Age estimation of common minke whales (*Balaenoptera acutorostrata*) in Icelandic waters by aspartic acid racemization (AAR),-AAR and earplug readings of Antarctic minke whales (*B. bonaerensis*) used as a reference.

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Summary
This study uses eye nuclei from 38 earplug-aged Antarctic minke whales (*Balaenoptera bonaerensis*) and eye nuclei from 21 fetuses of Icelandic minke whales (*B.acutorostrata*) to construct an AAR-model for estimating age of the Icelandic minke whale.
The racemization rate, *k*, of the Antarctic minke whale was found to be 0.00147±0.00006/y (SE) which is higher than that obtained for Icelandic fin whales, bowhead whales, and harp seals but lower than that found for harbour porpoises. The ratio of D and L at birth, $(D/L)_0$, found for the Icelandic minke whale fetuses and the regresssion of the transformed D/L-ratio of the Antarctic minke whales on earplug readings was found to be 0.0196±0.0009 (SE) which is in the lower range of values found for other marine mammals. Since Antarctic minke whale has more biological resemblance to the Icelandic minke whale than other marine mammalian species for which racemization rate has been estimated so far, the Antarctic minke whale is assumed to be the presently best model of the racemization behaviour in the lens nucleus of the Icelandic minke whale. However, further studies are needed to better understand what governs the values of racemization rate and $(D/L)_0$.
The age of Icelandic minke whale was found from the regression of age on transformed D/L-ratios for the Antarctic minke whale and the fetuses of Icelandic minke whale. The model is based on Antarctic minke whales in the age interval 2-48 years while the minimum and maximum age estimated for the Icelandic minke whales were 3 and 42 years, respectively. The standard prediction error is about 4 years while the SE of the left and right lens nuclei is on average between 2.6 and 3.1 years.

Introduction
Good estimates of age of animals is of great importance in all aspects of wildlife research as for example management of stocks, elucidating age structure, life-history, and catch-at-age history of populations (Olsen, 2002). However, it has been found difficult to determine age of common minke whales from the North Atlantic (*Balaenoptera acutorostrata*) by way of either earplugs (Sigurjónsson, 1980) or *bulla tympanica* (Olsen, 2002) in contrast to Antarctic minke whale (*B. bonaerenis*) and fin whales (*B. physalus*) for which earplugs are routinely used for age estimation. Therefore, an alternative method is needed for the common minke whales from the North Atlantic. A method based on the racemization of L-aspartic acid

enantiomer into its D-form in lens nuclei has gained some attention. The nucleus of the eye lens is used since it is metabolically inactive or of very low metabolic activity and therefore its proteins are long-lived and do not or only very slowly exchange their amino acids throughout the lifetime of the animals (Masters *et al*., 1977; Masters *et al*., 1978; Ritz-Timme and Collins, 2002). The method, called the aspartic acid racemization (AAR) technique, has been used for several marine mammals where age estimation of animals by growth layer groups (GLGs) has been compared with age estimation by way of AAR in order to establish a sort of calibration for other marine mammals. This has been done for fin whales (Nerini, 1983; Nielsen *et al*., 2012), harp seals (*Pagophilus groenlandicus*) (Garde *et al*., 2010), harbour porpoises (*Phocoena phocoena*) (Nielsen *et al*., 2012), and bowhead whales (*B. Mysticetus)* (Rosa *et al*., 2012). However, it was at first evaluated for humans for this purpose (Bada and Protsch, 1973; Masters *et al*., 1977; Masters *et al*., 1978; Ritz-Timme and Collins, 2002). The relationships between age and racemization for humans and fin whales together with additional information on racemization ratios at zero age have been used to estimate age of several marine mammals, *e.g*. bowhead whales (George *et al*., 1999), North Atlantic minke whales (Olsen and Sunde, 2002), and narwhals (*Monodon monoceros*) (Garde *et al*., 2007).
This study used eye lenses from 38 GLG age estimated Antarctic minke whales and eye lenses from 21 fetuses of Icelandic minke whales to construct an AAR-model for estimating age of the Icelandic minke whale. The study is a part of a wide ranging research programme on the biology and feeding ecology of minke whales in Icelandic waters (Marine Research Institute 2003).

Materials and methods

Sampling
A schematic diagram of an eye globe is shown in Figure 1.

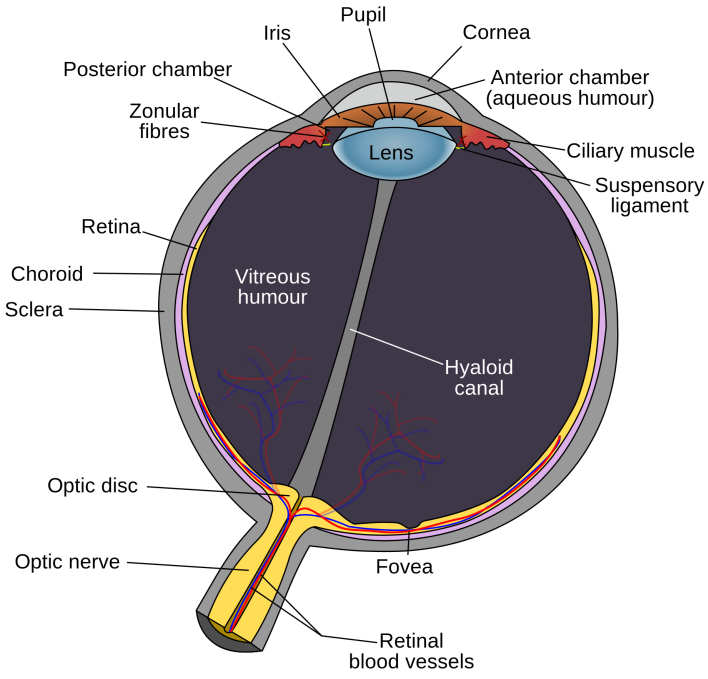

Fig. 1. Schematic diagram of vertebrate eye (Wikipedia)

