Cementochronology Protocol for Selecting a Region of Interest in Zooarchaeology

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Seasonal variations and changes in environmental conditions directly impacted social and economic activities of past human populations. Among various approaches, cementochronology has been developed to tackle such questions. It relies on the study of the dynamic patterns and rhythmicity in the deposition rate of the dental cementum observed microscopically on petrographic thin-sections of animal teeth recovered from archaeological contexts. However, while archaeologists have used this method for the past fifty years, no protocol of analysis, including the definition of acceptation of a specific Region of Interest (ROI) and the decision process in the identification of the last increment, has been published. Based on the confrontation of experiments of specialists from different archeological fields (past wildlife predation, animal husbandry, human burials), biological and optical criteria have been identified for the selection of optimal ROIs and their analysis. The reliability of this protocol has then been assessed through a blind-test performed by different analysts with different skill levels. Thirty thin-sections of modern reindeer teeth with known age and season at death were selected. Observations were conducted using the same microscope. Each observer provided the number of selected ROIs, their locations, the recorded images, and the main data about the cementum increments (number, nature of the last deposit). The comparative study of the results demonstrates here the accuracy of this new protocol and its replicability. It also underlines the impact of experience in the decision process for the acceptation or the reject of a ROI.

INTRODUCTION

Since the middle of the previous century, cementum analyses were developed for aging and assessing the season of death of varied taxa (Klevezal' 1996, **Buikstra this volume**). Applied in archaeology since the '60s, the method has been largely used to discuss subsistence strategies (Pike-Tay 1991; Rendu 2010), animal migrations (Burke 1993), mobility pattern (Rendu 2007; Delagnes and Rendu 2011), and livestock management (Gourichon 2004). More recently, applications on humans were conducted notably for estimating the age-at-death and demographic variables (Naji, Gourichon, and Rendu 2015; **Part 3, this volume**). While the method is now the most used for studying seasonality in archaeological contexts, there are still some methodological issues that limit part of the interpretations.

Even though most of the authors seem to agree on the general conditions of observation of the cementum increments, very few explicit criteria are defined to accept or reject an observation precisely. In particular, the criteria to select a region of interest (ROI) for observation are rarely detailed. Instead, only a general location is usually proposed, such as the "first third of the root", just below the enamel-cementum junction (Pike-Tay 1991; Klevezal 1996), where lies the regular and straight acellular-type cementum. Since only a small fraction of the tooth is analyzed, the proper selection of the ROI is, therefore, a critical initial step in the protocol. This issue is highly sensitive when dealing with archaeological teeth of ungulates since the Minimum Number of Individuals (MNI) is generally established on cheek teeth, and are thus preferentially selected for age and season of death analyses (Gordon 1988; Pike-Tay 1995; Naji, Gourichon, and Rendu 2015). For practical reasons, histological sections of molar and premolar roots are usually made longitudinally, following a mesio-distal axis (**Pubert et al., this volume**). However, the curvature of the root prevents the cut of a section to be strictly perpendicular to the increment deposits (as measured from the surface of the root), a necessary condition to avoid the superposition doubling effect of increments (Naji, Gourichon, and Rendu 2015; Cool et al. 2002). Consequently, contrary to the limited ROI obtained in transverse sections mostly used for canine and incisor human teeth (Naji et al. 2016), the portion of cementum observable on an ungulate longitudinal thin section can be very large, although only part of it is suitable for the analysis.

OBJECTIVE

To solve repeatedly raised issue (Roksandic et al. 2009), we decided to assess the inter-observer reliability regarding the three main steps of the microscopical analytical protocol in cementochronology: **step 1,** the selection of an optimal ROI, **step 2,** the estimation of the age-at-death, and **step 3**, the identification of the season of death estimations.

For the first step, we proposed a definition of the optimal ROI criteria that will be tested for clarity and reproducibility. For the second and third steps, agreement rates will be evaluated between documented age and season at death and the estimations proposed by the observers.

To define by common agreement the main criteria of ROI's selection, we used the White-Kaminuriak collection constituted of teeth of 999 members of the extant population of barren-ground caribou (*Rangifer tarandus groelandicus*) from Kaminuriak (Canada), with precise information about sex, age and date of death of each individual. These animals were killed in the late '60s by the Canadian Wildlife Service in Spring, Summer and Fall (namely in April, May, June, July, September, November and December). Thin sections were produced in the '90s by the Department of Anthropology at New York University and were analyzed in a previous study (Pike-Tay 1995).

