) that contains appositional growth (Monks 1981). Incrementally deposited annual structures are distinctive, self-contained additions to the previous growth with the most recent positioned on its immediate predecessor (Dammers 2006: 15–17; Reitz & Wing 2004: 80; Monks 1981: 193). Such features have been found in a variety of anatomical elements, including teeth (cementum and dentin) and bone (periosteum) (Hillson 2005; Lam 2008; Stallibrass 1982).

Equine check teeth are hypsodont or 'high-crowned'. They consist of a relatively larger enamel crown and smaller root system that occupies the apical region (Stromberg 2006). Much of the crown lies within the alveolus (termed the reserve crown). As the exposed occlusal enamel is worn away, gradual eruption occurs in response which causes the eventual appearance of the reserve crown (Kirkland et al. 1996; Stromberg 2006; Sahara 2014). For the purposes of this study, we refer to the sub-gingival reserve crown as part of the root portion, as it functions as such. It is covered by cementum (Beasely 1987). During crown formation and eruption, the peripheral enamel (adjacent to the alveolus) has layers of cementum embedded with Sharpey's fibres of the periodontal ligament for anchorage (Lieberman 1994; Sahara 2014). As the tooth erupts to maintain masticatory efficiency, it is the peripheral cementum in the sub-gingival region which is most active in cementum deposition compared to the more apical root area (Mitchell et al. 2003; Sahara, 2014). As a result, not all areas of sub-gingival cheek teeth in equids are equally promising for cementum analysis. Selecting precise and appropriate target locations is paramount for accurate age and season of death estimation.

Dental cementum in equids covers not only the root surfaces, but also much of the occlusal crown (Burke & Castanet 1995). Cementum increments in fossil teeth are best viewed as thin-sections under transmitted, polarised light microscopy. The descriptions of the various layers follow from the use of this technique. Generally speaking, cementum is characterised by alternating growth zones and annuli. A growth zone is lighter in colour, less dense, wider, and translucent. It is associated with a period of active and rapid cementum deposition and is usually relatively wider than the corresponding annuli. Following a growth period, cementum deposition either slows or ceases, creating the layers known as annuli and lines of arrested growth (LAG). The annuli are darker, denser, narrower, and opaque (Burke 1995; Lam 2008; Stallibrass 1982; Wedel 2007). LAGs, also known as chromophile discontinuities, are the result of growth cessation (Burke and Castanet 1995). In temperate climates, many terrestrial mammals generate the aforementioned morphological features on an annual basis (Klevezal 1995; Klevezal and Kleinenberg 1996; Grue & Jensen 1979).

Factors that affect the formation of these layers are both endogamous (internal genetic patterns) and exogenous (environmental influences). The principle endogamous component is the genetically determined rhythm that regulates deposition rate and structural expression (Grue & Jensen 1979). Exogenous cause(s) are linked to associated seasonal changes, such as nutritional abundance or deficit, biomechanical force of chewing related to fodder type, hormonal cycles, climate, temperature, and photoperiodicity (Lieberman 1994: 528-529; Pike-Tay 1991). These seasonal variables may act as 'triggers' causing the alternating banding phenomenon to be markedly pronounced. Despite the absence or reduction of external stimuli (i.e., impacted teeth in humans), cementum deposition still occurs (Bocutoglu and Yakan 1997), albeit with reduced expression and visibility. This seasonally caused banding effect has been observed in both wild and domesticated animals, whether living in the wild or within sheltered living quarters, raised on a seasonally variable (heterogeneous) or regulated (homogenous) diet. It has also been observed among both animals that hibernate and those that have been denied hibernation (e.g., door mice and hamsters) (Klevezal 1995). Also, the synchronic generation of several incrementally forming tissues is evident in the same organism, such as bone and cementum (Grue and Jensen 1979). It is clear that there is a plethora of complex and intermixed causal factors at play that regulate the periodicity of hard tissue mineralisation.

There are two types of cementum in equids: acellular and cellular (Burke 1995). As the tooth crown is approached, cementum becomes more acellular and contains fewer cementocytes on average. As the root apex is approached, cementum becomes more cellular in nature and contains more cementocytes. Acellular cementum increments are more compact, uniform and straighter. The difference in the physical nature of acellular and cellular cementum affects the study of cementum increments since acellular increments have different optical properties under different microscopy methods (see below). In consequence, it is more profitable to examine acellular cementum for ageand season-of-death analysis.

Method: determining season- and age-of-death with archaeological equid cementum

The first systematic study of the relationship between dental cementum and age- and season-of-death in archaeological horse remains was conducted by Burke (1995) and Burke and Castanet (1995). A modern control sample was created in order to establish the timing of horse cementum deposition, which could then be applied to an archaeological population. Subsequently, McKinley & Burke (2000) refined the relationship between dental cementum increments and age- and season-of-death with a further control sample from a living modern population of Equus caballus with known age- and season-ofdeath. They were able to demonstrate that there was a consistent relationship for Equus sp. between cementum increment growth and the age- and season-of-death, and that there was little inter-observer disagreement in increment assessments. Estimation of the precise month of death was not possible, however, and only rough season of death estimates were made. Burke (1995) determined that cementum growth of the outer increment occurs from May through October, or in seasonal terms from approximately sometime in spring through the beginning of winter. Dental cementum increments, therefore, could be used to both determine the season-of-death and the numerical age of the individual at death. The techniques for both of these are summarised next.

The first step in cementum annuli analysis is to identify the gingival sulcus (i.e., gum line) which is accomplished easily since the specimens were still within the jaws and this feature was recorded for each tooth. Identifying the sulcus is important for targeting the sub-gingival third of the tooth since this region is more active in cementum deposition than the apical portion (Mitchell et al. 2003; Sahara, 2014). Following this step, thin-section preparation and microscopy are implemented.

Next, the enamel-cementum junction (ECJ), which broadly partitions the crown from the peripheral cementum, is identified. This is relatively easy to accomplish due to the marked structural dissimilarity of these tissues and also the presence of the Tomes' Granular Layer and the ephemeral Hopewell-Smith's Hyaline Layer (Beasley 1987) in between the enamel and cementum. Once the Granular and Hyaline layers are identified, the first formed and subsequent cementum increments can then be ascertained

Season-at-Death

The goal of seasonality studies is to determine the time of the year in which an animal may have died (i.e., spring, summer, fall, winter or warm or cold season - Burke 1995, Burke & Castanet 1995, McKinley & Burke 2000; Monks 1981:178). It is rarely possible to refine the moment of death for archaeological specimens. Determining the season-of-death of an animal is difficult in most cases. One of the most reliable indicators of seasonality has been shown to be incremental dental structures, i.e., cementum. Dental cementum can be used to estimate the period or time of year at death because the increment(s) reflect and record the biological lifespan of the individual. The nature of cementum deposition varies through the year with more and faster growth taking place in the warm seasons and less and slower growth taking place during the cold seasons (Table 1). In temperate (as applicable to the study here) and sub-polar climates, the cold months of the year cause a visually distinctive dark/opaque/ thinner band to be produced and a light/translucent/ thicker band during the warm months. Season-of-death is reflected in mammalian cementum by whether the final cementum is deposited in the alternating light/dark, opaque/translucent, or thick/thin sequence. The light/ dark bands are visible when histological thin-sections are prepared and viewed under higher magnifications (Stallibrass 1982). The season-of-death is reflected by the location of the point where there is an interruption in the sequence of banding.

Traditionally, season-at-death was ascertained using non-digital techniques. For example, the outermost (ultimate) layer (increment) was compared with the penultimate (immediate predecessor full increment) layer of its kind. If the outer layer is dark, then it is compared to the previous dark increment. Estimation of the relative percentage of completeness between these layers enables the analyst to determine the time of year when the animal died. It is expressed as a proportion (ratio) of cementum growth (Klevezal 1995).

Age-at-Death

Estimating the age at the time of death is simpler than estimating the season-of-death because it involves simple counting of increments rather than having to measure the relative width of increments. The number of increments is added to the eruption age of the particular tooth in order to achieve the age-at-death for the individual (Burke 1995; McKinley & Burke 2000; Stallibrass 1982). On the other hand, simply counting bands is often not so straightforward as specimen diagenesis needs to be taken into account.

Diagenesis of cementum

Macroscopically, archaeological cementum is commonly stained, cracked and/or ablated (Bell, Boyde & Jones 1991) by taphonomic change. Cementum is the most sensitive of all dental tissues to post-depositional change because of its relative softness and porosity. The macroscopic changes can be a reflection of microscopic changes that can affect increment analysis since histological structures may become altered (Stutz 2002). An example of increment alteration by the burial environment is when the addition of replacement extraneous apatite crystals during diagenesis may cause a lack of distinction between contiguous increments, thereby giving the appearance of one band when in reality two bands are present. An example of increment mimicry is when diagenesis advances from the surface inward from a structural breach (e.g., crack). Then, the infiltration and removal of collagen may create new or add to previously existing increments. These altered increments interfere with the optical properties when viewed by thin-section under magnification. Fortunately, these features may sometimes be recognised through the use of polarising transmitted light microscopy (Burke 1995; Lieberman 1994).

