

## Update on age estimation of bowhead whales (*Balaena mysticetus*) using aspartic acid racemization

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### ABSTRACT

Ninety-eight eye globes (from 84 individual bowhead whales) were collected and analyzed to estimate ages of the whales using the aspartic acid racemization aging technique. Racemization rate ( $k_{Asp}$ ) was based on data from earlier studies of humans and fin whales; the estimate used was  $1.175 \times 10^{-3} \text{ yr}^{-1}$ . The D/L ratio at birth  $(D/L)_0$  was estimated using eyes from two term bowhead fetuses. The  $(D/L)_0$  value was 0.02708. Its variance, as well as the variance of the D/L ratios measured for whales older than age 0, was calculated via analysis of variance using multiple measurements from the same whale. Age estimates for each whale and standard errors (SE) of these age estimates were obtained using the delta method. It was found that five individuals exceeded 100 years of age; all of them were male. The SE increased with estimated age, but the age estimates had lower coefficients of variation for older animals. The SE of the age estimates could be reduced by improving the laboratory protocol for determining the D/L ratio. There are limited apparent gross and histologic changes that are indicative of aging in the >200 bowheads examined as well as a striking lack of evidence for senescence for the many gonads examined. This, along with the recovery of "traditional" whale hunting tools from five recently harvested whales, suggests that life spans in excess of 100 years may be possible.

KEYWORDS: AGE DETERMINATION; BOWHEAD WHALE; AGE AT SEXUAL MATURITY

## INTRODUCTION

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 simple photo re-identification to such methods as ear plug growth layer measurement, tooth growth layer group quantification, aspartic acid racemization in the teeth or eye lens nucleus and, to a limited extent, the aging of baleen. For a review of these methods, see Christensen *et al.* (1981), Schell *et al.* (1989) and Hohn *et al.* (1989).

In mysticete whales, specifically the bowhead whale (*Balaena mysticetus*), estimating age is challenging. Teeth are not present, ear plugs do not appear to form and baleen aging is reliable only up to approximately eleven years of age due to wear at the distal ends of the baleen plates. Recent research has found that this species of whale may routinely live over 100 years of age, with data showing ages possibly in excess of 200 years (George *et al.*, 1999). Aspartic acid racemization (AAR) aging of the eye lens nucleus is the only published method, to date, for directly estimating ages of bowheads over 12m in length (Schell and Saupe, 1993; George *et al.*, 1999).

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 is 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 animal is born (or even before in its 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 degrees 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, educated estimates for the racemization rates can be used.

As part of a larger health assessment study, the ages for 42 whales (harvested by Inupiat hunters between 1998 and the year 2000) have been determined by aspartic acid racemization of their eye lens nuclei via methods developed by George *et al.* (1999). These data have been added to the previously analyzed bowhead data from George *et al.* (1999) ( $n=42$ ) and the combined data ( $n=84$ ) reanalyzed.

The objective of this study was to extend the previous work, to further evaluate the aspartic acid racemization aging method in cetaceans via analysis and comparison of paired eyes from individual whales and, finally, to provide essential data for the bowhead whale health assessment project.

## METHODS

### Sample collection and preparation

Each eye (intact globe) was collected during the Inupiat bowhead whale subsistence hunt in Alaska between 1978 and 2000. Eyes were frozen immediately after collection and shipped by airfreight to Scripps Institution of Oceanography (SIO). Eye lenses were dissected from the globes (via sterile technique) and the lens nuclei identified and separated from the surrounding lens 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  $-20^{\circ}\text{C}$ . 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.

### Hydrolysis and estimation of D/L ratio of aspartic acid

To each of the lens splits in the glass tubes was added 1ml 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 6h. 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 were determined from the solutions using ion-exchange chromatography (Nerini, 1983; Bada, 1984). All other 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<sup>®</sup> 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 3ml 2 M NH<sub>4</sub>OH 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); 50µl 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 = 0.08) was analyzed before and after each analysis session in order to assess the consistency of the system.

A Phenomenex Luna phenyl-hexyl-column (250 x 4.60mm) 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 (see Appendix A).

### Calibration

In order to calibrate the measured D/L ratios for aspartic acid with the actual ones, 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 averages of the calculated D/L ratios were compared to the known values of the solutions. Based on these data, a calibration equation was determined by linear regression ( $R = 0.99617$ ). The resulting equation is

$$(D/L)_{act} = 1.3219 \times (D/L)_{meas} + 0.0031,$$

where  $(D/L)_{act}$  is the actual D/L ratio and  $(D/L)_{meas}$  is the measured one (Figure 1). This equation was then used to calculate the actual D/L ratios in the samples. These actual D/L values were used as in George *et al.* (1999) to determine the ages of the whales, as described in the next section.

### Estimating age and the relationship of length to age

The actual D/L ratios described in the previous section were used in estimating age from the following equation:

$$\text{age} = [\ln((1 + D/L)/(1 - D/L)) - \ln((1 + (D/L)_0)/(1 - (D/L)_0))] / [2k_{\text{Asp}}]$$

where  $k_{\text{Asp}}$  is the racemization rate for aspartic acid, and  $(D/L)_0$  is the D/L value at age 0. Ages for whales with both eyes sampled were estimated as the average of the age from the left eye and the age from the right eye. In one case in which we had two measurements from each eye, we were able to identify one as an outlier and omit it.

Growth rates were estimated by fitting the von Bertalanffy growth curve model to the data using nonlinear least squares. The fitted model was:

$$\text{length} = (L_{\text{max}} + I_F L_{\text{diff}}) [1.0 - \exp(-(k + I_F k_{\text{diff}})(t - t_0))]$$

where  $t$  = age,  $L_{\text{max}}$  = average maximum length for males,  $I_F$  is coded as 0 for males and 1 for females,  $L_{\text{diff}}$  = average difference between female and male maximum length,  $k$  = the growth rate constant for males,  $k_{\text{diff}}$  = the difference between male and female growth rate, and  $t_0$  = age at length 0. Note that  $t_0$  is not really an age but rather a constant analogous to the intercept in a linear model which permits the growth curve to fit even if some of the age estimates are negative and the smallest lengths are much greater than zero.

