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Comparison of age determination techniques for known-age Cape fur seals

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Known-age teeth were used to validate age determination techniques for the Cape fur seal (*Arctocephalus pusillus pusillus*). Thin sectioning and staining of decalcified teeth produced the poorest age estimates. For etched half canines, only upper canines could be used to estimate age with good results, and coating improved the accuracy. Scanning electron microscopy produced poor accuracy in age estimation. External ridges reflected age accurately only in younger age classes and should only be used to verify counts of internal growth layer groups, or when rapid, preliminary estimates of age are necessary. This study has highlighted the importance of comparing different age determination techniques and validating such techniques with known-age animals. The reliability with which age can be estimated for the Cape fur seal has also been improved.

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Laws (1962) and Casselman (1983) have emphasised the need for quantitative study and comparison of age determination methods. Furthermore, such methods require validation with known-age animals. As few such comparisons have been made, the aim of this study was to review three age determination methods using teeth of known-age Cape fur seals *Arctocephalus pusillus pusillus*, and compare their accuracy with the two methods described by Oosthuizen (1997) and Oosthuizen, Greyling & Bester (in press).

The methods to be compared are based on the counting of growth layer groups (GLGs) in teeth which are then related to the actual age of an animal (Bowen, Sergeant & Øritsland 1983). GLGs are a repetitive or semi-repetitive pattern of parallel incremental growth layers and are parallel within the formative surface of the tooth hard tissue (Perrin & Myrick 1980). The age determination methods to be compared were based on:
(i) acid-etching of half canine teeth
(ii) Scanning Electron Microscopy (SEM) of half canine teeth
(iii) counting external ridges on the canine teeth.

Methods

Both upper and lower canines were used and the results compared. Teeth were cut in half longitudinally and care was taken to expose surfaces along the midline so as to avoid interpretation problems of the GLGs (*cf.* Hohn, Scott, Randall, Sweeney & Irvine 1989; Oosthuizen 1997). For each age determination method, five independent replicate counts of GLGs in each canine were made by a single observer to exclude biases amongst readers (Anas 1970; McLaren & Smith 1985). The final age estimate was the mean number of GLGs counted and rounded off to the nearest full year. The median birth date of all seals was taken to be 1 December (Shaughnessy 1979).

A weighted least squares regression procedure (Press, Teukolsky, Vetterling & Flannery 1992) was used to evaluate the fit of a linear model to the results of each age determination method. The null hypothesis, that the estimated age equalled the true age, was rejected at the 95% confidence level if: (1) the intercept and slope of the model were different from zero and one respectively, (2) the linear model was inappropriate or (3) if the Runs test (Zar 1984), indicated that the residuals were serially correlated (following Oosthuizen 1997). Biases in prediction occur if residuals are serially correlated. The runs test was used to assign a probability to the hypothesis that the residuals were not serially correlated, and if this probability did not exceed 0.05, the fit was rejected.

(i) Etched half sections

The teeth were halved along the mid-longitudinal plane with a Buehler isomet low speed saw, as outlined in Oosthuizen *et al.* (in press). The half section containing the midline was first polished on a felt wheel until smooth, and then etched for 15 min in 5% formic acid with the cut side fully exposed to the acid. The acid solution, with a volume at least 10 times that of the teeth, was continually stirred to prevent disproportionate etching. After etching, the sectioned teeth were washed in water for 1 h, sonically cleaned on a Branson ultrasonic cleaner, and air dried. The etched halves were then read under reflected light using a dissecting microscope at low magnification (7–10×). To evaluate whether coating would improve the accuracy of the age estimation by enhancing the GLGs, the etched male upper canine (MUC) surfaces were coated with gold-palladium and re-examined.

(ii) Scanning electron microscopy (SEM)

Only MUC, already etched and coated with gold-palladium were examined, as they were reasonably straight and the most likely to provide adequate results. Each specimen was viewed on a Cambridge S 200 scanning electron microscope. The etched surfaces of the specimens were photographed at a 30 degree tilt at 10 kV, including at least one-half of the tooth from the midline to the periphery. Contact prints were made and the number of GLGs was counted directly from the prints.