Specimen preparation techniques and technologies

In this section, the manner in which the specimens used in our study were prepared and the techniques and tools of examination are described.

Season	Description	Increment ratio (ultimate to penultimate)
Spring	By the end of March, some specimens have experienced incipient deposition of a growth zone (i.e. <10% of the ultimate growth zone when compared to the penultimate growth zone). Incipient deposition of growth zone layer occurs in most samples before mid-June.	0-10%
Summer	By mid-June, individuals are into their growth period. From this time to approximately the end of August, the majority of individuals will exhibit 10–90% of the ultimate growth zone, compared with the penultimate growth zone.	10-90%
Fall	From the end of August to the end of December, most individuals will have 90–100% of a growth zone deposited compared to the previous year's growth zone.	90–100%
Winter	Between the end of December and the end of March, most individuals will experience a sig- nificant slow-down or arrest in growth.	100%

Table 1: General model of annual formation of cementum layering in accordance with time of year (Burke 1995; Burke and Castinat 1995; McKinley and Burke 2000; Adapted from: McKinley and Shead, unpublished report).

General specimen preparation

First, each alveolus and tooth to be analysed was photographed and measured and any relevant osteological and taphonomic information recorded prior to the application of any destructive techniques. Tooth type and side of the body were corroborated by comparison to modern adult domesticated horse specimens from the Zooarchaeology Laboratory of the Department of Anthropology at the University of Manitoba. Second, each tooth was removed from the surrounding alveolus (if required) using a hand saw and pliers; special care was taken to not touch the tooth with any tools in order to preserve the material. Next, each tooth was colour photographed at all anatomical angles with a digital camera (Olympus). Finally, the crown and root length and width were measured at all angles with a digital Vernier caliper (Mitutoyo). All measurements were recorded in millimetres. These data are presented and discussed below.

Thin-section preparation protocol

This study used the protocol as recommended in Arnold and Greenfield (2006), Burke (1995) and Pike-Tay (1991) for dehydrating, embedding and curing, and de-bulking and cutting of the specimens.

Cementum increment target location

The difference in location between acellular (coronal) and cellular (apical) cementum is important, particularly in equids, because it affects where it is most profitable to thin-section teeth in the investigation of cementum increments. Such increments are best viewed where the increments lie parallel to one another and this occurs rather predictably on the side of the tooth just below the gum line (Burke 1995). This location is preferable to the gum line or closer to the exposed crown because diagenetic change may cause cementum increments to be visually faint or indistinguishable from one another (Stutz 2002). The results may be a series of bands that appear as a single band, yielding lower age values for the specimen.



Figure 1: Anatomical tooth diagram for thin-section preparation; A) crown, B) ECJ, C) cementum location layer on root just below the gum line, and D) cementum location layer on upper mid-root (i.e., reserve crown). Right UP3, buccal aspect.

The coronal-most portion of the gum line is not as protected in comparison to the sub-gingival area just below or on the reserve crown. The cementum in this area is more susceptible to weathering and other taphonomic variables. In contrast, the areas below the gum line and the reserve crown above the root are more protected since they are sealed within the alveolar bone. In archaeological samples, one should always be aware of diagenetic change and exercise caution when assessing cementum increments that are visually damaged to any degree. In cases where there is



Figure 2: Thin-section cut orientation; A) initial cut perpendicular to the longitudinal axis of the upper-third of the tooth, B) section orientation that captures the gum line portion, C) section orientation that captures just below the gum line, and D) an illustration showing the cut perpendicular to the cementum surface on the long axis of the tooth (note that depending on the external surface, the plain of cut is adjusted so that the cementum increments are sliced as perpendicular as possible). Same tooth as **Figure 1**. Upper Right P3, buccal and occlucal aspects.

any evidence for damage to the gum line, the upper-most portion (as close as possible to the gum line) of the tooth is the best location for cementum analysis.

In summary, the target zone of data acquisition was prioritised based as follows: 1) if the area slightly below the gum line appeared free of damage, it was measured first, 2) if the area just below the gum line was damaged, then the regions apically to the gum line were assessed, and 3) if both the areas below the gum line and upper-most root appeared damaged, then cementum analysis was regarded as inappropriate and an alternative (non-incremental) indicator was used.

Number of thin-sections

In early studies of histologically prepared dental cementum samples, a substantial proportion of archaeological samples were found not to be suitable for cementum analysis. But, this has changed over time as technology and understanding of taphonomy and dental histology has improved. For example, Coy et al. (1982) sectioned Saxon cattle molars and found that only half of the sample yielded useable results. A decade later, techniques had improved to the point where cementum could be recovered in a larger number of teeth – i.e., Burke and Castanet (1995) found that a larger number were useable (8 out of 13 and 4 out of 5 teeth) in two archaeological samples. In the sample to be described below, all but one tooth was found to have usable dental cementum increments. Another factor that affects readability is the different types of cementum (cellular and acellular). The layering of cementum varies spatially within the same tooth and between teeth within an individual (Stallibrass 1982). Thin-sections consecutively cross-cut from the same tooth will show some slight variation in layering. For all of these reasons, multiple thin-sections from the same tooth should be obtained and examined.

One cut was made along the buccal-lingual plane and down from the occlusal surface through the mesial root, while the second went down through the distal root (**Figure 2D**). The net result was that two slides were made from each specimen for thin-sectioning. The mesial and central thirds of the tooth were mounted on separate slides and then polished. Both distal faces of both the mesial and central samples were mounted on slides on the distal faces, thereby exposing the mesial faces of both. This not only ensured comparability in reading between the two slides of the same specimen (Burke 1995), but also allowed for four potential surfaces for data acquisition from each tooth - both mesial and distal from both samples could be analysed, if necessary.

The mesial face of the sectioned specimens were mounted on a diamond polishing wheel and polished with successively finer grit sandpapers (600, 800, 1200) until transparent and no scratches were visible.

Slide Mounting

Slide mounting involved first placing a small amount of resin in a T-shape (Pike-Tay 1991) on each slide and then placing, in one motion, one side of the specimen into resin and flattening the specimen onto the slide. The specimen was moved in a circular motion to remove any possible trapped air between the slide and specimen. Once the slide-mounted specimens had cured for 24 hours, a large portion of each tooth was removed by a microtome and the remaining section was reduced to 0.5 millimetres by an Isomet grinder.

Polishing

The final step was to polish each slide with successively finer grit sand papers (600, 800, & 1200) to a 100 microns thickness. This was then reduced further, to around 50–70 microns with a 1200 grit sand paper. Final polishing was done with a diamond polishing wheel. It should be noted that Burke's (1995) recommended thin-section thickness was 30 microns, while the ones for this research were slightly thicker since the features were all visible by then. Each slide was then checked under a microscope for saw marks and/or scour lines. If any were present, the slide was re-polished.

Digital Image Acquisition

The type of light used for observation of cementum thinsections (transmitted or reflected) can affect interpretations of readings, especially with respect to age and season of death for archaeological specimens. Different types of light and filters (e.g., polarising) can yield different results. Transmitted polarised light has long been recognised as the best for zooarchaeological material since it makes the alternating layers appear much more pronounced and any taphonomic damage more visible. Also, staining and demineralisation of thin-sections, which may damage the specimens, is not required. As a result, the thin-sectioning protocol becomes simpler, more consistent, and less timeconsuming (Lieberman & Meadow 1992; Hillson 2005). This procedure was followed here.

The digital images were recorded for each sample slide at 10x (following Burke & Castanet 1995) and 4x magnification. In some cases, a 10x magnification did not allow for the complete width of cementum to be captured. In these cases, a 4x magnification was employed.

The digital image acquisition system used in this study included a video-fed camera mounted on a digital microscope (Olympus Transmitted Light Microscope model #BX51) linked to a personal computer. This system has become the standard for digital microscopy in recent years. This apparatus allowed the field of view of the microscope to be manipulated in real-time and to be recorded by means of digital images stored on a computer hard drive. Each image was recorded and saved as a grayscale, 1300x1300 sized pixel image.

The program used for image capture and analysis was Image-Pro Plus ver. 5.1 (Media Cybernetics) Image Analysis and Processing Software. The software allows for the importing and examination of digital images by a graphical user interface (GUI) with a variety of tools and features available to the user.

Analytical technique of dental cementum increments

Three major techniques of cementum analysis are tested in this paper. Two have been used previously for cementum analysis of equids, while the third is introduced for the first time for this purpose:

- Manual optical counting of increments

 Directly from the microscope aperture
 From the acquired computer digital images
- 2. Line profile histogram of increments a. Manual counting and/or measurement
- 3. Computer assisted summation of increments
 - a. Automatic increment addition
 - b. Automatic measurement of increment widths

Manual optical

While manual or optical analysis of cementum increments for age- and-season-of-death is theoretically simple, it may be difficult to successfully achieve for a number of reasons: 1) experience of the examiner, 2) precise definition of what is an increment, and 3) the basic complexity and variability of cementum may create an optical illusion. In this analysis, increments are ascertained and counted based on a digital image of cementum taken from immediately below the gum line.