### Estimating the racemization rate for aspartic acid

The racemization rate ( $k_{\text{Asp}}$ ) for aspartic acid is determined by regressing the natural log of  $(1+D/L)/(1-D/L)$  for a sample on the animals' ages (Masters *et al.*, 1977). The slope of the regression line estimates  $2k_{\text{Asp}}$ . In the case of humans and fin whales, the relationship appears to be linear. Masters *et al.* (1977) reported  $2k_{\text{Asp}}$  for humans, with body temperature 37°C, as  $2.50 \times 10^{-3} \text{ yr}^{-1}$  with a standard error (SE) of  $0.29 \times 10^{-3} \text{ yr}^{-1}$  based on a sample of 17 normal human eye lenses. George *et al.* (1999) determined  $2k_{\text{Asp}}$  for fin whales based on the sample of 16 whales given in Nerini (1983) (with average D/L used when two samples were available for the whale) as  $2.209 \times 10^{-3} \text{ yr}^{-1}$  with SE =  $0.716 \times 10^{-3} \text{ yr}^{-1}$ . Note that the maximum deep body temperature of fin whales (36.1°C) is lower than that of humans, and one would expect  $k_{\text{Asp}}$  to be lower (Brodie and Paasche, 1985). However, ages of the fin whales, based on ear plug data, are less precisely determined than human ages, increasing the variability of the estimate of  $2k_{\text{Asp}}$  for fin whales and perhaps leading it to be negatively biased. As in George *et al.* (1999), the human and fin whale estimates were averaged to allow for the possibility that whale  $2k_{\text{Asp}}$  is lower than human. The resulting estimate of  $2k_{\text{Asp}}$  is  $2.35 \times 10^{-3} \text{ yr}^{-1}$  with a variance  $V_k = 0.149 \times 10^{-6} \text{ yr}^{-2}$  and a standard error SE =  $0.39 \times 10^{-3} \text{ yr}^{-1}$ .

### Determining the $(D/L)_0$ (age 0) value

The  $(D/L)_0$  value is a critical variable in calculating the age estimates. Because they had an eye lens from only one bowhead fetus, George *et al.* (1999) estimated  $(D/L)_0$  and its variance  $V_0$  using D/L measurements from all the sampled bowhead whales assumed on the basis of their body length and baleen length to be age 2 or less. An eye from an additional term fetus was collected in 1999 and used with the D/L value from the earlier fetus to estimate  $(D/L)_0$ . The D/L values for the two fetal eye lenses analyzed were 0.027 and 0.02717. These two values, together with an additional D/L measurement from the second fetus, were used in an analysis of variance of  $\ln((1 + D/L)/(1 - D/L))$  with between-whale and within-whale variance components to estimate  $V_0$ .

### D/L ratio measurement error

To estimate the measurement error variance, paired D/L ratio measurements from 21 whales were analyzed. The data did not suggest that between-eye variability was higher than within-eye variability, so we did not distinguish in this analysis between measurements from different eyes and repeated measurements from the same eye. The residual mean squared error from an analysis of variance of  $\ln((1 + D/L)/(1 - D/L))$  with between-whale and within-whale variance components provided an estimated variance  $V$  of a single measured value of  $\ln((1 + D/L)/(1 - D/L))$ .

### Standard error for age estimates

As in George *et al.* (1999), we applied the delta method (Seber 1982, pp. 7-8) and a formula of Goodman (1960) for an estimate of the variance of a product of two independent random variables to calculate standard errors for the age estimates. This approach is necessary to treat the multiple sources of error in the estimates discussed above: measurement errors in the D/L ratios of the samples, variability in the D/L ratio at age 0, and error in the estimate of  $2k_{\text{Asp}}$ .

The equation for age given above can be written as the product of two independent random variables:  $\text{age} = xy$  where  $x = [\ln((1 + D/L)/(1 - D/L)) - \ln((1 + (D/L)_0)/(1 - (D/L)_0))]$  and  $y = 1/[2k_{\text{Asp}}]$ . These quantities are clearly independent since  $x$  is estimated from bowhead data and  $y$  from human and fin whale data. Since the two terms in  $x$  are independent for all the sampled whales except the two term fetuses, for which we do not estimate age since it is defined as zero, an estimate of the variance of  $x$  is given by  $V_x = V + V_0$ . The delta method gives us  $V_y = V_k/[2k_{\text{Asp}}]^4$  as an estimate of the variance of  $y$ . Then formula (5) of Goodman (1960) gives

$$V(\text{age}) = x^2V_y + y^2V_x - V_xV_y$$

where  $V(\text{age})$  is the estimated variance of the age estimate with the appropriate measured and estimated quantities used in computing  $x$  and  $y$ . The SE is just the square root of  $V(\text{age})$ .

## RESULTS

### Sampled bowheads

Eye lenses were collected from 84 bowhead whales. A distribution of sex and body length is given in Figure 2. Of these 84 whales, 46 (55%) were female and 38 (45%) were male; 31 (37%) were greater than 13m long and probably sexually mature. Fourteen of the 84 had paired right and left eye lenses analyzed.

We could not use a random sampling scheme to obtain the eyes used in this analysis. Eye globes were collected as whales were available. A hunt-based bias toward younger animals was expected. The International Whaling Commission (IWC) recommended that hunters should avoid taking mature animals in the early years of sampling. In addition, there is some hunter preference for smaller animals. However, the 37% estimated to be sexually mature in our sample does not differ greatly from the percentage of mature animals in the general population estimated from aerial photogrammetry at Barrow (41%, Angliss *et al.*, 1995).

### Ages and growth

Estimated ages for all 84 whales are given in Table 1. Results of the von Bertalanffy model fit to the age-length data are given in Table 2 and Figure 3.

The estimated ages varied considerably for a given length class. This is expected for young animals (<11m) based on work by Schell *et al.* (1989) and others. However, variability in  $k_{\text{Asp}}$  estimates, measured D/L ratios and  $(D/L)_0$  values also produce substantial error (Table 1). Uncertainty in the  $k_{\text{Asp}}$  rate is a major contributor to uncertainty, particularly in the older ages. D/L ratio measurement error accounts for most of the variability in the age estimates at the younger ages. In combination, these sources of error lead to large coefficients of variation (CV) for the age estimates, particularly for young animals. SE increased with age (Figure 4) while CV decreased; thus older animals have greater precision in a relative sense.

None of the whales <11m long had estimated ages that were implausible given their SE, with the possible exception of 00B16. However, published ages for whales in this size range are 11 years or less (Schell *et al.*, 1989). We therefore omitted whales with ages <1 (all of whom were <10m long) as well as all whales <10.5m long with ages of 20 or more from the von Bertalanffy fit. The fetuses were omitted because exploratory analyses indicated that the rapid growth in the first year of life could not be fit well. The estimated growth curve summarized in Table 2 indicates that females differ from males both in maximum length (17.6m vs 15.1m) and in growth rate. Females and males of the same age are estimated to be similar in length up to about age 10, but beyond this female lengths are estimated to exceed male lengths. Sexual maturity – at 12.5 to 13m for males (O'Hara *et al.*, 2002) and 13 to 14m for most females (Koski *et al.*, 1993; George *et al.*, 2004) – is estimated to occur in the mid to late twenties.