From this study, 50 thin sections were selected for observation by four experienced analysts (LG, SN, WR, EP). The aim was to share our experience, acquired on different taxa and contexts, in order to describe the minimum number of common criteria for including or rejecting an ROI (**Erreur ! Source du renvoi introuvable.**Table 1: Criteria for the inclusion of a region of interest. White-Kaminuriak collection.) suitable for age and season at-death estimation (Table 1):

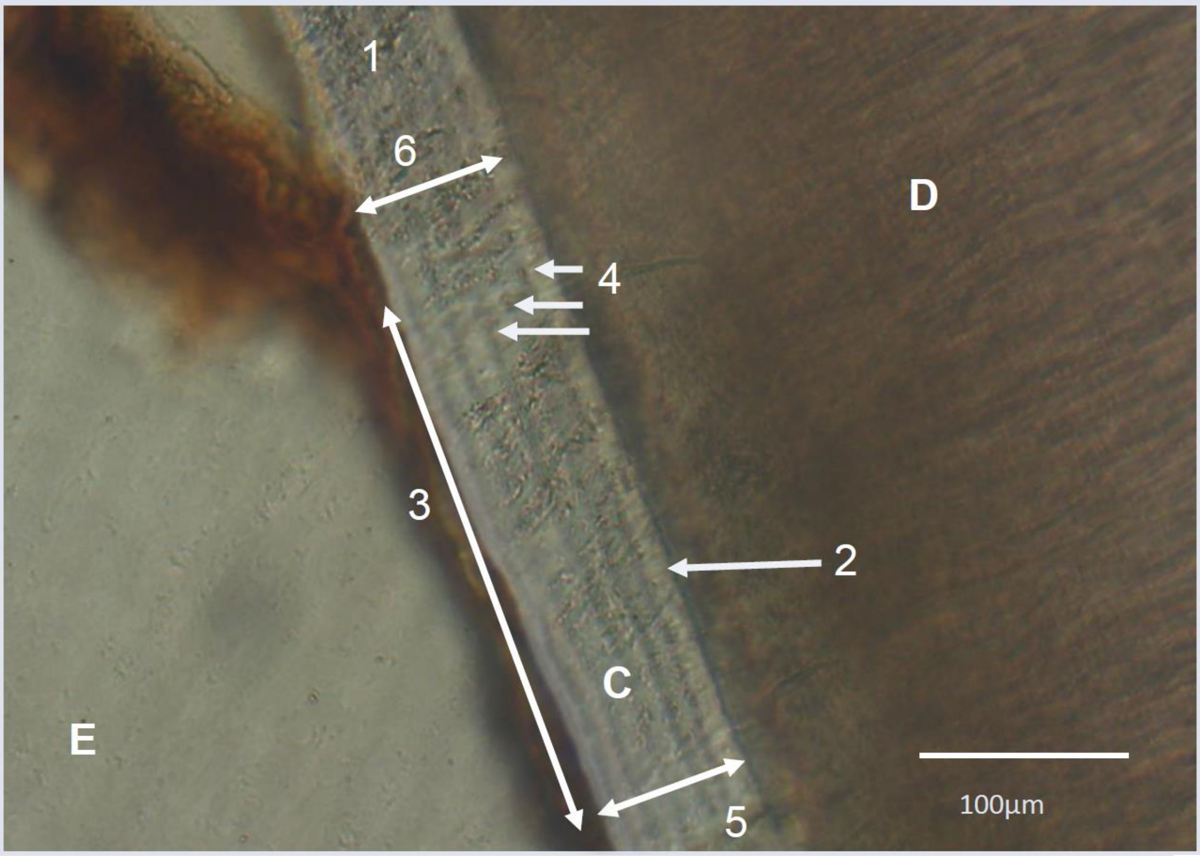

Figure 1: the six criteria for the selection of a ROI. Number refers to the criteria. Picture from the White-Kaminuriak collection.

#	CRITERIA - DEFINITION	GOAL
1	The observation must be made within the Acellular Extrinsic Fiber Cementum (AEFC), located generally *in the cervical third* of the root (see definition in **Foster et al., chap. X. this volume**).	avoids confusion with Cellular Intrinsic Fiber Cementum (CIFC) structures
2	Good visibility of the main histological structures in the cementum and the dentin: (i) Tomes' granular layer, (ii) hyaline layer of Hopewell-Smith (a.k.a. hyaline line), and (iii) dentin tubules.	confirms the nature of the AEFC; confirms that first and last increments are observable
3	Continuity of the AEFC increments on the whole ROI.	minimizes the presence of artifacts, increment splits or convergence
4	Comparable thickness for all increments: directly observed or measured with an image processing software (see Lieberman, Deacon, and Meadow 1990).	excludes CIFC inclusions (mixed cementum), or diagenetic changes
5	ROI should be at least twice as large as thick.	allows for repetitive observations and measurements
6	Edges of cementum (outer border and inner hyaline line) should be equidistant on the whole ROI.	confirms the expected shape of AEFC regular seasonal growth, as opposed to irregular CIFC reactive growth

Table 1: Criteria for the inclusion of a region of interest. White-Kaminuriak collection.