Age-at-death: Cementum annulation counts

Manual optical analysis or the use of a light optical microscope without any computer assisted tools is the most basic technique used in this study. It was used for the vast majority of studies since the inception of the technique for estimating age- or season-of-death from dental cementum (Klevezal 1995; Klevezal & Kleinenberg 1967). Estimation of time of death was accomplished by visually assessing images of either the 'light' or 'dark' lines and adding this number to the eruption age of the tooth in question. This method relies on the skill and knowledge of the researcher to determine the beginnings and ends of light and dark cementum layers. This relatively simple method has more disadvantages than advantages.

Season-at-death: pentultimate:ultimate increment ratio

Optical, visual, or manual scoring of season-at-death was accomplished by examining the outer increment and the like increment or predecessor from snapped images. Then, an approximate ratio between these was ascertained and recorded at several spots along the image.

Line histogram techniques

Burke (1995) and Lieberman (1994) utilised a line histogram tool to assess pixel intensity variations in cementum tissue for age- and season-at-death. This technique combines the manual optical analysis (described above) with a graphical representation of the pixel intensities of the cementum. A line profile is used to measure the numeric values per pixel which can then be plotted onto a histogram. It is a less subjective technique than manual optical counting in that it helps to standardise the point at which incremental layers are recognised.

It should be noted that the entire region of sub-gingival cementum was visually assessed for defects. These areas were strictly avoided for analysis. In many cases, only a small portion of the specimen could be used. Defects included cracks, staining, and/or cellular components. Several line profile histograms were plotted over as much of the surface as possible to gain the most data and to minimise the possibility of false reads.

Age-at-death

Manual line profile histogram increment counting for ageat-death was accomplished by Burke's (1995) method for identifying a cementum band and plotting a line histogram perpendicularly across the strip of tissue. This technique places a line histogram on the cementum band and counts the number of 'valleys' or dark bands. Each line histogram was placed over several spots on each cementum strip and several counts were made; after a satisfactory number of samples were taken with congruency in numerical 'valley' counts, the largest values were recorded. This was done to test for discrepancies between the two methods and to determine which method is more accurate.

Season-of-death

The same technique was followed as with age-of-death since both estimates rely upon the recognition and counting of bands (Burke 1995; Pike-Tay 1991; Lieberman & Meadow 1992; Lieberman 1994). The line profile histogram was plotted at several locations on the cementum strip in order to find a suitable area free of taphonomic damage and/or cellular components. The outermost layer pixel value was compared to the predecessor layer and respective value. The widths of these two layers expressed as variations of intensities in the line histogram were compared and a ratio between the two was assessed to determine the season-of-death.

Automated/computer assisted analysis (new method)

The introduction of digital images has proven to be highly useful in the analysis of mineralised tissues for several reasons: 1) a permanent copy is saved and can be referred to in the future, 2) it can be easily disseminated to other assessors, 3) it can provide measurements from images as opposed to direct microscopy scoring (which has repeatedly proved more accurate in recent research and has become routine), and 4) the digital images may be scored using a variety of virtual analytical techniques which improves visibility, accuracy, and precision. A luminance profile or line profile histogram detects the numeric intensity values of pixels and plots a histogram profile. In the case of incrementally generated tissue such as cementum, the histogram reflects the 'light' and' dark' pattern of apposition. This method has obvious advantages over traditional layer counting, except that it still leaves open the possibility of human error because the increments in the form of a 'valley' or 'peaks' are tallied manually.

The new method, described next, goes one step beyond traditional histogram counting and achieves automatic counting of 'peaks' and 'valleys'. One important advantage to this tool is that it automatically summarises the counts, which can then be saved indefinitely for future use and/ or be directly exported into other graphic, tabular and/or analytical programs (e.g., Excel). The single most important advantage this technique has over normal counting

of bands is that it can detect many structures that the eye cannot see at all or cannot consistently see. All images for this research were created using the Olympus Transmitted Light Microscope connected with a video feed to a computer.

Digital images of cementum were obtained from slightly below the gingival sulcus (Burke 1995) on an area of cementum that was free of physical ablation, taphonomic alterations, and biological defects, such as cementocytes, all of which introduce error during the analyses. The cementum area must also show clear and distinct undulating layering, be thin and transparent enough to reduce focal anomalies, and clearly display the enamel that underlies cementum – i.e., equid dental anatomy has enamel juxtaposed to cementum. After the digital images were captured from two slides per tooth, they were saved as a TIFF files and subsequently imported into the image analysis software Image-Pro 5.1.2.59 (Media Cybernetics – see above).

Age-of-death

Acquiring automated age-of-death data was accomplished by using the Caliper Tool that utilises a line profile histogram (similar to Burke 1995 and Lieberman 1994), with an automatic summary feature that tallies the spikes within the histogram. This feature allows the user to plot a line wherever desired and obtain intensity values based on average numeric pixel values and automatically makes counted distinctions (i.e., peaks or valleys). This tool also has the ability to count 'peaks' and 'valleys' that correspond to high or low grey values - the former is generated by lighter shades and the latter by darker shade values. The starting point was plotted at the ECJ where the tissues have a 'scalloped' appearance, which is the border between the two. Then the line was dragged until the peripheral edge of the cementum and mounting medium was met. The tool was set so that the 'peaks' or 'valleys' were counted that would coincide with the light or dark layers. Only the 'valleys' were counted to keep congruency with the previous two techniques. These lines were placed upon areas of cementum that were visually free from cracks, staining, cellular components and/or regions that appeared as uniform as possible. Up to ten lines, but no less than five, were plotted. An average number of identified lines was calculated and considered to be the 'true' number of increments. The count of the number of lines was based on data from both slides of each tooth, and included both buccal and lingual aspects of the slide to account for possible ablation of cementum by the burial environment. These counts were used to calculate the age of tooth at the time of the death of the organism.

Season-of-death

Season-of-death data were acquired by comparing the size and nature of the outermost increment to its immediate like-increment predecessor. This was accomplished with a combination of the Caliper and Line Drag tools (**Figure 3**). This method is similar to acquiring age-of-death data,



Figure 3: Caliper and Line Drag tools.

but more complex measurements are employed. The Caliper tool was used as a guide to follow the divisions of the increments, while the Line Drag tool was used to plot linear measurements of the penultimate and ultimate layers in pixels. Pixel units were chosen instead of absolute values because all images were taken with the same magnification (10x or 4x with multiple images) and is the finest metric value possible. The average of these two separate parameters was recorded, expressed as a ratio, and subsequently divided, thus yielding the percentage of difference. These percentages were then compared to those in the model in **Table 1** which indicates the warm or cold season correlates for the growth of the outer increment.

One advantage of using the digital microscopic and automated technique was that using polarised light did not make a difference in the readings, contrary to much of the earlier literature, which emphasised the need for polarised light. The dark bands remains dark, while the light bands remained light whether polarised or normal light was employed.

Advantages and disadvantages of the various cementum analysis techniques

It has been repeatedly demonstrated that the technique for acquiring age- and season-of-death through cementum increments directly influences the outcome in terms of accuracy and precision (Wittwer-Backofen et al. 2004; Czermak et al. 2006). All of the techniques discussed above have associated drawbacks and advantages. For example, optical assessment offers the most flexibility in that the microscope focus may be adjusted to reveal the possibility of 'hidden' increments, but at the cost of severe eye strain, significantly reduced data acquisition speed, and severe inter-and-intra-observer error (Wittwer-Backofen et al. 2004). The precise definition of an increment has come under increasing scrutiny between researchers. Instead of hand-counting layers or approximating the penultimate/ultimate increment ratio, the histogram approach allows for much greater accuracy in defining what is an increment (for counting/age estimation). It also allows for a quasi-metric assessment width

measurements of the increments (seasonality estimation) (Lieberman 1994; Burke 1995) which is a positive direction for objectivity and reduction in training. Despite the obvious advantages of increased speed and reduced error in using a line profile histogram, it still relies on manual counting and also subjectivity in approximating differing sizes of 'peaks' or 'valleys' to true increments. Automated digital counting and measurement is at the forefront of age-and-season-of-death estimation. The advantages include rapid quantification and dissemination of data, reduced intra-and inter-observer error, increased accuracy and precision, and universality of technology and technique. In other words, it allows researchers to be on a level playing field in the acquisition and reconstruction of demographic profiles.

Analytical assemblage

The teeth from several wild horses (*Equus ferus*) that were used in this research originate from the site of Dullenried (Germany), which was occupied during the Central European Late Neolithic period.

The site and its research history

The settlement area is situated at a distance of 700 metres east of Bad Buchau in between the southern and the central part of the Federsee basin. Unlike any other of the known Neolithic sites, the Dullenried settlement was founded far "off shore" in the wetlands (Figure 4). Its formerly supposed location on a peninsula (Reinerth 1929) was in fact caused by younger transgressions of the Federsee that shaped the settlement area. To a large extent, the site has been destroyed by peat mining and the subsequent excavations in 1920, 1928 and 1929 directed by H. Reinerth, but the photographic documentation has allowed a re-evaluation of the timber construction and the settlement pattern (Bollacher 2001). The remains of at least nine houses with different orientations and partially superimposed (Figure 5) suggest that there was a multi-phase occupation of only a few of the houses at the same time (Figure 6). In 2001, a small-scale excavation of a multi-phase hearth (Bollacher 2002) yielded several samples of organic material for scientific analyses (e.g., archaeobotany - Herbig 2009a, b; radiocarbon dating -Schlichtherle 2004, figs. 12 and 13). Unfortunately, the radiocarbon data (Table 2) fall into a 250-year plateau of the calibration curve resulting in a low high-calibration certainty (Table 2, Figure 7). The pottery from Bad Buchau-Dullenried bears strong resemblance to assemblages from Swiss lake-pile dwellings at Lake Constance dated by dendrochronology from 3280 to 3150 BC, cal. (Bollacher 1999; Schlichtherle 2004: 21).

