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Age estimates based on aspartic acid racemization for bowhead whales (*Balaena mysticetus*) harvested in 1998–2000 and the relationship between racemization rate and body temperature

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Abstract

Fifty-two eyes were collected and analyzed to estimate ages of 42 bowhead whales using the aspartic acid racemization aging technique. Between-eye and within-eye variance components for the ratio of the D and L optical isomers (D/L ratio) were estimated *via* analysis of variance using multiple measurements from nine whales with both eyes sampled and analyzed. For whales with more than one $(D/L)_{act}$ value, an inverse variance weighted average of the values was used as $(D/L)_{act}$ for the whale. Racemization rate (k_{Asp}) and D/L ratio at birth $(D/L)_0$ were estimated using $(D/L)_{act}$ from 27 bowhead whales with age estimates based on baleen or ovarian corpora data and two term fetuses. The estimates were $k_{Asp} = 0.977 \times 10^{-3}$/yr and $(D/L)_0 = 0.0250$. The nonlinear least squares analysis that produced these estimates also estimated female age at sexual maturity as ASM = 25.86 yr. SE(age) was estimated via a bootstrap that took into account the SE of $(D/L)_{act}$ and the variances and covariance of k_{Asp} and $(D/L)_0$. One male exceeded 100 yr of age; the oldest female was 88. A strong linear relationship between k_{Asp} and body temperature was estimated by combining bowhead data with independent data from studies of humans and fin whales. Using this relationship, we estimated k_{Asp} and ASM for North Atlantic minke whales.

Key words: age determination, bowhead whale, *Balaena mysticetus*, age at sexual maturity.

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Accurate age estimates are crucial to interpreting many aspects of marine mammal health, biology and population assessment. Confident estimates of the ages of sexual maturity, senescence and life span are critical to population modelers. In the field of health assessment, age can affect many organismal characteristics ranging from tissue chemical composition to the gross and histological appearance of tissues.

The age of a marine mammal may be determined by various methods, ranging from photo reidentification to such methods as counts of ear plug growth layers, quantification of tooth growth layer groups, aspartic acid racemization in the teeth or eye lens nucleus, and baleen growth increments. For a more detailed review of these methods, see Christensen (1981), Hohn *et al.* (1989), Schell *et al.* (1989), George *et al.* (1999) and Lubetkin *et al.* (2008).

In mysticete whales, and specifically for the bowhead whale (*Balaena mysticetus*), estimating age is challenging. Teeth are not present, ear plugs do not appear to be readable and baleen aging is reliable only for relatively young whales, most likely only those <20 yr old, due to wear at the distal end of the baleen plates (Lubetkin *et al*. 2008). Recent research has found that bowhead whales may routinely live over 100 yr, with data showing ages possibly in excess of 200 yr (George *et al*. 1999, 2011). While ages of sexually mature bowhead females can be estimated from ovarian corpora counts (George *et al*. 2011), aspartic acid racemization (AAR) aging of the eye lens nucleus is the only published method for directly estimating ages of bowheads of both sexes in their 20s or older.

Briefly, the aspartic acid racemization aging technique is based on the fact that amino acids exist in two different optical isomers (the D and L enantiomers), which rotate plane polarized light in opposite directions, but at equal absolute values. Living organisms biochemically produce only the L-enantiomers of the amino acids, which are important for the functionality of enzymes, for example (Bada *et al.* 1980). These organisms maintain the disequilibrium state by continuous biosynthesis during metabolism. In the absence of metabolic activity, in tissues such as teeth and eye lens proteins in mammals, a chemical process called "racemization" begins immediately after the tissue is formed prior to parturition in the fetal stage. In the racemization reaction, the L amino acids are converted interchangeably into the D enantiomer and vice versa until both enantiomers have equal concentrations, *i.e.*, a D/L ratio of 1.0 (this is called a racemic mixture). The rate at which racemization occurs varies for each amino acid and is also temperature dependent, with higher temperatures leading to a higher reaction rate. Aspartic acid is an amino acid with a high racemization rate and can be used for age determination in the range of tens of years (humans, dolphins, and other cetaceans). It should be noted that the D/L value of amino acids even in metabolically inactive fetal tissue $[(D/L)_0]$ is not zero and must therefore be determined in order to use this technique. In most mammals, the average body temperature is held steady at 37°C. Since the racemization rates k_{AA} for amino acids such as aspartic acid (k_{Asp}) have been determined for this temperature from a sample of mammals with known ages and body temperature (Masters *et al.* 1977), the age of other animals can be estimated from the D/L ratio, assuming they have a similar body temperature (Bada *et al.* 1980). If the body temperature is not known accurately, estimated racemization rates can be used but will be less accurate.

As part of a larger health assessment study, eyes from 42 bowheads harvested by Inupiat hunters between 1998 and 2000 were collected so that ages could be

determined from aspartic acid racemization of their eye lens nuclei using methods developed by George *et al.* (1999). George *et al.* (1999) obtained ages for 42 whales harvested prior to 1998.

Objectives of the 1998–2000 data collection, in addition to estimating the ages of the whales whose eyes were sampled, were to extend the previous work by estimating the racemization rate (k_{Asp}) for bowheads using whales among the 84 with AAR data for which ages had been obtained by a different method and to further evaluate the AAR aging method *via* analysis and comparison of paired eyes from individual bowhead whales.

Methods

Sample Collection and Preparation

Each eye (intact globe) was collected during the Inupiat bowhead whale subsistence hunt in Alaska. Eyes were frozen at −20°C immediately after collection and shipped by airfreight to Scripps Institution of Oceanography (SIO). At time of dissection, the eyes were partially thawed. The cornea was reflected and lens removed and placed on a gas sterilized frosted glass plate mounted on a block of dry ice. Each lens was then dissected to the central nucleus by rolling it and trimming it carefully from one section of the sterile plate to another (the external layers would freeze/adhere to the plate), carefully avoiding contamination of the internally located nucleus with external (younger) tissue. Each lens nucleus from eyes collected between 1998 and 2000 was split in half, and the two halves were stored in an Eppendorf vial and a sterilized glass tube, respectively, at −20°C. The halves that were stored in the glass tubes were processed to determine D/L ratios of aspartic acid, while those in the Eppendorf vials are still archived. The analysts determining the D/L ratios did not know the lengths or sexes of the whales.

Hydrolysis and Estimation of D/L Ratio of Aspartic Acid

To each of the lens splits in the glass tubes was added 1 mL of doubly distilled (dd) 6 M HCl to hydrolyze the protein, and the tube was flame sealed using a torch. The tubes were placed in an oven at 100°C for 6 h. Under these conditions, no racemization of aspartic acid is expected. After removal, the vials were broken and placed in a centrifuge evaporator to remove the HCl under vacuum. Each of the residues was taken up in 1mL dd water and stored at −20°C. A variety of colors and turbidities were observed for the resulting solutions. The aspartic acid D/L ratios for eyes collected in 1978–1981 had been determined from the solutions using ion-exchange chromatography (Nerini 1983*a*, *b*; Bada 1984). All subsequent aspartic acid D/L ratios were determined using high performance liquid chromatography (HPLC) (Zhao and Bada 1995). Some further details regarding the processing of the sample solutions from the eyes collected between 1998 and 2000 are given in the following sections.

Desalting

Following unsuccessful experiments to analyze the raw extracts (interference with the derivatization reaction), the solutions were purified with an analogous

method as used routinely for the desalting acid-hydrolyzed meteorite and sediment extracts. The sample solutions were added to a BIO-RAD AG 50W-X8 cation exchange resin (prepared in sterilized Pasteur pipettes) and rinsed with dd water. The amino acids that remained on the resin were then eluted with 3 mL 2 M NH_4OH solution into small glass tubes. These purified lens extracts were dried down under vacuum, and the residues were taken up in 200 μL dd water and stored at −20°C until analysis.

Derivatization and HPLC Analysis

To 10 μL of the sample solutions were added 10 μL of 0.4 M Borate buffer, and this solution was dried under vacuum to remove traces of ammonia remaining from the purification procedure. Then 20 μL of dd water was added, followed by 5 μL of OPA/NAC reagent (Zhao and Bada 1995). After one minute derivatization time, the reaction was quenched with 475 μL acetate buffer (pH 5.5); 50 μL of the resulting solution were injected into the HPLC column. The signals for D- and L-aspartic acids were identified by comparison with retention times of known standards. One standard (D/L = 0.06) was analyzed before and after each analysis session in order to assess the reliability of the system.

