The suitability of mandible growth layers in the common minke whale (*Balaenoptera acutorostrata*) for age determination

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ABSTRACT

Ovaries from 82 female minke whales (30 from 1999 and 52 from 2001) caught in the North Atlantic were examined macroscopically and the number of *corpora lutea, c. albicatia* and *c. artretica* determined by two or three readers. From these whales and an additional 19 males (13 from 1999 and 6 from 2001), the number of GLGs in the buccal wall of the anterior part of both mandibles were counted. Mandible GLGs were counted by either examining digital images of haematoxylin stained 200-500μm segments, or from high-resolution X-ray images of 3mm thick unstained segments examined by two readers. The readers agreed completely when counting ovarian *corpora lutea*, but there was disagreement with the interpretation of *c. albicantia* and *c. artretica* in some ovaries. The average CV of the number of ovulations ($n_{c.lutea} + n_{c.albicantia}$) was 6%; when counting only *c. albicantia* the CV was 16.7%, and 64.9% when counting only *c. artretica.* The precision when counting mandible GLGs using the digital images was poor, with mean CV of 82%, compared to 41% using the X-ray images. There was poor agreement between the repeated readings of the X-ray images by each reader, as well as between the readers. Mean GLG count using either method did not correlate with the number of ovulations, and provided biologically unreasonable von Bertalanffy growth models. This study shows that there is some uncertainty when examining ovaries, although this is small compared to the variability and bias associated with counting mandible GLGs. New bone is deposited in the mandible in such a way that growth layers do not continuously accumulate, or cannot be distinguished using present technology and methods.

KEYWORDS: COMMON MINKE WHALE; ATLANTIC OCEAN; AGE DETERMINATION; REPRODUCTION; OVULATION

INTRODUCTION

Age determination of baleen whales is more difficult than ageing other mammals due to their lack of teeth. Many methods have been attempted, and most species are now routinely aged by counting annual Growth Layer Groups (GLGs) in the wax-like earplug (Purves, 1955; Kato *et al*., 1991). However, earplugs seldom form in the North Atlantic common minke whales, *Balaenoptera acutorostrata* (Christensen, 1992) and there have been attempts to determine age by counting GLGs in the periosteal layer of the tympanic bulla (Christensen, 1981). However, bulla age estimates have low precision (Christensen, 1995) and are so heavily biased (Olsen, 2002) that these are of little practical use. In sperm whales, annual GLGs have been found in the mandibular walls (Laws, 1960), and these correlate well with the age estimate from counting GLGs in the teeth until the attainment of physical maturity (Nishiwaki *et al*., 1961). A study of mandible GLGs in the white whale (*Delphinapterus leucas*) yielded similar results (Brodie, 1969). GLGs are found in the mandible of many other mammals and birds (Klevezal and Kleinenberg, 1967). Klevezal and Mitchell (1972) attempted to determine the age of fin (*Balaenoptera physalus*) and sei whales (*Balaenoptera borealis*) by counting mandibular laminations, but were unable to detect clear growth zones. However, this method had not been attempted on minke whales, and it was conceivable that in this short-lived species mandible GLGs were formed. In addition, during the three decades since the Klevezal and Mitchell (1972) study, technological advances in imaging and image analysis have provided new tools to identify possibly diffuse growth layers.

Beamish and McFarlane (1983) and later Campana (2001) stressed the need for validating possible ageing methods, preferably using animals of known age or by using mark-recapture experiments. Records of known-age animals are lacking for common minke whales and there has been no mark-recapture programme in place for the last 20 years. To test if mandibular GLGs are useful in ageing, an indirect approach was therefore required. Mandibular GLG counts were compared with body length, and for females, with the number of ovulations as determined by counting *corpora lutea* and *c. albicantia* in the ovaries[1]. Both indices increase with age, body length following a curvilinear growth with age, usually modelled by a growth equation (e.g. Gompertz or von Bertalanffy). Most mysticete species have been shown to have a regular ovulation and birth cycle, giving birth to one young every 1-3 years depending on species (Lockyer, 1984b). Mature female minke whales have a ~90% pregnancy rate (Jonsgård, 1951; Chrstensen, 1975; Larsen, 1984 and Olsen, 1997). It is also assumed that minke whales have a regular ovulation rate, as Laws (1958) observed in fin whales. These observations imply that the numbers of ovulations increase linearly with age after attainment of sexual maturity. Accordingly, unbiased and precise age estimates would be expected to follow these relationships when compared with body length or the number of ovulations. It was therefore important to investigate the precision of the indices, particularly the counting of ovarian *corpora*, which had not been done before. The aim was to quantify the precision of counting ovarian *corpora*, and use the *corpora* counts together with body length to test if mandible GLGs are useful for age determination of North Atlantic common minke whales.

MATERIALS AND METHODS

The samples used in this study were collected in 1999 (30 females; 13 males) and 2001 (52 females; 6 males) on commercial whaling vessels operating in the Norwegian Economic Zone along the coast of Northern Norway, Spitsbergen, the North Sea and the Norwegian Sea east of Jan Mayen. Standard body length was measured as the

[1] In cetaceans, the corpus albicans generally persists on the ovaries throughout life (Perrin and Donovan, 1984).

distance in a straight line from the tip of the snout to the notch in the fluke. Ovaries were removed during flensing, labelled, and stored in 4% buffered formaldehyde for later laboratory examination. In the laboratory, excess connective tissue was removed and the ovaries cut into 3mm slices. Two or three persons trained and experienced in examining ovaries of cetaceans examined these independently without any accessory information. Each reader counted the number of *corpora lutea, c. albicantia* and *c. artretica* in each ovary. The number of times a female had ovulated was calculated as the sum of *c. lutea* and *c. albicantia* in each pair of ovaries, and is henceforth referred to as the number of ovulations. Variance between the readers when counting the different *corpora* and the variance of the numbers of ovulations was expressed as CV to facilitate comparison between individuals and with other studies.