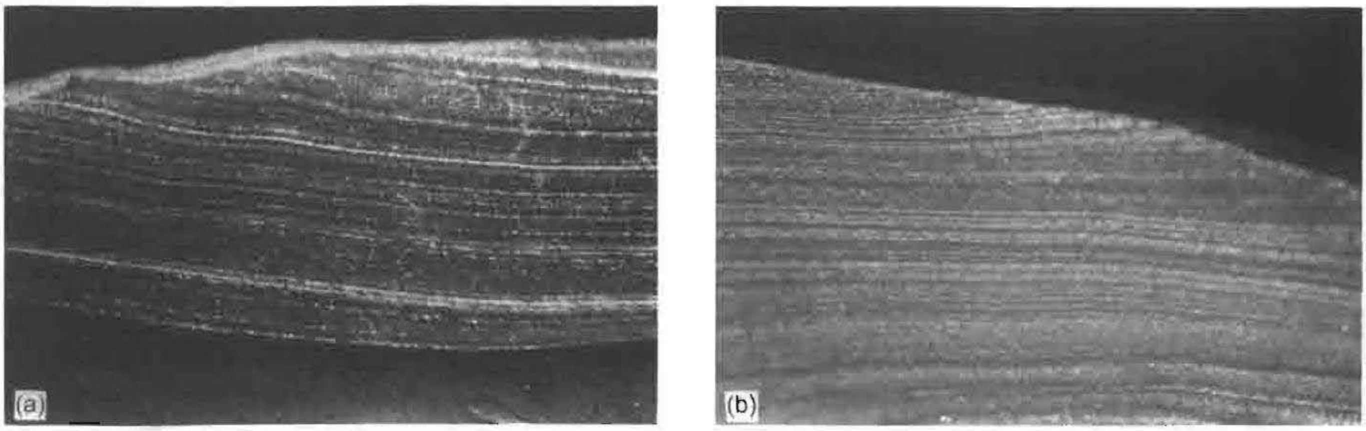

Figure 1 Longitudinal etched section of the canine of (a) a 6.5-year-old male to show the presence of GLGs in the dentine and (b) a 3.5-year-old female to show the absence of GLGs in the cementum.

(iii) External ridges

The surface of the roots of upper and lower canines of both sexes was examined with the unaided eye under a strong light and the number of ridges counted. The teeth did not require any preparation other than cleaning.

Results

(i) Etched half canines

The GLGs in the dentine showed up as clear valleys and ridges on the surface of the etched canines, but the GLGs in the cementum were unreadable (Figure 1). The estimated mean number of GLGs counted in the dentine, fitted to the true ages (Figure 2) provided the parameters a (intercept) and b (slope) with their associated error estimates (Table 1).

For female lower canines (FLC), the null hypothesis was rejected as the intercept (+ 2 *SE*) of the linear regression was smaller than zero and the probability of the Chi-square value being exceeded by chance was too small (Table 1). The accuracy of the age estimates was poor, with the largest difference between the estimated and true age being six years for one 11- year-old animal (Figure 3a). For female upper canines (FUC) the null hypothesis was accepted on all grounds (Table 1). There were few errors in age estimation, but when errors were evident, the difference between the estimated and true age was as high as five years (Figure 3b).

For male lower canines (MLC) the null hypothesis was rejected as the residuals were serially correlated (Table 1). The errors in age estimation tended to be over-estimations by as much as two years and not one seal older than five years was correctly aged (Figure 3c). In the male upper canines the null hypothesis was accepted on all grounds (Table 1). The difference between the estimated and true age was never more than two years, and the tendency was to over-, rather than under-estimate the true age (Figure 3d). Coating the MUC reduced the number of age estimation errors (Figure 3e), and improved the regression fit (Table 1).

(ii) SEM

The GLGs in the dentine were clearly visible but were unreadable in the cementum (Figure 4). The null hypothesis was rejected as the intercept (+ 2 *SE*) of the linear regression