INSERER FIGURE 1

MATERIAL AND METHOD

SAMPLE SET

To test our criteria, we prepared a new set of thin sections from the White-Kaminuriak collection. To reduce the number of parameters, the sample was composed of 80 teeth from the same population of caribous. Ten teeth for each of the recorded seven months of death represented in the corpus were selected to account for all potential growth stages of the last AEFC increment. One or two thin sections were then produced for each tooth by one of us (EP) following the protocol described by Pubert and colleagues (**this volume**).

The teeth were embedded in epoxy resin using a vacuum chamber and then cut transversally in the mesio-distal plane with a Buehler Isomet 5000 saw. After polishing the cut surface, the embedded teeth were glued on a 30X45mm glass. Finally, the samples were grounded to a thickness of 100 µm and polished. A total of 115 thin sections were produced.

For the blind test, 30 thin sections were sampled by two of us (WR&LG) based on the presence of acellular cementum alone, identified with a polarized microscope at low magnification (X40).

TESTING AN OPTIMAL ROI SELECTION

Four observers with different skill levels in cementum analysis (A & B: experienced; C: intermediate; D: beginner) were asked to conduct the observation by pursuing three-step goals:

1) identify all the possible ROIs in each section.
2) count the number of light incremental deposits (in order to evaluate the age at death).
3) determine the nature and stage of growth of the last increment for estimating the season of death.

Regarding the last step, the procedure followed the method usually applied in zooarchaeology for the identification of the nature and growth stage of the last increment (Pike-Tay 1995; Gourichon 2004; Naji, Gourichon, and Rendu 2015; Sánchez-Hernández et al. 2019):

First, the **nature of the last deposit** is identified as either **(i)** a growth “**zone**,” usually formed during the summer (wide, translucent in natural light, or light in cross-polarized light); or **(ii)** a rest layer or “**annulus**”, usually formed in winter (thin, opaque in natural light or dark in cross-polarized light) (**See Naji, chap X this volume for a definition**).
Second, the **growth stage** of the last deposit is estimated. For a growth **zone**, three stages are defined by comparing the thickness of the last increment proportionally to the previous growth zone: **Z1-** *beginning* (width lesser than 1/3 of the previous one), **Z2-** *middle* (width lesser than 2/3 and greater than 1/3), and **Z3-** *late* (width greater than 2/3). For an **annulus**, the increment’s width is usually too thin to subdivide.

During the blind test, observations have been performed under the same conditions for all analysts, with the same Leica DM2500P microscope and Leica MC120HD camera. Analysts were asked to examine each of the 30 thin sections with a X400 magnification. Following Stutz (2002), pictures had to be recorded using three illuminations: bright-field (“natural” henceforth), cross-polarized, and cross-polarized with a full wave retardation plate (λ-plate). The observation under the λ-plate was required to identify diagenetic transformations, which is being recommended for all cementum analyses applied to archaeological context (Naji, Gourichon, and Rendu 2015).

For the first goal**,** the analysts identified the coordinates of the ROIs using a mechanical XY stage, based on the six criteria defined in figure 1. Analysts systematically photographed the ROI for an inter-observer comparison. The *number* of identified ROIs and their *locations* for each thin section were compared using their coordinates as well as visual histological features on the micrograph.
For the second and third goals, the increment *count*, and the identification of the nature and *stage of growth* of the last increment was compared between the analysts and checked against the information available for each caribou.
Analysis of all the collected data was done by one of the authors (WR).