Collection of mandibles

While the whale was flensed on deck, the mandibles were cut loose at the jaw joint and the anterior 50cm of both mandibles were cut off using a saw. Blubber, muscle and connective tissue were removed using a knife and the mandible sections were frozen on board at -23°C. The mandibles were thawed in the laboratory and segments were cut of the buccal (outer) wall of both mandibles 45cm posterior to the tip of the jaw using a dual-bladed saw. The segments cut from the 1999 samples were 200-500μm thick, while those from 2001 were 3mm thick. In a pilot-study of whale mandibles sampled in 1997 and 1998, what appeared to be GLGs were observed in the buccal wall of the mandible, and it was found that these were most clear in the area 40-50cm from the tip. Most of the mandible of baleen whales consists of a highly spongiose bone matrix filled with fat, with an outer edge of highly ossified bone also infused with fat. The segments from the 1999 whales were examined using visible light microscopy, while the segments from 2001 were examined using X-ray imaging. To increase the contrast of the segments examined using visible light, they were stained with haematoxylin. The high fat content of the bone prevented first attempts of staining the sections, but soaking the segment in concentrated HCl for about 30 seconds alleviated this. The segments were then rinsed in water, followed by ethanol and lastly stored in glycerin in small containers (they were too large to fit available microscope slides). One such segment was prepared from both mandibles of all whales (except for two whales where one of the mandibles was lost). For 13 whales sampled in 1999, four additional segments of the same thickness were prepared from each mandible to investigate if the same GLG pattern found in one segment could be detected in other segments cut within 5cm of the first. The mandibles collected in 2001 were to be analysed using X-ray techniques. X-ray imaging did not need staining or fat-removal, but a pilot-study had shown that the segments needed to be ~3mm thick to yield sufficient contrast when X-rayed. These segments were cut in the same manner as those for the visible light analysis, but stored in 4% buffered formaldehyde as this was thought to alter the chemical structure of the bone to the least extent.

Analysis of mandibles

Due to their size, it was difficult to examine the mandible segments in the limited field of view of the microscopes used. Instead, the segments were placed on a light table and a picture of each was taken using a *Nikon Coolpix* 990 digital camera. Pictures were taken at maximum resolution (2048 × 1536 pixels) in colour mode, and stored as TIFF (Tagged Image File Format) files for conservation of all image information. Each picture was later analysed using *ImagePro Plus* 4.0 software. In the image-analysis, an initial attempt was made to enhance the contrast and clarity of the pictures using several different filters and techniques. Eventually, the brightness and contrast of each colour channel (red, green and blue) were manipulated separately to achieve the best contrast of the GLGs (Fig. 1). Following image-enhancement, two readers cooperated in determining where the potential GLGs were placed in the segment, and marked and measured these using the software's tools. Prior to the analysis, all image files had been renamed by an independent observer to prevent the readers from using additional knowledge or recognising individual whales. The mean GLG count was calculated for all age estimates of the same whale.

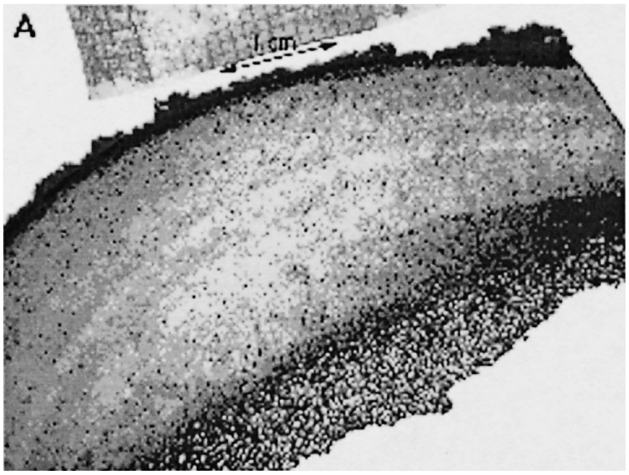

Fig. 1. Image of haematoxylin stained segment of the buccal mandible wall of a female minke whale. The colour balance has been manipulated to enhance the contrast of possible GLGs.

X-ray imaging

High resolution X-ray images of the 2001 sampled whales were taken using a human mammography X-ray apparatus (*Siemens Mammomat 3000*) at Haukeland University Hospital in Bergen, Norway. The pictures were taken using *Kodak* Min R 2000 X-ray film and, after some trial and error, the highest contrast was found at 25kV and 28mAs settings of the apparatus. Each segment was rinsed in water and images were taken at 2× magnification. Ordinary (higher intensity) X-ray technique as well as ultra-sound imaging were attempted, but the resolution of these was too low to discern any GLGs or fine structure in the mandible. Similar resolution was obtained when using mammography X-ray as when using visible light and digital camera (Fig. 2). The X-ray images were examined independently, twice by two readers to identify and count GLGs. Both readers were experienced in counting GLGs from other marine mammals, and made a subjective classification of the readability of each segment examined. Prior to the analysis, both readers and the first author examined 10 segments together to agree on criteria of how to interpret the observed structures in the mandibles.

Control of mandible aging using mandible and tooth from sperm whale

In April 1999 a male sperm whale (*Physeter macrocephalus*) stranded on a beach in Sola in southwestern Norway; sections of both the mandible and teeth of this animal were

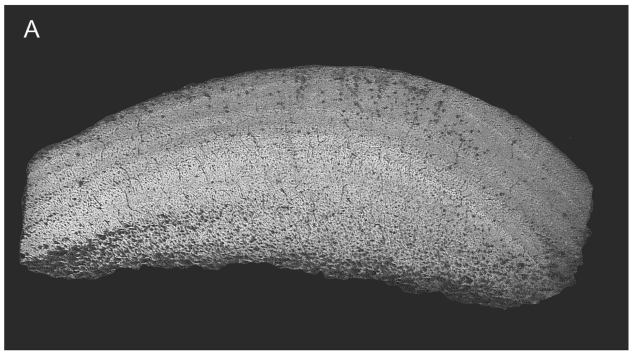

Fig. 2. Scanned X-ray (mammographic X-ray equipment) of buccal mandible wall from a female minke whale. The image was photographed at 25 kV and 28 mAs settings using *Kodak* X-ray film. Growth layers can be seen and followed through the length of the segment.

obtained. GLGs have previously been found in the mandible of sperm whales (Nishiwaki *et al*., 1961), and this sample allowed verification of whether the preparation and examination techniques used for this study were appropriate to identify the GLGs in the mandible. Three segments of the sperm whale mandible were prepared and stained in the same way as for the minke whale (Fig. 3); one tooth was cut longitudinally and the surface polished to verify whether the mandible GLG count corresponded with the tooth GLG count. Two readers independently examined the two sides of the tooth and mandible segments visually using a magnifying glass.

