MARINE MAMMAL SCIENCE, 21(2):346–354 (April 2005)

COMPARISON OF BETWEEN-TOOTH AGE ESTIMATES OF ATLANTIC WALRUS (*ODOBENUS ROSMARUS ROSMARUS*)

ROBERT E. A. STEWART
Department of Fisheries and Oceans, Freshwater Institute,
501 University Crescent, Winnipeg, Manitoba R3T 2N6, Canada
E-mail: stewartre@dfo-mpo.gc.ca

BARBARA E. STEWART
Sila Consultants, 1218 Marchand Road,
Howden, Manitoba R5A 1J6, Canada

The use of layers in hard structures to estimate ages of marine mammals is well established, as are the requirements to use the appropriate tissue and methods of preparation (Hohn *et al.* 1989, Bernt *et al.* 1996, Stewart *et al.* 1996, Hohn and Fernandez 1999). Walrus (*Odobenus rosmarus*) are customarily aged by counting growth layer groups in thin, longitudinal sections of the lower canine (Fay 1955, 1982, Mansfield 1958, Garlich-Miller *et al.* 1993) although other teeth are sometimes used (Born 2003). Most biological samples used to determine ages of walrus are derived from subsistence hunts (*e.g.*, Fedoseyev and Goltsen 1990, Fay *et al.* 1989, Outridge *et al.* 2003) and canines are valued by Inuit artisans. The teeth preferred for age estimation may be damaged or absent. An alternative tooth is often required for age estimation, but there has been no systematic study to determine if the canine provides the most precise and accurate age information. We used a sample of Atlantic walrus (*O. r. rosmarus*) to compare age estimates among teeth to assess the applicability of canines and postcanines for age determination.

Jaws were collected from 100 Atlantic walrus killed in the summer by Inuit subsistence hunters between 1996 and 1999 at five communities in Nunavut, Canada. Jaws were frozen until they were processed in the lab. A full complement of mandibular teeth including a canine (C) and 3 postcanines (PC1–3) was present in 98 jaws (two jaws lacked PC3). PC3 in one of these jaws was too small to section. The three jaws with the small or missing PC3 teeth were not processed further. Data on sex were available for only 66 of the 97 (34 females, 32 males). There are sex-related differences in the appearance of incremental lines (Fay 1955, Mansfield 1958), and detailed analysis was limited to the known males and known females (Table 1).

The jaws were bisected at the mandibular symphysis and the right ramus was simmered gently in water to facilitate tooth extraction. Extracted teeth were mounted on wood blocks with hot melt glue. Longitudinal sections (~0.35 mm) were cut from the middle of the tooth, roughly parallel to the ramus, using a Micro-matic Precision Wafering Machine with a water-cooled, 13-cm diameter, high concentration diamond wafering blade (Buehler Ltd. No. 11-4245)

Table 1. Sources of collected walrus mandibles used to compare age estimates from different teeth (C, PC1-3).

Community	Formerly known as[a]	Location (N/W)	Year	Sample size	
				Males	Females
Sanirajak	Hall Beach	68°47′/81°13′	1996	8	15
lglulik	lgloolik	69°23′/81°40′	1996	15	13
Ausuittuq	Grise Fiord	76°35′/83°14′	1996	3	2
			1998	1	1
			1999	3	2
Qausuittuq	Resolute Bay	74°41′/94°50′	1996	2	1

[a] Many communities changed their names leading up to and in conjunction with the formation of Nunavut on 1 April 1999.

(Wainwright and Walker 1988). Sections were stored in glycerin: alcohol: water (1:1:1, Pueck and Lowe 1975) and allowed to clear for a week. Wet sections were viewed with a variable-magnification dissecting microscope using transmitted light. Cementum growth layer groups (GLGs, Perrin and Myrick 1980) were counted in three to five blind replicates (Johnston *et al.* 1987) by one reader (BES). Three identical readings or the median of five readings were taken as the final age estimate (Johnston *et al.* 1987, Garlich-Miller *et al.* 1993). If fewer than three readings were the same, outliers were removed from the five readings using maximum normed residuals (Snedecor and Cochran 1980).