### Estimate of $(D/L)_0$

The mean value of  $\ln((1 + (D/L)_0)/(1 - (D/L)_0))$  for the two term fetuses was 0.05418, corresponding to  $(D/L)_0 = 0.02708$ , close to the average of the fetal D/L values 0.027 and 0.02717. The estimated variance of the  $\ln((1 + (D/L)_0)/(1 - (D/L)_0))$  estimate was  $V_0 = 0.4907 \times 10^{-4}$ .

### D/L ratio measurement error

There were 44 D/L ratio measurements from 3 fin (Nerini, 1983) and 18 bowhead whales (including left and right eye globes from 17 animals). A paired *t*-test between left and right globes for the bowhead whales indicated only a minor difference in D/L ratio between eyes (left eyes having a D/L ratio higher on average by 0.0055 than right eyes,  $P = 0.02$ ). The residual mean square from an analysis of variance of  $\ln((1 + D/L)/(1 - D/L))$ , with each whale with paired data constituting a group, was  $V = 0.6075 \times 10^{-3}$ . Thus the standard error of a single  $\ln((1 + D/L)/(1 - D/L))$  measurement was 0.0246.

The measurement error variance for the whales sampled since 1996 was significantly higher than the corresponding estimate of George *et al.* (1999) ( $0.8385 \times 10^{-3}$  versus  $0.7946 \times 10^{-4}$ ,  $P = 0.002$ ). This may be because the new pairs covered a broader range of D/L values. In addition, although most of the paired data values reflect relatively small measurement error, the new data pairs include three outliers with differences between the paired values 2 to 4 times as large as the differences in the other pairs. Such outliers could result from sample contamination. There were no such outliers in the paired data of George *et al.* (1999), perhaps because with fewer pairs, there was a lower probability of outliers being present. Pending further investigation of measurement errors, we opted to use the value of  $V$  given above for estimating standard errors of age estimates from both the old and new data.

## DISCUSSION

### Growth, age at sexual maturity and longevity

George *et al.* (1999) reported growth, age at sexual maturity and longevity related topics with respect to the AAR technique based on 42 bowhead whales. Our results, with an addition of 42 whales, support these findings and will not be revisited in depth in this paper. In brief, the additional data support the findings of an initial high rate of growth following birth, followed by an interrupted period of growth after weaning. This period is succeeded by steady growth up until sexual maturity is attained, at 12.5-13m for males (O'Hara *et al.*, 2002) and 13-14m for females (Figure 3) (Koski *et al.*, 1993; Schell *et al.*, 1989; George *et al.*, 2004). This roughly correlates with 24-29 years of age in males and 23-30 years of age in females.

In addition, with the larger sample size, the results from the nonlinear least squares von Bertalanffy fit (Table 2) show that differences exist not only between the male and female  $L_{\max}$  but also between the male and female  $k$ ; all parameters had significant *t* values. This indicates that males and females differ in growth rate as well as in maximum length.

### Viability of the AAR technique for aging whales

The AAR technique is most appropriate for whales over approximately 20 years of age. Age estimates below 26 in Table 1 had estimated SE = 11 unless both eyes were sampled so that an average with SE = 8 could be computed. As discussed below, improving the accuracy and precision of the HPLC analyses could reduce standard errors. Olsen and Sunde (2002) reported lower SE for AAR estimates of minke whale ages. For older bowhead whales, even the standard errors estimated from our data do not prevent the age estimates for individuals from being useful. Our AAR ages for young bowheads can be used in estimating life history parameters even though they provide little information about the ages of individuals. Progress is being made in the area of baleen aging for the younger whales (Lubetkin *et al.*, 2004).

### Implications for cetacean health assessment studies

Age affects a majority of biological health parameters, including tissues (histological interpretation), toxicology (bioaccumulation of contaminants), reproduction and basic hematological parameters such as biochemistry panels and complete blood counts (which often differ between mature and immature members of mammalian species). The bowhead whale health assessment project depends upon AAR and baleen aging to accurately assess these parameters *with respect to age*. As an example, bowhead whales exhibit little pathology at a gross or microscopic level. After hundreds of necropsies and review of thousands of histology slides, the few incidences of pathology that have been noted are remarkable. The discovery of renal (kidney) fibrosis in numerous whales, which can have a marked effect upon individual whale health,

was a significant but yet unexplained finding. This change was initially thought to be related to an increase in renal cadmium concentration, but it is now believed to be attributed mainly to age (data still undergoing analysis) (Willette *et al.*, 2002). This is just one example of the importance of reasonably accurate age estimates to the interpretation of results. Another noteworthy example is the fact that, presently, there is no strong evidence of reproductive senescence in the bowhead whale. The implications of this are far-reaching and may be another unique feature of the bowhead whale.

#### **Future improvements to AAR methodology / future research directions**

From this analysis, we have several suggestions to improve cetacean AAR analysis. First, we recommend the development of a standard laboratory protocol and certified standards. Samples should be analyzed in random order with no prior knowledge of body length, sex or reproductive status. Also, a clear protocol for repeat analyses of samples should be decided upon prior to data collection. Data from repeated runs for the 42 whales sampled most recently are available and will be used to design such a protocol. These data can also be used in estimating V for each eye analyzed instead of a single value applied to all eyes, permitting more accurate estimation of the SE of the age estimated from each eye. With an adequate number of repeated runs for each sample, statistical techniques for outlier detection can be used to eliminate discrepant measurements, and measurement error can be reduced considerably by averaging.

Second, we now have enough data to estimate  $(D/L)_0$  only from term fetuses. However, efforts should be made to collect additional fetal eyes in order to strengthen the estimate.

Finally, the collection of samples that are of interest due to their utility in the process of aging cetaceans should be collected under a set protocol. This includes eyes, baleen (for aging young whales), collagen (for aging method development) and ovaries (for corpora counts).

#### **Other techniques**

In conjunction with this study, other methods of aging are being pursued in bowheads. Two promising approaches include baleen aging using stable isotopes (Lubetkin *et al.*, 2004) and collagen aging (Rosa *et al.*, 2001). These techniques may fill the gap that exists and provide ages for younger whales, where the standard errors tend to be too large to infer age from the AAR estimates. Additionally, as an adjunct method, standard counts of ovarian corpora albicantia and corpora lutea from harvested females can provide important reproductive information that can be useful in age determination. This technique is currently being investigated to assess its utility in bowhead whales (George *et al.*, 2004).