RESULTS

STEP 1. SELECTION OF THE ROI

Sample information				Analyst A			Analyst B			Analyst C			Analyst D			Comparisons		
tth	**NB**	**Age**	**SEASON**	Number of ROI	Age	SeasonC	Number of ROI	Age	SeasonB	Number of ROI	Age	SeasonA	Number of ROI	Age	SeasonD	Analysts finding the same ROI	Nb Age Disagreement	Nb Season Disagreements
P4	304	**8,42**	Z3	2	0	Z3	1	0	Z3	1	1	A	1	N/A	N/A	ABCD	0	0
P3	310	**7,42**	Z3	2	0	Z3	2	0	Z3	1	0	Z1	1	1	Z	ABCD	0	1
P4	436	**7**	Z1	3	0	Z1	1	0	Z1	1	0	A	2	1	Z1	ABCD	0	1
P4	437	**5**	Z1	1	0	Z1	3	0	Z1	2	0	Z	1	2	N/A	ABCD	1	0
P4	440	**7**	Z1	2	-1	Z1	2	0	Z1	1	N/A	Z1	1	N/A	A	ABCD	0	2
M2	441	**7**	Z1	1	0	Z1	1	1	Z1	1	0	Z1	3	1	Z1	ABCD	0	0
M2	441B	**7**	Z1	2	0	Z3	2	0	Z3	1	0	Z3	1	2	Z3	ABCD	1	0
P4	500	**7**	Z1	1	0	Z1	1	0	Z1	1	-1	A		1	A	ABCD	0	1
P4	507	**6**	Z1	1	0	Z1	4	0	Z1	1	0	Z	2	1	A	ABCD	0	2
M2	**521**	**6,25**	Z2	1	0	Z2	1	0	Z2	1	-1	A	1	-1	Z3	ABCD	0	2
P3	566A	**2,25**	Z2	2	1	Z2	3	1	Z2	2	0	Z	3	3	N/A	ABCD	1	0
M2	602	**6,42**	Z3	1	0	Z3	1	1	Z3	1	-1	A	1	-1	N/A	ABCD	0	0
P4	607	**4,42**	Z3	1	1	Z3	2	1	Z3	2	0	Z(3)	1	3	N/A	ABCD	1	0
P4	**608A**	**3,42**	Z3	R			1	1	Z3	2	3	A	1	4	N/A	BC	2	0
P4	**608B**	**3,42**	Z3	1	3	A	1	1	Z3	1	3	A	1	3	Z1	ABC	3	0
M2	652	**8,42**	A	1	-1	A	1	0	A	1	0	Z	1	-1	N/A	ABCD	0	1
M2+P4	657	**3,42**	A	1	0	Z3	2	0	A	2	0	Z1	1	0	A	ABCD	0	2
M2	667	**4,42**	A	R			1	0	A	1	0	Z1	1	0	N/A	BCD	0	1
M2	684	**4,42**	A	2	0	A	1	0	A	1	0	A	1	-1	Z3	ABCD	0	1
M2	704	**4,42**	A	2	0	A	1	1	A	2	0	A	1	1	N/A	ABCD	0	0
M2	716	**6,83**	A	R			R			R			R			R	0	0
M2	754	**8,83**	A	1	-1	Z	1	1	A	1	1	Z	1	2	N/A	ABCD	1	2
P4	774	**5,83**	A	1	1	A	1	2	A	1	2	A	1	1	N/A	ABCD	2	0
P4	777	**5,83**	A	1	1	A	1	1	A	2	N/A	N/A	3	N/A	N/A	ABCD	0	1
M2	783	**6,93**	A	1	1	A	1	1	A	2	1	A	2	2	Z1	ABCD	1	0
M2	**793b**	**9,83**	A	1	-1	Z1	1	-1	Z1	1	-2	Z1	1	-2	Z3	ABCD	2	1
P4	920	**9,08**	Z2	2	1	Z2	3	0	Z2	1	0	Z1	2	0	N/A	ABCD	0	1
M2	926	**10,08**	Z2	1	0	Z2	1	-1	Z2	1	0	Z1	1	1	A	ABCD	0	2
P4	935	**5,08**	Z2	1	0	Z2	1	0	Z2	1	1	Z	1	0	N/A	ABCD	0	0
P4	992	**7,08**	Z2	1	0	Z2	1	0	Z2	1	-1	Z1	1	-1	N/A	ABCD	0	2

Table 2: Results of the blind test by thin section and analyst. Age is expressed in year; the season is expressed by the theoretical last stage of AEFC increments growth (Z1: Zone Beginning of Growth; Z2: Zone Middle of Growth; Z3: Zone End of Growth; A: Annulus, R: rejected; N/A: no answer). Age estimation for each analyst represents the deviation with the real age.

All 30 thin sections were observed by the four analysts blindly (Table 2). One of the thin sections (#716) was rejected by all analysts on the bases of the absence of criteria 2, 3, 4, and 6.

The number of identified ROIs (Table 2 and 3) does not strictly reflect the degree of experience of the analysts, since one of the most experienced (B) identified a total of 43 ROIs, while the other observers identified only 37 or 38 ROI. A range between one and three ROIs was identified per thin sections, with a mean of 1.4.

For 26 thin sections, all analysts found at least one common ROI (Table 2 & Fig. 2**Erreur ! Source du renvoi introuvable.**).

In two cases (#608A and #667), analyst A considered that criteria 1 was not respected, and in one of these cases (# 608A) analyst D selected an ROI rejected by the three other observers based on the absence of criteria 4 and 6.

Finally, for thin section 608B, analyst D selected a different ROI than analysts B and C, which appeared to disregard criteria 1 (Table 2 & Fig. 3).

	Analyst A	Analyst B	Analyst C	Analyst D
4 agreements	26	26	26	26
3 agreements	1	2	2	1
2 agreements		1	1	
Rejected teeth	3	1	1	1

Table 3 : Number of occurrences of agreements for all analysts and per specific analyst.