In the absence of known-age material, accuracy of age estimates was assessed on the basis of possible underestimates due to occlusal tooth wear that may erode GLGs. We used the presence or absence of fetal dentine, indicating wear, to determine if an unknown number of GLGs may also have been missing, resulting in an underestimate of age. Each of the four teeth provided an age estimate for each walrus and the frequency with which a tooth (C, PC1–3) produced the maximum age estimate was recorded. The frequency of underestimates and maximum age estimates were examined using chi-square.

The precision in estimated age may be related to how clear the GLGs appear in the section. This, in turn, depends both on the preparation of the section or trauma and the inherent properties of the tooth that affect GLG definition. We subjectively scored section quality as 1 if it was the proper and uniform thickness, showed no saw marks, and was in the middle of the tooth. It was scored as 0 if it showed sectioning artifacts, was too thick, or was offset. For each section, we also subjectively rated the clarity of the GLG definition on a poor to excellent scale, expanded with a plus or minus for each descriptive category (*e.g.*, a section rated poor^{+} was a little more damaged and harder to read than one rated good^{-}). These ratings were assigned numerical scores ($1 =$ poor^{-} to $8 =$ excellent) for statistical analysis. Section quality and GLG clarity combine to make the section more or less difficult to read, reflected in the number of replicate readings (four to five) required to obtain a final age estimate. For the four tooth positions, we compared the frequency of three, four, and five readings, comparable to the Precision Index of

Garlich-Miller (1997). The frequency of required replicates (three to five) and the distribution of clarity scores were examined using chi-square.

The age estimate data were not normally distributed and median values were compared instead of means for each tooth location. One-way repeated-measures ANOVA on median final ages derived from the four teeth was used to test the hypothesis that age estimates based on postcanine teeth were representative of those derived from canine teeth. The relationship between PC-age estimates and C-age estimates was examined using multiple linear regressions that included section quality and clarity as dependent variables. Statistical outliers were detected among the four within-animal age estimates using extreme Studentized deviates (Snedecor and Cochran 1980), and between paired teeth using the 95% predictive confidence interval of linear regressions. Data for outliers was further scrutinized for explanatory factors such as section clarity. Normality and homogeneous variance were tested at $P = 0.01$, all others at $P = 0.05$.

Males—The median age estimates derived from the four teeth were not significantly different ($n = 32$ animals). Known underestimates indicated by an absence of fetal dentine appeared to be slightly more common in canines (75%) than in PC3 (41%), but the differences were not significant ($\chi^2 = 3.4$, df = 2, $P > 0.05$). Maximum estimates were obtained slightly more often from canine teeth (56%) and less often from PC2 (34%) but differences among teeth were not significant ($\chi^2 = 4.5$, df = 2, $P > 0.05$).

Nearly all sections were of good quality and quality scores are retained hereafter only to explore their effect on extreme age estimates. The clarity of canines was rated "excellent$^-$" to "excellent" in 12% of the males, compared to 6% of PC1 and 3% of PC2 and PC3. Slightly more (22%) PC2 clarity scores were poor$^+$ or less compared to canines (15%) and PC1 and PC3 (both 19%), but the frequency distribution of clarity scores did not change significantly with tooth location ($\chi^2 = 10.1$, df = 9, $P > 0.05$, categories combined to achieve valid expected values: poor$^-$ to poor$^+$; good$^-$ to good, and good$^+$ to excellent). Tooth position had no significant effect on the number of blind replicates required for each tooth. Overall, approximately 33% of tooth sections produced three identical readings in three replicates, 21% required four replicates, and 46% required five replicates. In multiple linear regressions relating postcanine age estimates to canine age estimates and including quality and clarity scores, section quality, and clarity made no significant contribution ($P > 0.26$).

Linear regressions relating postcanine age estimates to canine age estimates were significant. None had a slope significantly different from 1 or an intercept significantly different from 0 (Fig. 1).