#### **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.

Another factor that could result in AAR-based ages being over-estimates would be the presence of more asparagine residues (in comparison to humans and other mammals, including several marine species) in the eye lens nucleus proteins of bowhead whales. Asparagine racemizes several times faster than aspartic acid (Geiger and Clarke, 1987; Brinton and Bada, 1995). Thus, if there are higher abundances of asparagine residues in the bowhead lens proteins, and these would be hydrolyzed to aspartic acid during 6 M HCl treatment, this would generate an apparently higher D/L ratio for aspartic acid. The apparent extent of aspartic acid racemization in the bowhead whale eye lens nucleus samples would thus be greater than in eye lens nucleus samples from other mammals of similar age. Using the human and fin whale based racemization rates would then give age estimates for bowhead whales that were too old. However, it seems unlikely that bowhead whales would differ from other cetacean species in this regard.

We recognize that specific aspartic acid residues in  $\alpha A$  and  $\alpha B$ -crystallin (lens proteins) racemize at different rates in humans which could lead to inaccuracies in age estimates (Fujii *et al.*, 1994a; Fujii *et al.*, 1994b). However, since the approach of Zhao and Bada (1995) measures the overall D/L value, the racemization rates should not differ significantly between animals.

The AAR age estimates would be biased *downwards* if: a) the average temperature experienced by the eyeglobe was held at a lower body temperature than humans or fin whales, or b) the samples were contaminated with blood or “modern” tissue. The cornea is in contact with very cold water throughout the year and it is possible that the internal temperature of the globe is lower than deep body temperature. Sub-normal temperatures would slow racemization and subsequently the AAR aging technique would *under-*

estimate age to some degree. If surrounding tissue or blood contaminates the sample (lens nucleus) during dissection, the D/L ratio could be dramatically lowered resulting in a gross underestimate (George *et al.*, 1999).

### **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 principle factors in determining the quota. As background, the subsistence harvest of bowhead whales is regulated at international, national and local levels. The strike quota is established by the IWC (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 100yrs 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 situation of the population and harvest is within the range tested in the trials.

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Table 1

Basic data for bowhead whales used in this study with age estimates determined using aspartic acid racemization. Whale identification number indicates: year, village (B = Barrow, G = Gambell, WW = Wainwright, H = Pt. Hope, S = Savoonga) and sequential harvest number. Age and its standard error (SE) were set to zero for the two fetuses used in  $D/L_0$  calculations. All other age estimates were estimated from D/L ratios given in Appendix A, with SE obtained by the delta method. When D/L ratios from both eyes were available, the two age estimates were averaged, resulting in a smaller SE. Females (F) are shown on the left and males (M) on the right.

NA = not available.

| Whale id | Sex | Length<br>(m) | Baleen<br>(cm) | Age | SE | Whale id | Sex | Length<br>(m) | Baleen<br>(cm) | Age | SE |
|----------|-----|---------------|----------------|-----|----|----------|-----|---------------|----------------|-----|----|
| 99B18F   | F   | 4             | 11             | 0   | 0  |          |     |               |                |     |    |
| 95B8     | F   | 4.1           | 10             | 0   | 0  |          |     |               |                |     |    |
| 96B2     | F   | 7.6           | 67             | 9   | 11 |          |     |               |                |     |    |
| 99KK1    | F   | 7.7           | 85             | 1   | 11 |          |     |               |                |     |    |
| 00B9     | F   | 7.9           | 70             | 6   | 8  |          |     |               |                |     |    |
| 99B19    | F   | 8.1           | 96             | 1   | 8  | 96B15    | M   | 8.1           | 70             | 4   | 11 |
| 78B1     | F   | 8.5           | 146            | 3   | 11 | 78B3     | M   | 8.4           | 95             | -5  | 11 |
| 96B1     | F   | 8.5           | 126            | 20  | 11 | 94B14    | M   | 8.4           | 76             | 0   | 11 |
| 95B4     | F   | 8.6           | 102            | 2   | 11 | 79B1     | M   | 8.7           | 75             | 0   | 11 |
| 00B8     | F   | 8.6           | NA             | 2   | 11 | 94B11    | M   | 8.7           | 151            | 2   | 11 |
| 00KK3    | F   | 8.8           | 112            | 1   | 11 | 99B24    | M   | 8.8           | NA             | 9   | 11 |
| 00B15    | F   | 8.9           | NA             | 7   | 8  | 00B1     | M   | 8.9           | NA             | 3   | 11 |
| 00B18    | F   | 8.9           | NA             | 10  | 11 | 99B5     | M   | 9             | NA             | -1  | 11 |
| 99B20    | F   | 9             | 130            | 5   | 8  | 79H3     | M   | 9.1           | 105            | -2  | 8  |
| 99B12    | F   | 9.2           | 174            | 7   | 11 | 93B18    | M   | 9.3           | 200            | 9   | 11 |
| 00KK1    | F   | 9.2           | 150            | 7   | 11 | 99B9     | M   | 9.3           | 193            | 6   | 11 |
| 78H1     | F   | 9.3           | 150            | -2  | 11 | 00B13    | M   | 9.4           | 189            | -4  | 11 |
| 00B10    | F   | 9.4           | 166            | 9   | 11 | 00B17    | M   | 9.5           | NA             | 9   | 8  |
| 98B25    | F   | 9.7           | NA             | 35  | 12 | 78H2     | M   | 9.7           | 164            | 8   | 11 |
| 99B22    | F   | 9.7           | 175            | 9   | 8  | 99B21    | M   | 10.5          | 174            | 10  | 8  |
| 00B14    | F   | 9.9           | 191            | 6   | 11 | 00B12    | M   | 10.8          | 193            | 13  | 8  |
| 00B16    | F   | 10            | NA             | 45  | 13 | 99B23    | M   | 10.9          | 192            | 12  | 11 |
| 96B18    | F   | 10.1          | 202            | 23  | 11 | 96B16    | M   | 11            | 212            | 27  | 12 |
| 96B24    | F   | 10.9          | 243            | 23  | 8  | 99B8     | M   | 11            | 217            | 8   | 11 |
| 98B12    | F   | 11.3          | 174            | 8   | 11 | 96B22    | M   | 11.6          | 199            | 23  | 8  |
| 96B9     | F   | 12.1          | 240            | 32  | 12 | 00KK2    | M   | 12.1          | 214            | 34  | 12 |
| 95B12    | F   | 12.3          | NA             | 11  | 11 | 96B19    | M   | 12.9          | 249            | 32  | 12 |
| 99B6     | F   | 12.6          | 236            | 22  | 11 | 95B11    | M   | 13            | 263            | 20  | 11 |
| 96B6     | F   | 12.7          | 235            | 31  | 12 | 96B17    | M   | 13.3          | 269            | 35  | 12 |
| 99B18    | F   | 13            | 342            | 29  | 12 | 78G1     | M   | 13.8          | 298            | 23  | 8  |
| 96B10    | F   | 13.4          | 320            | 26  | 12 | 00B11    | M   | 13.8          | NA             | 60  | 14 |
| 96B4     | F   | 14.4          | 300            | 43  | 13 | 95B16    | M   | 14.1          | NA             | 92  | 19 |
| 00B2     | F   | 14.5          | 273            | 26  | 8  | 99B13    | M   | 14.1          | 303            | 46  | 13 |
| 79WW1    | F   | 14.6          | 316            | 58  | 14 | 99B14    | M   | 14.2          | NA             | 57  | 14 |
| 00B3     | F   | 14.6          | NA             | 41  | 13 | 95B15    | M   | 14.5          | 289            | 58  | 14 |
| 99B16    | F   | 14.8          | NA             | 39  | 12 | 95WW5    | M   | 14.6          | NA             | 213 | 36 |
| 95B10    | F   | 15            | 320            | 20  | 11 | 99B15    | M   | 14.6          | NA             | 70  | 16 |
| 99B7     | F   | 15.4          | NA             | 71  | 16 | 80S1     | M   | 14.7          | 229            | 48  | 13 |
| 00B4     | F   | 15.4          | NA             | 52  | 14 | 00B6     | M   | 14.7          | 232            | 17  | 11 |
| 81G1     | F   | 15.5          | 297            | 35  | 12 | 99B17    | M   | 14.9          | NA             | 113 | 22 |
| 80G1     | F   | 15.6          | 291            | 27  | 12 | 78WW2    | M   | 15            | 319            | 136 | 25 |
| 81WW1    | F   | 16.2          | NA             | 29  | 12 | 95B7     | M   | 15.2          | 305            | 160 | 28 |
| 78WW1    | F   | 16.3          | 322            | 70  | 16 | 95B9     | M   | 17.4          | 384            | 174 | 30 |
| 95B13    | F   | 16.5          | NA             | 48  | 13 |          |     |               |                |     |    |
| 81S1     | F   | 16.8          | NA             | 39  | 12 |          |     |               |                |     |    |
| 00B5     | F   | 18.9          | 331            | 66  | 15 |          |     |               |                |     |    |