The zooarchaeology of Bad Buchau-Dullenried

According to H. Reinerth (1929), the settlement at Dullenried yielded a large number of animal remains, which are stored at the Stuttgart State Museum of Natural History. The assemblage numbers 137 specimens with a total weight of approximately 12 kg. Due to the coarse excavation techniques, the animal bone assemblage is biased towards larger fragments (**Table 3**).



Figure 4: Archaeological sites located in the Federsee Basin: 1 - Bad Schussenried-Riedschachen I-III; 2 - Bad Schussenried-Aichbühl; 3 - Bad Buchau-Taubried; **4 Bad Buchau-Dullenried**; 5 - "Wasserburg" Buchau; 6 - Bad Buchau-Siedlung Forschner; 7 - Alleshausen-Ödenahlen; 8 - Alleshausen-Hartöschle; 9 - Seekirch-Stockwiesen; 10 - Seekirch-Achwiesen; 11 - Alleshausen-Grundwiesen; 12 - Alleshausen-Täschenwiesen; 13 - Bad Buchau-Torwiesen II; 14 - Bad Buchau-Torwiesen I; 15 - Bad Buchau-Bachwiesen III; 16 - Bad Buchau-Bachwiesen I; 17 - Bad Buchau-Bachwiesen II; 18 - Bad Buchau-Bachwiesen IV; 19 - Oggelshausen-Bruckgraben. © and reprinted with permission from Landesamt für Denkmalpflege Baden-Württemberg. Adapted from Schlichtherle et al. 2013, figure 7b. Illustrator: Almut Kalkowski.

Zoologist Richard Vogel, curator at the Württembergische Naturaliensammlung, the predecessor of the Stuttgart State Museum of Natural History, conducted the primary analysis (Schüz 1955). His report contains vague information regarding the proportion of wild and domestic mammal species identified among the animal remains from Dullenried (Vogel 1929), merely implying that hunted animals were more common. Reinerth (1929) presents further zoological information based on Vogel's analysis: Among the large wild mammals, red deer was the most common, followed by roe deer, wild boar, bear, moose, aurochs and bison. Fur bearing animals included beaver, badger and otter. Among the wild birds and fish species were grey heron, pike and catfish. Domestic cattle, horse, sheep, goat and dog were also identified. According to Vogel (1929), there is evidence for small domestic ruminants, excluding domestic pig.

Reanalysis of the faunal assemblage confirmed Vogel's analysis: the clear predominance of wild mammal species, the absence of domestic pigs, and the presence of domestic cattle and dog (**Table 3**). But a comparison of the list of animal species given above with the results of the first analysis (Vogel 1929) and the results of the revision (Steppan 2004, 194ff.) show that there are some discrepancies (**Table 3**). Bison, roe deer and moose were neither identified by Vogel (1929) nor by the one of the authors (KS). The thirty-four perforated canine teeth mentioned by Vogel (1929) were revised by Baumeister and Steppan (2006) as follows: badger (n=20), dog (n=5), otter (n=5), red fox (n=3) and wild cat (n=1). Bird bones are lacking completely and all fish bones derive from catfish.

The most interesting aspect, already stressed by Vogel, is the abundance of horse bones at the "Dullenried" site. They are considered to be wild given their size. There is no evidence of the size and gracilisation typical of domestic



Figure 5: Bad Buchau-Dullenried: Intra-site spatial organisation of the site with the wooden floor constructions of at least nine houses (cf. Bollacher 2001, Abb. 31). © and reprinted with permisison from Landesamt für Denkmalpflege Baden-Württemberg. Illustrator: Helmut Schlichtherle.

horses. The numerous remains of wild horses from the site of Bad Buchau-Dullenried suggest that subsistence activities were dominated by hunting and fishing (as the presence of the few hand-collected fish remains suggest). The teeth from these horse remains are used in the ensuing analysis.

Taphonomy

Despite the wide use of cementum for seasonality and ageing purposes, not all zooarchaeological samples are appropriate for such analyses. The degree that cementum in archaeological samples survives the various taphonomic agents that cause attrition are manifold and must be controlled (Lyman 1994). There is a quite variable level of randomness in cementum preservation even within the same sample (inter- and intra-individual) (Hillson 2005). Cementum is the softest dental tissue and most susceptible to destruction despite being protected within the alveolus. Destruction can occur through leaching of the tooth's organic component, physical perturbation, mineral staining, or sediment infiltration. Post-depositional forces may cause the undulating banding effect to be optically diminished (Burke & Castanet 1995). The various taphonomic agents that modified each sample are described in Appendix 5.

Sample description

The sample consists of ten horse teeth from four individuals (labelled Du-A-1 through 4 – **Figure 8**, **Table 4**). All of the data described in the tables are syntheses of the multiple slides and analysed faces from each sample. Two thin-sections were made from each tooth, allowing four faces to be analysed if necessary. The resulting data are a synthesis of all four faces because not all data were obtainable from each thin-section due to various agents of destruction.

Results

The results of each technique for age and season of death for each tooth are discussed separately below.

Age-of-Death

In order to calculate the age-of-death using tooth eruption and wear, the age of eruption into the oral environment for the various teeth had to be estimated. Modern analogues for extant equids (Hoppe et al. 2004) indicate that the P2 and P3 erupted at 2.5–3 years of age, P4 at 3.5–4 years, M1 at 8–12 months, M2 at 22–26 months, and M3 at 3.5–4 years (**Table 5**). Mandibular teeth usually erupt at the same time or slight earlier (by a few months) than maxillary teeth (Hoppe et al. 2004: 357). These modern age estimates must be kept in mind while we review the results of the various methods below.

Du-A-1

There is a wide range of age estimations between the various techniques. Based on tooth eruption patterns alone, this individual would be aged at older than 3.5 years. Based on the incisor eruption and wear pattern, this specimen should be 5–6 years of age at the time of death.



Figure 6: Bad Buchau-Dullenried: Reconstruction of four hypothetical occupation phases: Similarly oriented houses are supposed to be built and used simultaneously. © and reprinted with permisison from Landesamt für Denkmalpflege Baden-Württemberg. Original publication: Schlichtherle 2004, page 19.

Species	Element	LabCode	14C Age BP
	Charcoal	Hd-21892	4463 ± 22
	Charcoal	Hd-22209	4496 ± 25
Equus ferus	Mandible	UtC-11467	4355 ± 48
Equus ferus	Scapula	UtC-11468	4450 ± 50
Equus ferus	Metacarpal III	UtC-11469	4480 ± 50
Equus ferus	Mandible	Erl-9766	4420 ± 48
Equus ferus	Mandible	Erl-9767	4384 ± 48
Equus ferus	Mandible	Erl-9768	4461 ± 46
Equus ferus	Mandible	Erl-9769	4443 ± 47

Table 2: Radiocarbon dated charcoal and horse bone samples.

Based on Levine's cheek tooth wear measurement system, this individual would be 7-10 years, with an average of 8.5 years (**Table 5**).

The manual counting of annual cementum increments technique suggests that the various teeth from



Figure 7: Bad Buchau-Dullenried: 2-D Dispersion Calibration of radiocarbon data on horse bones (cf. Weniger et al. 2011). Image created by Karlheinz Steppan.

this specimen range from 8.5 to 14 years of age (**Table 6**, **Figures 9–11**). A centre point for the age of the individual is c. 11.25 years. It is not clear why such a large range of variation exists. Two out of the three specimens with gum line preservation were unable to be scored using the manual technique because of damage to the cementum. However, this did not apply to the upper root portions.

The line histogram technique of cementum annual increments yielded a range from 10 to 13 years (**Table 6**). In contrast, the upper root digital line histogram technique yielded a wider range, from 8.5 to 13 years. The centre point for this individual was 11–12 years of age. The difference in age estimations between the two locations is probably a result of damage to cementum layers at the gum line, where it is more exposed to various attritional forces. It would appear that the upper root is a better location for cementum analysis with archaeological material since it affords better protection.

The automated technique yielded slightly more refined age estimations – 10.5–13 years for the gum line specimens and 13.5–14 for the upper root specimens, which yield an average of 11.5–14 years of age (**Table 6**). It found more annuli than were visible to the eye using traditional microscopic light optical manual counting techniques.

Each tooth yielded widely varying estimates for the age of the individual based on the different ways of counting cementum increments (**Table 7**). Furthermore, there was a great deal of variation between teeth using the same cementum increment technique. When the techniques which yielded relatively narrow age estimation ranges were averaged for this individual, an age range of 10.8 to 13.6 years was obtained. The incisor and Levine's techniques were not included in the average because they consistently yielded far younger age estimates for individuals than the various cementum analyses, which would have skewed the results. All of the cementum analyses fell into the same general age range.