A Phenomenex *Luna* phenyl-hexyl-column (250 × 4.60 mm) including a guard system was used in the analysis. The pump was a Hitachi L-6200 HPLC pump with low-pressure mixing. Eluents: Buffer A: Methanol; Buffer B: 50 mM sodium acetate buffer, pH 8. Elution was isocratic on buffer B at the retention time of aspartic acid, and buffer A was used to rinse the column after the analysis. The detector was a Shimadzu RF-530 fluorescence detector. The data were automatically integrated on a Hitachi D-7500 integrator. These raw data were used to calculate the D/L ratios of aspartic acid in the samples.

Calibration and D/L Ratio Measurement Error

In order to calibrate the measured D/L ratios for aspartic acid $(D/L)_{meas}$ with the actual ones $(D/L)_{act}$, a set of standards was analyzed. First, a pure 10 mM solution of L-aspartic acid was prepared and analyzed to check for D-contamination in the purchased batch. No contamination was found. Then, 1 mM solutions of aspartic acid were prepared with the following D/L ratios: D/L = 0.20, 0.15, 0.10, 0.08, 0.06, 0.04, 0.02, 0.01. Each of these standards was injected three times, and the average $(D/L)_{mean}$ of the $(D/L)_{meas}$ values was calculated. Based on these data, calibration equations were determined by linear regression. George *et al.* (1999) found that D/L ratio measurement error was generally relatively small but that there were some obviously discrepant measurements (outliers). We therefore used a robust regression routine, lmRobMM in S-PLUS (Venables and Ripley 1999), which identifies and downweights outliers.

For the calibration that applied to most of the D/L ratios calculated for eyes collected from 1998 through 2000 (Calibration 1), only the average $(D/L)_{mean}$ value obtained for each of the eight $(D/L)_{act}$ calibration values was available. Thus the calibration equation was $(D/L)_{act} = \beta_0 + \beta_1 \times (D/L)_{mean}$. The 0.20 average was identified as an extreme outlier and given no weight in the regression. Calibration 1 applied to D/L ratios obtained up to 12 November 2002.

The remaining D/L ratios were not obtained until the spring of 2003 and thus required a new calibration (Calibration 2). It was recognized that using individual

measurements rather than averages in fitting the Calibration 2 equation would be preferable since it would allow better determination of outliers. For example, if only one Calibration 2 $(D/L)_{meas}$ value at a given calibration value was identified as an outlier, the remaining measurements at that $(D/L)_{act}$ calibration value could be included in determining the calibration equation.

A careful review of chromatograms and other information in laboratory journals for both whale and calibration data, particularly those obtained in spring 2003, was carried out by two of the authors (OB and MZ). They were able to identify some suspect measurements that needed to be omitted during this review. Reasons for omissions included mislabeled samples and poor quality data from chromatograms with no baseline separation of the peaks. The review also determined the date of each spring 2003 measurement. Comparison of calibration data from different days suggested the existence of systematic between-day differences secondary to daily changes in the GC system related to variables such as the output of the lamp, the detector response at different concentrations of Asp, flow rates, separation power of the column etc. For that reason, calibration data from 7–8 May 2003, with the only calibration measurements at 0.06 and no whale measurements, were omitted. All other spring 2003 calibration measurements were considered in determining the Calibration 2 equation. Whales with two or more $(D/L)_{meas}$ values in spring 2003 were also used as described below. The steps in determining the Calibration 2 equation were as follows:

(1) Use lmRobMM to identify the two most extreme outliers, one the 0.20 $(D/L)_{meas}$ value on 23 May and the other one of the 22 April measurements for whale 99B7. These were omitted from analyses to estimate day effects since they could have undue influence on the estimates. For example, the 22 April outlier was one of only five measurements on that date.
(2) Estimation of a day effect was only attempted for days with at least four calibration and/or whale measurements. Day effects were modeled as simple shifts in $(D/L)_{meas}$. The full model for $(D/L)_{meas}$ under these constraints was $(D/L)_{meas} = \alpha_0 + \alpha_1 \times (D/L)_{act} + \alpha_{422} \times I_{422} + \alpha_{501} \times I_{501} + \alpha_{502} \times I_{502} + \alpha_{513} \times I_{513} + \alpha_{514} \times I_{514} + \alpha_{519} \times I_{519} + \alpha_{520} \times I_{520} + \alpha_{521} \times I_{521} + \alpha_{522} \times I_{522} + \alpha_{527} \times I_{527} + \varepsilon$, where α denotes a parameter to be estimated, ε represents measurement error and $I_{mdd} = 1$ if $(D/L)_{meas}$ was obtained on month m and day dd, $I_{mdd} = 0$ otherwise.
(3) Determine the best model for the $n = 34$ calibration measurements only using lmRobMM. Each model was evaluated using a robust version of the Bayesian Information Criterion (BIC) in which the robust *scale* computed by lmRobMM replaced root mean squared error: $BIC = n\log_e(scale^2 \times dof/n) + n_{par}\log_e(n)$ where dof is residual degrees of freedom and n_{par} is the number of model parameters. The model with lowest BIC is best. This was $(D/L)_{meas} = \alpha_1 \times (D/L)_{act} + \alpha_{501} \times I_{501} + \alpha_{502} \times I_{502} + \alpha_{513} \times I_{513} + \alpha_{527} \times I_{527} + \varepsilon$.
(4) From the best model for the calibration measurements compute adjusted $(D/L)_{meas}$: $(D/L)_{madj} = (D/L)_{meas} - \alpha_{501} \times I_{501} - \alpha_{502} \times I_{502} - \alpha_{513} \times I_{513} - \alpha_{527} \times I_{527}$. Then fit the calibration equation $(D/L)_{act} = \beta_1 \times (D/L)_{madj}$ after using BIC to determine that a model with no intercept is best. Then compute $(D/L)_{act}$ for each $(D/L)_{madj}$ from each whale eye as $(D/L)_{act} = \beta_1 \times (D/L)_{madj}$ and the median of each eye's $(D/L)_{act}$ values. Then replace the $(D/L)_{act}$ values for each eye with their median to obtain a robust estimate of that eye's true $(D/L)_{act}$.

(5) Add the whale measurements to the calibration measurements and compute the best model for day effects as in 3) except that there are now $n = 72$ data points. The final best model was $(D/L)_{meas} = \alpha_1 \times (D/L)_{act} + \alpha_{422} \times I_{422} + \alpha_{501} \times I_{501} + \alpha_{502} \times I_{502} + \alpha_{513} \times I_{513} + \alpha_{514} \times I_{514} + \alpha_{521} \times I_{521} + \alpha_{527} \times I_{527} + \varepsilon$.

(6) Compute $(D/L)_{madj} = (D/L)_{meas} - \alpha_{422} \times I_{422} - \alpha_{501} \times I_{501} - \alpha_{502} \times I_{502} - \alpha_{513} \times I_{513} - \alpha_{514} \times I_{514} - \alpha_{521} \times I_{521} - \alpha_{527} \times I_{527}$ for each calibration and whale measurement from the parameter estimates obtained in (5). Then determine the best Calibration 2 equation as $(D/L)_{act} = \beta_0 + \beta_1 \times (D/L)_{madj}$ with β_0 set to zero since BIC indicated that a zero-intercept model was best.

The Calibration 2 equation determined in step (6) was used to compute $(D/L)_{act}$ for all spring 2003 whale measurements.

Several sources of variability need to be considered in estimating the standard error (SE) of $(D/L)_{act}$. First are between-eye and within-eye variability in $(D/L)_{meas}$ for Calibration 1 or $(D/L)_{madj}$ for Calibration 2 for each whale. These were estimated as variance components V_{eye} and V_{err} using a nested random effects analysis of variance with eye nested within whale and measurements from nine bowheads with both eyes sampled in 1999 or 2000. The two most extreme outlier measurements were omitted from these analyses because V_{eye} and V_{err} were intended to represent typical variability. These outliers were identified by examining sample variances for each eye of each of the nine whales.

Alternative estimates of within-eye variance could be computed as the sample variance of the measurements for each whale/eye/calibration combination. However, the sample size for 81% of these computations was 2 or 3, and never more than 6, so the estimated error variance would be very imprecise. Nevertheless, to account for higher variances due to outliers in some cases, the sample variance was computed. For spring 2003 measurements, the variances of the estimated day effects used in computing $(D/L)_{madj}$ needed to be taken into account. Each spring 2003 $(D/L)_{madj}$ involved at most one day effect since each was made on a particular day. The estimated variances of α_{422}, α_{501}, α_{502}, α_{513}, α_{514}, α_{521} and α_{527} from the model fit in step 5) provided variance adjustments for measurements made on those days. These were added to the appropriate sample variances. The adjusted sample variances were used as V_{err} when they were significantly higher than V_{err} based on an F-test at the 0.05 level.