Table 2

Results of von Bertalanffy curve fit.

 $L_{\max}$  = average maximum length for males,  $k$  = growth rate constant for males,  $t_0$  = age at length 0; $L_{\text{diff}}$  = average difference between female and male maximum length, so $L_{\max} + L_{\text{diff}}$  = average female maximum length; $k_{\text{diff}}$  = difference between male and female growth constant, so $k + k_{\text{diff}}$  = growth rate constant for females.

| Parameter         | Value   | SE     | <i>t</i> -value |
|-------------------|---------|--------|-----------------|
| $t_0$             | -16.23  | 3.34   | -4.86           |
| $L_{\max}$ (male) | 15.07   | 0.42   | 36              |
| $k$ (male)        | 0.0439  | 0.0072 | 6.10            |
| $L_{\text{diff}}$ | 2.551   | 1.025  | 2.49            |
| $k_{\text{diff}}$ | -0.0099 | 0.0047 | -2.11           |

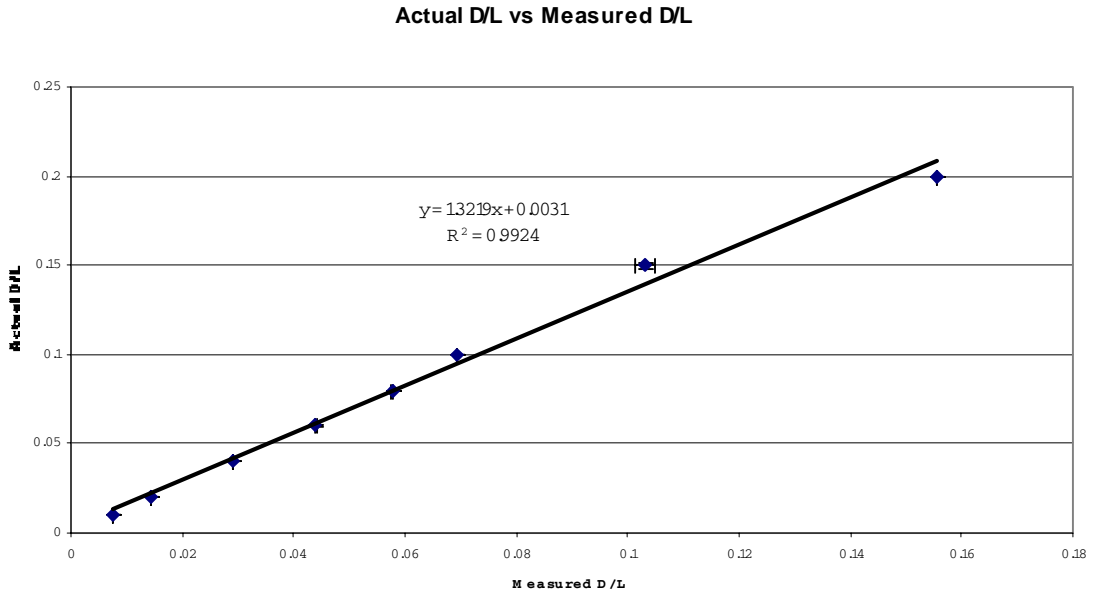


Figure 1. Linear regression of the measured D/L ratios versus the actual values for the standard aspartic acid solutions used to calibrate the sample data.