In three of the 32 males (9.4%), the final age estimate of every tooth was identical (ages 1, 11, and 13). There were statistically significant outliers ($P > 0.05$) among the four final estimates in 12 males all between 12 and 23 yr of age. Four involved deviations at the canine and eight involved other tooth positions (four at PC1, two at PC2, and two at PC3). The extreme Studentized deviate test is sensitive to the variance in the sample, and nine statistical outliers identified by this test were deviates of 1 yr compared to three identical readings from the other teeth.

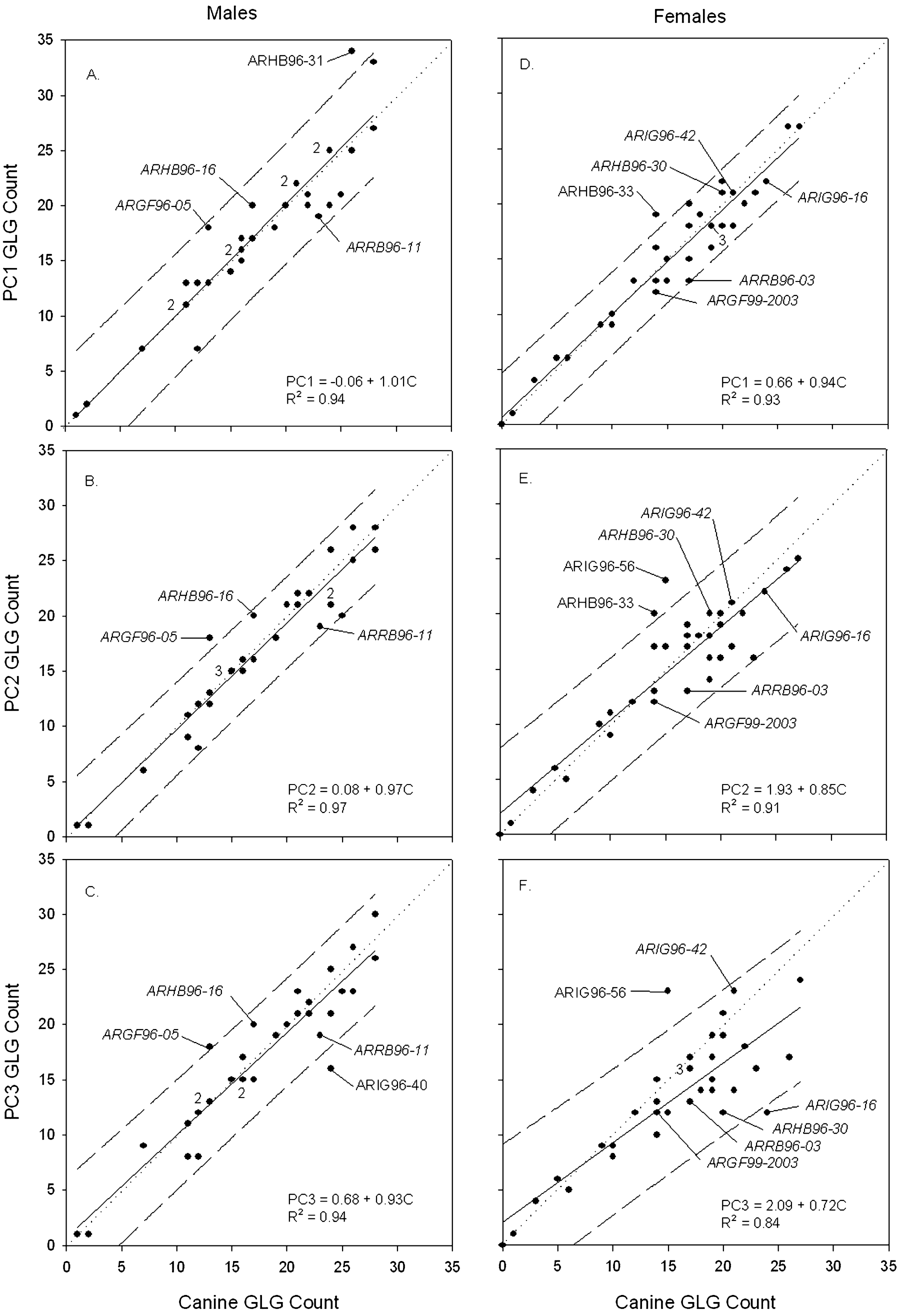

Figure 1. Age estimates based on PC1–3 as a function of C estimate for males and females. Solid lines are the regression flanked by the 95% predictive confidence intervals. Each dot represents one walrus unless otherwise indicated by an accompanying number. The dotted line is 1:1 correspondence (slope 1, intercept 0). Sample numbers of outliers are provided for values outside the 95% predictive intervals as determined using the extreme Studentized deviates test (in italics).