Finally, the uncertainty in the calibration must be taken into account in estimating the variance of $(D/L)_{act}$. The estimated covariance matrix of the estimates (β_0, β_1) in the Calibration 1 equation must be taken into account. For Calibration 2, the variance of β_1 must be incorporated.

For each whale/eye/calibration combination, the median measured D/L ratio (adjusted as described above for spring 2003 measurements) was used to compute an actual D/L ratio from the appropriate calibration equation. The estimated variance of the median, which included all the variance components described above except those related to the calibration equation, was also computed.

The variances of the median measured D/L ratio and the calibration equation terms were accounted for via a bootstrap using 200 bootstrap replicates. First, 200 Calibration 1 replicate calibration equations were generated from a bivariate normal distribution with mean (β_0, β_1) from the actual Calibration 1 equation. The covariance matrix for the bivariate normal contained the estimated variances

and covariance of (β_0, β_1). For Calibration 2, 200 replicate calibration equations were generated, with intercept $\beta_0 = 0$ and slope normally distributed with mean β_1 from the actual Calibration 2 equation and variance its estimated variance.

Second, for each whale/eye/calibration combination, 200 values were generated from a normal distribution with mean the median measured D/L ratio and variance the estimated variance of that median. The appropriate generated calibration equation was used to convert each of these generated values to an actual D/L ratio. The standard deviation (SD) of the 200 generated actual D/L ratios was used as the SE of the actual D/L ratio for that whale/eye/calibration.

Estimating Age

The actual D/L ratios described in the previous section were used in estimating age from the following equation (George *et al.* 1999):

$$\text{Age} = \left(\log_e[(1 + \text{D/L})/(1 - \text{D/L})] - \log_e\{[1 + (\text{D/L})_0]/[1 - (\text{D/L})_0]\}\right)/2k_{\text{Asp}}$$

where k_{Asp} is the racemization rate for aspartic acid, and $(\text{D/L})_0$ is the D/L value at age 0. For whales with more than one whale/eye/calibration actual D/L value, an inverse variance weighted average actual D/L ratio for the whale and its estimated variance were computed.

Estimating $(\text{D/L})_0$, k_{Asp} and Female Age at Sexual Maturity (ASM) for Bowhead Whales

The age equation in the previous section can be rearranged to give

$$\log_e[(1 + \text{D/L})/(1 - \text{D/L})] = \log_e\{[1 + (\text{D/L})_0]/[1 - (\text{D/L})_0]\} + 2k_{\text{Asp}} \times \text{Age},$$

so that $2k_{\text{Asp}}$ can be estimated as the slope of the line determined by regressing $\log_e[(1 + \text{D/L})/(1 - \text{D/L})]$ on age. The intercept from that regression estimates $\log_e\{[1 + (\text{D/L})_0]/[1 - (\text{D/L})_0]\}$. Actual D/L ratios and ages determined for term fetuses or from baleen data (Lubetkin *et al.* 2008, 2012) or ovarian corpora counts (George *et al.* 2011) are available for 29 bowhead whales. Actual D/L ratios for eleven of the 29 are from George *et al.* (1999) and the rest are described in this paper. A description of ovarian corpora count methodology can be found in George *et al.* (2011). A problem with age estimates based on ovarian corpora counts is that they require an estimate of female age at sexual maturity (ASM), *i.e.*, the age at which the female first ovulated. In order to estimate ASM, corpora ages were first computed assuming ASM = 20. The revised equation

$$\log_e[(1 + \text{D/L})/(1 - \text{D/L})] = \log_e\{[1 + (\text{D/L})_0]/[1 - (\text{D/L})_0]\} + 2k_{\text{Asp}} \times \left(\text{Age} + \Delta_{\text{age}} \times \text{cor}\right)$$

was then solved using nonlinear least squares (nls in S-PLUS) for $(\text{D/L})_0$, k_{Asp}, and Δ_{age}; cor = 1 if age was estimated from ovarian corpora counts, cor = 0 otherwise. Thus estimated ASM = 20 + Δ_{age} (George *et al*. 2011).

First, with corpora ages computed assuming ASM = 20, lmRobMM was used to estimate $2k_{\text{Asp}}$ as the slope and $\log_e\{[1 + (\text{D/L})_0]/[1 - (\text{D/L})_0]\}$ as the

intercept *via* weighted linear regression. Outliers identified and downweighted by lmRobMM had large positive or negative residuals, *i.e.,* they were far from the line determined by the majority of the data points. A residual with magnitude greater than the residual scale estimate (*scale*) was used to identify a whale as a potential outlier. While weighted regression downweights points when the estimated variance of $\log_e[(1 + \mathrm{D/L})/(1 - \mathrm{D/L})]$ is large, errors in the age estimates can also result in outliers. For this reason, it is important to use a method like lmRobMM that downweights outliers even when the estimated variance of $\log_e[(1 + \mathrm{D/L})/(1 - \mathrm{D/L})]$ is small.

Because nls is sensitive to outliers, we omitted the two largest outliers identified by lmRobMM from the nls analysis. Both had residuals with magnitude more than double *scale*. One of the remaining four potential outliers was also omitted because the whale's age was estimated using the corpora count from only one instead of both ovaries. SE(age) for that whale was larger than for the other potential outliers. Weighted nls on the remaining 26 whales was used to estimate $(\mathrm{D/L})_0$, k_{Asp}, and Δ_{age}. The results of omitting some or all of the remaining potential outliers, as well as the oldest whale, an influential point though not an outlier, were also examined. Plots were used to assess how well the bulk of the data were fit. It was concluded that omitting the three outliers just described was sufficient. Results were relatively insensitive to the presence or absence of the oldest whale.

Standard Errors for Age Estimates

Standard errors SE(Age) were computed *via* a bootstrap in order to take account of the variance of the actual D/L ratio obtained for the whale and of the uncertainty in $(\mathrm{D/L})_0$ and k_{Asp}. The number of bootstrap replicates used was 2,000. First, 2,000 replicate pairs of $(\mathrm{D/L})_0$ and k_{Asp} were generated from a bivariate normal distribution with mean values the actual estimates from nls and covariance matrix as estimated by nls. Then, for each whale, 2,000 replicate D/L ratios were generated from a normal distribution with mean that whale's actual D/L ratio and standard deviation its SE. Then 2,000 replicate values of Age for the whale could be computed from the replicate pairs of $(\mathrm{D/L})_0$ and k_{Asp} and the corresponding replicate D/L ratios. The SD of the 2,000 replicate values of Age provides the estimate of SE(Age). With 2,000 replicate values, estimation of a confidence interval for Age is possible.

Results

Sampled Bowheads

Eye lenses were collected from 41 bowhead whales and one fetus in 1998, 1999, and 2000. Paired right and left lenses were collected from 10 of the 42. During processing, labels were lost from the single eye obtained from one whale and one of two eyes obtained from another, leaving 40 whales with identified samples to be analyzed (9 with both eyes) along with the fetus.

We could not use a random sampling scheme to obtain the eyes used in this analysis. Eye globes were collected as whales were available. Including the fetus, there were 22 females, 18 males, and 1 whale of unknown sex. A hunt-based bias toward younger animals was expected. Although calves are never deliberately

taken, there is some hunter preference for smaller animals. Of the 40 whales, 13 (32.5%) were $\geq$13 m in length compared to 39.7% in the general population estimated from aerial photogrammetry at Barrow (Koski *et al.* 2006). Thus, although population age and sex structure cannot be inferred from the data in this paper alone, subadults and adults of both sexes are well represented.

From the 41 identified individuals, 179 $(D/L)_{meas}$ values were obtained. These represented 58 unique whale/eye/calibration combinations. Of the 58, 35 had three $(D/L)_{meas}$ values, 12 had only two, 8 had four, and 3 had six.