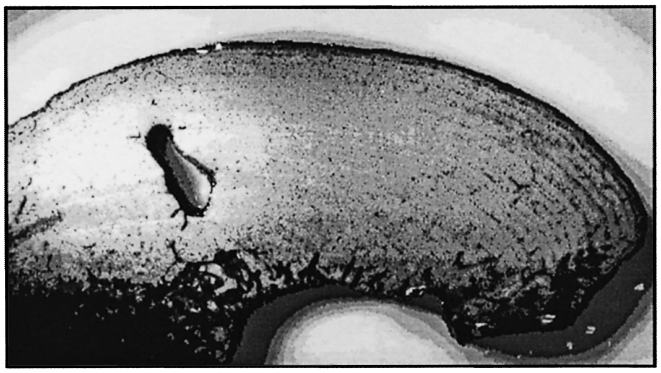

Fig. 3. Colour enhanced image of haematoxylin stained section of sperm whale mandible.

Statistical analysis

Precision of counting mandible GLGs was measured as the degree of agreement between successive readings of the same segment, or between different segments from the same animal. Precision could thus be assessed at two levels: the first at the individual reader level; and secondly between readers. In the visible light analysis the segments had been read only once, and only allowed analysis of precision between different segments, while the X-ray analysis allowed for both intra- and inter-reader analysis of precision. Possible bias of the mandible GLG count in relation to true age was examined by plotting the mean GLG count against the standard body length and number of ovulations. Body length would show a logistic growth with age levelling off around a sex-specific maximum body length, which was modelled with a von Bertalanffy growth equation:

$$Length = L_{MAX}[1 - e^{-k(age - t_0)}] \quad (1)$$

L_{MAX} is the maximum body length, k is the growth rate, and t_0 is the age at length 0.

Linear least square regression models were fitted to the number of ovulation plots and the correlation together with the slope and intercept were examined to determine if mandible GLG counts were unbiased in relation to true age.

RESULTS

Ovary examinations

The largest group in the sample from 1999 and 2001 was females with 0 ovulations (Fig. 4) constituting > 20% of the sample. One female was estimated to have had 39 ovulations, but the majority of the sample had less than 15 ovulations. All readers agreed completely when classifying *c. lutea*, while the CV when counting *c. albicantia* was 16.7%, and 64.9% when classifying *c. artretica* (Table 1). There were some slight differences in CV between the right and left ovaries when classifying *c. artretica* and *c. albicantia*, but this was not significant. From Fig. 5 it appears there is an increase in CV with length, but there is large variability in CV for the larger (and older) whales. There was complete agreement amongst readers on the number of ovulations up to and including six ovulations, but with more ovulations, CV ranged from 0 to 44%, with a mean of 11% as compared to 6% for all females examined. This showed that as the *c. albicantia* became smaller and more numerous it was easier to misinterpret them. There seemed to be some misinterpretation of *c. albicantia* as *c. artretica* and vice versa, but in general it seemed that *c. artretica* were easily overlooked, probably due to their small size.

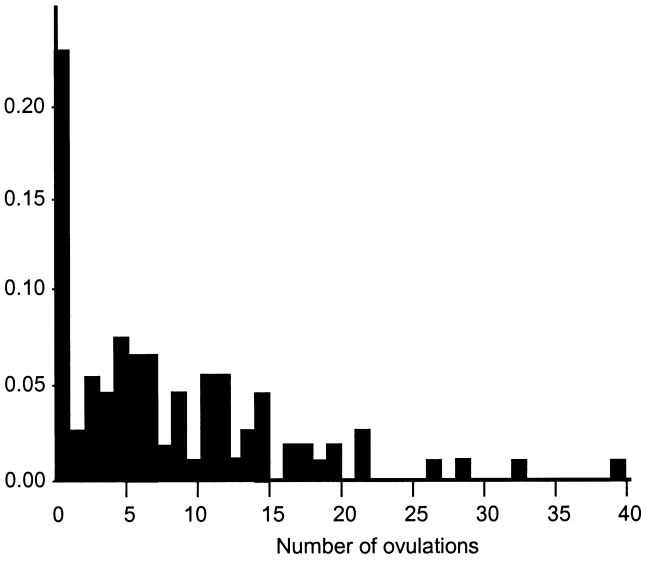

Fig. 4. Relative frequency of the number of ovulations of common minke whales caught in the North Atlantic in 1999 and 2001.

Table 1

CV of counting *corpora lutea*, *c. albicantia* and *c. artretica* in ovaries from minke whales caught in the North Sea and Norwegian Sea in 1999 and 2001. Each ovary was examined by two or three readers.

	C. lutea		*C. albicantia*		*C. artretica*		# Ovul.
	Right	Left	Right	Left	Right	Left	
All	0.0 %	0.0 %	14.8 %	18.7 %	70.6 %	61.9 %	7 %

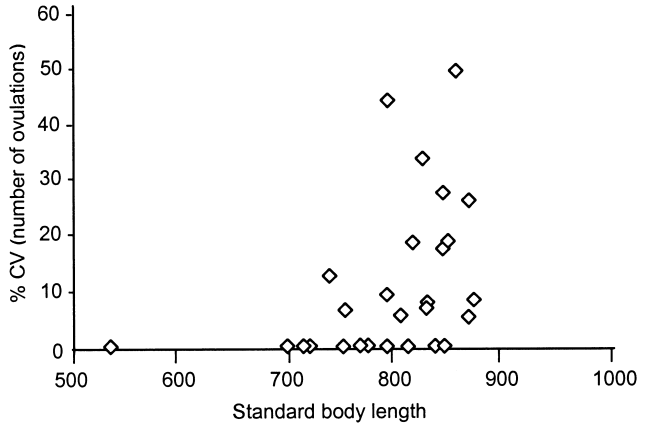

Fig. 5. CV of the number of ovulations versus standard body length.