Du-A-2

Based on tooth eruption patterns alone, this individual would be aged at older than 3.5 years. Based on the incisor wear, this specimen should have been aged at c. 5–6 years. Levine's cheek tooth wear technique suggested a range of 7–8 years, with a centre point of 7.5 years (**Table 5**). Unfortunately, the single tooth available from this individual for cementum analysis was heavily damaged. The cementum layer was chipped and cracked on the gum line and totally absent from the root body. The specimen was thin-sectioned in an attempt to determine if any usable cementum was present, but the tissue had a 'shredded'

Species	NISP_1	NISP_1 %	NISP_1 %	Weight [g]	Weight %	Weight %	Mean weight
Indet.	1	0.79		1.10	0.01		1.10
Indet. pig size	1	0.79		2.10	0.02		2.10
Total indet.	2	1.59		3.2	0.03		1.60
Red deer (Cervus elaphus)	7	5.56	5.65	130.60	1.18	1.18	18.66
Aurochs (Bos primigenius)	25	19.84	20.16	3463.80	31.42	31.43	138.55
Wild horse (Equus ferus)	59	46.83	47.58	5521.40	50.08	50.09	93.58
Total wild mammals	91	72.22	73.39	9115.8	82.68	82.70	100.17
Wild boar or domestic pig	3	2.38	2.42	39.90	0.36	0.36	13.30
Aurochs or cattle	9	7.14	7.26	427.50	3.88	3.88	47.50
Large wild or domestic ruminants	11	8.73	8.87	234.60	2.13	2.13	21.33
Small wild or domestic ruminants	1	0.79	0.81	4.30	0.04	0.04	4.30
Total wild or domestic mammals	24	19.05	19.35	706.3	6.41	6.41	29.43
Dog (Canis familiaris)	1	0.79	0.81	4.50	0.04	0.04	4.50
Cattle (Bos taurus)	8	6.35	6.45	1195.50	10.84	10.85	149.44
Total domestic mammals	9	7.14	7.26	1200	10.88	10.89	133.33
Total det.	124	98.41	100.00	11022.1	99.97	100.00	88.89
Total	126	100.00		11025.3	100.00		87.50
Red deer - shed antler	7			626.90			89.56
Catfish (Silurus glanis)	4			18.40			4.60
Total	137			11670.6			85.19

Table 3: Number and weight of the analysed animal bones.

appearance often being unattached to the cementum enamel junction. As a result, no dental cementum increment information could be obtained.

Du-A-3

Based on tooth eruption patterns alone, this individual would be aged at older than 4 years. No incisor tooth wear data were available for this specimen. Levine's cheek tooth wear technique indicated a relatively young age for the specimen: 5 to 6 years with a centre point of 5.5 years of age (**Table 5**).

Each tooth yielded variable cementum layer counts using the manual band counting technique, indicating that the specimens ranged in age from 8.5 to 13 years of age (**Table 6**). The average manually calculated age estimate was 10–11 years of age. When the gum line and upper root estimated ages were combined and then averaged, the estimated age became more congruent. Essentially, the variability in increment counts per specimen slide was lower than when either location was assessed separately. For example, the increment counts for the gum line and upper root were added together and divided by two.

The line histogram technique for cementum analysis of both the gum line and upper root yielded a wide age range of 10–14 years (**Table 6**; **Figures 12–14**). When these were averaged, a narrower age range of 10.5–14 years was produced. Unfortunately, two out of the three



Figure 8: Photograph of the four horse samples before thin-sectioning. A) Du-A_1, B) Du-A_2, C) Du-A_3, D) Du-A_4.

specimens from this individual were unable to be scored using the line histogram technique due to damage at the gum line area. For this individual, the upper root samples proved more reliable in terms of their readability.

The automated technique produced an age range of 9.5–13 years for the gum line and 10–13 years for the upper root (**Table 6**). When these were averaged, the age range became more congruent (i.e., less variation between

Catalogue #	Other #	Element	Tooth Type	Side	Root #
Du-A-1	NA	Maxilla	P2	R	3
Du-A-1	NA	Maxilla	Р3	R	3
Du-A-1	NA	Maxilla	P4	R	3
Du-A-2	NA	Maxilla	P2	L	3
Du-A-3	Du-B/A-Cu	Mandible	M1	R	2
Du-A-3	Du-B/A-Cu	Mandible	M2	R	2
Du-A-3	Du-B/A-Cu	Mandible	M3	R	3
Du-A-4	Du-B/A-Cu	Mandible	M1	R	2
Du-A-4	Du-B	Mandible	M2	R	2
Du-A-4	Du-B	Mandible	М3	R	3

NA - Not applicable.

Table 4: Anatomical description of all elements.

multiple slides for each tooth specimen), and a narrower age range was achieved (i.e., 10–11.5 years of age).

There was a wide range of age estimates when all techniques were compared for the same, and even between different teeth. When all of the dental cementum age techniques for this individual were averaged, an age range of 10.3–11.5 years was obtained and were very different than results obtained with Levine's and the tooth eruption technique for ageing (**Table 7**).

Du-A-4

Based on tooth eruption patterns alone, this individual would be aged at older than 4 years. No incisor tooth wear data were available for this specimen. Levine's check tooth wear technique yielded an age of 4 to 6 years with a centre point of 5 years (**Table 5**). It consistently underestimated the age vis-à-vis the other techniques.

The manual counting technique produced an age range of 11–13 years for gum line specimens and 10.5 years for upper root specimens (**Table 6**, **Figures 15–17**). When averaged, the age range was 12.5–13 years. The line histogram technique yielded 10–13 years for the gum line and 12.5–13 years for the upper root. When averaged, 12.5–13 years was the age range (**Table 6**). The automated counting technique produced 11.5 to 12 years for the gum line and 10.5–11 years for the upper root (**Table 6**). When the results of the cementum techniques were averaged, an age range of 11.5–14 years of age was produced (**Table 7**). It should be noted that the upper root portion of the M1 was damaged which may produce an age bias.

A wide age range was obtained between techniques and even between teeth. When all techniques (except Levine's) were averaged, an age range of 10.3–13 years was produced for this specimen. Based on cementum analysis, it would appear that the individual was probably in the range of 12–13 years of age at the time of death.

Summary

The results of the various age-at-death techniques employed in this study are summarised in **Table 7**. The tooth eruption and incisor wear techniques (i.e., Hoppe et al. 2004; Silver 1969) produced age much lower estimates than the other techniques. The crown height (Levine 1982) technique yielded ages that were only slightly better than the incisor wear technique. All cementum-based techniques indicate that the age-of-death was substantially older for each specimen, often by a difference of 4 years.

There were some discrepancies between the ages acquired by each of the cementum analytical techniques. In general, the manual technique produced younger ages, the line histogram yielded slightly older ages, and the automated technique generated the oldest age estimates. Because the archaeological specimens are not living specimens with recorded ages, knowing the true chronological age of each individual is impossible. Additionally, selecting a technique which minimises the subjective nature of human error is critical because archaeological dental tissues can be challenging to observe. We propose that our automatic counting technique has advantages over others in that it uses a chronometric tissue for age estimation and removes some aspects of human error associated with dental cementum increment counting analysis and produces less variability in results. It will only eventually be through the use of age-controlled analyses that these issues can be resolved.

Season-of-Death

The season-of-death was able to be determined for three out of the four individuals. For all individuals, the outer increment was 'dark' or translucent on both the gum line and upper root portions of all slides examined. That the histogram technique yielded a 'valley' on all specimens further indicated the presence of a final 'dark' layer.

Du-A-1

The results from each of the techniques varied quite widely (**Table 6**; **Figures 9–11**). The gum line optical technique manual ratio yielded a range in increment ratios between the various teeth from 0.8 to 0.9 between the ultimate and penultimate increments (**Table 8** – P2=0.9, P3=0.8, P4=UN/Unknown). According to the general model, this