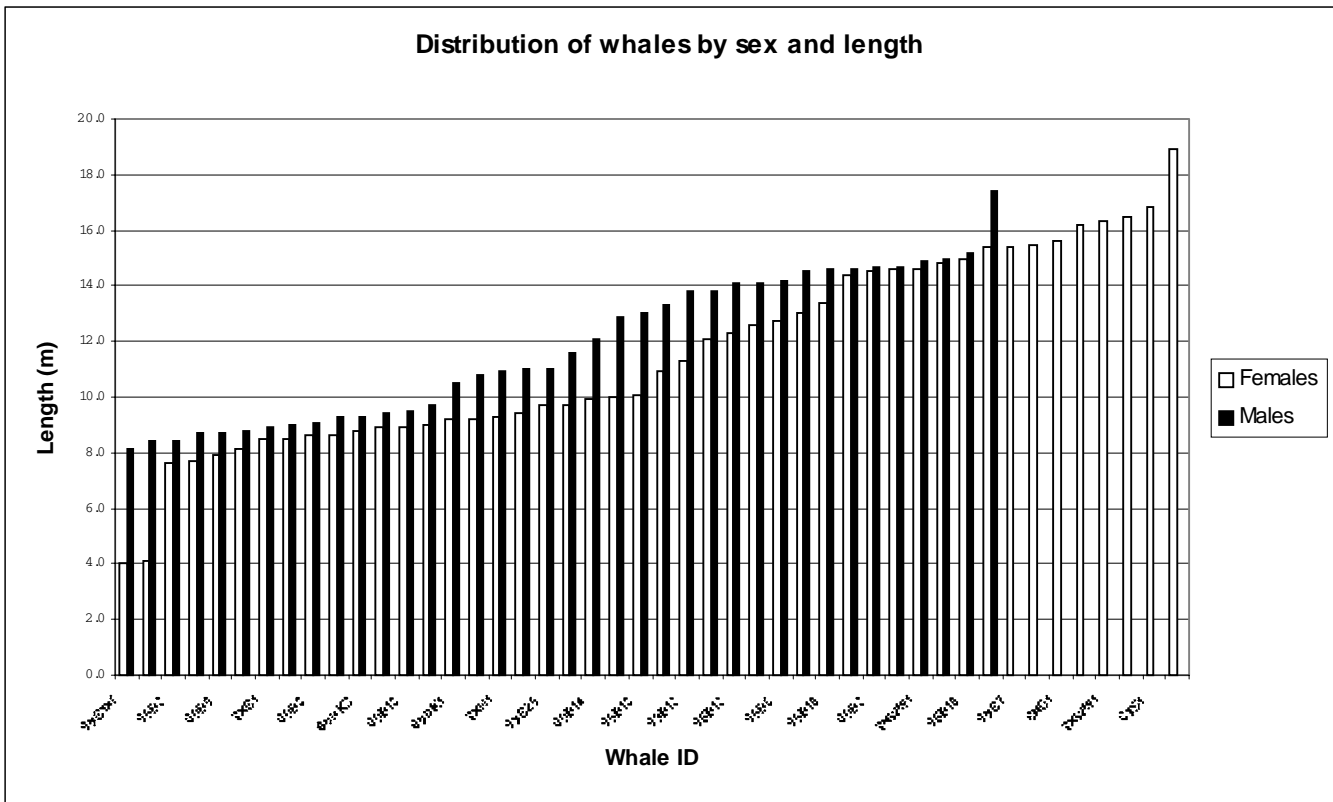


Figure 2. Distribution of sex and body length of bowhead whales sampled for AAR analysis.

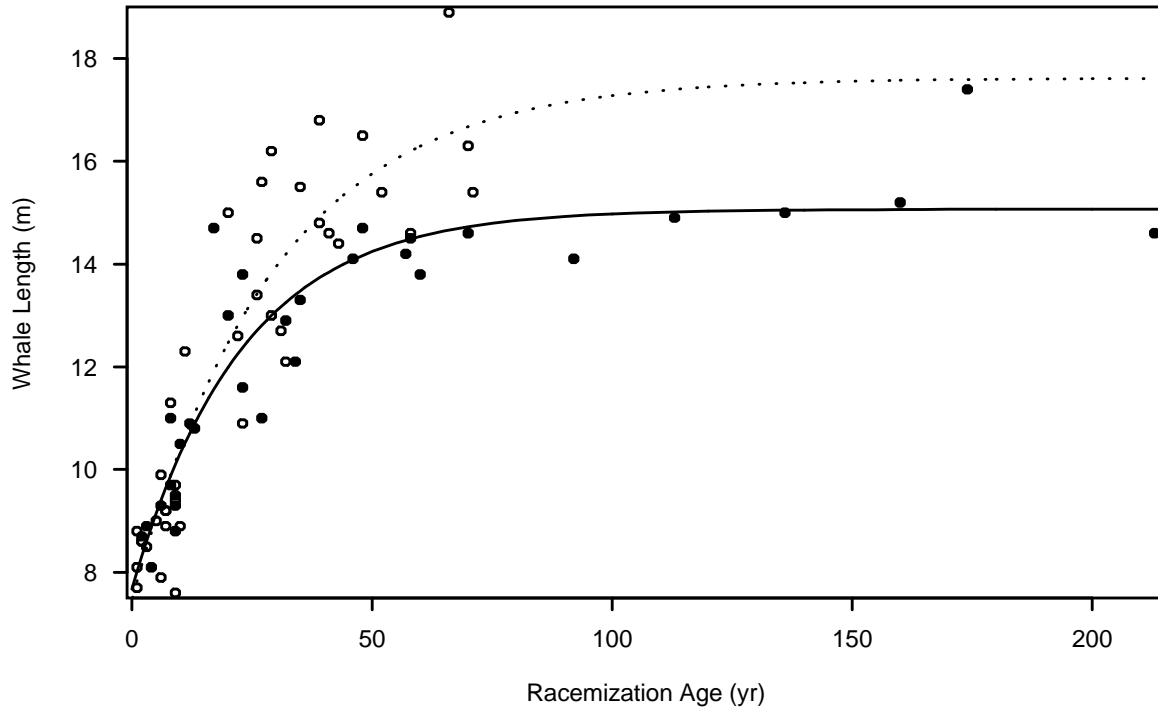


Figure 3. Estimated age-at-length for bowhead whales using the aspartic acid racemization technique. The von Bertalanffy growth curves are shown for females (upper curve) and males (lower curve).