One or both eye globes of the Icelandic minke whale (*B. acutorostrata*) were collected whole from the animal as soon after catch as possible. The time from death till sampling varied from few minutes to couple of hours. The whole eye was kept frozen at -20 °C until they were prepared for analysis of L- and D-aspartic acid.
In the laboratory of the Marine Researh Institute (MRI), the eyes were allowed to thaw at 0-4°C for about 2-3 days or until convenient to work with. The preparation took place in a room at about 4 °C. All equipments used at the laboratory were of stainless steel and thoroughly washed with soap, flushed with distilled water, and salt water.
Firstly, the eye globe was cut open at the back of the lens and the lens removed with a spoon. However, since the sclera is very tough, this was a difficult and time consuming process. This procedure was therefore altered at later stages whereby the eye lens was reached by cutting through the cornea and then, by following the outermost edge of the iris and pupil, the lens was made accessible by cutting the zonular fibres and/or ciliary muscle. Thereby, the lens could easily be removed with a spoon. This method proved to be the most convenient and the risk of damaging the lens was minimal.
The lens was washed in dilute saltwater and put on clean glass plate. Using clean tools the lens was rolled in zig-zag track over the glass plate whereby the layers were peeled off while frequently changing to new and clean tools as the lens nucleus was approached.
When about third of the eyes had been prepared, a solution of 75% ethanol and 5% isopropanol was used instead of the water to wash the tools since that made it easier to wash off the sticky lens tissue.

The eyes of the Antarctic minke whales (*B.bonaerenis)* were treated in the same way. The eyes were generously provided by the Institute of Cetacean Research, Tokyo, Japan, who also provided data on earplug readings, biological and catch data for each animal. However, no measure of variance or other measures of dispersion in readings was provided. The earplug readings were carried out according to Lockyer (1984).

Hydrolysis and high performance liquid chromatography (HPLC)

Hydrolysis of the samples and the following HPLC analysis were done mainly by following the procedures of Zhao and Bada (1995). The analysis was carried out at two laboratories. Most of the analytical work was carried out at the University of Copenhagen: all the 38 eyes lenses of the Antarctic minke whale and 111 of the lens nuclei of the Icelandic minke whales. The rest of the analytical work, 34 lenses, was carried out at the Icelandic Fisheries Laboratories. Additionally, lenses of 31 animals were analysed at both laboratories, *i.e.* left and right eyes lenses.

At the University of Copenhagen, an Agilent 1100 Series HPLC system was used for the analysis. The system consisted of a G1379A degasser, a G1376A capillary pump, an ALS G1313A autosampler, a G1316 A column compartment, a G1321A fluorescence detector (operated at ex = 340 nm, em = 450 nm) and a MWD G1365B multi-wavelength detector. The column used was a Zorbax Eclipse XDB-C18, 4.6 x 150 mm, with particle size of 3.5 µm. The HPLC system was managed by Chemstation Software system, version A.08.03. When preparing the OPA-NAC, a 0.1 M sodium borate buffer (pH 9.4) was used as suggested by Olsen & Sunde (2002) instead of the 0.4 M borate buffer used by Zhao and Bada (1995). Also, a sodium citrate buffer (pH 5.6) was used in the mobile phase instead of the sodium acetate buffer (pH 5.6) used by Zhao and Bada (1995). This modification resulted in a better separation of the D- and L-enantiomers and thereby facilitating a more accurate integration of

the peak areas. The derivatisation of the amino acids in the hydrolysate was performed automatically just before injection into the HPLC column using the autosampler of the HPLC system.
D- and L- aspartic acid peak areas were integrated at preset parameters by the computer programme of the Chemstation software or manually if the peak areas were too small for the programme to integrate automatically. For calibration of the D/L ratios measured by the HPLC, the following D/L standards were prepared (10^{-4} M): 0.5/99.5, 1/99, 2/98, 5/95, 10/90, 15/85. These standards were run at the beginning and at the end of each batch of eye lenses and a calibration line was made by regression of the actual ratios (of the standards) as a function of the measured ratio. The measured D/L ratios of each eye-lens batch were corrected using the calibration line obtained for that batch.

At the Icelandic Fisheries Laboratories, the analysis was carried out by a Kontron Instrument HPLC equipment (autosampler and pump) connected to a Supelcosil LC-PAH column (4.6x250mm; 5 µm particle size) and Jasco 820-FP fluorescence detector. The system was managed by KROMA software. The method by Olsen and Sunde (2002) was used as described. Peak heights instead of peak areas were used for calculation of the D/L-ratio according to Olsen and Sunde (2002).