		Tooth eruption (Hoppe et al. 2004)	Incisor wear (Silver 1969)				Levine	(1982) chee	k tooth wea	ır system aç	e.		
Catalogue #	Tooth Type [*]	Age inter	-val**	MD Crown (mm)1	BL Crown Height (mm)1	M Crown Height (mm) 1	D Crown Height (mm) 1	B Crown Height (mm)1	L Crown Height (mm) 1	Saddle Height (mm)2	Maximum tooth height (mm)3	Estimated age",4	Average age**
Du-A-1	UP2	>2.5-3 Y	5 - 6 Ү	36.25	24.56	NA	10.59	10.93	10.81	31.29	42.22	8-10 Y	8Υ
Du-A-1	UP3	>2.5-3 Y	5 - 6 Ү	29.37	24.8	6.27	9.11	8.51	NA	46.48	55.59	Υ 9-7	8Υ
Du-A-1	UP4	>3.5-4 Y	5 - 6 Ү	27.9	26.98	0	0	3.28	NA	58.12	61.4	7–8 Y	8Υ
Du-A-2	UP2	>2.5-3 Y	5 - 6 Ү	36.05	24.61	NA	NA	NA	4.49	48.46	52.95	7–8 Y	7.5 Y
Du-A-3	LM1	>8-12 M	NA	32.82	34.75	NA	4.94	4.33	6.23	71.7	77.93	5–6 Y	5Υ
Du-A-3	LM2	>22-26 M	NA	27.71	15.7	4.91	7.56	6.74	9.91	67.79	77.7	4-5Υ	5Υ
Du-A-3	LM3	>3.5-4 Y	NA	29.3	13.44	NA	8.14	6.27	4.54	64.24	72.38	5–6 Y	5Υ
Du-A-4	LM1	>8-12 M	NA	28.62	17.43	10.33	8.08	7.99	3.28	66.71	77.04	4–5 Y	5Υ
Du-A-4	LM2	>22-26 M	NA	27.06	17.2	9.83	13.82	6.37	10.46	62.63	76.45	4–5 Y	5Υ
Du-A-4	<m3< td=""><td>>3.5-4 Y</td><td>NA</td><td>15.03</td><td>30.76</td><td>14.83</td><td>7.45</td><td>9.56</td><td>7.73</td><td>59.9</td><td>74.73</td><td>5–6 Y</td><td>5Υ</td></m3<>	>3.5-4 Y	NA	15.03	30.76	14.83	7.45	9.56	7.73	59.9	74.73	5–6 Y	5Υ
NA - Not appli B-buccal, L-lii	cable; * - 'I ngual; 2 - c	U' indicates m distance of sad	axillary po dle - crown	sition, 'L' ir at ECJ; 3 - 6	idicates mai excluding ro	ndibular posit ot - saddle-cro	ion; ** - 'Y' wn height;	indicates ye 4 - See Levi	ars, 'M' ind ne 1982, ap	icates mon pendix IIIa	ths 1 - anatomic , p. 249.	al abbreviaitons:	M-mesial,

Table 5: Age distribution based on incisor and cheek tooth eruption and wear systems.

			Gumlin	ne data		Manual (counting of c	ementum aı	nnuli ageing		Line histogi ing of cer techni	ram count- mentum ique [*]	Digital auto	omated counti	ng of cement	ım ageing [*]
Catalogue #	Element	Tooth Type	Gumline Specimen Thickness Mes (µ m)	Gumline Specimen Thickness Dis (µm)	Gumline Optical Manual Counts"	Upper Root Optical Manual Counts	Gumline Optical Manual Counts Estimated Ages (Years)	Upper Root Optical Manual Counts Estimated Ages (Years)	Gumline Digital Line Histogram Counts	Upper Root Digital Line Histogram Counts	Gumline Digital Line Histogram Estimated Ages (Years)	Upper Root Digital Line Histogram Estimated Ages (Years)	Gumline Digital Automated Increment Counts	Upper Root Digital Automated Increment Counts	Gumline Digital Automated Increment Estimated Ages (Years)	Upper Root Digital Automated Increment Estimated Ages (Years)
Du-A-1	Maxilla	P2	1.62	1.063	9	2	8.5 to 9	9.5 to 10	7	6	9.5 to 10	11.5 to 12	∞	10	10.5 to 11	12.5 to 13
Du-A-1	Maxilla	P3	1.075	1.06	8	7	10.5 to 11	9.5 to 10	6	6	11.5 to 12	11.5 to 12	10	8	12.5 to 13	10.5 to 11
Du-A-1	Maxilla	P4	1.78	1.06	10	6	13.5 to 14	12.5 to 13	10	10	13.5 to 14	13.5 to 14	10	10	13.5 to 14	13.5 to 14
Du-A-2	Maxilla	P2	1.067	1.81	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN
Du-A-3	Mandible	M1	1.051	1.063	NN	10	NN	11	NN	6	NN	10	NN	6	NN	10
Du-A-3	Mandible	M2	1.08	1.07	8	7	10.5 to 10	9.5 to 10	8	6	13 to 14	11.5 to 12	11	10	11.5 to 13	11.5 to 12
Du-A-3	Mandible	M3	1.08	1.76	ſ	6	8.5 to 9	12.5 to 13	UN	6	NN	12.5 to 13	9	6	9.5 to 10	12.5 to 13
Du-A-4	Mandible	M1	1.81	1.067	10	NN	11	NN	6	NN	10	NN	6	NN	10	NN
Du-A-4	Mandible	M2	1.08	1.76	6	8	11 to 12	10.5 to 11	10	6	12.5 to 13	10.5 to 11	10	6	11.5 to 12	10.5 to 11
Du-A-4	Mandible	M3	1.08	1.73	6	8	12.5 to 13	12.5 to 13	10	6	12.5 to 13	12.5 to 13	10	10	13.5 to 14	13.5 to 14
Notes: * Or UN - Unide	ıly the nuı ntifiable.	nber of	dark incre	ements ar	e countec	÷.										

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Table 6: Age of specimens based on thin-section techniques (manual, line histogram and automated).

			Tooth eruption age	Incisor tooth erup- tion and wear	Levine che eruption a	ek tooth ind wear	Manual con cementun ageir	unting of 1 annuli 1g°	Line histogr ing of cer techni	am count- nentum ique [*]	Digital aut counting of c ageir	omated cementum 1g*	All ce	mentum age	ing techniq	ues combined	<u>.</u>
Catalogue #	Element	Tooth Type	Tooth erup- tion age (Hope et al. 2004)	Age based upon incisor tooth wear (cf. Silver 1969)	Estimated age based on Levine method (1982, appendix IIIa)	Average age esti- mated for indi- vidual based on Levine	Gumline Optical Manual Counts Ages (Years)	Upper Root Optical Manual Counts Ages (Years)	Gumline Digital Line Histogram Estimated Ages (Years)	Upper Root Digital Line Histogram Estimated Ages (Years)	Gumline Digital Automated Increment Estimated Ages (Years)	Upper Root Digital Automated Increment Ages (Years)	Average Estimated Age-All Techniques	Average Estimated Age for Individual (Manual)	Average Estimated Age for Individual (Line)	Average Estimated Age for Individual (Digita/ Automated)	Probable age (all cemen- tum tech- niques aver- aged)
Du-A-1	Maxilla	P2	>2.5–3 Y	5-6 Y	8–10 Y	8Υ	8.5 to 9	9.5 to 10	9.5 to 10	11.5 to 12	10.5 to 11	12.5 to 13	10.3 to 10.8	9 to 9.5	10.5 to 11	11.5 to 12	10.825
Du-A-1	Maxilla	P3	>2.5–3 Y	5-6 Y	7-9 Υ	8Υ	10.5 to 11	9.5 to 10	11.5 to 12	11.5 to 12	12.5 to 13	10.5 to 11	11 to 11.5	11.25 to 12	11.5 to 12	11.5 to 12	11.59375
Du-A-1	Maxilla	P4	>3.5-4 Y	5-6 Ү	7–8 Y	8Υ	13.5 to 14	12.5 to 13	13.5 to 14	13.5 to 14	13.5 to 14	13.5 to 14	11.6 to 13.8	13 to 13.5	13.5 to 14	13.5 to 14	13.6375
Du-A-2	Maxilla	P2	>2.5-3 Y	5-6 Y	7–8 Υ	7.5 Υ	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN
Du-A-3	Mandible	M1	>8-12 M	NA	5—6 Ү	5Υ	NN	11	NN	10	NN	10	10.3	11	10	10	10.325
Du-A-3	Mandible	M2	>22-26 M	NA	4–5 Y	5 Ү	10.5 to 10	9.5 to 10	13 to 14	11.5 to 12	11.5 to 13	11.5 to 12	11.25 to 11.8	10	12.25 to 13	11.5 to 12.5	11.5375
Du-A-3	Mandible	M3	>3.5-4 Y	NA	5-6 Y	5Υ	8.5 to 9	12.5 to 13	NN	12.5 to 13	9.5 to 10	12.5 to 13	11 to 11.5	10.5 to 11	12.5 to 13	11 to 11.5	11.5
Du-A-4	Mandible	M1	>8-12 M	NA	4–5 Y	5Υ	11	NN	10	NN	10	NN	10.3	11	10	10	10.325
Du-A-4	Mandible	M2	>22-26 M	NA	4–5 Y	5 Ү	11 to 12	10.5 to 11	12.5 to 13	10.5 to 11	11.5 to 12	10.5 to 11	11 to 11.6	10.75 to 11.6	11.5 to 12	11 to 11.5	11.5125
Du-A-4	Mandible	M3	>3.5-4 Y	NA	5-6 Ү	5 Ү	12.5 to 13	12.5 to 13	12.5 to 13	12.5 to 13	13.5 to 14	13.5 to 14	12.8 to 13.3	12.5 to 13	12.5 to 13	13.5 to 14	13.075
Notes: NA	- Not app Aldeidie	licable	.:														

UN - Unidentifiable. * Only the number of dark increments are counted. ** All ageing techniques combined excludes incisor and cheek tooth wear.

Table 7: All techniques for estimation of age of specimens.





Figure 9: Thin-section of DU-A-1, UP2: A) gum line image, B) mid-root image.



Figure 10: Thin-section of DU-A-1, UP3: A) gum line image, B) mid-root image.