Mandible GLGs

Mandible GLGs in the segments examined using visible light proved elusive and indistinct (Fig. 1) relative to those found in the sperm whale mandible (Fig. 3). The count of mandible GLGs (16) agreed with the tooth GLG count (16) by the three readers who examined these. Minke whale GLGs were by comparison indistinct and difficult to follow through even a small part of the segment. These difficulties resulted in high CVs of up to 165%, with an average of 82% (Table 2). There was no significant correlation between the mandible GLG count of the females and the number of ovulations, as is evident from Fig. 6. Similarly the plots of mandible GLG count versus body length (Figs 7a and b) showed a poor fit, and the model parameters for the fitted von Bertalanffy growth function indicated a maximum body length for males of 754cm, and 841cm for females. The poor fit was especially evident for the males (Fig. 7b) with very wide confidence intervals. The maximum body lengths estimated by the von Bertalanffy models were reasonable compared with the expected maximum body length from the 10% largest males and females caught from 1945 to 1994 (812cm for males and 826cm for females). However, the models would imply unrealistic lengths at birth (females: 643cm and males: 483cm), and juvenile growth rates, as well as a much larger difference in growth rate between the sexes than expected.

Interpretation of the X-ray images (Fig. 2) was easier and the GLGs observed were more distinct and easier to follow through the whole segment than in the visible light analysis. Although this increased the precision of the age estimates as compared with the visible light analysis, with CV of the GLG count averaging 41% (Table 3), it is still poor. From Fig. 8 it is evident that both readers had difficulties in interpreting the same segment in the same way in both readings. The correlation between the first and second reading was significant for both readers, but the slope of the regression line was different from the expected (1) in both cases. Neither was there any increase in deviation from the equivalence line with increasing GLG count. Fig. 9 shows

Table 2

Standard body length (L), numbers of ovulations with CV, number of age readings, mean GLG count with CV of 30 female and 13 male minke whales (*Balaenoptera acutorostrata*) sampled in the Spitsbergen (ES), Norwegian Sea (CM) and North Sea (EN) small management areas in 1999. GLG counts were made using visible light microscopy.

		Ovulations		Mandible		
Whale	L	Count	CV%	n	GLGs	CV%
Males						
K14	730	-	-	2	10.0	80
K18	769	-	-	2	10.0	40
K19	762	-	-	2	4.5	67
K20	730	-	-	2	7.5	40
K24	485	-	-	2	2.0	0
K25	840	-	-	2	4.5	111
K26	740	-	-	2	5.0	40
K29	832	-	-	1	9.0	
K31	820	-	-	2	1.5	67
K33	700	-	-	2	6.5	15
K35	845	-	-	2	3.5	86
N9	640	-	-	2	5.5	18
U1	701	-	-	2	7.0	29
Average	738	-	-		5.9	46
Females						
F1	620	Missing ovaries		3	7.0	0
F2	760	1.0	0	3	9.3	11
F7	760	0.0	0	3	8.0	65
F9	780	3.0	0	3	8.3	24
K1	857	12.0	0	1	4.0	
K2	840	8.7	6	2	11.0	36
K3	810	11.0	20	3	9.0	33
K4	885	14.3	8	6	11.8	107
K5	870	10.0	16	6	15.8	104
K6	776	3.0	0	6	5.7	101
K7	855	13.7	16	6	7.5	120
K8	801	10.0	61	8	6.6	161
K9	868	9.0	44	6	14.0	88
K10	845	5.0	0	6	11.0	73
K11	705	0.0	0	2	11.5	61
K12	802	11.7	40	6	10.0	118
K13	855	18.0	12	6	18.5	56
K15	858	4.0	0	6	7.8	118
K17	760	0.0	0	2	5.0	0
K21	675	0.0		2	8.0	75
K22	725	0.0	0	2	14.5	7
K23	726	0.0	0	3	5.0	69
K27	760	6.0	0	6	6.5	165
K28	835	10.0		6	8.2	108
K30	822	4.0		6	8.2	136
K34	788	6.0	0	6	7.0	70
N10	580	0.0		2	3.5	29
U2	593	0.0		2	5.5	91
U3	788	3.0		2	11.5	9
U4	822	2.0		2	7.5	13
Average	782	5.6	12		9.2	82

that there was no relationship between the GLG count by reader B when reading the same segments as reader A. With knowledge of the poor precision and low agreement between the readers it was not surprising that a large bias was present. In the plot of GLG count versus the number of ovulations (Fig. 6) there is no correlation between the variables and a large variability in GLG count for a given number of ovulations. This variability was lower than for the visible light analysis, but still large. A von Bertalanffy growth model could not be fitted to the male data (Fig. 7b) as the sample only numbered six males. The fit to the female data was better than using the visible light GLG count, but the model parameters were biologically unrealistic with estimated length at birth of –3.43cm (1999) and 0.008cm

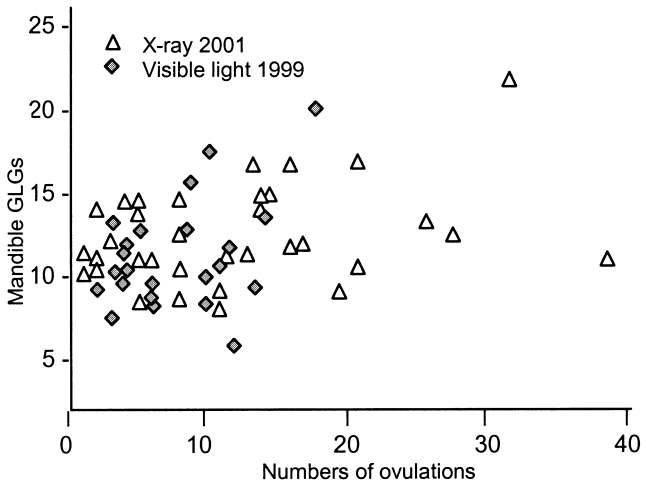

Fig. 6. Plot of ageing accuracy expressed as the relationship between the numbers of ovulations and mean mandible GLG count of females with one or more ovulations. Counts of GLGs using visible light microscopy (1999 samples) and mammographic X-ray (2001 samples) are shown.