Estimation of racemization rate, $(D/L)_0$ and age

Racemization of aspartic acid is assumed to occur according to the non-enzymatic, reversible and first order rate reaction of the L-enantiomer to the D-enantiomer:

$$L \leftrightarrow D \tag{1}$$

The rate is the same in both directions, *i.e.* the rate constant, *k*, is the same in both directions resulting in an equilibrium constant equal to one. This means that the concentration of both the L- and D-forms is equal when equilibrium is reached (a racemic mixture). At any time *t*, the concentration of the L-enantiomer has decreased by x and thereby the D-form has increased by the same amount. Therefore the following equations are assumed to prevail:

$$[L] = [L]_0 - x \tag{2}$$

$$[D] = [D]_0 + x \tag{3}$$

where the brackets denote concentration and the subscript 0 denotes concentration of the L- and D-enantiomers at the time when exchange of blood with it ceases to occur ($t = 0$). For the sake of simplicity, the brackets will not be used below. Equations 2 and 3 mean that no degradation of aspartic acid is assumed to occur during the lifetime of the animal since addition of 2 and 3 gives

$$L + D = L_0 + D_0 \tag{4}$$

The rate equation becomes

$$-\frac{dL}{dt} = \frac{dx}{dt} = kL - kD = k(L_0 - x) - k(D_0 + x) = k(L_0 - D_0) - 2kx \tag{5}$$

The solution to this equation is the following

$$e^{2kt}\left(x-\frac{L_0-D_0}{2}-\right)=-\frac{L_0-D_0}{2} \tag{6}$$

Applying equations 2 and 3 to obtain expression for x, *i.e.*

$$x=\frac{L_0-D_0}{2}-\frac{L-D}{2} \tag{7}$$

equation 6 becomes

$$e^{2kt}=\frac{L_0-D_0}{L-D}=\frac{L_0-D_0}{L-D}\times\frac{L+D}{L_0+D_0}=\frac{1+D/L}{1-D/L}\times\frac{1-(D/L)_0}{1+(D/L)_0} \tag{8}$$

after equation 4 is used to separate the terms into terms containing either D/L or $(D/L)_0$. Therefore, the age is obtained as a function of the enantomer ratios by the following equation 9:

$$2kt=\ln\left(\frac{1+D/L}{1-D/L}\right)-\ln\left(\frac{1+(D/L)_0}{1-(D/L)_0}\right)\cong\ln\left(\frac{1+D/L}{1-D/L}\right)-2(D/L)_0 \tag{9}$$

where the second term is approximated, *i.e.* $\ln(1+(D/L)_0))\approx(D/L)_0$ and $\ln(1-(D/L)_0))\approx-(D/L)_0$ since $(D/L)_0<<1$. The racemization rate, *k*, and the ratio of enantiomers at birth, $(D/L)_0$, are obtained from the slope and intercept, respectively, by regressing $\ln\left(\frac{1+D/L}{1-D/L}\right)$ on earplug age for the Antarctic minke whales, *i.e.*

$$\ln\left(\frac{1+D/L}{1-D/L}\right)\cong 2kt+2(D/L)_0 \tag{10}$$

where D/L is the result of the analysis of the L- and D-enantiomers by HPLC of the lens nuclei of the Antarctic minke whales while *t* is the age estimated from the earplugs. The values of $(D/L)_0$ from fetuses of the Icelandic minke whales are included in this regression. The term $(D/L)_0$ actually represents both this ratio at birth plus additional racemization occurring during acid hydrolysis of the samples (Waite *et al*., 1999).

For the age estimation of the Icelandic minke whales, age of the Antarctic minke whales and Icelandic minke whale fetuses is regressed on their $x=\ln\left(\frac{1+D/L}{1-D/L}\right)$:

$$t = \frac{\ln\left(\frac{1+(D/L)}{1-(D/L)}\right) - \ln\left(\frac{1+(D/L)_0}{1-(D/L)_0}\right)}{2k} = bx + a \qquad (11)$$

whereupon the age of the Icelandic minke whale is calculated by inserting their values of $x = \ln\left(\frac{1+D/L}{1-D/L}\right)$ into equation 11.

Error of the age so estimated is obtained as the standard error of prediction:

$$SE(age) = s\sqrt{1 + \frac{1}{n} + \frac{(x-\bar{x})^2}{\sum(x-\bar{x})^2}} \qquad (12)$$

where s is the standard error of the regression.

Results and discussions

Estimation of $(D/L)_0$ *and the racemization rate, k*

The GLG-age of the Antarctic minke whale ranged between 2 and 48 years. No standard deviation or any other measure of variation was obtained for the GLG-age determination. Table 1 shows the data on the Icelandic minke whale fetuses while data on the Antarctic minke whales are found in Table 2.

Table 1. Data on 21 fetuses from Icelandic minke whale. The assigned age of the fetuses is zero

Specimen ID	D/L
A0510F	0.0218
A0603FV	0.0227
A0610FV	0.0180
B0506F	0.0195
B0610FV	0.0156
B0608FV	0.0181
B0507F	0.0219
B0608FHR	0.0232
C0205F	0.0204
C0506FV	0.0214
C0604FV	0.0219
C0605FHR	0.0181
C0607F	0.0140
C0611FH	0.0201
C0611FV	0.0203
C0612FHR	0.0160
D0601F	0.0157
D0611FH	0.0182
D0612FV	0.0187
D0609FHR	0.0375
D0703F	0.0220