These outliers have not been considered further. Among the remaining three, the canine estimate was significantly higher than the PC1, PC2, and PC3 for one animal (sample ARRB96-11: 23 *vs.* 19, 19, 19, respectively) and significantly lower than the other three teeth for two animals (ARGF96-05:13 *vs.* 18, 18, 18 and ARHB96-16: 17 *vs.* 20, 20, 20, Fig. 1). The PC1 estimate for walrus ARHB96-31 (age estimates 26, 34, 28, 27, respectively) was above the 95% predictive confidence interval of the PC1-C regression analysis (Fig. 1) and was included in an examination of explanatory variables.

Section quality was "good" for all these outliers. Wear patterns within an animal were consistent among teeth: all four teeth had fetal dentine in one outlier and all four were missing fetal dentine in the other three walrus. The canine ARGF96-05 required five readings compared to three readings for the other teeth in this walrus, but the number of readings did not vary by tooth location in the three other walrus. All the clarity scores were "good" or "good^{-}".

In four males (12.5%, including ARHB96-31), the four teeth produced four different age estimates. Within individuals, the four estimates differed by 5–8 yr (mean ±1 SD: 6.5 ± 1.5 yr). The minimum age estimate was 8 yr and the maximum 34 yr. Of the 16 final estimates, one required three readings, three required four readings, and the rest required five readings. All but PC2 and PC3 for one walrus were minimum estimates with missing fetal dentine. The clarity of the sections from the youngest of these males, aged from 8 to 13 yr, was "poor^{-}" to "poor". The other three scored "good^{-}" to "good^{+}".

Females—The median age estimate for PC3 was significantly lower than those from the other three teeth C = PC1 = PC2 = 17 yr, PC3 = 13.5 yr; Friedman repeated measures ANOVA, $\chi^2 = 19.4$, df = 3, $P < 0.001$, $n = 34$ animals). Known under-estimates indicated by an absence of fetal dentine occurred more frequently in canines (76%) than in PC1 (35%) PC2 (24%), and PC3 (21%) ($\chi^2 = 17.4$, df = 2, $P < 0.05$). Maximum estimates were obtained slightly more often from canine teeth (47%) and slightly less often from PC3 (21%), but the differences were not significant ($\chi^2 = 3.5$, df = 2, $P > 0.05$).

The quality of most sections was good and section quality was not examined further except as an explanatory variable in assessing statistical outliers. Section clarity was rated "excellent^{-}" to "excellent" in 3% of the canine, PC1, and PC3 sections and 0% in PC2. More (76%) PC2 clarity scores were "poor^{+}" or less compared to canines (59%), PC1 (56%), and PC3 (64%), but the frequency distribution of clarity scores did not change significantly with tooth location ($\chi^2 = 3.5$, df = 8, $P > 0.05$, categories combined to achieve valid expected values: poor^{-} to poor, poor^{+} to good^{-} to excellent). Tooth position had no significant effect on the number of blind replicates required for each tooth. Overall, approximately 31% of tooth sections produced three identical readings in three replicates, 17% required four replicates and 52% required five replicates. In multiple linear regressions relating postcanine age estimates to canine age estimates and including quality and clarity scores, section quality and clarity made no significant contribution ($P > 0.08$).

Linear regressions relating postcanine age estimates to canine age estimates were significant, but only PC1 estimate as a function of canine estimate had a slope not significantly different from 1 and an intercept not significantly different from 0 ($P < 0.05$) (Fig. 1).