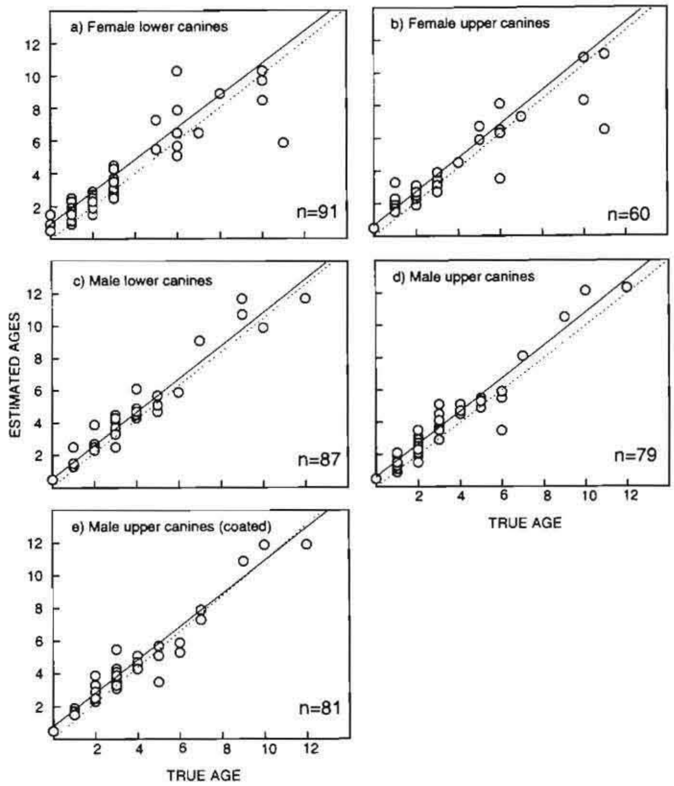

Figure 2 The means of the replicate estimated ages of the etched sections of canines fitted to the true ages using a maximum likelihood estimator (solid line) and compared to a line with a slope of one and intercept of zero (broken line).

was smaller than zero (Figure 5) and the probability of the Chi-square value being exceeded by chance very small (Table 2). The residuals were not serially correlated (Table 2), but there was a slight tendency to over-estimate the age in the younger age classes (Figure 6). The accuracy of determining the true age was poor with the largest difference between the true age and the estimated age being two years (Figure 6).

(iii) External ridges

The external ridges showed up clearly on the surface of the roots of the canines (Figure 7). The estimated means calculated from the counts of external ridges were fitted to the true

Table 1 Comparison of intercept and slope parameters, their standard error estimates and Chi-square and Runs test values for etched half upper and lower canines of both sexes

	Female lower	Female upper	Male lower	Male upper	Male upper coated
Intercept (a)	−0.179	−0.129	−0.015	−0.038	−0.063
SE of a	0.066	0.101	0.073	0.087	0.086
$a - 2(SE)$	−0.311	−0.330	−0.162	−0.211	−0.234
$a + 2(SE)$	−0.047	0.072	0.131	0.136	0.108
Slope (b)	0.999	0.992	1.016	0.954	1.014
SE of b	0.023	0.033	0.025	0.029	0.030
$b - 2(SE)$	0.953	0.926	0.964	0.895	0.954
$b + 2(SE)$	1.045	1.057	1.069	1.013	1.074
Chi-square	152.36	48.454	30.114	47.386	44.788
Q	<0.001	>0.005	>0.9	>0.1	>0.1
Runs Test	−1.373	−1.275	−2.079	−1.584	−0.741
Z	0.170	0.202	0.038	0.113	0.459
N	91	60	87	79	81

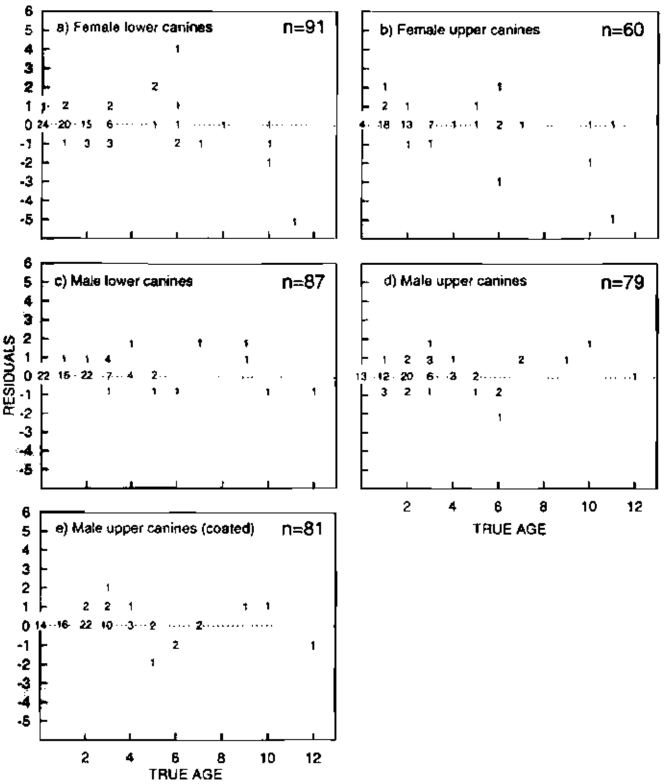

Figure 3 Residuals of actual age minus estimated age from etched sections for each year class.