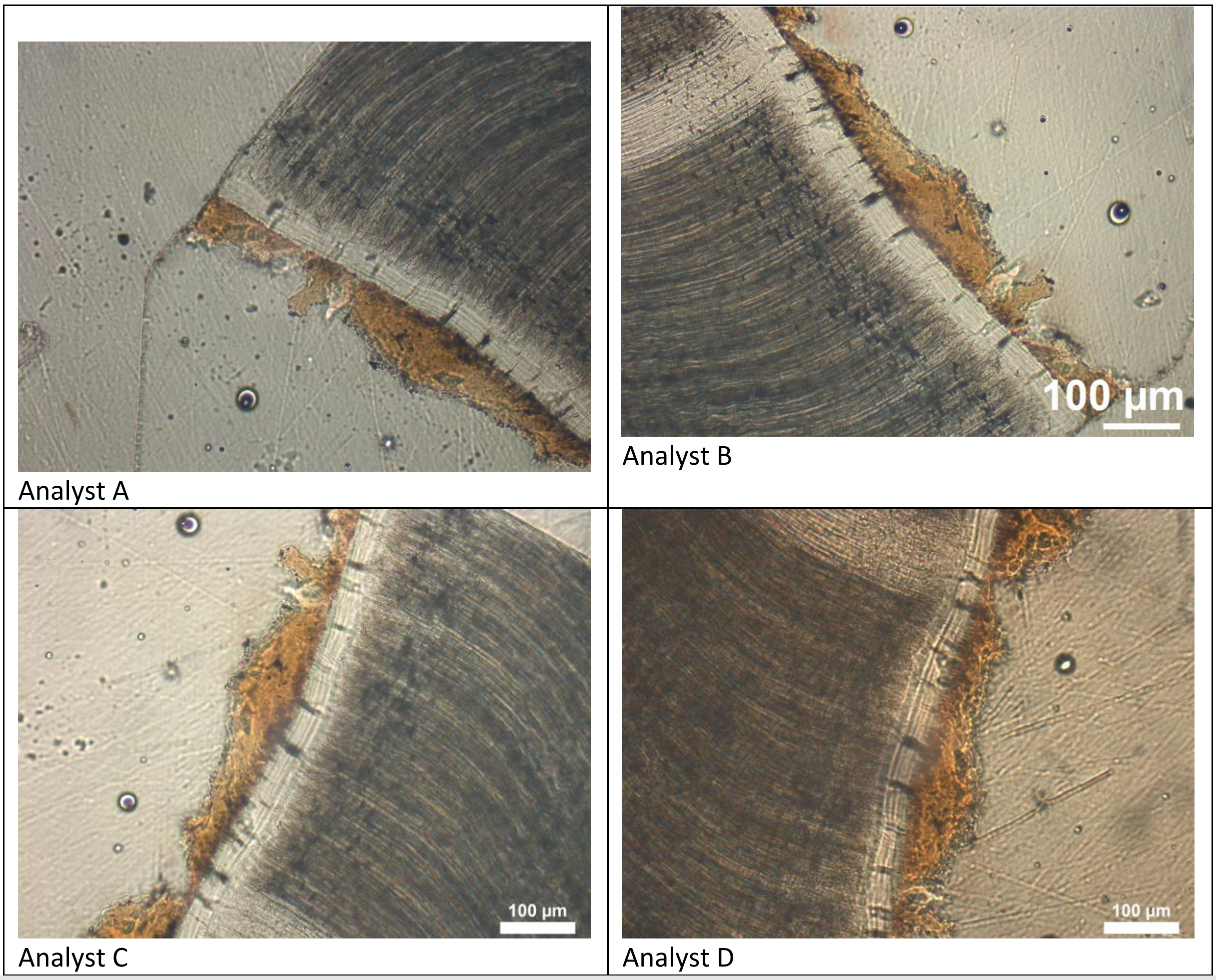

Figure 2 : Example of agreement in the identification of ROI. Here, the four analysts selected the same portion of the thin section to make their analysis.