(2001) (Table 4). Each GLG count was accompanied by a readability assessment of the segment in question. These were averaged across the readers and readings, and the combined 'quality' of a segment versus CV was plotted (Fig. 10). If the quality was related to precision, one would expect high quality segments to have a lower CV than low quality ones, and this was observed. However, little could be gained from this as the low-quality segments showed a wide spread in CV, including the CV range for the high-quality ones. Plotting only high-quality segments versus their number of ovulations or body length did not improve the correlation in Fig. 6. In addition, only 16.4% of the segments were given a combined quality score greater than 0, showing low subjective assessment of the possibility to correctly count GLGs in the mandible. Finally, the relationship between GLG count and the number of ovulations for each reader separately was evaluated, including only those segments with a CV (based on two readings by the same reader) of less than 15%. This resulted in significant positive correlation for the left mandible segments read by reader B (Fig. 11a), while for the right mandible of reader B (Fig. 11b) and both mandibles of reader A (Fig. 11c and d) the relationship was not significant. Reducing the CV criterion to 10% did not improve this result. Although Fig. 11a showed a significant positive correlation, the slope of the fitted regression line was only 0.52, implying an annual ovulation rate of 2.5 — much higher than expected for minke whales.

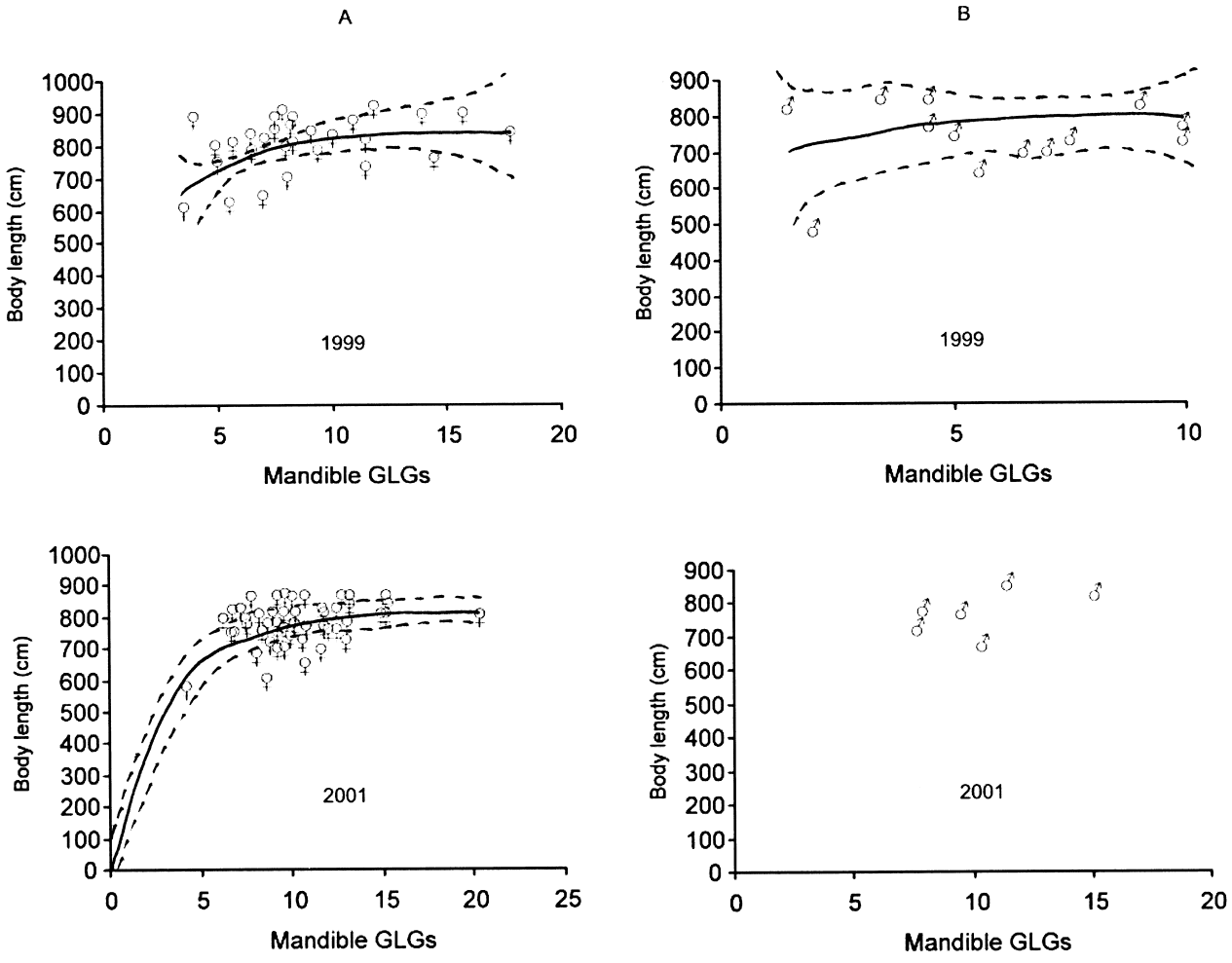

Fig. 7. Plot of mandible GLG count versus standard body length. Von Bertalanffy growth functions with 95% confidence intervals are fitted to the plots.

Table 3

Standard body length (L), numbers of ovulations with CV, number of age readings, mean GLG count with CV of 52 female and 6 male minke whales (*Balaenoptera acutorostrata*) sampled in the Spitzbergen (ES), Norwegian Sea (CM) and North Sea (EN) small management areas in 2001. GLG counts were made from high-resolution X-ray images.