Figure 4 Longitudinal section of a 1.5-year-old male half canine examined with a scanning electron microscope to show the presence of GLGs in the dentine and the absence of GLGs in the cementum.

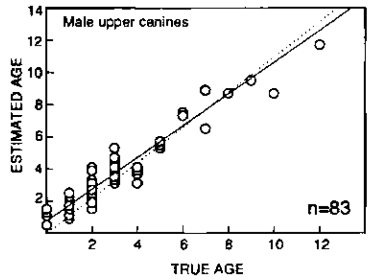

Figure 5 The means of the replicate estimated ages taken from the scanning electron microscope photographs of male upper canines fitted to the true ages using a maximum likelihood estimator (solid line) and compared to a line with a slope of one and intercept of zero (broken line).

ages (Figure 8). For FLC the null hypothesis was accepted on all grounds (Table 3). The largest difference between the age estimates and the true ages was three years. There was a slight tendency to over-estimate age especially in the age classes 2 to 7 years (Figure 9a). For FUC, the null hypothesis was rejected as the intercept (+ 2 SE) and slope (+ 2 SE) of the regression were smaller than zero and one respectively (Table 3). The largest difference between the age estimates and the true age for ≤ 5-year-old seals was one year, with a tendency to over-estimate, but for the age classes ≥ 6 years, the age was under-estimated by as much as five years (Figure 9b).

Table 2 Comparison of intercept and slope parameters, their standard error estimates and Chi-square and Runs test values for upper male canines examined under a SEM

	Male
Intercept (a)	−0.204
SE of a	0.083
$a - 2(SE)$	−0.370
$a + 2(SE)$	−0.038
Slope (b)	1.052
SE of b	0.027
$b - 2(SE)$	0.997
$b + 2(SE)$	1.107
Chi-square	71.221
Q	< 0.001
Runs test	−0.114
Z	0.909
N	83

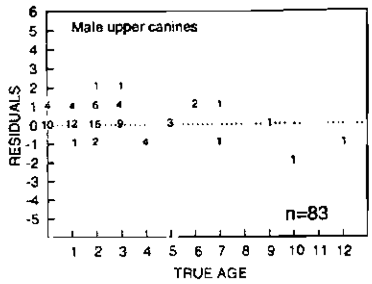

Figure 6 Residuals of actual age minus estimated age from scanning electron microscope photographs of male upper canines for each year class.

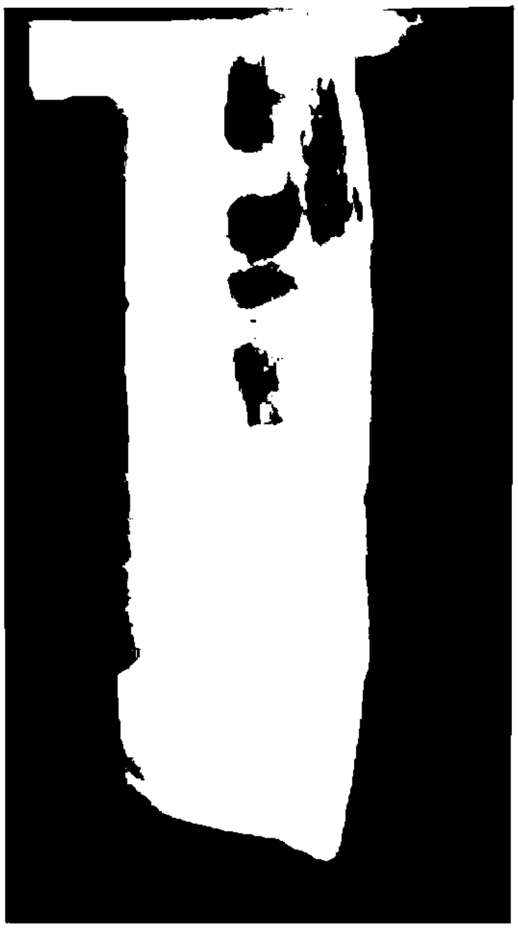

Figure 7 Whole upper canine of a 5.5-year-old male to show the presence of external ridges on the root.