Table 2. Data on 38 Antarctic minke whales

Specimen No.	Catch date	Body length (m)	Sex	GLG age (y)	D/L
01/02-001	30-Nov-01	5.4	M	2	0.02341
01/02-002	01-Dec-01	8.8	M	22	0.04571
01/02-045	23-Dec-01	8.9	F	18	0.04816
01/02-062	24-Dec-01	9.7	F	28	0.06402
01/02-073	25-Dec-01	9.1	F	28	0.05585
01/02-087	26-Dec-01	5.5	F	2	0.02302
01/02-088	27-Dec-01	5.8	F	2	0.02307
01/02-090	27-Dec-01	6.3	M	3	0.02629
01/02-093	27-Dec-01	6.2	M	3	0.02364
01/02-096	29-Dec-01	9.1	M	44	0.07837
01/02-097	29-Dec-01	8.8	M	27	0.06374
01/02-100	29-Dec-01	5.4	M	2	0.02222
01/02-103	31-Dec-01	7.5	F	5	0.02696
01/02-104	31-Dec-01	5.7	M	2	0.02111
01/02-112	07-Jan-01	6.1	M	3	0.02138
01/02-114	07-Jan-01	5.6	M	2	0.02132
01/02-115	07-Jan-01	5.1	M	2	0.02451
01/02-116	07-Jan-01	9.7	F	22	0.05154
01/02-121	08-Jan-01	7.8	F	8	0.03024
01/02-122	08-Jan-01	8.4	M	48	0.11388
01/02-129	08-Jan-01	9.1	M	35	0.05150
01/02-133	09-Jan-01	8.1	M	14	0.03734
01/02-151	10-Jan-01	7.9	F	5	0.02584
01/02-153	11-Jan-01	8.8	F	15	0.03813
01/02-170	15-Jan-01	7.4	M	5	0.02428
01/02-187	18-Jan-01	7.6	M	6	0.02511
01/02-190	18-Jan-01	7.1	M	5	0.02322
01/02-193	19-Jan-01	7.4	F	6	0.02575
01/02-214	24-Jan-01	6.4	F	4	0.02261
01/02-218	27-Jan-01	5.5	F	2	0.02545
01/02-221	27-Jan-01	9.0	F	30	0.06255
01/02-225	28-Jan-01	6.9	F	5	0.02572
01/02-226	28-Jan-01	5.7	F	2	0.03876
01/02-228	28-Jan-01	8.3	F	7	0.03019
01/02-231	29-Jan-01	6.1	M	3	0.02296
01/02-323	10-Feb-01	9.2	M	32	0.06650
01/02-364	16-Feb-02	9.1	M	35	0.07208
01/02-034	17-Dec-01	7.8	M	8	0.02477

The data of Tables 1 and 2 are plotted in Figure 2 in accordance with equation 10. Figure 2 indicates that the D/L-ratios of the Icelandic minke whale fetuses fit well with the data on Antarctic minke whales. The intercept has a value of 0.0391±0.0018 (SE) and thus the $(D/L)_0$ is 0.0196±0.0009 (SE), which is the same value as the D/L-ratio of the 20 fetuses (0.0194±0.0040 (SE)) when the apparent outlier shown in Figure 2 is omitted (t-test; p=0,75) (the regression residual for this apparent outlier is more than three times the standard error from the regression line). Even if the apparent outlier for the fetus is included, there is not a significant difference between the value of $(D/L)_0$ obtained from the regression intercept and the average value of the 21 D/L-ratios of the fetuses (0.0202±0.004 (SE)) (t-test; p = 0.22).

The values of $(D/L)_0$ estimated for marine mammals vary somewhat, being 0.0285 for bowhead whales (George *et al*., 1999), 0.0282 for fin whales (Nielsen *et al*., 2012), 0.0231 for harbour porpoises (Nielsen *et al*., 2012), and 0.0193 for harp seals (Garde *et al*., 2010). Recently, Rosa *et al.* (2012) estimated $(D/L)_0$ of bowhead whales to be 0.0250 and Garde *et al.* (2007) estimated $(D/L)_0$ of narwhals to be 0.0288. Our value is in the lower range of these values. However, this value may be affected by the hydrolysis during sample preparation, *i.e.* increase (Waite *et al*., 1999), which may differ from one study to another. Additionally, here it has been assumed that the fetuses are of age zero since exchange of blood with the lens nucleus may occur during pregnancy, even until birth. However, if minus ages were assigned to the fetuses (estimated from their lengths) a slightly lower estimate of $(D/L)_0$ is obtained but it is not significantly different from the value obtained by assigning fetuses the age of zero.
Another factor that might result in low estimate of $(D/L)_0$ is possible contamination of the nucleus with tissues surrounding the nucleus or contamination by blood or other young tissues since these tissues have lower D/L-values. However, the nuclei of both the Antarctic and Icelandic minke whales were prepared in the same manner, and therefore this would not or to a very little extent affect the age estimation of the Icelandic minke whales described below.

Only two regression residuals fall outside the range of three times the standard error of the regression line (apart from the fetus lens above), one at a GLG of 35 and one at GLG of 48 (the oldest animal), both easily spotted in Figure 2.

The racemization rate, k, is obtained from the slope in Figure 2. The value of $2k$ is 0.00294±0.00012/y (SE). To test if this value may differ for young and old animals, regression was carried out by adding dummy variable to equation 10 resulting in equation 13:

$$\ln\left(\frac{1+D/L}{1-D/L}\right) = 2kt + 2(D/L)_0 + \beta Z \qquad (13)$$

where Z is zero if t≤7 but equal to one if t>7. β was found to be not significantly different from zero (p = 0.9). If this regression was carried out without the (D/L)-values for the fetuses, β was still found to be not significantly different from zero (p = 0.14), also when values for the fetuses and the two outliers above were omitted (p = 0.48).