		Ovulations		Mandible		
Whale	L	Count	CV%	n	GLGs	CV%
Males						
A17	670	-	-	8	10.4	36
A20	765	-	-	8	9.5	33
A25	720	-	-	8	7.8	20
A30	775	-	-	8	7.9	4
K17	816	-	-	8	15.1	81
K23	849	-	-	8	11.4	27
Mean	766				10.33	34
Females						
B1	760	6.0	-	7	6.6	87
B2	870	16.0	-	7	10.0	79
B3	830	8.0	-	8	6.9	108
B4	805	11.0	-	8	7.4	18
B5	870	13.0	-	8	9.6	41
B6	810	5.0	-	8	11.9	17
B7	760	5.0	-	8	6.8	13
B8	827	14.0	-	8	12.4	61
B9	793	4.0	-	8	9.9	40
B10	782	4.0	-	8	10.1	19
B11	697	0.0	-	8	8.0	53
B12	756	8.0	-	8	8.6	68
B13	825	17.0	-	7	10.1	55
B14	738	5.0	-	8	9.3	27
B15	794	4.0	-	8	9.5	30
B16	741	0.0	-	8	9.9	48
B17	803	2.0	-	7	8.4	54
B18	659	0.0	-	8	10.8	60
B19	822	26.0	-	8	11.6	44
B20	803	11.0	-	8	6.4	30
B21	591	0.0	-	8	4.3	53
A1	770	2.0	0	8	12.4	35
A2	720	1.0	0	8	9.6	11
A3	780	8.0	0	8	10.8	23
A4	760	11.5	6	8	9.5	73
A5	880	14.5	5	12	13.2	42
A6	730	3.0	0	8	8.6	20
A7	800	6.0	0	8	9.3	28
A8	765	2.0	0	8	9.3	20
A9	815	13.5	5	8	15.0	20
A10	740	3.0	-	8	10.4	40
A11	815	21.0	-	4	9.0	52
A12	765	1.0	-	8	8.4	52
A13	865	6.0	-	8	7.8	31
A14	870	4.0	-	8	12.8	25
A15	820	21.0	-	7	15.1	27
A16	845	14.0	-	8	13.1	24
A18	870	16.0	-	8	15.0	12
A19	845	10.0	-	8	15.1	27
A21	790	2.0	-	8	7.5	103
A22	780	14.0	-	8	11.9	25
A23	710	0.0	-	8	11.5	36
A24	805	6.0	-	8	7.4	66
A26	615	0.0	-	8	8.5	33
A27	805	32.0	-	8	20.4	27
A28	780	8.0	-	8	13.0	26
A29	740	5.0	-	8	12.9	31
A31	870	28.0	-	8	10.8	14
K18	775	5.0	0	8	12.1	34
K19	836	19.5	33	8	7.3	70
K20	783	4.0	0	8	8.6	26
K21	881	39.0	25	7	9.3	90
Mean	788	9.212	6		10.3	41

Table 4

Model parameters for von Bertalanffy growth model fitted to plots in Fig. 9.

	1999		2001	
Parameter	♂	♀	♂	♀
L_{MAX}	754	841	n.a.	809.7
k	0.42	0.22	n.a.	0.4096
t_0	-4.56	-3.93	n.a.	0.0080

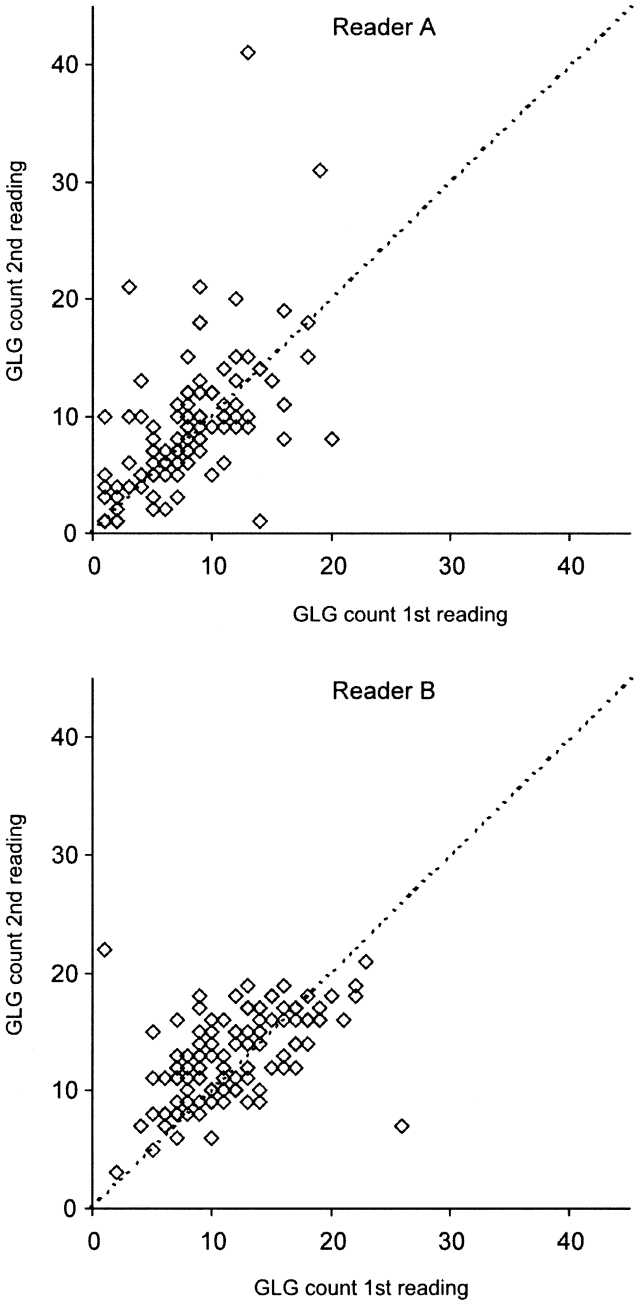

Fig. 8. Plot of intra-reader variation when counting mandible GLGs from X-ray images. The expected 1:1 equivalence line is shown.

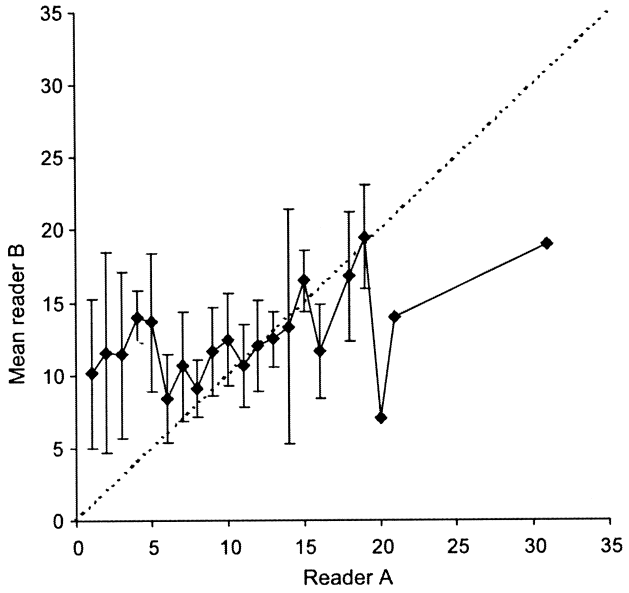

Fig. 9. Inter-reader bias plots of mandible GLG count by two readers. The *Y* axis represents the mean GLG count by reader B of all whales assigned age X by reader A. Error bars represent the standard deviation of the mean. The dotted line indicates the 1:1 equivalence line.