Calibration and D/L Ratio Measurement Error

The Calibration 1 equation used for $(D/L)_{meas}$ obtained up to 12 November 2002 was $(D/L)_{act} = -0.0022 + 1.4617 \times (D/L)_{meas}$. The SE of the intercept was 0.0012, the SE of the slope was 0.0208 and the correlation between the slope and intercept was −0.832. The Calibration 2 equation used for $(D/L)_{madj}$ obtained in spring 2003 was $(D/L)_{act} = 1.4748 \times (D/L)_{madj}$. The SE of the slope was 0.0052. Thus the equation derived from individual calibration measurements adjusted for day effects was more precise than the one derived by using means of measurements.

Figure 1 shows the calibration data and robust regression fits for both calibrations. The outliers at $(D/L)_{act} = 0.20$ were downweighted by the robust regression routine lmRobMM, leading to a much better fit to the remaining data than would have been possible using ordinary regression. All measured bowhead D/L ratios to which these calibration equations were applied were less than 0.12, so the downweighted outliers in Figure 1 with measured D/L ratios exceeding 0.15 are not a cause for concern.

The estimated day effects $\alpha_{mdd} \pm$ SE for the model chosen using BIC were 0.001763 ± 0.000919 for 422, −0.005193 ± 0.000748 for 501, −0.000778 ± 0.000580 for 502, −0.002149 ± 0.000855 for 513, −0.002357 ± 0.000910 for 514, 0.000547 ± 0.000618 for 521 and −0.002705 ± 0.000589 for 527. The effects for 501 and 527 were highly significant ($P < 0.001$) and those for 513 and 514 were significant ($P < 0.02$). The effects for 422 ($P = 0.059$) and 502 ($P = 0.184$) were not significant. It is not surprising that 502 was retained since 502 and 527 were the days on which most of the calibration measurements were obtained. An attempt was made to drop 521 ($P = 0.38$) from the model, but lmRobMM failed in that attempt, so BIC could not be computed.

The variance components V_{eye} and V_{err} that are part of the variance of a measured D/L ratio were estimated to be $V_{eye} = 3.315 \times 10^{-6}$ and $V_{err} = 1.921 \times 10^{-6}$. The sum of V_{eye} and V_{err}, 5.236×10^{-6}, is the variance of a single measured D/L ratio that is not an outlier based on the results of the nested random effects analysis of variance. However, V_{err} may underestimate the variability of the measured D/L ratios for a particular whale/eye/calibration combination if outliers are present and/or several day effects are involved. If the sum of the sample variance and the day effect variances for a given whale/eye/calibration combination was significantly greater than V_{err} at the 0.05 level, this sum was used in place of V_{err} in computing the variance of a single measured D/L ratio for that whale/eye/calibration combination. This occurred for 19 of the 58 unique whale/eye/calibration combinations; the 19 resulting estimates of the variance of a single measured D/L ratio ranged from 9.099×10^{-6} to 1.843×10^{-4}.

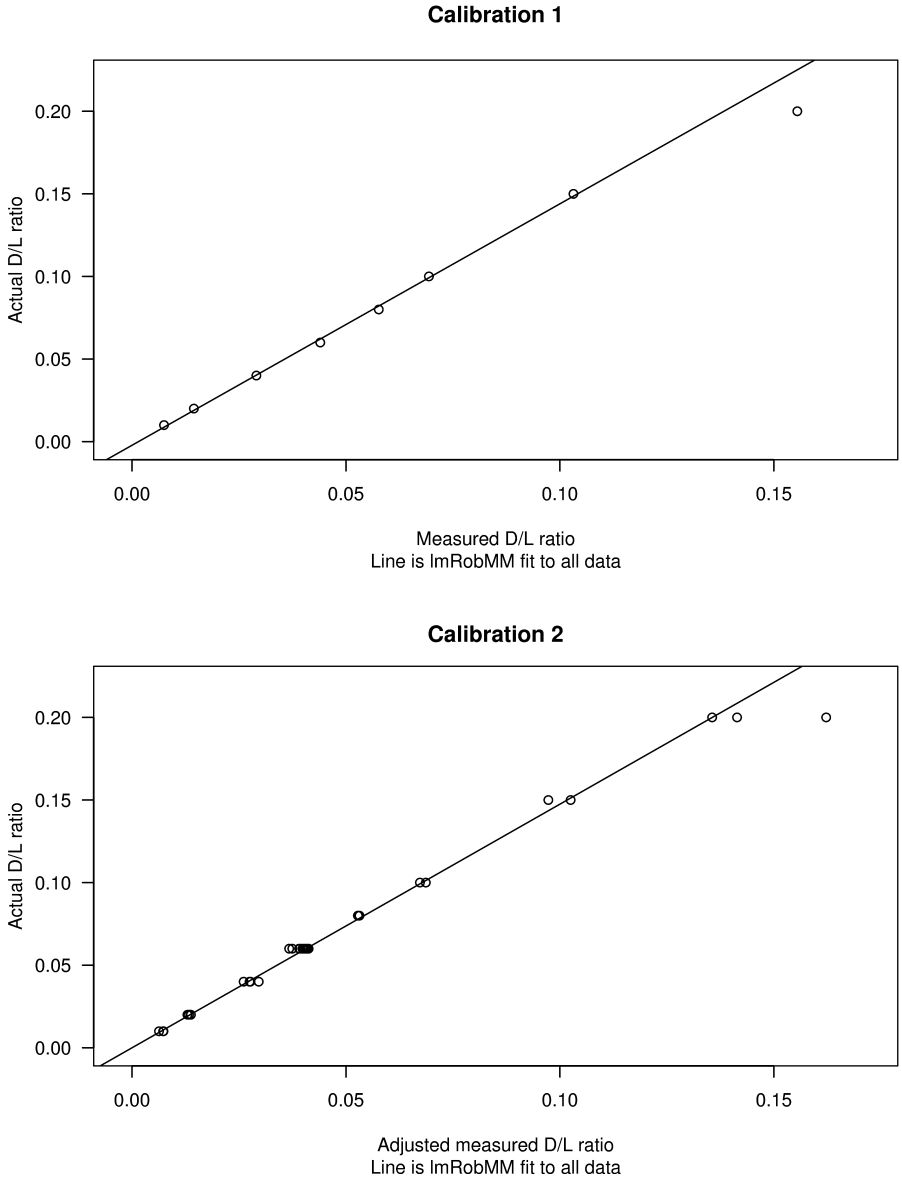

Figure 1. Data and calibration lines determined using robust regression for Calibration 1 (for D/L ratios obtained before 2003) and Calibration 2 (for D/L ratios obtained in spring 2003). The robust regression routine downweighted outliers with actual D/L = 0.2, providing a better fit for measured D/L values covering the range of the bowhead data. The measured D/L values shown for Calibration 2 were adjusted for day effects. Such an adjustment was not possible for Calibration 1 because dates were not available for calibration measurements obtained before 2003.

The variances of the median measured D/L ratios for the 58 unique whale/eye/calibration combinations were estimated to range from 1.371×10^{-6} to 9.650×10^{-5}. The estimated SE of the actual D/L ratios computed from the median measured D/L ratios for the whale/eye/calibration combinations and the appropriate calibration equation ranged from 0.0019 to 0.0139.

Bowhead D/L Ratios, Ages and SE

Actual D/L ratios and estimated ages for all 41 individuals are given in Table 1 along with their SE. Table 1 also shows whale identifier, which includes year of harvest and ends with F for the fetus. Whale length (m) and, when available, sex and baleen length (cm) are also included. The table is ordered by whale length.

Estimates of $(D/L)_0$, k_{Asp}, Δ_{age} and ASM for Bowheads with SE and Correlations

Figure 2 shows the robust regression line fit by lmRobMM to the available whales with both D/L ratio data and an age estimate obtained by another method, assuming ASM = 20 and a linear relationship between age and $\log_e[(1 + D/L)/(1 - D/L)]$. D/L is the actual D/L ratio computed for the whale. The intercept of the regression is $\log_e\{[1 + (D/L)_0]/[1 - (D/L)_0]\}$ and the slope is $2k_{Asp}$. The lmRobMM fit was used to identify potential outliers. Three of the potential outliers were removed before using nls to estimate the parameters $(D/L)_0$, k_{Asp} and Δ_{age}. The parameter estimates ± SE obtained from nls were $(D/L)_0 = 0.0250 \pm 0.0013$, $k_{Asp} = 0.000977 \pm 0.000145$ and $\Delta_{age} = 5.857 \pm 5.868$. Thus female age at sexual maturity was estimated as ASM = 25.857 with the same SE as Δ_{age}. Figure 2 also shows the data points used in nls and the line determined by the estimated parameters with ASM = 25.857 used for the ages based on ovarian corpora counts. There was a positive correlation of 0.123 between $(D/L)_0$ and Δ_{age}, while k_{Asp} was negatively correlated with both $(D/L)_0$ (−0.360) and Δ_{age} (−0.883).