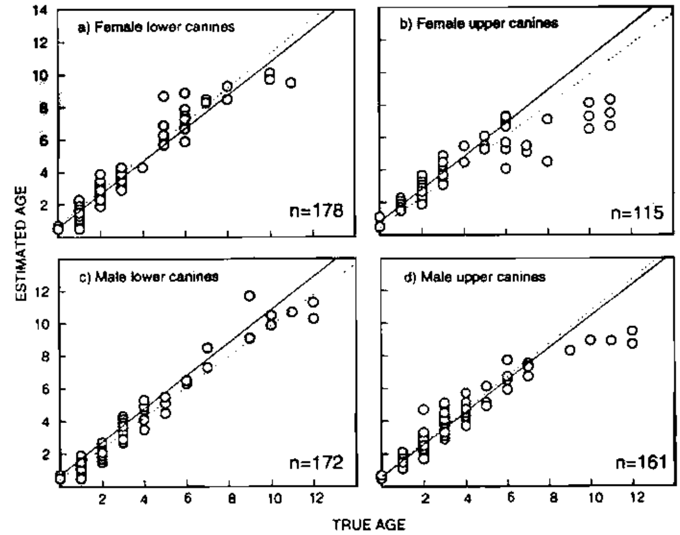

Figure 8 The means of the replicate estimated ages from the external ridges of the canines fitted to the true ages using a maximum likelihood estimator (solid line) and compared to a line with a slope of one and intercept of zero (broken line).

Table 3 Comparison of intercept and slope parameters, their standard error estimates and Chi-square and Runs test values from examination of the external ridges on the upper and lower canines of both sexes

	Female lower	Female upper	Male lower	Male upper
Intercept (*a*)	–0.004	–0.504	–0.027	–0.090
SE of *a*	0.045	0.067	0.047	0.057
a – 2(*SE*)	–0.095	–0.637	–0.068	–0.205
a + 2(*SE*)	0.087	–0.370	0.122	0.025
Slope (*b*)	1.018	0.951	0.931	1.016
SE of *b*	0.020	0.017	0.023	0.019
b – 2(*SE*)	0.980	0.916	0.886	0.978
b + 2(*SE*)	1.056	0.985	0.976	1.055
Chi-square	69.250	302.56	47.989	90.032
Q	>0.9	>0.1	>0.999	>0.1
Runs Test	–1.267	–0.089	–5.186	–5.186
Z	0.205	>0.001	>0.001	>0.001
N	178	115	172	161

For MLC, the null hypothesis was rejected as the slope (+ 2 *SE*) of the regression was smaller than one and the residuals were serially correlated (Table 3). The residuals also showed a trend with the estimated age being too low (Figure 9c). In the MUC the null hypothesis was rejected as the residuals were serially correlated (Table 3). The accuracy with which true age was estimated for ≤ 7-year-old seals was reasonable (within two years), but in the ≥ 9 age classes it under-estimated the age by as much as four years (Figure 9d).

Discussion

Etching of teeth can be used to determine age in the Cape fur seal from GLGs in the dentine, as for Weddell seals *Leptonychotes weddellii* (Stirling 1969), New Zealand fur seals *Arctocephalus forsteri* (Mattlin 1978) and Ross seals *Ommatophoca rossii* (McCann 1993). With the exception of counting external ridges, etching also has an advantage compared with other methods, in that the time-consuming preparation of sections is obviated (Perrin & Myrick 1980). However, the distinct disadvantage of etching is that one could not follow the midline of the tooth along the curve, especially in the heavily curved lower canines. This leads to erroneous readings which can be out by as much as five years. The upper canines are normally less curved and give more accurate age estimates, but the few that are excessively curved lead to incorrect age estimates. The coating of the MUC improved the accuracy of