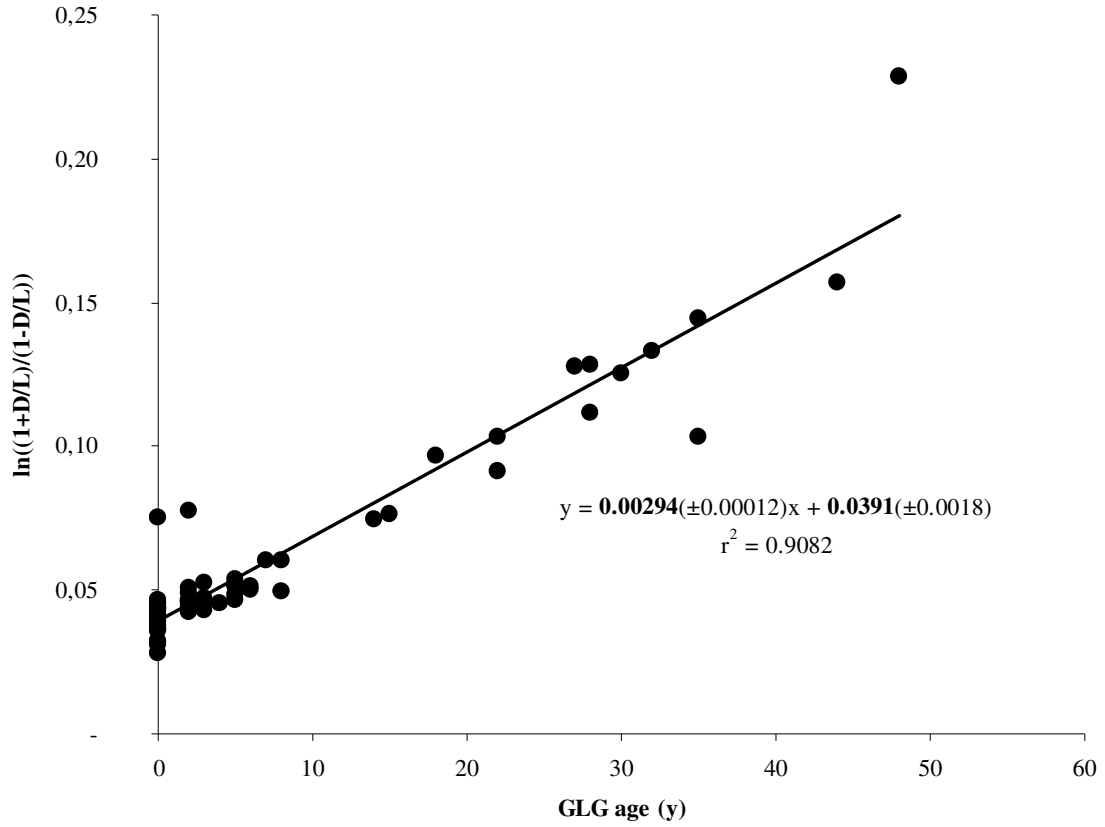

Fig. 2. Transformed D/L-ratios of Antarctic minke whales (n = 38) and Icelandic minke whale fetuses (n = 21; age = 0) as a function of GLG age. The slope is twice the racemization rate, *i.e.* 2*k*, and the intercept is twice the $(D/L)_0$ value according to equation 10. The values in parantheses are the standard errors of the estimated parameters.

The value of 2*k* in this study is higher than that found for fin whales (0.00220 in Nielsen *et al*., 2012) and 0.00232 in Nerini (1983)), harp seals (0.00260 in Garde *et al*. (2010)), and bowhead whales (0.00195 in Rosa et al. (2012)). The 2*k* is also higher than 0.0025 in humans found by Masters *et al*. (1977). However, the racemization rate for the Antarctic minke whales is slower than that found for harbour porpoises (0.00610 found by Nielsen *et al*. (2012)).

k increases with temperature (Waite *et al*., 1999; Olsen and Sunde, 2002; Rosa *et al*., 2012) but may also be affected by various parameters during sampling, sample preparation and analysis (Waite *et al*., 1999). The core temperature of minke whales is lower than those of both fin whales (Olsen and Sunde, 2002; Rosa *et al*., 2012) and humans, but higher than that of bowhead whales (Rosa *et al*., 2012) and therefore the *k* of the minke whales would have been expected to be lower than those found for humans and fin whales but higher than that of bowhead whales. Rosa *et al*. (2012) found a good linear relationship between *k* and core body temperature of fin whales, bowhead whales and humans, and by this relationship predicted the 2*k* of the North Atlantic minke whales to be 0.002101, which is about 70 % of the 2*k* found here for the Antarctic minke whales. Thus, temperature does not seem to explain the higher estimate of racemization rate found here for the Antarctic minke whales than that found for fin whales and humans, *i.e.* other factors than temperature seem to affect the racemisation rate. Various physicochemical factors within and outside the proteins (in which the racemization of aspartic acid takes place) may affect the racemization rate (Ritz-Timme