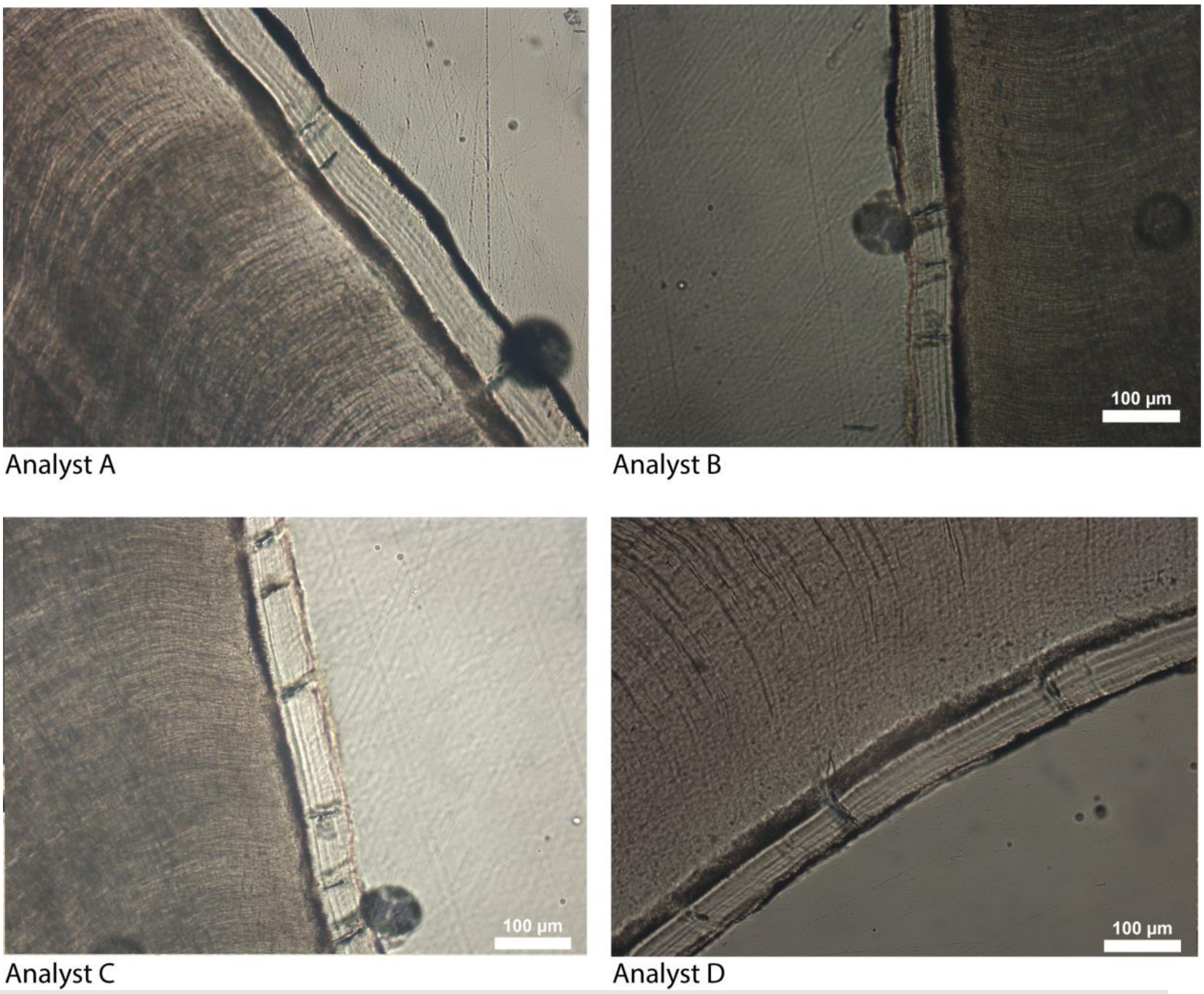

Figure 3 : Example of disagreement in the identification of ROI. Here, three analysts selected the same portion of the thin section to make their analysis, while Analyst D selected another region rejected by the others.

STEP 2. AGE ESTIMATION

While the ROIs were successfully identified most of the time, it was not possible for the different analysts to propose a valid increment count in every case. For these specific cases, the analysts identified some problems in the thin section preparation (e.g., grounding too intense) that limited their confidence in counting the increments. Results presented here are thus proposed for sections with identified ROIs only.

Out of the 29 thin sections in which one or more ROIs were selected by the analysts, from 26 to 29 provided increment counts, i.e. 108 observations which can be used for estimating the individual age. The observed counts were compared to the expected number of increments for each tooth, number calculated by subtracting the eruption age of the tooth from the documented age of the individual. Considering the variability in the eruption dates (Canadian Wildlife Service and Miller 1974), and considering that most of the biologists agree that a margin of error of ±1 year is acceptable for the age estimation based on cementum count (Matson et al. 1993), we have defined a "success" when the estimation is equal to the expected increment number or has a deviation of only one unit (above or below) from the latter.

Table 4 shows a large variability in the results with a total of 86% (93/108) increment counts in agreement with the expected age. Overestimations are more frequent (12%, 13 cases) that underestimations (1.9%, 2 cases).

The discrepancies between the analysts are of interest since their reliability is ranked by experience, with the most experienced observers (A and B) scoring very high success rates (>95%); the intermediate observer (C) scoring 85%, and the least experienced (D) scoring 65%. This ranking confirmed previously published recommendations that proper training should be set up before data observation (Naji et al. 2016, **Wittwer-Backofen *et al.*, this volume**). If we only include the experienced analysts, the success rates are 96 and 97% for analysts A and B, respectively, with no undervaluation, and only a 1.9% overestimation by one or 2 years only above the usual confidence interval.

Difference between the counting and the expected results.	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**NA**	**Success rate**
Analyst A		4	16	6		1		3	96%
Analyst B		2	16	10	1			1	97%
Analyst C	1	4	15	4	1	2		3	85%
Analyst D	1	5	4	8	4	3	1	4	65%

Table 4: number of discrepancies between the observed and the expected numbers of increments per analyst.

Based on the recorded observations on problematic ROIs, the one to two-year underestimations seems to be linked to the misidentification of the first increment after the hyaline line, when it is a thin summer band (i.e. when teeth are in occlusion at the end of the root formation).