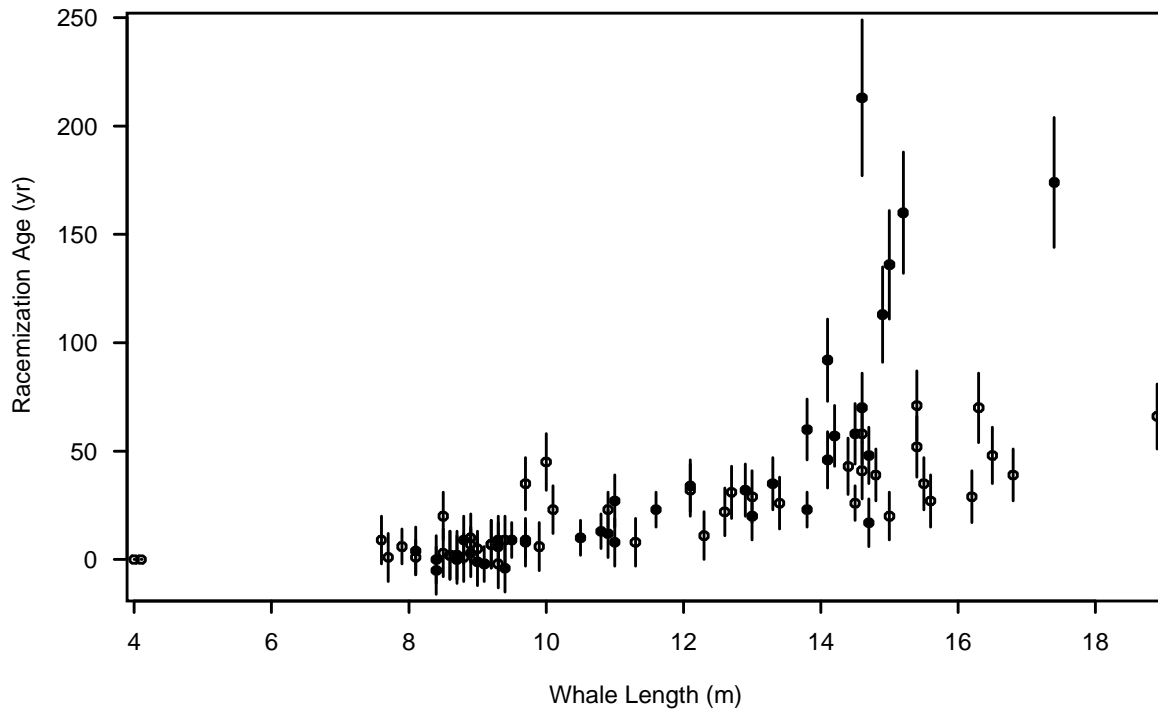


Figure 4. Age estimates (yr) by whale length (m) shown with estimated standard errors. Note that the standard error increases with age. Open circles females, closed circles males.

### Appendix A

Basic data for bowhead whale eyes used in this study with age estimates determined using aspartic acid racemization. Whale identification number indicates year, village and sequential harvest number; F indicates a fetus. Info is L (left), R (right), A (unknown whether left or right), B (second eye in pair with orientation unknown), 1 (first measurement on the eye if there are two measurements), 2 (second measurement on the eye). The D/L ratios for the samples obtained in the 1978-1981 harvest were determined using ion exchange chromatography (Bada, 1984); all other D/L ratios were determined using the HPLC based method (Zhao and Bada, 1995). NA = not available.

| Whale id | Info | Length (m) | Sex | Baleen (cm) | D/L     | Village    | Age  | SE   |
|----------|------|------------|-----|-------------|---------|------------|------|------|
| 78B1     | A    | 8.5        | F   | 146         | 0.0308  | Barrow     | 3.2  | 10.8 |
| 78B3     | L    | 8.4        | M   | 95          | 0.0216  | Barrow     | -4.7 | 10.8 |
| 78G1     | L    | 13.8       | M   | 298         | 0.0510  | Gambell    | 20.4 | 11.3 |
| 78G1     | R    | 13.8       | M   | 298         | 0.0569  | Gambell    | 25.4 | 11.5 |
| 78H1     | A    | 9.3        | F   | 150         | 0.0246  | Point Hope | -2.1 | 10.8 |
| 78H2     | R    | 9.7        | M   | 164         | 0.0369  | Point Hope | 8.4  | 10.8 |
| 78WW1    | L    | 16.3       | F   | 322         | 0.1090  | Wainwright | 70.1 | 15.8 |
| 78WW2    | R    | 15.0       | M   | 319         | 0.1850  | Wainwright | 136  | 24.8 |
| 79B1     | L    | 8.7        | M   | 75          | 0.0270  | Barrow     | -0.1 | 10.8 |
| 79H3     | L    | 9.1        | M   | 105         | 0.0260  | Point Hope | -0.9 | 10.8 |
| 79H3     | R    | 9.1        | M   | 105         | 0.0240  | Point Hope | -2.6 | 10.8 |
| 79WW1    | A    | 14.6       | F   | 316         | 0.0955  | Wainwright | 58.5 | 14.4 |
| 80G1     | A    | 15.6       | F   | 291         | 0.0590  | Gambell    | 27.2 | 11.6 |
| 80S1     | R    | 14.7       | M   | 229         | 0.0830  | Savoonga   | 47.7 | 13.3 |
| 81G1     | A    | 15.5       | F   | 297         | 0.0680  | Gambell    | 34.9 | 12.2 |
| 81S1     | A    | 16.8       | F   | NA          | 0.0724  | Savoonga   | 38.7 | 12.5 |
| 81WW1    | R    | 16.2       | F   | NA          | 0.0615  | Wainwright | 29.4 | 11.8 |
| 93B18    | R    | 9.3        | M   | 200         | 0.0380  | Barrow     | 9.3  | 10.9 |
| 94B11    | R    | 8.7        | M   | 151         | 0.0300  | Barrow     | 2.5  | 10.8 |
| 94B14    | R    | 8.4        | M   | 76          | 0.0270  | Barrow     | -0.1 | 10.8 |
| 95B10    | L    | 15.0       | F   | 320         | 0.0510  | Barrow     | 20.4 | 11.3 |
| 95B11    | A    | 13.0       | M   | 263         | 0.0510  | Barrow     | 20.4 | 11.3 |
| 95B12    | A    | 12.3       | F   | NA          | 0.0400  | Barrow     | 11.0 | 10.9 |
| 95B13    | A    | 16.5       | F   | NA          | 0.0830  | Barrow     | 47.7 | 13.3 |
| 95B15    | L    | 14.5       | M   | 289         | 0.0950  | Barrow     | 58.0 | 14.4 |
| 95B16    | L    | 14.1       | M   | NA          | 0.1350  | Barrow     | 92.5 | 18.6 |
| 95B4     | A    | 8.6        | F   | 102         | 0.0300  | Barrow     | 2.5  | 10.8 |
| 95B7     | R    | 15.2       | M   | 305         | 0.2120  | Barrow     | 160  | 28.4 |
| 95B8F    | A    | 4.1        | F   | 10          | 0.0270  | Barrow     | -0.1 | 10.8 |
| 95B9     | R    | 17.4       | M   | 384         | 0.2270  | Barrow     | 174  | 30.5 |
| 95WW5    | A    | 14.6       | M   | NA          | 0.2700  | Wainwright | 213  | 36.5 |
| 96B1     | A    | 8.5        | F   | 126         | 0.0510  | Barrow     | 20.4 | 11.3 |
| 96B10    | R    | 13.4       | F   | 320         | 0.0580  | Barrow     | 26.4 | 11.6 |
| 96B15    | R    | 8.1        | M   | 70          | 0.0320  | Barrow     | 4.2  | 10.8 |
| 96B16    | R    | 11         | M   | 212         | 0.0590  | Barrow     | 27.2 | 11.6 |
| 96B17    | R    | 13.3       | M   | 269         | 0.0680  | Barrow     | 34.9 | 12.2 |
| 96B18    | R    | 10.1       | F   | 202         | 0.0540  | Barrow     | 22.9 | 11.4 |
| 96B19    | L    | 12.9       | M   | 249         | 0.0645  | Barrow     | 31.9 | 12.0 |
| 96B2     | A    | 7.6        | F   | 67          | 0.0380  | Barrow     | 9.3  | 10.9 |
| 96B22    | L    | 11.6       | M   | 199         | 0.0580  | Barrow     | 26.4 | 11.6 |
| 96B22    | R    | 11.6       | M   | 199         | 0.0490  | Barrow     | 18.7 | 11.2 |
| 96B24    | L    | 10.9       | F   | 243         | 0.0570  | Barrow     | 25.5 | 11.5 |
| 96B24    | R    | 10.9       | F   | 243         | 0.0520  | Barrow     | 21.2 | 11.3 |
| 96B4     | R    | 14.4       | F   | 300         | 0.0770  | Barrow     | 42.6 | 12.8 |
| 96B6     | L    | 12.7       | F   | 235         | 0.0630  | Barrow     | 30.6 | 11.9 |
| 96B9     | R    | 12.1       | F   | 240         | 0.0650  | Barrow     | 32.3 | 12.0 |
| 98B12    | A    | 11.3       | F   | 174         | 0.03668 | Barrow     | 8.2  | 10.8 |