Dependence of k_{Asp} on Body Temperature

As already mentioned, the racemization rate for aspartic acid is dependent on temperature. Human body temperature, like that of most mammals, is approximately 37°C. Therefore George *et al*. (1999) estimated k_{Asp} as the average of values obtained from human (0.00125/yr, Masters *et al.* 1977) and fin whale (*Balaenoptera physalus*) data. The D/L data from fin whale eye lenses and ages from ear plug laminations (Nerini 1983*a*) used by George *et al*. (1999) contained obvious outliers. George *et al*. (1999) used ordinary least squares rather than a robust technique to estimate k_{Asp} from these data and therefore probably obtained a negatively biased value for fin whales. Nerini (1983*b*) estimated $k_{Asp} = 0.00116$/yr for fin whales from a data set without large outliers. Fin whale deep body temperature is 36.1°C (Brodie and Paasche 1985) while bowhead deep body temperature is 33.8°C (George 2009). Our bowhead $k_{Asp} = 0.000977$ given in the previous section is therefore, as expected, lower than the fin whale and human k_{Asp} values cited above.[5] It was

[5]The effect of mammalian body temperature was evaluated in known age rhesus monkeys and k_{Asp} was found to be higher than the human value, consistent with the higher body temperature of the monkeys. This result, along with our present work, shows that without D/L and age data for k_{Asp} estimation, body temperature is needed for accurate racemization ages (Bada and Brown 1985).

Table 1. Actual D/L ratios and AAR ages (yr) estimated from them, with standard errors (SE). The identifier for each whale and body length (m) are also shown. Sex, baleen length (cm) and alternate age information are shown if available. Methods used for the alternate age estimates are FETUS, CC2 = counting of annual baleen growth increments (cycles) by two people (Lubetkin *et al.* 2008), B12 = baleen length and harvest date for whales 1–2 yr old (Lubetkin *et al.* 2012) and COR = ovarian corpora counts (George *et al.* 2011). The whales with alternate age estimates in this table not used for k_{Asp} estimation were omitted because they were outliers. The whales used for k_{Asp} estimation not shown in this table were those with $(D/L)_{act}$ data from George *et al.* (1999) and with alternate age estimates like those shown here.

Whale	Sex	Length (m)	Baleen (cm)	$(D/L)_{act}$	SE of $(D/L)_{act}$	AAR Age		Alternate Age			Used for k_{Asp}?
						Age	SE	Age	SE	Method	
99B18F	M	4	11	0.0229	0.0016	−2.2	2.3	−0.01	0.03	FETUS	Yes
99KK1	F	7.7	85	0.0259	0.0022	0.9	2.6	2	0.3	CC2	Yes
00B9	F	7.9	70	0.0315	0.0022	6.7	2.8	1.3	0.42	B12	Yes
99B19	F	8.1	96	0.0254	0.0021	0.4	2.7	1.25	0.3	CC2	Yes
99B24	M	8.8	NA	0.0368	0.0027	12.1	3.4				
00KK3	F	8.8	112	0.0252	0.0025	0.2	3	2.63	0.3	CC2	Yes
00B8	?	8.9	NA	0.0271	0.0027	2.2	3.1				
00B15	F	8.9	NA	0.0332	0.0019	8.4	2.5				
00B18	F	8.9	NA	0.0374	0.005	12.7	5.5				
99B5	M	9	NA	0.0213	0.0044	−3.8	5.1				
99B20	F	9	130	0.0264	0.0015	1.4	2	2.25	0.3	CC2	Yes
99B12	F	9.2	174	0.0331	0.0026	8.3	3.2				
00KK1	F	9.2	150	0.0368	0.0046	12.1	5.3				
99B9	M	9.3	193	0.0324	0.0027	7.6	3.2				
00B1	M	9.4	116	0.0281	0.0026	3.2	3.1				
00B10	F	9.4	166	0.0339	0.0013	9.1	2.1				
00B13	M	9.4	189	0.0187	0.0028	−6.5	3.5	7.25	1.07	CC2	Yes
00B17	M	9.5	NA	0.0331	0.0016	8.3	2.2				
98B25	F	9.7	NA	0.0692	0.0025	45.3	7.4				
99B22	F	9.7	175	0.0302	0.0027	5.3	3.3	5.25	1.07	CC2	Yes
00B16	F	10	NA	0.0756	0.002	51.9	8.3				

(Continued)

Table 1. (Continued)

Whale	Sex	Length (m)	Baleen (cm)	$(D/L)_{act}$	SE of $(D/L)_{act}$	AAR Age		Alternate Age			Used for k_{Asp}?
						Age	SE	Age	SE	Method	
99B21	M	10.5	174	0.0377	0.0019	13	2.9	9.5	1.07	CC2	Yes
00B12	M	10.8	193	0.0406	0.0018	16	3				
99B23	M	10.9	192	0.0411	0.0025	16.5	3.6	9.25	1.07	CC2	Yes
99B8	M	11	217	0.0351	0.0027	10.3	3.4				
98B12	F	11.3	208	0.0349	0.0023	10.1	3				
00KK2	M	12.1	214	0.0675	0.0053	43.6	8.6				
99B6	F	12.6	236	0.0533	0.002	29	4.7	26	6	COR	Yes
99B18	F	13	342	0.0483	0.0022	23.9	4.1	30	6	COR	Yes
00B11	M	13.8	NA	0.1012	0.0019	78.3	12.4				
99B13	M	14.1	303	0.0806	0.0032	57.1	9.4				
99B14	M	14.2	NA	0.0982	0.0026	75.2	12.2				
00B2	F	14.5	273	0.0571	0.0018	32.9	5.3	58	12	COR	No
99B15	M	14.6	NA	0.1058	0.0139	83.1	20				
00B3	F	14.6	NA	0.0792	0.0038	55.6	9.4	71	16	COR	Yes
00B6	M	14.7	232	0.0461	0.0034	21.6	4.8				
99B16	F	14.8	NA	0.0764	0.0071	52.8	11.4	33	6	COR	No
99B17	M	14.9	NA	0.1658	0.0018	145.7	23.2				
99B7	F	15.4	NA	0.0618	0.0053	37.7	8	38	7	COR	Yes
00B4	F	15.4	NA	0.0906	0.0032	67.4	10.8	55	11	COR	Yes
00B5	F	17.5	331	0.1108	0.0111	88.3	18.5	89	22	COR	Yes