In two of the 34 females (5.9%), the final age estimate of every tooth was identical (ages 0 and 1). There were statistically significant outliers ($P > 0.05$) among the four final estimates in 10 females. The significant deviation was the canine estimate in five walrus and at the other tooth locations (one at PC1 and at PC2, and three at PC3) in five walrus. Five of these deviations were 1 yr compared to three identical readings from the other teeth and were not included in further analysis. In two females the canine estimate was greater than the other three (ARGF99-2003: 14 *vs.* 12, 12, 12, and ARRB96-03: 17 *vs.* 13, 13, 13). The other large deviates occurred at PC3 and included one high outlier (ARIG96-42: 21, 21, 21 *vs.* 23) and two low outliers (ARHB96-30: 20, 21, 20 *vs.* 12 and ARIG96-16: 24, 22, 22 *vs.* 12) (Fig. 1). Additionally, walrus ARHB96-33 had PC1 and PC2 estimates, and walrus ARIG96-56 had PC2 and PC3 estimates that exceeded the 95% predictive confidence level based on canine estimates (Fig. 1).

Section quality, wear, required replicates, and clarity of these six outliers were assessed. The quality of sections was good for all but one outlier (ARIG96-56) in which it did not relate to the deviation. Instead, both the canine and PC1 lacked fetal dentine and produced estimates well below the unworn PC2 and PC3. All but one of the large outliers required five replicates to generate a final estimate, reflecting the generally poor clarity scores. In four of the six walrus, the outlier values were associated with clarity scores of “poor” to “poor^{-}”. In ARRB96-03, the high canine value was obtained from a section with a “good^{-}” rating, but both PC2 and PC3 scored “poor” and may have influenced the statistical result. Of the 24 estimates derived for these six walrus, 14 (58.3%) scored “poor^{+}” or less.

In eight females (23.5%), the four teeth produced four different age estimates. Among these females, within individuals the four estimates differed by 3–7 yr (5.4 ± 1.3 yr). The minimum age estimate was 10 yr and the maximum 21 yr. Of the 32 final estimates, three required three readings, seven required four readings, and the rest (22) required five readings. Eighteen of these readings were from teeth with fetal dentine, although all of the canines produced minimum estimates. Most (26/32) of the clarity scores were “poor^{-}” to “poor^{+}”. The other six scored “good^{-}” to “good”.

Various mandibular teeth have been selected for age estimation in walrus based on varying assumptions relating to the maximum counts available (Born 2003) and GLG clarity (Fay 1955, Mansfield 1958, Working Group 1 1993). However, these assumptions had not been tested, and there was speculation about variation in GLG counts within a tooth-row.

PC3 was missing in 2% of our sample ($n = 100$), similar to a sample of 69 Pacific walrus (*O. r. divergens*, 1.4%, PC4 in Fay 1982, table 10). When it is present, it is often small and bent, making sectioning difficult and leading to underestimates. PC3 displayed poor repeatability (46% and 52% required five readings in males and females, respectively) and agreed with the canine estimate in only 30% of the males and 18% of female walrus. PC3 generally gave lower age estimates in females but not in males.

There was a tendency for the fetal dentine to be worn away more often in canines than postcanines in both sexes, but the difference was significant only for females. Regressions relating canine estimates to PC estimates differed from equality (intercept $= 0$, slope $= 1$) for females at tooth positions PC2 and PC3.

The highest final age estimate is a function of absolute growth layer deposition, dental wear, and clarity, which in turn relies on the quality of the section, the size of the tooth, and the inherent visual definition of the GLGs. Although statistically significant only for females, there was a tendency for fetal dentine to disappear from canines, introducing an unknown negative bias, at an earlier age than in PC1 and PC2, resulting in a greater proportion of canine ages being underestimates. There was a non-significant tendency for canines to provide the maximum age for an animal. More canine sections received high clarity scores, especially for males, suggesting that maximum estimates resulted from greater clarity despite greater wear. It may be that, although the fetal dentine disappears from most canines, relatively few cemental GLGs are lost. The first cemental GLG extends below the level of the fetal dentine and will persist for some unknown time after the fetal dentine disappears. It is more probable that the large size of the canine allows better visual definition of GLGs because the lines are larger. Cementum deposition is affected by the mechanical forces operating on the tooth (Klevezal 1996), and if these forces are greater or act differently on the canine than on post-canines, the larger GLGs that result would be easier to discern and count.