The count overestimations systematically occurred with the same thin sections. Based on the micrographs, it was possible to evaluate that in at least half of the cases, there was a microscope focus problem that led to an doubling optical effect due to the increment superposition throughout the thickness of the section (Naji, Gourichon, and Rendu 2015; Naji et al. 2014; Cool et al. 2002). This is specifically true for the thin section 608B where analysts C and D both overestimated by over 3 years

For 608A, two analysts selected the same ROI which does not follow criteria 1 and 4 (presence of cellular cementum appositions) (Figure 4).

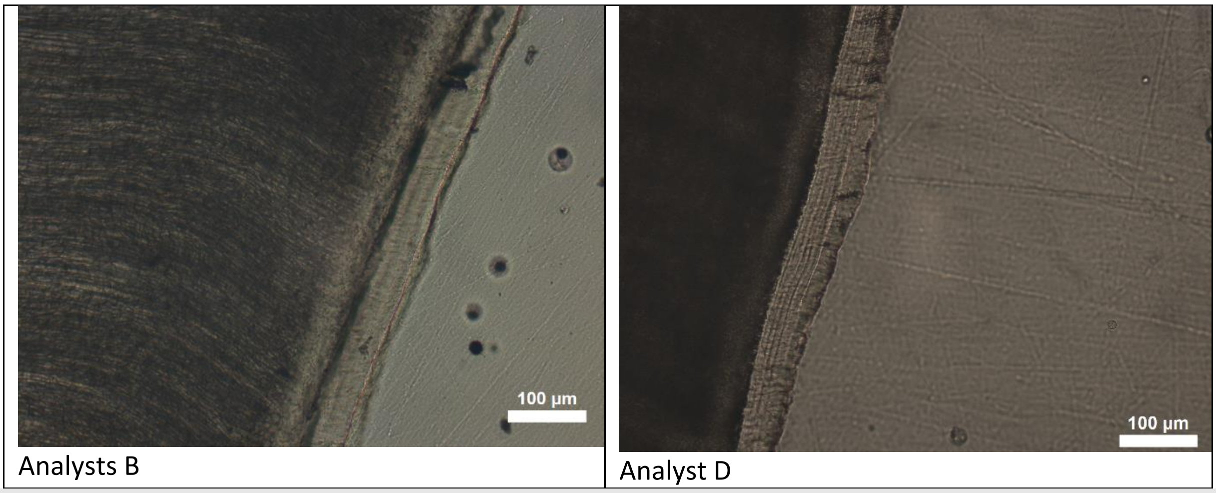

Figure 4: Thin section 608A. Analyst D selected an ROI with an extra apposition of cellular cementum, leading to an overestimation of the age at death. Analyst B, who selected an ROI by properly following the guidelines, obtained the right estimated age.

Moreover, analyst D frequently used low magnifications for some of the observations, contrary to the test requirements.

Thus, the definition of the original criteria appears to not have been clear enough for unexperienced analysts, which led to misinterpretations and inaccurate observations.

STEP 3. IDENTIFICATION OF THE SEASON OF DEATH

The identification of the last increments for the season of death estimate is known to be a difficult task which directly depends on the experience of the observer (Naji, Gourichon, and Rendu 2015; Lubinski and O'Brien 2001). Thus, it is not surprising to observe that this estimation was made for only 86% (98/114) of the selected ROI. However, this result masks a large disparity since the analysts A, B and C proposed a season estimation for most of the ROIs selected (up to 100% for analyst B), whereas the analyst D, probably less confident than the others, barely reaches 50%.

Annulus

Between the two seasonal appositions (growth/rest), the annulus (rest band) was misidentified more often, specifically by the two least experienced analysts (Table 5). In two cases, analyst C identified a complete growth zone instead of an annulus for an animal that died in early December, at the time of initiation of the annulus period (Pike-Tay 1995). Similarly, for sample 793 that died in the same period, three of the analysts identified the last increments as a first stage growth zone (Z1). In this case, it is possible that the cementum cycle of this specific individual was slightly in advance compared to the theoretical patterns. Indeed, small variations exist within a population, and the transition from one phase of cementum deposit to the other can be spread over several weeks (for this specific population see (Pike-Tay 1995).

	NTO	ANALYST A	ANALYST B	ANALYST C	ANALYST D
ANNULUS	11	3	1	5	3
GROWTH ZONE	19	1	0	7	4
No estimation		4	1	2	16

Table 5: Number of errors made by the analyst regarding the identification of the last increment (which is estimated for each tooth on the theoretical growth pattern established on the same collection by (Pike-Tay 1995)). NTO: Number of teeth observed. No estimation: tooth for which no estimation was given.

Zone

The growth zones were accurately identified by the two experienced (A&B) analysts (error rate: A: 1/18 = 5.6%; B: 0%; Tables 2 and 6). Whereas the two least experienced observers (C&D) had a significantly high error rate (C: 7/19 = 36.8%; D: 4/10 = 40%; Tables 2 and 6), and even higher when considering the estimation of the growth stage (C: 11/19 =58%; D: 7/10 = 70%).