Figure 11: Thin-section of DU-A-1, UP4: A) gum line image, B) mid-root image.

Catalogue #	Element	Tooth Type	Microscopic Taphonomic Damage	Manual se techn	asonality ique*	Our analysis	Line histogram technic	seasonality que*	Our analysis	Digital automated seasonality technique*
				Gumline Optical Manual Season Ratio	Upper Root Optical Manual Season Ratio	Seaon of death	Gumline Line Histogram IncrementRatio (Burke and/or Lieberman)	Upper Root Line Histogram Increment Ratio (Burke and/or Lieberman)	Seaon of death	Gumline Digital Pentultimate Increment Width (pixels)
Du-A-1	Maxilla	P2	0	0.9	UN	Very late Summer/ early Fall	0.8	0.8	Late Summer	35
Du-A-1	Maxilla	Р3	0	0.8	UN	Very late Summer/ early Fall	0.8	0.9	Late Summer	32
Du-A-1	Maxilla	Ρ4	0	UN	UN	UN	UN	0.7	UN	26
Du-A-2	Maxilla	P2	Specimen damaged beyond usability	UN	UN	UN	UN	UN	UN	UN
Du-A-3	Mandible	M1	0	UN	1	Winter	UN	0.8	Late Summer/ Late Fall	UN
Du-A-3	Mandible	M2	0	0.9	0.9	Winter	0.8	0.7	Late Summer/ Late Fall	55
Du-A-3	Mandible	М3	0	1	1	Winter	0.9	0.9	Late Summer/ Late Fall	25
Du-A-4	Mandible	M1	Increments difficult to see	0.9	UN	Winter	0.8	0.8	Late Summer/ early Winter	47
Du-A-4	Mandible	M2	0	UN	1	Winter	UN	0.9	Late Summer/ early Winter	UN
Du-A-4	Mandible	М3	0	0.9	UN	Winter	0.7	0.8	Late Summer/ early Winter	18

Table 8: Seasonality related data based on thin-sectioning of cementum techniques (manual, line histogram and automated).

Digital	automate	ed seasona	lity techni	ique*	Our analysis	Seas	son of death (a	all technique	s)	Our analysis
Upper Root Digital Pentultimate Increment Width (pixels)	Gumline Digital Ultimate Increment Width (pixels)	Upper Root Digital Ultimate Increment Width (pixels)	Gumline Digital Increment Ratio	Upper Root Digital Increment Ratio	Seaon of death	Gumline Pentultimate Increment Colour	Upper Root Pentultimate Increment Colour	Gumline Outer Increment Lumenance Profile	Upper Root Outer Increment Lumenance Profile	Probable Seaon of death
UN	22	UN	0.99	UN	Late Fall or early Winter	Dark	Dark	Valley	Valley	Late Fall or early Winter
34	27	21	0.84	UN	Late Fall or early Winter	Dark	Dark	Valley	Valley	Late Fall or early Winter
UN	22	UN	0.84	UN	Late Fall or early Winter	Dark	Dark	Valley	Valley	Late Fall or early Winter
UN	UN	UN	UN	UN	UN	Dark	Dark	UN	UN	UN
UN	UN	9	UN	9	Late Fall/ Early Winter	Dark	Dark	Valley	Valley	Late Fall/ early Winter
13	46	12	0.61	0.9	Late Summer/ Late Fall	Dark	Dark	Valley	Valley	Late Summer/ early Winter
22	23	15	1	0.7	Late Summer/ Late Fall	Dark	Dark	Valley	Valley	Late Summer/ early Winter
35	40	35	0.73	0.7	Late Summer/ early Winter	Dark	Dark	Valley	Valley	Late Summer/ early Winter
24	UN	21	UN	0.9	Late Summer/ early Winter	Dark	Dark	Valley	Valley	Late Summer/ early Winter
UN	16	UN	0.73	UN	Late Summer/ early Winter	Dark	Dark	Valley	Valley	Late Summer/ early Winter

Notes: NA - Not applicable.

* Only the number of dark increments are counted.

UN - Unidentifiable.

Table 8: Seasonality related data based on thin-sectioning of cementum techniques (manual, line histogram and automated).



Figure 12: Thin-section of DU-A-3, LM1: A) gum line image, B) mid-root image.



Figure 13: Thin-section of DU-A-3, LM2: A) gum line image, B) mid-root image.



Figure 14: Thin-section of DU-A-3, LM3: A) gum line image, B) mid-root image.



Figure 15: Thin-section of DU-A-4, LM1: A) gum line image, B) mid-root image.



Figure 16: Thin-section of DU-A-4, LM2: A) gum line image, B) mid-root image.



Figure 17: Thin-section of DU-A-4, LM3: A) gum line image, B) mid-root image.

indicates the individual died in very late summer or early fall.

The line histogram technique produced a ratio range of 0.7-0.8 for both the gum line and upper root portions (**Table 8** – P2=0.8, P3=0.8–0.9, P4=0.7). According to the general model, this indicates the individual died in late summer.

The automated measurement technique yielded a width value of 30-35 pixels for the penultimate increment and 21-22 pixels for the ultimate increment from all teeth. The ratio of these was calculated to be 0.84-0.99, respectively (**Table 8** – P2=0.99, P3=0.84, P4=0.84). According to the general model, this technique indicates that the individual died in late fall or early winter.

The average increment ratio of this individual was found to between 0.8–0.99. This individual died during a dark increment, which means it was a cold season death. When the ratios from each of the techniques are averaged, a ratio value of 0.85 was produced. Based on this value, the individual is estimated to have died during the late fall or early winter.

Du-A-2

The season of death for this individual was not determinable due severe damage to the cementum.

Du-A-3

The outer increment for this individual was determined to be dark or translucent on both the gum line and upper root portions (**Figures 12–14**). The optical manual method indicated an increment ratio of 0.9-1.0 (**Table 8** – M1=1, M2=0.9, M3=1). These ratios indicated a season-of-death estimated to be during the winter.

The line histogram technique yielded an increment ratio of 0.7-0.9 (**Table 8** – M1=0.8, M2=0.8 to 0.9, M3=0.9). Referring to the general model, this individual was estimated to die between late summer and late fall.

The automatic measurement technique yielded a pixel width between 46–55 and 23–25 for the gum line and 12–13 and 15–25 for the upper root (**Table 8** – M1=0.73, M2=0.9, M3=0.73) The automated increment ratio was determined to be between 0.7–0.9. According to this technique, the season-at-death was estimated to be between late fall or early winter.

When the average ratios of all the techniques were calculated, a value of 0.88–0.9 was produced. This estimate would place this individual's death during the late fall or early winter.

Du-A-4

The outer increment was determined to be dark or transparent on both the gum line and the upper root areas (**Figures 15–17**). The optical manual technique produced an increment ratio between 0.9-1 (**Table 8** – M1=0.9, M2=1, M3=0.9). This suggests that the season-at-death should be during the winter.

The line histogram technique yielded a ratio of 0.7-0.8 (**Table 8** – M1=0.8, M2=0.9, M3=0.7 to 0.8). This suggests that the season-at-death was during the late summer or early winter.

The automated measurement technique produced pixel values of 21–24 and 35–40 pixels, which yielded an increment ratio between 0.7–0.9 (**Table 8** – M1=0.73, M2=0.9, M3=0.7). This technique would place this individual's death during the late summer or early winter.

The average ratio of all techniques was determined to be between 0.8–0.9. This would place the season-of-death of the individual during late fall or early winter.

Summary

The season-at-death data are summarised in **Table 8**. The sample overwhelmingly indicates that all individuals died during the colder months of the year. This conclusion is supported by the colour of the ultimate increment being 'dark' which suggests cold season growth. Furthermore, the ultimate:pentultimate increment ratio for the majority of the sample suggests late fall or early winter deaths.

Conclusions

The aim of this study was to compare and test the consistency between different techniques for determining age- and season-at-death for equids, including tooth eruption and wear, crown height and dental cementum increments, for archaeological specimens (Burke 1995; Levine 1982; Silver 1969). In particular, it was designed to assess the validity of a new automated technique, which should improve the speed of preparation and the accuracy of analyses. Previously employed techniques included assessing cementum increments by eye where each increment is manually counted, or by luminance profile histograms where scoring is accomplished by measuring or counting the relative size of incremental 'peaks' and 'valleys'. In an automated technique, the number and relative thicknesses of each increment, or part thereof (peaks and valleys) is automatically counted, measured and calculated.

Summary

Some of the more important results of our study for ageing of adult equid dental remains through tooth eruption and wear, crown height measurements, manual increment counting of cementum increments include the following:

- 1. There was a great deal of variation between teeth using the same cementum increment technique. Each technique that was employed on multiple teeth from the same individual yielded widely varying estimates for the age of the individual. This is not surprising given the range of variation obtained from even tightly controlled chronological samples (Wedel 2007).
- 2. The tooth eruption and wear and crown height measurement techniques yielded the lowest age estimations and were not useful for seasonality determination. Simply based upon comparison with modern tooth eruption analogues, all of the ages of the individuals under analysis would have been underestimated.
- 3. The manual increment counting age estimation technique yielded wider variation in age estimation than the other thin-sectioning techniques, and

older age estimation than tooth wear/eruption or crown height measurements.