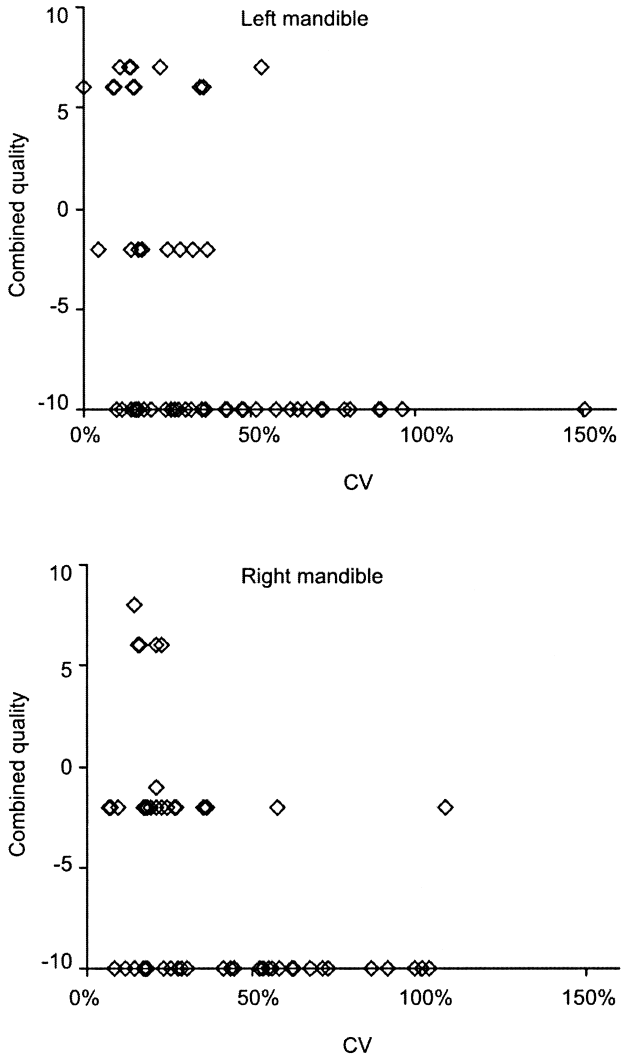

Fig. 10. The combined quality score of reader A and B for each segment from the left and right mandible plotted against the CV of the mean GLG count for each segment.

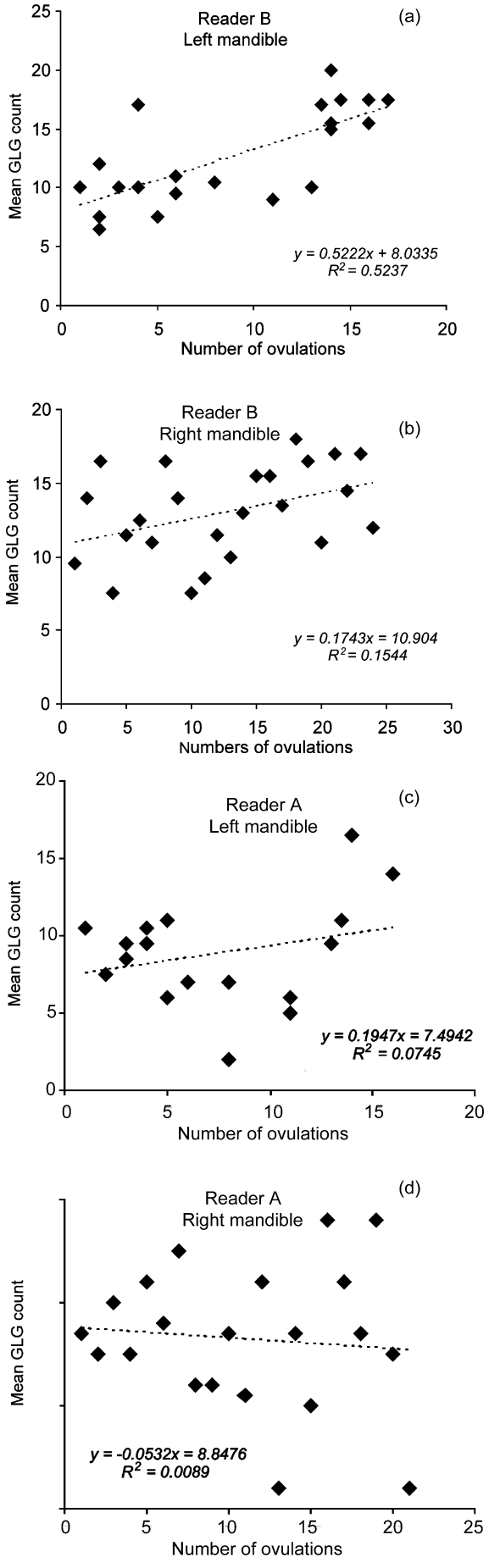

Fig. 11. The mean of two GLG counts of left (a) and right (b) mandible examined by reader B and left (c) and right (d) examined by reader A versus the numbers of ovulations. Only females with 1-19 ovulations and a CV < 15% were included.

DISCUSSION

The ovary reading experiment did reveal some variability in the way ovaries were interpreted by the readers. As expected, there was complete agreement for ovaries with few *corpora*, while variability increased with increase in total *corpora* count. A CV of 8% was found for the mature animals (excluding whales with 0 ovulations). Much of the reason for this variability appears to be caused by differences in how readers interpret small *corpus albicans* and *artreticum*, which are sometimes hard to distinguish and easy to overlook. The latter are usually smaller than *c. albicantia* (sometimes 1-2mm) and this is the most probable explanation for very high CV when counting them (Table 1). Larger *c. artretica* and *c. albicantia* can sometimes be confused with each other, as some *c. albicantia* have an orange colour, which is usually typical of *c. artretica*. However, the observed variability in corpora counts is too small to have any major implications on using the number of ovulations as an independent index of age in the comparison with GLG count. Estimated 95% confidence intervals averaged only ±2.4 *corpora* for the females where the readers did not agree on the *corpora* count. Thus the present study has shown the need for care when examining ovaries, as there is some error associated with counting *corpora*. However, this error is small, and of the same magnitude as that observed when ageing fish which are considered easy to age (e.g. haddock, *Melanogrammus aeglefinus*; Campana *et al*., 1995).