and Collins, 2002), factors that might be different from one species to another. For example, extensive replacement of aspartic acid by asparagine (that converts to aspartic acid during hydrolysis) in the proteins of the lens nuclus of Antarctic minke whales would result in higher apparent *k* value since asparagine racemizes faster than aspartic acid (George *et al*., 1999). However, it seems unlikely that the nuclei of different mammalian species differ in this respect (George *et al.,* 1999). Furthermore, animals with cataract may result in increased rate of aspartic acid racemization in their lens nuclei (Masters *et al*., 1977; Masters *et al*., 1978). Cataract was not examined in the eyes of neither the Antarctic minke whales nor the Icelandic minke whales. However, to our knowledge, cataracts have not been found in marine mammals and studies on 50 eyeballs of bowhead whales did not show any obvious signs of cataract (George *et al*., 1999) nor did eyeballs from one fin whale, two narwhals and one beluga reveal catarct in these individuals (Nielsen *et al*., 2012). Finally, if the GLG-age of the Antarctic minke whales is negatively biased with age (and therefore the bias in absolute age is greater the older the animal is), the 2*k* obtained by equation 10 will be higher than the true value. This hypothesis, however, warrants further studies to be confirmed or rejected.
Since Antarctic minke whale has more biological resemblance to the Icelandic minke whale than other marine mammalian species for which *k* has been estimated so far (Icelandic fin whales (Nerini, 1983; Nielsen *et al*., 2012), Greenlandic harbour porpoises (Nielsen *et al*. 2012) Greenlandic harp seals (Garde *et al*., 2010), and bowhead whales (Rosa *et al*., 2012)), the regression of Figure 2 is assumed to be the presently best estimate of the racemization behaviour in the lens nucleus of the Icelandic minke whale.

Age estimation of the Icelandic minke whales

For estimating age, regression in terms of equation 11 was carried out, *i.e.* age was regressed on $x = \ln\left(\frac{1+D/L}{1-D/L}\right)$ with the following result (all values included, *i.e.* also the apparent outliers above; n=59):

$$Age = -11.30(\pm 0.96) + 308.6(\pm 13.1)x \qquad (14)$$

where values within brackets are standard errors of the estimates. The standard error of prediction is obtained in accordance with equation 12:

$$SE(age) = \sqrt{168.8(x - 0.06366)^2 + 14.71} \qquad (15)$$

The smallest standard deviation in age is obtained for the average x, 0.06366, of the 38 Antarctic minke whales and the 21 Icelandic minke whale fetuses. This value corresponds to the age of 8.3±3.8 (SE) years.

For evaluation of the method, both left and right lens nuclei were analysed at both laboratories. 11 pairs were analysed by the Icelandic Fisheries Laboratories resulting in a non-significant difference between right and left eye (paired one-tailed t-test; p = 0.26) where the average variance results in a SE of 2.6 years (range: 0.2-4.2 years). At the University of Copenhagen, 55 pairs were analysed, and no significant difference was found (paired one-sided t-test; p = 0.46) and an average variance resulting in SE of 2.8 years (range: 0.01-14.7 years). When the AAR age from the left lens was regressed on AAR age from right lens for

the two laboratories in a nested model, no significant difference was found between the two laboratories.
Furthermore, lens nuclei of 31 animals were analysed at both laboratories and there was not a significant difference between the right and the left eye determined by the two laboratories (27 pairs with known left and right sides) when the results of one laboratory were regressed on the results of the other, *i.e.* a slope of unity was obtained (95% CI of slope: 0.97-1.17) and an intercept not significantly different from zero (p = 0.09). Average variance for the two laboratories results in SE of 3.1 years (n = 30 where one apparent outlier was excluded) (range: 0.94-7.1). For these reasons, age estimation based on results from both laboratories were treated as equivalent and the average age for the left and right lens nuclei were used when both results were available.

Figure 3 shows the relationship between left and righ lenses for all the pairs analysed by both laboratories (n = 94). The agreement between the right and left nuclei is good as reflected by the fact that the regression line has a slope which is not significantly different from unity (95% CI: 0.936-1.012) and an intercept that is not significantly different from zero (p = 0.10) when two .outliers are excluded (with residual deviations 6.2 and 6.7 times the regression standard error)