- 4. The line histogram technique yielded narrower range of age estimation than manual observations, but yielded a slightly older range.
- 5. The automated age estimation technique yielded the narrowest range of age estimation. However, age values were slightly higher in age than the manual or line histogram results. It was by far the most efficient in terms of time and effort, and the most accurate when comparing teeth from the same individual.

Could the higher ages of the automated ageing technique be because the technique is counting all increments, including those that may be intra-year increments? It is possible that the analyst might filter out some of these smaller increments (such as from natural variability in cementum or diagenetic artefacts) with the manual counting technique. However, this is unlikely given that it is only slightly higher ages, with the narrowest range of variability.

Some important results from our seasonality analysis include the following:

- 1. All of the thin-sectioning techniques yielded a similar range for the season-of-death of the animal. Broadly congruent values were derived even when multiple target areas on each slide were considered. When only a single target area was considered, there was much more divergence that was most likely due to the variable nature of cementum as its thickness can vary in three dimensions. This was because of the variability in tooth cementum morphology and diagenesis (see below). For these reasons, multiple target areas are necessary for more accurate analyses.
- 2. With the automated ageing technique, it is possible to begin to pinpoint when in cold or warm seasons the specimen may have died. This is a result of the introduction of systematic metrical analysis of increment widths.
- 3. Automated seasonality measurement estimation yields estimates are broadly congruent for the entire sample. The vast majority of individuals indicate that they died in the late fall or early winter. These were most likely early cold season deaths, and not from the deep or late winter.

Taphonomic or diagenetic variables may have affected the potential for analysing some of the thin-sections. Cracking and mineral staining often obscured cementum increments. For example, in some cases, the entire cementum layer was so stained that no increments could be observed. In these cases, mineral staining had also leached into the enamel. In other cases, the entire outer increment from the gum line to the upper root was stained and/or absent. In these situations, it was not possible to determine the season-of-death or age-at-death. Where the gum line was stained, sometimes the upper root was not stained. In these situations, it was possible to use the upper root to determine season- and age-of-death.

Technically, when the suggested 30 micron thickness (Burke 1995) for slides was attempted, there was damage to some part of the cementum in almost each case (i.e., part of the cementum was removed during sanding or polishing). This precluded definitive analysis since the cementum increments became visually less distinct (faint) with increasing specimen thinness. It was found that a slightly thicker slide yielded more consistent results (50–80 microns).

Discussion of significance

Which technique is better? The tooth eruption/wear and crown height measurement techniques (Silver 1969; Levine 1982) consistently underestimate age when compared to all the thin-sectioning techniques. The line histogram is an improvement, but is time consuming and subject to operator error. The automated digital technique is the most rapid and consistent technique. It requires the least knowledge by the observer, since the counting and measurement is automatically generated. A good technician can accomplish all of the work relatively rapidly and more efficiently by using the automated digital technique.

However, the automated technique may overestimate the age of some of the teeth because it removes the element of human observation and judgment. In some cases, it may be picking up taphonomic and cellular components not easily visible to the eye, or are being ignored because the analyst has learned that they are not relevant for estimating season- or age-of-death. On the contrary, it could be picking up increments so fine that the human eye cannot see them and that the age estimations may be most accurate. This issue remains to be resolved. It should be stressed that when available, more than one age- and season-of-death indicator should be used and compared to minimise error - any age marker is subject to a multitude of biological and taphonomic errors and subjectivities.

Another means to further improve the technique and remove possible overestimation due to 'noise' is digital filtering. Increments in horse cementum are heterogeneous (contain more cellular components), thick and wavy. They are more difficult to discriminate as a result. Digital filtering has been demonstrated as an effective means to reduce the amount of noise. For example, such filters have been effectively tested with accurate results in studies of human dental increments (Czermak et al. 2006). The identification of human cementum increments is much more difficult than in equids because they are much more homogenous and have thinner increments. Hence, the automated technique is probably picking up "real" increments in equids.

In this paper, we investigated the congruency of various analytical techniques for age- and season-at-death of equids. It was shown that the automated digital technique used in analysing dental incremental structures (cementum) is more objective and replicable than other techniques. In the future, this new technique needs to be tested extensively on a large control sample with specimens from known ages. While this study focused on equids, the results can be more widely applied and should be extended to other commonly exploited species (e.g., sheep, goat, cattle, etc.).

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Appendix 5 - Sample description

In this appendix, the sample of ten horse teeth from four individuals (labelled Du-A-1 through 4 – **Figure 8**) used in the analysis are described in detail.

Du-A-1

There are three teeth from this specimen: UPM2, UPM3, and UPM4.

P2

This specimen is a maxillary right second premolar (**Appendix 1A**). The specimen has minor damage in the form of broken root tips and cracked cementum; this cracking is most apparent around the ECJ. The crown exhibits light wear which does not breach the dentin on all sides.

Ρ3

This tooth is a maxillary right third premolar (**Appendix 1B**). Some minor damage to the cementum sheath is present in the form of cracking and pitting. The crown shows moderate wear that almost extends into the dentin; the only damage is broken root tips. Overall this specimen is intact.

P4

This tooth is a maxillary right fourth premolar (**Appendix 1C**). The specimen exhibits damage to the lingual-distal root that is fractured two-thirds towards the crown; dentin and enamel can be easily observed. The crown shows heavy wear and only remnants may still be seen. This tooth is largely free of post-depositional damage as the cementum is intact and free of cracks.

Du-A-2

There was only one tooth from this specimen available for sectioning: UPM2. This tooth is a maxillary left second premolar (**Appendix 2**). This specimen is heavily damaged and most, if not all, of the cementum is ablated from the surface. The root tips are also broken and the internal



Appendix 1A: Photograph of specimen Du-A-1, UPM2, various views.



Appendix 1B: Photograph of specimen Du-A-1, UPM3, various views.

dentin is removed. Crown wear is not uniform and tilts distally. The distal portion is heavy, while the mesial portion is light in wear.

Du-A-3

There are three teeth from this individual: LM1, LM2 and LM3.

M1

This tooth is a right mandibular first molar (specimen image unavailable). This specimen is in excellent condition and exhibits no damage except for a minor chip on the mesial root apex. Crown wear is moderate and does not breach the cementum or dentin.

M2

This specimen is a right, mandibular second molar (**Appendix 3A**). The specimen is in excellent condition save for minor random cracks along the cementum surface. These cracks are random in direction and orientation along the root body and concentric closer to the crown. One peculiar feature is that the root is rough and almost 'fuzzy' in appearance, like sandpaper. The crown exhibits moderate wear and the cusp tips are still intact. The root and root tips are largely intact; the very apex is hollow which is indicative of a still growing root. Root etching is present represented by scour lines at random locations along the root.



Appendix 1C: Photograph of specimen Du-A-1, UPM4, lingual view.



Appendix 2: Photograph of specimen Du-A-2, LPM2, various views.

М3

This tooth is a right, mandibular third molar (**Appendix 3B**). As with the previously described teeth from this individual, this specimen exhibits excellent preservation and is whole. Minor damage includes some small concentric cracking around the gum line and the presence of 'fuzzy' crystal-like growth on the cementum surface. No root etching is present. Crown wear is heavier that the M1 and M2 and is tilted buccally. Wear is more pronounced in that the cusp tips are absent, but not totally worn to expose the dentin.

Du-A-4

There are three teeth from this individual: LM1, LM2, and LM3.

M1

The specimen is a right mandibular first molar (**Appendix 4A**). The tooth is intact and in good condition. It is an example of staining as its colour is darker than others.



Appendix 3A: Photograph of specimen Du-A-3, LM1, various views.



Appendix 3B: Photograph of specimen Du-A-3, LM2, various views.

Crown wear was difficult to assess as the occlusal surface was damaged by a large crater on the surface. Besides this damage, the crown exhibits only moderate wear as the cusp tips are intact. Damage includes cracking along the root body. These cracks are mostly small, but some medium size cracks radiate from these. The root tips are still intact, but the central portion is gouged out and obliterated as with the crown.

M2

The tooth is a mandibular, right first molar (**Appendix 4B**). This specimen is in moderate condition since there are minor and major cracks in the cementum. Some of the cementum has been ablated and removed, which can be seen along the lingual surface. It also appears that the tooth has been broken axially at mid-root, but glued and repaired at some point in the past. The root tips are intact and still open. Crown wear is light since the cusp tips are still present and extend above the dentin. This tooth is the second most damaged specimen in the sample.



Appendix 3C: Photograph of specimen Du-A-3, LM3, various views.



Appendix 4A: Photograph of specimen Du-A-4, LM1, various views.

M3

The specimen is a mandibular right third molar (**Appendix 4C**). The tooth is in excellent condition except for minor cracking of the cementum on the coronal distal aspect and random minor cracking along the root surface. Some minor ablation of cementum that does not penetrate the dentin may be seen over the entire surface. The crown is only moderate in wear and the cusps are high with no penetration into the dentin. The crown wear is tilted buccally.

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Appendix 4B: Photograph of specimen Du-A-4, LM2, various views.



Appendix 4C: Photograph of specimen Du-A-4, LM3, various views.

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