Using GLGs in the mandible of common minke whales proved to be more difficult and time-consuming than first anticipated. The sections could not be prepared or stained in a manner that brought forth the faint and elusive GLGs with acceptable clarity and distinctiveness. The experiments with the sperm whale mandible and tooth (Fig. 3) and with the harbour porpoise (*Phocoena phocoena*) teeth and mandibles (E. Olsen, unpublished results) indicated that the technique was not at fault. Rather, it appeared that common minke whales, like their larger cousins (fin, and sei whales, Klevezal and Kleinenberg, 1967) do not form GLGs in the mandibles that are clear and distinct under visible light. X-ray imaging of mandible segments gave higher contrast, and increased the readability of the GLGs, as was evident from the higher precision (low CV) of this method. From a practical perspective, X-ray imaging was simpler than the visible light analysis, as the segments used were thicker and thus easier to cut and required less treatment or staining than for the visible light analysis. The only other study using X-ray methods to elucidate GLGs was by Lockyer (1974), who attempted to use X-rays to image the earplug of sei whales with little success. In studies of GLGs in bone, it seems that X-ray methods are more appropriate and should be attempted as supplement to traditional visible light analysis. Mandible GLG counts had higher CVs than the CV of age estimates of sablefish (*Anoplopoma fimbria*; Heifetz *et al*., 1998) or Greenland halibut (*Reinhardtius hippoglossoides*; Bowering and Nedraas, 2001), two species of fish considered difficult to age.

The mandibles were thicker in larger animals, and the highly ossified buccal wall was thicker in larger than in small whales. In some minke whale mandibles, clear GLGs of two types were found. The first being narrow bands similar to those observed by Klevezal and Kleinenberg (1967) in the buccal wall of sei and fin whales. These were structurally similar to GLGs in the sperm whale and harbour porpoise mandibles examined. Also found were broader, less distinct bands using both X-ray imaging and visible light analysis. When observed, these GLGs could be followed through the whole segment, and were found within the whole highly ossified outer layer. Such wide GLGs were found either alone or together with a thin band of narrow GLGs in the outer wall. Interpretation of the observed structures was therefore difficult, and the GLG count of a segment was therefore the sum of all GLGs observed, narrow or broad. Had the GLG count been unbiased in relation to true age, one would have expected to see a linear increase in GLG count with the number of ovulations. In the case that one type of GLG was correlated with age, while the other could be considered random noise, one would still expect to find a correlation between the number of ovulations and GLG count. Such correlations were not found (Fig. 6, r^2 visible light = 0.175, r^2 X-ray = 0.134), irrespective of the method used to examine the segments.

Bone growth in the mandible is linked with absorption of bone tissue in the mandible canal (Nishiwaki *et al*., 1961) and with bone drift and compression of growth layers with increasing age (Brodie, 1969) as well as bone mobility during foetal growth and lactation. Nishiwaki *et al.* (1961) found mandibular GLGs to correlate with tooth GLGs up to 14 GLGs after which absorption seemed to equal formation of new GLGs. Bone absorption in minke whale mandibles does not necessarily start at 14 GLGs, but assuming this and an age at sexual maturity of ~8 years (Olsen, 1997), one would expect mandible GLGs and the numbers of ovulations to correlate up to 6 ovulations. However, the estimated correlation between GLG count and the number of ovulations was not significant for either examination method, in fact it was lower than when using the whole dataset. This poor fit could be explained by large variations in ovulation rate and age at sexual maturity, assumptions which are not fulfilled. Olsen (1997) found a pregnancy rate of 98% in the Northeastern Atlantic for the period 1972-1979, which would allow for some variability in ovulation rate from the hypothesised 1/yr, but far less than that needed to explain the lack of correlation in Fig. 6. Studies of Antarctic minke whales (*Balaenoptera bonaerensis*; Kato, 1983; Thomson *et al*., 1999) have not shown any short-term variability in the age at sexual maturity necessary to explain the variability in Fig. 6. Neither have there been any large-scale environmental changes in the North Atlantic which could explain a rapid increase in age at sexual maturity which would be necessary to explain the poor correlation observed. The modelled von Bertalanffy growth equations fitted to plots in Figs 7a-d yielded wide confidence intervals and biologically unrealistic parameter estimates. Fitting growth curves to such data where most animals were fully grown and few animals were sampled during the phase of most active growth limited the use of these analyses. There is some room for random error associated with the length data, but we find it highly unlikely that this is so large and biased that it could explain the poor fit of the length/GLG plots. We therefore interpret the poor fit as indicative of a large but unspecified bias in the mandible GLG count.

Lockyer (1984a) showed that there was disagreement between five readers for ~70% of a set of Antarctic minke whale earplugs examined, and Kato (1984) pointed out that 4% of all whole collected earplugs are classified as unreadable. Assigning a ‘readability’ criteria has been the standard procedure when reading earplugs, and a similar procedure was therefore attempted when reading the mandible segments. Introducing such a readability or quality criteria did not improve the analysis, as the GLG count of high quality segments did not have a better correlation with

the number of ovulations or length than the rest of the dataset. In addition the high-quality segments constituted only 16% of all samples, which would imply that a large sample collection scheme would be needed to acquire a sufficient annual sample size for ageing.

While clearly there is continual growth in common minke whale mandibles, new bone is deposited in such a way that useful growth layers are not formed, or cannot be observed using the present technology and methods. The poor definition of mandibular GLGs in minke whales could possibly be attributed to the variable duration of the feeding season (Brodie, 1975), and the highly variable diet of North Atlantic common minke whales (Olsen and Holst, 2001).

ACKNOWLEDGEMENTS

This project was funded as part of Norwegian Research Council project number 127211/120. We wish to acknowledge the invaluable help given by Dr Audun Braathen and radiographer Tone Lønning at Haukeland Hospital in Bergen, Norway for allowing us access to the X-ray equipment and help with imaging. We also wish to thank Steinar Olsen for commenting on an earlier version of the manuscript.

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Date received: February 2002
Date accepted: April 2003