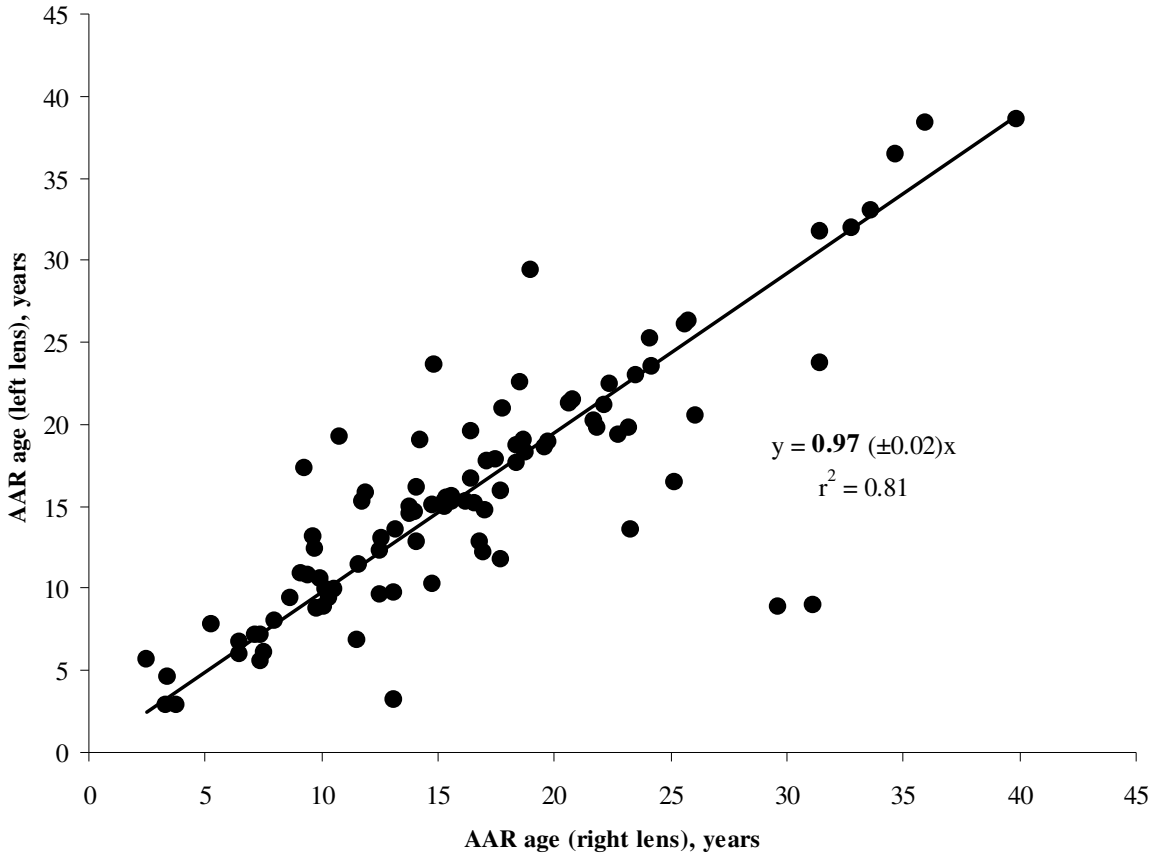

Fig. 3. The relationship between AAR ages of the right and left lenses of Icelandic minke whales (n = 94; regression with n = 92). The value within bracket is the standard error of the parameter estimate when the intercept was set equal to zero and the outliers at right lenses of AAR ages 29.6 and 31. 1 years were omitted (which have residual deviatons 6.2 and 6.7 times the regression standard error).

Table 3 shows the data for all the 176 Icelandic minke whales for which age was estimated.

Table 3. Data on 176 Icelandic minke whales.

Whale ID	AAR age (y)	SE(L/R)*	SE (predictive) (y)**	Lab
A0303	8,8		3,8	2
A0306	14,7		3,8	2
A0307	13,8		3,8	2
A0308	11,6		3,8	2
A0309	12,4		3,8	2
A0310	15,5		3,8	2
A0311	8,7		3,8	2
A0312	11,0	2,1	3,8	2
A0313	6,5	1,8	3,8	2
A0401	10,2		3,8	2
A0403	13,9	2,8	3,8	2
A0404	14,3		3,8	2
A0405	12,5	3,2	3,8	2
A0406	14,8	2,8	3,8	2
A0501	11,1	1,9	3,8	1&2
A0502	16,6	3,4	3,9	1&2
A0503	6,7		3,8	1
A0504	9,3	0,7	3,8	1&2
A0506	7,2	0,0	3,8	1&2
A0507	10,3	0,4	3,8	1&2
A0508	11,4	2,4	3,8	1&2
A0509	8,2	7,1	3,8	1&2
A0510	20,8	6,1	3,9	1&2
A0601	20,9	1,1	3,9	1
A0602	22,9		3,9	1
A0603	12,4	0,1	3,8	1
A0604	6,6	0,2	3,8	1
A0605	14,4		3,8	1
A0606	22,4	0,0	3,9	1
A0607	3,1	0,3	3,8	1
A0608	11,5	0,1	3,8	1
A0609	18,9	0,2	3,9	1
A06-101	15,9		3,8	1
A0610	10,0	1,3	3,8	1
A0611	6,2	0,3	3,8	1
A0701	10,0	0,2	3,8	1
A0702	25,9	0,3	3,9	1
A0703	15,6	0,0	3,8	1
A0704	15,0	6,0	3,8	1
A0705	19,0		3,9	1
A0706	35,6	1,2	4,0	1
A0707	15,9	1,6	3,8	1
A0708	16,5	0,2	3,9	1

Table 3. Continued

Whale ID	AAR age (y)	SE(L/R)*	SE (predictive) (y)**	Lab
A0709	23,2	0,3	3,9	1
A0710	21,8		3,9	1
B0301	11,9		3,8	2
B0302	11,0		3,8	2
B0303 I	25,1		3,9	2
B0307	12,0		3,8	2
B0309	7,8		3,8	2
B0310	9,1		3,8	2
B0311	29,5		3,9	2
B0312	26,3		3,9	2
B0401	41,7		4,1	1
B0402	9,8		3,8	1
B0403	20.0	15.7	3,9	1&2
B0404	20,5	2,8	3,9	1&2
B0405	25,2		3,9	1
B0406	13,3	5,7	3,8	1&2
B0407	35,3		4,0	1
B0408	4,1	2,2	3,8	1&2
B0409	22,7		3,9	1
B0410	14,5		3,8	1
B0411	10,9		3,8	1
B0413	26,6	6,1	3,9	1&2
B0501	18,0	2,2	3,9	1&2
B0502	27,6	5,5	3,9	1&2
B0503	13,4	0,9	3,8	1&2
B0504	4,8		3,8	1
B0505	11,4	2,4	3,8	1&2
B0506	26,4		3,9	1
B0508	6,6		3,8	2
B0509	21,5		3,9	2
B0510	14,4	1,9	3,8	1&2
B0511	15,5	0,2	3,8	1&2
B0601	16,6		3,9	1
B0602	15,5		3,8	1
B0603	13,5	2,5	3,8	1
B0604	9,9	0,6	3,8	1
B0605	10,3	0,5	3,8	1
B0606	32,4	0,6	4,0	1
B0607	22,6		3,9	1
B0608	21,1	0,5	3,9	1
B0609	39,2	0,9	4,1	1
B0610	10,4		3,8	1
B0611	14,9	0,2	3,8	1
B0612	8,2		3,8	1