In general, tooth sections from females appeared more difficult to read than those from males. There were more extreme deviations among females than males. A greater proportion of sections from females required five readings to determine a final age (71% *vs.* 59% of males), although the proportions requiring three, four, or five readings did not differ significantly between sexes ($\chi^2 = 1.2$ df $= 2$, $P > 0.05$). Approximately 6% of sections from males were “excellent^{-}” to “excellent” in clarity while only about 2% scored as well for females. Conversely, while only 19% of male sections scored in the lowest three clarity categories, 63% of sections from females were “poor^{-}” or worse. Generally, all the teeth from an animal usually had the about same clarity rating; if the canine was difficult to read, the PCs were also difficult to read.

PC1-3 appear to be suitable replacements for the canine for ageing male Atlantic walrus. Although large outliers (3–5 yr) did appear among males, the minimum age for any of these males was 13, most were 17–28 yr old, well past the age of maturation (Born 2003). Errors of 3–5 yr would not likely have any impact on other biological comparisons.

PC1 appears to be a suitable replacement for canines of females. Differences in estimates from canines and PC1 were greater than 1 yr for three females but, as with males the animals were ≥ 12 yr old—likely past the age of maturation (Garlich-Miller and Stewart 1999). In the absence of known-age material, the highest age cannot be taken as unbiased. The larger canine section may allow better definition of subannual lines and introduce a positive bias. However, analysis of the outliers suggests this may be unlikely. The canine estimate was a statistical outlier greater than 1 yr by either test in four males and seven females, but was higher in only two females. Usually, GLG counts will tend to negatively biased estimates because it is more likely for lines to be lost or not detected than for significant supernumery lines to be counted. To obtain the highest count possible, Born's (2003) approach using the maximum count obtained from any of these three teeth might be appropriate, though more laborious than using one tooth.

ACKNOWLEDGMENTS

We thank the Inuit hunters of Ausuittuq, Iglulik, Kingnait, Qausuittuq, Salliq, and Sanirajak, Nunavut, and Blair Dunn, Freshwater Institute (FWI), for collecting the samples. Reviewers Steve Ferguson and John Babaluk, both of FWI, provided useful comments on the manuscript, as did two anonymous reviewers. The research was funded by the Department of Fisheries and Oceans and the Nunavut Wildlife Research Trust.

LITERATURE CITED

BERNT, K. E., M. O. HAMMILL AND K. M. KOVACS. 1996. Age estimation of grey seals (*Halichoerus grypus*) using incisors. Marine Mammal Science 12:476–482.

BORN, E. W. 2003. Reproduction in male Atlantic walruses (*Odobenus rosmarus rosmarus*) from the North Water (N Baffin Bay). Marine Mammal Science 19:819–831.

FAY, F. H. 1955. The Pacific walrus: Spatial ecology, life history and population. Ph.D. thesis, University of British Columbia, Vancouver, BC. 171 pp.

FAY, F. H. 1982. Ecology and biology of the Pacific walrus, *Odobenus rosmarus divergens* Illeger. North America Fauna No. 74.

FAY, F. H., B. P. KELLY AND J. L. SEASE. 1989. Managing the exploitation of Pacific walruses: A tragedy of delayed response and poor communication. Marine Mammal Science 5:1–16.

FEDOSEYEV, G. A., AND V. N. GOLTSEN. 1990. Age-sex structure and reproductive capacity of the Pacific walrus population. Canadian Translation of Fisheries and Aquatic Sciences 5503, 13 typescript pages, Available from: Canada Institute for Scientific and Technical Information, National Research Council, Ottawa, Ontario, Canada, K1A 0S2. [Original title: Vozrastno-polovaya struktura i vosproizvoditelnaya sposobnost populyatsii tikhookeanskogo morzha. 1969 *in* Zoologicheskiy Zhurnal (Zoological Journal) 48:407–413.]