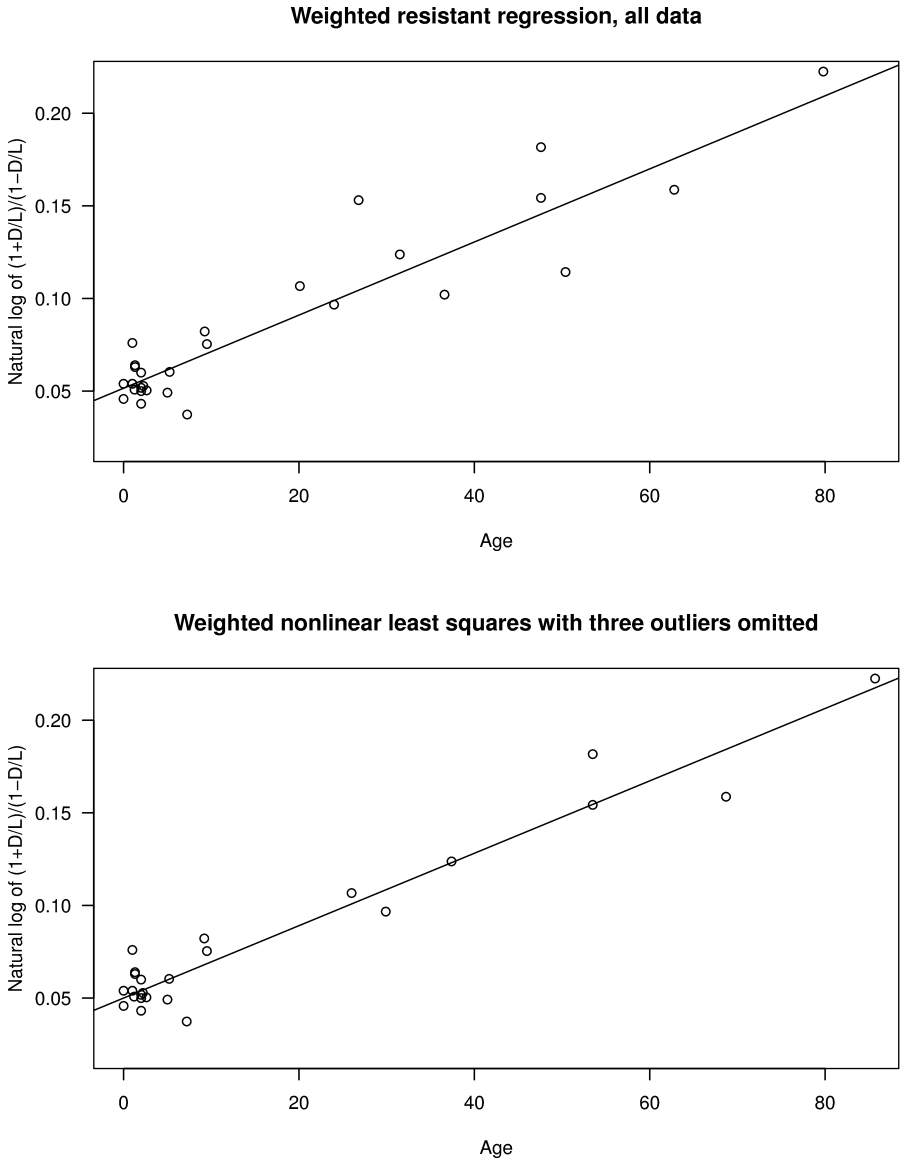

Figure 2. Data and regression lines showing the relationship between age and D/L ratio for whales with both D/L data and age estimates based on baleen or ovarian corpora data. The robust regression line fit to all these data to identify outliers is shown in the top plot. Three outliers were removed before using nonlinear least squares to estimate the parameters that determine age from D/L ratio. The line obtained by using these parameters is shown in the bottom plot.

computed from the first bowhead samples for which D/L ratios were determined and independent age data were also available. The correlation between k_{Asp} determined without considering body temperature for humans, fin whales, and

bowheads and their body temperatures is 0.999. This suggests that we could estimate k_{Asp} for another mammalian species with unknown k_{Asp} but known body temperature from the relationship between k_{Asp} and body temperature for these three species and use that estimated k_{Asp} to obtain ages from D/L ratio measurements from that species.

This approach was used by Olsen and Sunde (2002) for North Atlantic minke whales (*Balaenoptera acutorostrata*) based on the data available to them. Here we reanalyze their data based on the bowhead, fin, and human data given above and a more recent estimate of North Atlantic minke deep body temperature (34.7°C, Folkow and Blix 1992) than the one used by Olsen and Sunde. Since $2k_{Asp}$ is needed to compute age, we follow Olsen and Sunde (2002) in fitting a linear model for $2k_{Asp}$ as a function of body temperature. The equation obtained for predicting $2k_{Asp}$ is

$$2k_{Asp} = 0.00016836 \times °C - 0.00374122$$

so predicted $2k_{Asp} = 0.002101$ for these minke whales. Predicted values for bowhead and fin whales and humans are within 0.000017 of the data values obtained by doubling the k_{Asp} estimates given above. Since the minke data were inadequate for estimating $(D/L)_0$, we used our bowhead $(D/L)_0 = 0.0250$ for estimating minke ages.

Olsen and Sunde (2002) had D/L values for 6 immature and 25 sexually mature female minke whales. The two smallest immature whales were estimated by length to be more than 1 yr old. The sexually mature whales had ovarian corpora counts from which ages can be estimated if age at sexual maturity (ASM) and ovulation rate (OR) are known or can be estimated. Olsen and Sunde (2002) cite data supporting annual ovulation, *i.e.*, OR = 1. We estimated ASM = 9; this was the value that produced the median difference between AAR and corpora ages closest to zero and the minimum 75th percentile of the magnitude of the differences. We used the 75th percentile rather than minimizing over all the differences because of several obvious outliers in the data. With these values of OR and ASM, the two smallest minke whales had AAR ages of about 3 yr and the rest 5.7–36.5 yr.

Olsen and Sunde (2002) estimated ASM from their AAR ages by two different methods, obtaining 5.8 yr and 7.8 yr. These were compared with an earlier estimate of 8 yr, believed to be negatively biased, based on age estimates from *bulla tympanica* growth layer groups. They suggested that the $(D/L)_0$ and $2k_{Asp}$ values they had used may have been inappropriate. Our ASM = 9 yr is larger than the negatively biased estimates just cited, suggesting our $(D/L)_0$ and $2k_{Asp}$ are more appropriate.

In sensitivity runs, we explored $(D/L)_0$ and ASM values ranging from 0.024 to 0.0285 and from 7 yr to 11 yr, respectively. Both $(D/L)_0 = 0.024$ with ASM = 10 yr and $(D/L)_0 = 0.026$ with ASM = 8 yr performed similarly to $(D/L)_0 = 0.025$ with ASM = 9 yr. Values of $(D/L)_0 \geq 0.027$ gave negatively biased ages for the two smallest minkes and ASM (Olsen and Sunde 2002). We agree with Olsen and Sunde that $(D/L)_0$ for North Atlantic minke whales cannot be estimated directly and precisely without more AAR data from very young (small) animals. Data from term fetuses would also contribute to avoiding positively biased estimates of $(D/L)_0$. If fetuses and very small minke whales could be sampled, it would be worthwhile to explore other age estimation methods

(Olsen and Øien 2002; Lubetkin *et al*. 2008, 2012) as well as AAR using these animals with the goal of obtaining ages and D/L data for minke whales covering a wide enough range of ages to permit estimation of $2k_{Asp}$ and $(D/L)_0$ directly for North Atlantic minke whales.

Discussion

Estimated Ages and SE

The estimated ages in Table 1 vary considerably for bowheads of a given length or very similar in length. This is expected for young animals ($\leq$11.3 m) and physically mature animals based on work by Schell *et al.* (1989), George *et al.* (1999), and Lubetkin *et al.* (2008). D/L ratio measurement error accounts for most of the variability in the age estimates at the younger ages. Uncertainty in the k_{Asp} rate is a major contributor to uncertainty, particularly in the older ages. SE increased with estimated age, but by far the largest coefficients of variation (CV) were associated with the youngest ages similar to the earlier work (George *et al*. 1999).

Lubetkin *et al.* (2008) noted that baleen length is a better indicator of age than body length for bowheads <9 m long or with <200 cm of baleen. They concluded that age estimates based on annual baleen growth increments defined by stable isotopes were more precise than age estimates based on AAR for bowheads in their teens or younger. Baleen-based age estimates for bowhead whales $\leq$11.3 m long made by Schell *et al*. (1989) and Lubetkin *et al.* (2008) never exceeded 18.1 yr. Yet two of the 26 AAR-based age estimates in Table 1 for such whales exceed 45 yr. These biologically implausible Table 1 values support the conclusions of Lubetkin *et al.* (2008).

The age estimates in Table 1 for whales >12 m long all exceed 20 yr. The oldest male, 99B17, was also the longest at 14.9 m. Its age ± SE was estimated to be 145.7 yr ± 23.2. It was the only one of the 41 whales in Table 1 estimated to be over 100 yr old. George *et al.* (1999, 2011) summarized evidence that ages over 100 yr are plausible for bowheads. The oldest female, 00B5, was also the longest and was pregnant when harvested. Her age ± SE was estimated to be 88.3 yr ± 18.5. Ages based on ovarian corpora counts are available for eight of the mature females. Six were used in estimating $(D/L)_0$, k_{Asp}, and Δ_{age} and two were not used because they were outliers. The agreement between AAR and corpora ages for the six used in both AAR and corpora analyses is not surprising. Even for the two outliers, the differences between the AAR and corpora ages are not significant.

Comparison with Other Estimates of $(D/L)_0$ from Eye Lens Data

Nerini (1983*a*) estimated $(D/L)_0 = 0.0276$ based on D/L data from three bowheads believed on the basis of body length to be 1 yr old. George *et al.* (1999) estimated $(D/L)_0 = 0.0285$ for bowheads as the mean of $(D/L)_{act}$ values from one term fetus and seven whales assumed, based on the length of their longest baleen plates, to be between ages 0 and 2. Olsen and Sunde (2002) used the estimate of George *et al.* (1999), which was close to the D/L values of the two youngest minke whales they sampled. Those two whales were probably over 1 yr old given

their body lengths. Garde *et al.* (2007) estimated $(D/L)_0 = 0.0288$ by regressing D/L data from eyes of 15 young narwhals (*Monodon monoceros*) on ages based on lengths of the whales and date of capture. Since all these estimates are based primarily on whales 1 yr old or older, they are likely to be positively biased. Our estimate $(D/L)_0 = 0.0250$ does not share this potential for bias and is slightly smaller than their estimates.