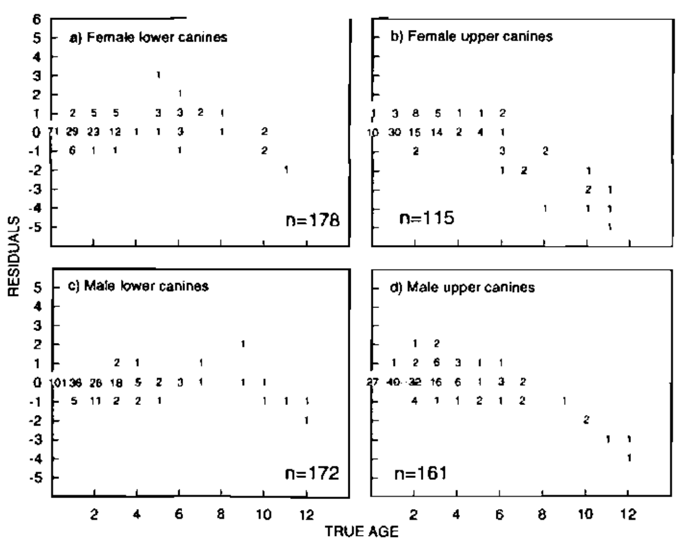

Figure 9 Residuals of actual age minus estimated age from external ridges for each year class.

age determination; a result which is in agreement with Perrin & Myrick (1980), who used gold-palladium coating with good results for the harbour porpoise *Phocoena phocoena*.

On the other hand, the accuracy with which age was estimated from the GLGs in the dentine with SEM was poor and this method could not be used. This was mainly a result of the relief of the finer detail being enhanced to such a degree that it proved difficult to distinguish GLGs from incremental layers, especially in young age classes. This accords with the finding of Goren, Brodie, Spotte, Ray, Kaufman, Gwinnett, Sciubba & Buck (1987), who used three methods to count GLGs in the teeth of known-age beluga whale *Delphinapterus leucas*, and judged SEM to be the least useful technique. SEM is also too expensive and time consuming to be used routinely. However, SEM produces excellent resolution of fine details of the GLGs (Hohn 1980; Pierce & Kajimura 1980; this study).

According to Payne (1978) external ridges originate from, and correspond to, the number of GLGs in the dentine, and can be used to verify the number of internal GLGs (Fiscus, Baines & Wilke 1964; Boyd & Roberts 1993). However, although the external ridges were prominent on the upper and lower canines, they were more pronounced in males than in females. In some canines the external ridges were barely discernible, a pattern similar to that observed in the northern fur seal *Callorhinus ursinus* (Scheffer 1950). This is in agreement with Laws's (1962) statement that the clarity of the ridges varied and that they were less pronounced in females.

The external ridges can be used to estimate age in the Cape fur seal with reasonable accuracy up to about 10 years of age for both sexes. The tendency of the ridges to be laid down closer together with increasing age, and the increasing deposition of cementum which obscured the ridges, made it progressively more difficult to estimate age in older animals. This accords with Anas (1970), who determined age reliably in female northern fur seals only up to seven years, and Payne (1978; 1979) who estimated age up to seven years in male, and six years in female Antarctic fur seals. Furthermore, the accuracy with which the age was estimated was better with sectioned teeth than external ridges in the Cape fur seal (Oosthuizen 1997; Oosthuizen, *et al.*, in press). This is in contrast to northern fur seal females ≤ 7 years old, where age was more accurately estimated from whole teeth than from sections (Anas 1970).

Compared to the age estimation procedures examined in the present study, estimates obtained by counting GLGs in the dentine of ground thin canine sections gave the best results (Oosthuizen 1997). Furthermore, in contrast to the perception that histological procedures produce the most consistent results (Stoneberg & Jonkel 1966; Roberts 1978; Boyde 1980; Fancy 1980), Oosthuizen, *et al.* (in press) found that histologically prepared thin, stained, decalcified sections should not be used in the Cape fur seal as they gave poor results. However, all methods based upon incremental lines in dentine are hampered by the fact that the deposit of dentine into the pulp cavity gradually fills it until it closes the cavity at about 13 years of age. The further formation of dentine GLGs is prevented and it becomes impossible to determine age (McCann 1993; Oosthuizen 1997). In such cases cementum GLGs in ground thin sections of postcanines can be used, but the same accuracy will not be obtained (Oosthuizen 1997).

Conclusions

The recommended age determination method to use in both female and male Cape fur seals, is to count dentine GLGs in upper canine ground sections for age classes where the pulp cavity is still open, and cementum GLGs in postcanine ground sections for older animals (Oosthuizen 1997). The second best method is the etching of half teeth, and external ridges should be used only in cases where rapid, preliminary estimates of age are required. SEM and thin stained, decalcified sections should be avoided as techniques to determine age in the Cape fur seal (Oosthuizen, *et al.*, in press; this study).

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