However, we have to emphasize that one shared misinterpretation of a Z1 stage is related to tooth 441B which was interpreted by all analysts as a Z3 stage. Since everyone agreed on the observation, we can hypothesize that we may be dealing with an individual deviating from the expected pattern of cementum deposition.

GROWTH STAGE	NTO	ANALYST A	ANALYST B	ANALYST C	ANALYST D
Z1	7	1	1	5	4
Z2	6	0	0	6	2
Z3	6	1	0	5	2
No estimation		1	0	0	9

Table 6: Number of errors made by the analyst regarding the identification of the stage of growth of the last increment when it was a growth zone (estimated for each tooth on the expected growth pattern identified on the same collection by (Pike-Tay 1995)). NTO: Number of teeth observed; No estimation: teeth for which no estimation was given.

The error rate in the identification of the nature and stage of growth of the last increments is relatively high for the two least experimented analysts (C and D). As mentioned above, it appears that analyst D did not apply the protocol rigorously, and made several observations using a low magnification (X100), instead of the recommended X400. This difference probably explains the high number of thin sections rejected or for which no estimation could be proposed (Table 7).

Similarly, analyst C tried to give an estimation of the season of death systematically, even though in five cases all the criteria were not met and doubts about the estimation were expressed. For these cases, results were in disagreement with the expected pattern.

	Nb without estimation	Nb agreement	Nb disagreement	Error rate
Analyst A	3	22	5	**81%**
Analyst B	1	27	2	**93%**
Analyst C	2	7 (+4)	17	**23%**
Analyst D	16	3 (+1)	10	**21%**

Table 7: number of agreements and disagreements with the expected results of the nature and stage of growth of the last increments. In parentheses are added the cases when a zone was properly identified but without evaluation of its stage of growth

Since most of the analysts found the same ROIs, the choice of the ROIs does not seem to influence the agreement rate for this final step. The disagreements seem to be mostly about the winter increment (annulus), frequently not recognize by the least experienced analysts.

IMPLICATIONS

STEP 1, ROI: The criteria proposed for the **identification of optimal ROIs**, were based on the aggregated experiences of various specialists, and relied on the proper identification of optical and histological structures. In most cases, the implementation of the six criteria allowed for the identification of the same ROIs (26/29 = ca. 90% success rate). This is a central point. Firstly, this result attests to the reproducibility of the observations, even for analysts with limited experience. Secondly, because the selected region is strictly the same, the criteria allow for results comparison. In 10% of the cases however, one observer made a decision different than the others. In these three cases, criteria 4 (same thickness of the increments), and 6 (equidistant cementum edges) were not followed. This deviation could indicate that these two criteria are less likely to be understood, or simply implemented, while the other four are easily integrated by the observers.

Thus, by applying the criteria rigorously, and following the protocol precisely, optimal ROI selection can be obtained systematically. Documentation including images of typical or problematic cases would support initial training for this fundamental step.

STEP 2, age-at-death: the agreement rates for the age estimation, based on the number of growth zones observed, were very high for the most experienced (A&B) analysts with 96% and 97% respectively. For the least experienced observers (C&D), the rates were significantly lower (85% and 65% respectively). The difference in success is clearly due to experience, even when the optimal ROI is accurately selected at the previous step. Miscounting seemed to have been mostly influenced by microscope focal adjustments leading to blurred lines.

STEP 3, season of death: As expected, the identification of the nature and stage of growth of the last increment was more difficult. The two most experienced analysts obtained a correct success rate (>80%). Conversely, the two least trained analysts departed from the protocol, and reached a poor agreement rate (<24%).

In conclusion, **applying the criteria rigorously, and following the protocol precisely** solves the initial step of selecting an optimal ROI. Following that step, our inter-observer test confirms previous reports (Lubinski and O'Brien 2001; Colard et al. 2015; Charles et al. 1986; Naji et al. 2016, and **Wittwer-Backofen and Engel, this volume**) emphasizing that accurately identifying and counting the cementum increments requires **proper training** on a documented collection before the analysis. However, as shown by the results obtained by the "beginner" and "intermediate" analysts, increment counting seems to be a relatively straightforward task to perform correctly and whose improvements, apart from training, can be made by adjusting the microscopic focus and using image processing software. Conversely, the identification and interpretation of the last cementum increment for estimating the season at death, which is a critical information for zooarchaeology and other disciplines, probably remains the most difficult step to control. This suggests that if the first parts of the analytical procedure in cementochronology can be carried out by little experienced observers, extra precautions should be taken for the last step. In view of the error rate observed with modern specimens, this caveat is all the more relevant when dealing with archaeological material, particularly because of the additional taphonomic issues (Gourichon et al., **this volume**).

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