GARLICH-MILLER, J. 1997. Age, sex, and reproductive status of Pacific walrus harvested in the Bering Strait region, 1994–1996. United States Fish and Wildlife Service Technical Report MMM 97-1:iii + 26 pp.

GARLICH-MILLER, J. L., AND R. E. A. STEWART. 1999. Female reproductive patterns and fetal growth of Atlantic walrus (*Odobenus rosmarus rosmarus*) in Foxe Basin, Northwest Territories, Canada. Marine Mammal Science 15:179–191.

GARLICH-MILLER, J. L., R. E. A. STEWART, B. E. STEWART AND E. A. HILTZ. 1993. Comparison of mandibular with cemental growth-layer counts for ageing Atlantic walrus (*Odobenus rosmarus rosmarus*). Canadian Journal Zoology 71:163–167.

HOHN, A. A., AND S. FERNANDEZ. 1999. Biases in dolphin age structure due to age estimation technique. Marine Mammal Science 15:1124–1132.

HOHN, A. A., M. D. SCOTT, R. S. WELL, J. C. SWEENEY AND A. B. IRVINE. 1989. Growth layers in teeth from known age, free-ranging bottlenose dolphins. Marine Mammal Science 5:315–342.

JOHNSTON, D. H., D. G. JOACHIM, P. BACHMANN, K. V. KARDONG, R. E. A. STEWART, L. M. DIX, M. A. STRICKLAND AND I. D. WATT. 1987. Aging furbearers using tooth structure and biomarkers. Pages 228–243 *in* M. Novak, J. A. Baker, M. E. Obbard and B. Malloch, eds. Wild furbearer management and conservation in North America. Ontario Ministry of Natural Resources, Toronto.

KLEVEZAL, G. A. (translated by M. V. Mina and A. V. Oreshkin) 1996. Recording structures of mammals determination of age and reconstruction of life history. A. A. Balkema, Rotterdam, The Netherlands.

Mansfield, A. W. 1958. The biology of the Atlantic walrus, *Odobenus rosmarus rosmarus* (Linnaeus) in the eastern Canadian Arctic. Fisheries Research Board Canada Manuscript Report Series (Biology) No. 653.

Outridge, P. M., W. J. Davis, R. E. A. Stewart and E. W. Born. 2003. Investigation of the stock structure of Atlantic walrus (*Odobenus rosmarus rosmarus*) in Canada and Greenland using dental Pb isotopes derived from local geochemical environments. Arctic 56: 82–90.

Perrin, W. F., and A. C. Myrick Jr., eds. 1980. Report of the Workshop: Proceedings of the International Conference on Determining Age of Odontocete Cetaceans and Sirenians. Report of the International Whaling Commission (Special Issue 3):1–50.

Pueck, Z., and P. W. Lowe. 1975. Age criteria in small mammals. Pages 55–72 *in* F. B. Golly, K. Petruseweiz and L. Ryszkowski, eds. Small mammals: Their productivity and population dynamics. IBP No. 5. Cambridge University Press, Cambridge, U.K.

Snedecor, G. W., and W. G. Cochran. 1980. Statistical methods. Seventh edition. The Iowa State University Press, Ames, IA.

Stewart, R. E. A., B. E. Stewart, I. Stirling and E. Street. 1996. Counts of growth layer groups in cementum and dentine of ringed seals (*Phoca hispida*). Marine Mammal Science 12:383–401.

Wainwright, K. L., and R. S. Walker. 1988. A method for preparing beluga (white whale), *Delphinapterus leucas*, teeth for ageing. Canadian Manuscript Report Fisheries and Aquatic Sciences No. 1967:iv + 15 pp.

Working Group 1. 1993. Ageing. Pages 20–23 *in* R. E. A. Stewart, P. R. Richard and B. E. Stewart, eds. Report of the 2nd Walrus International Technical and Scientific (WITS) Workshop, 11-15 January 1993, Winnipeg, Manitoba, Canada. Canadian Technical Report of Fisheries and Aquatic Sciences 1940:viii + 91 pp.

Received: 31 March 2004
Accepted: 14 November 2004