Table 3. Continued

Whale ID	AAR age (y)	SE(L/R)*	SE (predictive) (y)**	Lab
B0613	15,1	0,3	3,8	1
B0614	18,6	0,2	3,9	1
B0615	14,2	0,6	3,8	1
B0616	12,8	0,3	3,8	1
B0617	15,3	0,0	3,8	1
B0618	13,0		3,8	1
B0701	19,0		3,9	1
B0702	13,4	0,3	3,8	1
B0703	29,3		3,9	1
B0705	23,2		3,9	1
B0706	23,8	0,5	3,9	1
B0707	22,6		3,9	1
B0708	20,0	2,4	3,9	1
B0709	24,7	0,8	3,9	1
B0710	29,8		3,9	1
C0302	31,6	0,2	4,0	2
C0303	31,7		4,0	2
C0305	19,4	2,2	3,9	2
C0306	14,8	4,2	3,8	2
C0307	10,1	0,9	3,8	2
C0308	20,6		3,9	2
C0309	9,2	3,3	3,8	2
C0311	21,5	2,5	3,9	2
C0312	29,2		3,9	2
C0401	23,3	4,0	3,9	1&2
C0402	12,0		3,8	1
C0403	7,9		3,8	1
C0404	6,8	1,0	3,8	1&2
C0405	16,8	1,2	3,9	1&2
C0406	18,8		3,9	1
C0407	7,6		3,8	1
C0501	13,8		3,8	2
C0502	14,6	3,4	3,8	1&2
C0503	3,9	0,8	3,8	1&2
C0504	18,5	0,3	3,9	1&2
C0505	26,0	0,4	3,9	1&2
C0506	42,1		4,1	1
C0507	19,6	3,3	3,9	1&2
C0508	19,3	0,6	3,9	1&2
C0509	6,5	1,3	3,8	1&2
C0510	19,1	0,7	3,9	1&2
C0511	29,6		3,9	1
C0512	19,3	14,7	3,9	1
C0514	33,9		4,0	1
C0601	39,0		4,0	1
C0602	8,2		3,8	1

Table 3. Continued

Whale ID	AAR age (y)	SE(L/R)*	SE (predictive) (y)**	Lab
C0603	15,7	0,7	3,8	1
C0604	11,5		3,8	1
C0605	17,4	0,5	3,9	1
C0606	33,4	0,4	4,0	1
C0607	37,2	1,7	4,0	1
C0608	20,8	1,5	3,9	1
C0609	15,1	1,4	3,8	1
C0610	22,4		3,9	1
C0611	20,9	0,4	3,9	1
C0612	13,7		3,8	1
C0613	11,8		3,8	1
C0614	27,0		3,9	1
C0615	14,9		3,8	1
C0701	12,4		3,8	1
C0702	7,3	0,1	3,8	1
C0705	21,7	0,7	3,9	1
C0706	9,2		3,8	1
C0707	8,0	0,0	3,8	1
C0708	6,6		3,8	1
C0709	26,4		3,9	1
C0710	17,7	0,3	3,9	1
D0601	9,5	0,9	3,8	1
D0602	19,3	6,2	3,9	1
D0603	14,3	0,5	3,8	1
D0604	22,8		3,9	1
D0605	15,4	0,1	3,9	1
D0606	15,9		3,8	1
D0607	18,4	6,8	3,9	1
D0608	20,0		3,9	1
D0609	24,2	7,3	3,9	1
D0610	16,3		3,8	1
D0611	15,9	1,0	3,8	1
D0612	32,3		4,0	1
D0613	7,3		3,8	1
D0701	14,4	0,8	3,8	1
D0702	9,0	0,5	3,8	1
D0703	18,0	0,5	3,9	1
D0705	10,1		3,8	1
D0706	24,6		3,9	1
D0707	20,1		3,9	1
D0708	14,6		3,8	1
N0501	6,8		3,8	1
N0601	3,3	0,7	3,8	1&2

*Standard deviation in the estimation of age from both the right and the left lens nuclei.
**Predictive SE according to equation 15.

Conclusion

Since Antarctic minke whales have more biological resemblance to the Icelandic minke whales than other marine mammalian species for which racemization rate has been estimated so far, the Antarctic minke whales are assumed to be the presently best model of the racemization behaviour in the lens nucleus of the Icelandic minke whales. However, further studies are needed to better understand what governs the values of racemization rate and $(D/L)_0$.

For future work, a standardised protocol for sampling and sample preparation of eye lenses would be of much help as well as for the analytical method. A certified reference material with known D/L-ratio of aspartic acid would be of great help in future studies of age determination by the aspartic acid racemization technique.

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