Of course, $(D/L)_0$ values differ between species (Masters *et al.* 1977). Considering this, it is somewhat surprising that the fin, minke, and narwhal values are as close as they are to bowhead values. As pointed out by Garde *et al.* (2007), $(D/L)_0$ includes both D enantiomers present at birth and racemization that occurs when samples are hydrolyzed in HCl. Similar laboratory processing of samples from different species may be contributing a common component to all these $(D/L)_0$ estimates.

Table 1 includes six bowheads with $0.025 < D/L < 0.029$. Of these, four have alternate age estimates between 1.25 and 2.63 yr, one has AAR age 2.2 yr and the last is likely more than 2 yr old based on baleen length (Lubetkin *et al*. 2012). These data support our estimate $(D/L)_0 = 0.025$ for bowheads better than the larger values cited above for bowhead, fin, and minke whales and narwhals.

Dependence of k_{Asp} on Body Temperature

Our results quantify the relationship between racemization rate and temperature and suggest that it is linear, at least over the range of body temperatures considered. It is interesting, if not remarkable, that analyses of data from four different species produced such strong results. It is better to estimate k_{Asp} for the species being studied, as we have done for bowheads, than to use values based on other species. For investigators like Olsen and Sunde (2002) who have D/L and body temperature data but inadequate data for calculating k_{Asp} and $(D/L)_0$, we have provided evidence that age estimates can be obtained using the relationship between k_{Asp} and body temperature and using $(D/L)_0$ from a species with similar body temperature.

Comparison with Other Estimates of k_{Asp}

As mentioned in the last subsection of Results, our estimate of $k_{Asp} = 0.000977$/yr is based on the first bowhead samples for which D/L ratios were determined and independent age data were available. Species-specific estimates computed similarly from human ($k_{Asp} = 0.00125$/yr, Masters *et al*. 1977) and fin whale ($k_{Asp} = 0.00116$/yr, Nerini 1983*b*) data have been published. Most estimates of k_{Asp} other than the bowhead whale, human, and fin whale estimates just mentioned have been based on other species without accounting adequately for body temperature. This can lead to biased age estimates, particularly for older whales. For example, George *et al*. (1999) used human and fin whale data without accounting for bowheads' lower body temperature, which had not yet been measured. They lacked the D/L and independent age data from the same bowheads from which we estimated k_{Asp}. If our k_{Asp} estimate is used in place of theirs to calculate ages from their D/L data, the resulting ages are 1.2 times as high as their ages. For whales they estimated to be <35 yr old, the differences

between their ages and the recalculated ages are less than their SE, but for older whales equal to or greater than their SE.

More recent investigations[6] have attempted to estimate k_{Asp} as a function of *in situ* temperature of the bowhead eyeball. That approach has several problems. First, *in situ* measurements of whale eyeball temperatures are difficult if not impossible. Second, that approach requires complex heating experiments that may not be completely applicable to the racemization process in living animals.

We believe that the approach used by Masters *et al*. (1977) to estimate human k_{Asp}, by Nerini (1983*b*) to estimate fin whale k_{Asp} and by us to estimate bowhead k_{Asp} is the best approach for any species for which D/L data over an adequate range of ages and the corresponding independent age data can be obtained. We determined a strong relationship between k_{Asp} and body temperature for these three species. Our reanalysis of North Atlantic minke whale data (Folkow and Blix 1992, Olsen and Sunde 2002) provided further validation of this relationship and showed that it could be used in estimating ages for other similar mammalian species with body temperature and D/L data but without adequate age data for a direct species-specific estimate of k_{Asp}.

Age at Sexual Maturity

More is known about female than male bowhead size and age at sexual maturity because harvested pregnant females are recognized as sexually mature and examination of the ovaries can determine whether and how many times a female has ovulated. In addition, whales accompanied by calves are assumed to be the mothers and can be measured from aerial photographs.

The size of male bowheads at sexual maturity is estimated to be 12.5–13 m (O'Hara *et al.* 2002). George *et al*. (1999) estimated an age of 19 yr (SE 6) for the only whale in that size range. The largest male with an age determined by George *et al.* (1999) and known from the data of O'Hara *et al.* (2002) to be sexually immature was 11.6 m long and estimated to be 21 yr old. The smallest male known to be sexually mature with an age in George *et al.* (1999) was listed as 13.1 m long and 31 yr old. Lubetkin *et al.* (2008) estimated males around 13 m long to be in their mid teens. These data suggested that sexual maturity for male bowheads is reached between the mid teens and late twenties.

Table 1 includes no 12.5–13 m males. The closest to this range, at 12.1 m, is estimated to be nearly 44 yr old, and the large SE associated with this age as well as its anomalously high value for a whale this size flags it as an outlier. Thus it cannot be assumed to provide additional information.

Most female bowheads are believed to reach sexual maturity at 13–13.5 m (Koski *et al.* 1993, George *et al.* 2011). The only female in this size range aged by George *et al.* (1999) was estimated to be 25 yr old (SE 7). Among whales known to be mature by the presence of ovarian corpora, the lowest estimated ages obtained by George *et al*. (1999) were 19 yr (SE 6) and 26 yr (SE 7). The largest female bowhead whose age was estimated by Lubetkin *et al.* (2008) was 12.3 m long and known to be immature because her ovaries were examined and no corpora were found. Its age was estimated to be 19.8 yr (SE 3.7). These data

[6]Personal communication from T. Sformo, North Slope Borough Department of Wildlife Management, PO Box 69, Barrow, AK 99723, November 2011.

suggest that sexual maturity for female bowheads is most likely reached in the late teens to mid twenties.

However, the results of this paper provide new information for females. Most importantly, female age at sexual maturity was estimated as ASM = 25.857 yr (SE 5.868), so we can compute rough normal theory confidence intervals for female age at sexual maturity. An 80% confidence interval for female age at sexual maturity is 18.3–33.4 yr. The AAR age estimate (Table 1) for 99B6, 12.6 m long and known to have reached sexual maturity in the year in which she was harvested, was 29 yr (SE 5). Estimated AAR age for 99B18, 13 m long and estimated to be 4 yr past ASM via ovarian corpora data, was approximately 24 yr (SE 4). All other females in Table 1 known to be mature were $\geq$14.5 m long and had both AAR and corpora ages exceeding 32 yr.

Age estimates (0.3–159 yr) for 10 male bowheads, each of which is known to be either sexually immature or sexually mature, are available. Two are known to be immature because they were calves when harvested. The remaining eight had their testes examined to determine sexual maturation (O'Hara *et al.* 2002). None are 11.7–13 m in length, so these data provide only limited information for estimating male age at sexual maturity. Age (1.3–41 yr) and sexual maturity data (7 immature, 13 mature) for 20 female bowheads were added to the data from the 10 males. The resulting data set was used to estimate age at sexual maturity for male and female bowheads via logistic regression. The age at which the chance is 50% that a male or female bowhead is sexually mature was estimated to be 24.8 yr. It was estimated that 80% of bowheads reach sexual maturity between the ages of 18.2 yr and 31.4 yr. These results suggest that male and female bowheads may reach sexual maturity at about the same age, but more data from males are required before any firm conclusions can be drawn.

Problems with AAR Age Estimates

AAR age estimates (based on the nucleus of the lens) will over-estimate age if the animal has cataracts (brunnescent group IV) (Masters *et al.* 1977). Cataracts have not been reported in bowhead whales (Philo *et al.* 1993) and there was no evidence of cataracts noted during gross dissection of the eye lenses obtained in this study. If surrounding tissue or blood contaminates the sample (lens nucleus) during dissection, the D/L ratio could be dramatically lowered, resulting in underestimates of age (George *et al.* 1999).

A number of problems recognized by George *et al.* (1999) are solved in the present paper by estimating $(D/L)_0$, k_{Asp}, and Δ_{age} simultaneously from bowhead data only and taking account of the resulting correlations among these parameter estimates *via* bootstrap estimation of variances. Our method of estimating $(D/L)_0$ reduces the possibility of positive bias in that parameter compared to the method used by George *et al*. (1999), as well as reducing the number of negative age estimates.

However, we have not solved all the problems. Because the data available to George *et al*. (1999) were less detailed than ours, they used a delta method variance calculation that led to SE(Age) = 6 whenever Age < 20 yr. Their SE gradually increased to approximately the value we have for Age = 145.7 when their Age = 135. Our values of SE(Age) have the advantage of being able to reflect the apparent precision of a particular whale's measurements by considering the sample variance of those measurements. They are often more realistic than the

corresponding values of George *et al*. (1999), especially for the youngest whales. However, outlier Age values may have too few and/or consistent measured D/L ratios to be reflected in SE(Age), which may therefore be too small. These issues require further consideration. It would be helpful, but very expensive, to obtain more D/L measurements for each whale.

Although we omitted three outliers from the data used by nls to estimate k_{Asp}, it is possible that k_{Asp} could still be negatively biased because of outliers. We did not omit more potential outliers because the plotted fit looked good, and we did not wish to throw away data unnecessarily. It is also notable that Δ_{age} is estimated very imprecisely. This can only be remedied by including more whales with known ages in the analysis, particularly whales with baleen-based ages between 10 and 20 and whales with ages between 20 and 30 computed from ovarian corpora counts under the assumption that ASM = 20.

Implications for Bowhead Whale Management

As noted in George *et al.* (1999), the longevity of bowhead whales has relatively minor direct implications for the management of the aboriginal hunt by Alaskan Eskimos. Population abundance and trend and subsistence need are the principal factors in determining sustainable harvest levels. As background, the subsistence harvest of bowhead whales is regulated at international, national and local levels. The strike quota is established by the International Whaling Commission (IWC 1982) based upon the nutritional and cultural needs of the Eskimo communities. In past years, quota level was estimated using assessment models under the provisions of Paragraph 13a of the IWC schedule (IWC 1982). Currently, the quota request is evaluated by the *Bowhead SLA* (Strike Limit Algorithm) (IWC 2003). The *Bowhead SLA* was developed by members of the IWC Scientific Committee (SC) and tested in trials, each simulating 100 yr of bowhead management, covering a broad range of assumptions about the bowhead population and subsistence harvests. The SC agreed that the *Bowhead SLA* is the "best tool for providing management advice for this stock" (IWC 2003, p. 28). The *Bowhead SLA* determines whether the quota request can be met based on current and past population abundance and harvests (IWC 2003). However, age information is taken into account in periodic *Implementation Reviews* that evaluate whether the population size and harvest is within the range tested in the trials.

Acknowledgments

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head whales. Bowhead whale samples were collected under the authority of permit #932-1489-00 for the MMHSRP program (Dr. Teri Rowles). We dedicate this paper to Tom Warner.

Literature Cited

Bada, J. L. 1984. In vivo racemization in mammalian proteins. Posttranslational Modifications, Methods in Enzymology 106:98–115.

Bada, J. L., and S. Brown. 1985. In vivo racemization in teeth and the ocular lens nucleus. Pages 91–100 *in* R. T. Davis and C. W. Leathers, eds. Behavior and pathology of aging in rhesus monkeys. Monographs in Primatology. Volume 6. Alan R. Liss, New York, NY.

Bada, J. L., S. E. Brown and P. M. Masters. 1980. Age determination of marine mammals based on aspartic acid racemization in the teeth and lens nucleus. Report of the International Whaling Commission (Special Issue 3):113–118.

Brodie, P., and A. Paasche. 1985. Thermoregulation and energetics of fin and sei whales based on postmortem, stratified temperature measurements. Canadian Journal of Zoology. 63:2267–2269.

Christensen, I. 1981. Age determination of minke whales, *Balaenoptera acutorostrata*, from laminated structure in the tympanic bullae. Report of the International Whaling Commission 31:245–253.

Folkow, L. P., and A. S. Blix. 1992. Metabolic rates of minke whales (*Balaenoptera acutorostrata*) in cold water. Scandinavian Journal of Physiology 146:141–150.

Garde, E., M. P. Heide-Jørgensen, S. H. Hansen, G. Nachman and M. C. Forchhammer. 2007. Age-specific growth and remarkable longevity in narwhals (*Monodon monoceros*) from West Greenland as estimated by aspartic acid racemization. Journal of Mammalogy 88:49–58.

George, J. C. 2009. Growth, morphology and energetics of bowhead whales (*Balaena mysticetus*). Ph.D. thesis, University of Alaska, Fairbanks, AK. 168 pp.

George, J. C., J. Bada, J. Zeh, L. Scott, S. Brown, T. M. O'Hara and R. Suydam. 1999. Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. Canadian Journal of Zoology 77:571–580.

George, J. C., E. Follmann, J. Zeh, M. Sousa, R. Tarpley, R. Suydam and L. Horstmann-Dehn. 2011. A new way to estimate the age of bowhead whales (*Balaena mysticetus*) using ovarian corpora counts. Canadian Journal of Zoology 89:840–852.

Hohn, A. A., M. D. Scott, R. S. Wells, 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.

IWC (International Whaling Commission). 1982. Aboriginal subsistence whaling (with special reference to the Alaska and Greenland fisheries). Report of the International Whaling Commission (Special Issue 4). i–v + 86 pp.

IWC (International Whaling Commission). 2003. Report of the Scientific Committee. Journal of Cetacean Research and Management (Supplement 5):1–92.

Koski, W. R., R. A. Davis, G. W. Miller and D. E. Withrow. 1993. Reproduction. Pages 239–274 *in* J. J. Burns, J. J. Montague and C. J. Cowles, eds. The bowhead whale. Special Publication Number 2, The Society for Marine Mammalogy.

Koski, W. R., D. J. Rugh, A. E. Punt and J. Zeh. 2006. An approach to minimize bias in estimation of the length-frequency distribution of bowhead whales (*Balaena mysticetus*) from aerial photogrammetric data. Journal of Cetacean Research and Management 8:45–54.

Lubetkin, S. C., J. E. Zeh, C. Rosa and J. C. George. 2008. Age estimation for young bowhead whales (*Balaena mysticetus*) using annual baleen growth increments. Canadian Journal of Zoology 86:525–538.

Lubetkin, S. C., J. E. Zeh and J. C. George. 2012. Statistical modeling of baleen and body length at age in bowhead whales (*Balaena mysticetus*). Canadian Journal of Zoology 90:915–931.

Masters, P. M., J. L. Bada and J. S. Zigler. 1977. Aspartic acid racemization in the human lens during ageing and in cataract formation. Nature 268:71–73.

Nerini, M. K. 1983a. Age determination techniques applied to mysticete whales. M.S. thesis, University of Washington, Seattle, WA. 50 pp.

Nerini, M. K. 1983b. Age determination of fin whales (*Balaenoptera physalus*) based upon aspartic acid racemisation in the lens nucleus. Report of the International Whaling Commission 34:447–448.

O'Hara, T. M., J. C. George, R. J. Tarpley, K. Burek and R. S. Suydam. 2002. Sexual maturation in male bowhead whales (*Balaena mysticetus*) of the Bering-Chukchi-Beaufort Seas stock. Journal of Cetacean Research and Management 4:143–148.

Olsen, E., and M. Øien. 2002. A comparison of age determination methods when applied to North Atlantic minke whales. Paper SC/54/RMP7 presented to the International Whaling Commission Scientific Committee.

Olsen, E., and J. Sunde. 2002. Age determination of minke whales (*Balaenoptera acutorostrata*) using the aspartic acid racemization technique. Sarsia 87:1–8.

Philo, L. M., E. B. Shotts, Jr. and J. C. George. 1993. Morbidity and mortality. Pages 275–307 *in* J. J. Burns, J. J. Montague and C. J. Cowles, eds. The bowhead whale. Special Publication Number 2, The Society for Marine Mammalogy.

Schell, D. M., S. M. Saupe and N. Haubenstock. 1989. Bowhead (*Balaena mysticetus*) growth and feeding as estimated by $d^{13}C$ techniques. Marine Biology 103:433–443.

Venables, W. N., and B. D. Ripley. 1999. Modern applied statistics with S-PLUS. 3rd edition. Springer-Verlag New York, Inc., New York, NY.

Zhao, M., and J. L. Bada. 1995. Determination of α-dialkylamino acids and their enantiomers in geological samples by high-performance liquid chromatography after derivatization with a chiral adduct of *o*-phthaldialdehyde. Journal of Chromatography A 690:55–63.

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