| Whale id | Info | Length (m) | Sex | Baleen (cm) | D/L     | Village  | Age  | SE   |
|----------|------|------------|-----|-------------|---------|----------|------|------|
| 98B25    | A    | 9.7        | F   | NA          | 0.06767 | Barrow   | 34.6 | 12.2 |
| 99B5     | L    | 9.0        | M   | NA          | 0.02585 | Barrow   | -1.1 | 10.8 |
| 99B6     | L    | 12.6       | F   | 236         | 0.05275 | Barrow   | 21.9 | 11.3 |
| 99B7     | L1   | 15.4       | F   | NA          | 0.10998 | Barrow   | 70.9 | 15.9 |
| 99B7     | L2   | 15.4       | F   | NA          | 0.05928 | Barrow   | 27.5 | 11.7 |
| 99B8     | R    | 11.0       | M   | 217         | 0.03681 | Barrow   | 8.3  | 10.8 |
| 99B9     | R    | 9.3        | M   | 193         | 0.03472 | Barrow   | 6.5  | 10.8 |
| 99B12    | L    | 9.2        | F   | 174         | 0.03579 | Barrow   | 7.4  | 10.8 |
| 99B13    | R    | 14.1       | M   | 303         | 0.08127 | Barrow   | 46.3 | 13.2 |
| 99B14    | L    | 14.2       | M   | NA          | 0.09413 | Barrow   | 57.3 | 14.3 |
| 99B15    | A    | 14.6       | M   | NA          | 0.10894 | Barrow   | 70.0 | 15.7 |
| 99B16    | L    | 14.8       | F   | NA          | 0.07233 | Barrow   | 38.6 | 12.5 |
| 99B17    | A1   | 14.9       | M   | NA          | 0.15869 | Barrow   | 113  | 21.5 |
| 99B17    | A2   | 14.9       | M   | NA          | 0.17269 | Barrow   | 125  | 23.2 |
| 99B18F   | A1   | 4.0        | F   | 11          | 0.02717 | Barrow   | 0.1  | 10.8 |
| 99B18F   | A2   | 4.0        | F   | 11          | 0.02222 | Barrow   | -4.1 | 10.8 |
| 99B18    | A1   | 13.0       | F   | 342         | 0.06095 | Barrow   | 28.9 | 11.8 |
| 99B18    | A2   | 13.0       | F   | 342         | 0.05022 | Barrow   | 19.7 | 11.2 |
| 99B19    | L    | 8.1        | F   | 96          | 0.03147 | Barrow   | 3.7  | 10.8 |
| 99B19    | R    | 8.1        | F   | 96          | 0.02484 | Barrow   | -1.9 | 10.8 |
| 99B20    | L    | 9.0        | F   | 130         | 0.04519 | Barrow   | 15.4 | 11.0 |
| 99B20    | R    | 9.0        | F   | 130         | 0.01997 | Barrow   | -6.1 | 10.8 |
| 99B21    | L    | 10.5       | M   | 174         | 0.03947 | Barrow   | 10.6 | 10.9 |
| 99B21    | R    | 10.5       | M   | 174         | 0.03739 | Barrow   | 8.8  | 10.9 |
| 99B22    | L    | 9.7        | F   | 175         | 0.04265 | Barrow   | 13.3 | 11.0 |
| 99B22    | R    | 9.7        | F   | 175         | 0.03227 | Barrow   | 4.4  | 10.8 |
| 99B23    | R    | 10.9       | M   | 192         | 0.04151 | Barrow   | 12.3 | 10.9 |
| 99B24    | A    | 8.8        | M   | NA          | 0.03714 | Barrow   | 8.6  | 10.8 |
| 99KK1    | L    | 7.7        | F   | 85          | 0.02819 | Kaktovik | 0.9  | 10.8 |
| 00B1     | L    | 8.9        | M   | NA          | 0.03081 | Barrow   | 3.2  | 10.8 |
| 00B10    | L1   | 9.4        | F   | 166         | 0.07944 | Barrow   | 44.7 | 13.0 |
| 00B10    | R1   | 9.4        | F   | 166         | 0.03720 | Barrow   | 8.6  | 10.8 |
| 00B10    | L2   | 9.4        | F   | 166         | 0.03865 | Barrow   | 9.9  | 10.9 |
| 00B10    | R2   | 9.4        | F   | 166         | 0.03285 | Barrow   | 4.9  | 10.8 |
| 00B11    | A    | 13.8       | M   | NA          | 0.09664 | Barrow   | 59.5 | 14.5 |
| 00B12    | L    | 10.8       | M   | 193         | 0.04386 | Barrow   | 14.3 | 11.0 |
| 00B12    | R    | 10.8       | M   | 193         | 0.03981 | Barrow   | 10.8 | 10.9 |
| 00B13    | A    | 9.4        | M   | 189         | 0.02222 | Barrow   | -4.1 | 10.8 |
| 00B14    | R    | 9.9        | F   | 191         | 0.03459 | Barrow   | 6.4  | 10.8 |
| 00B15    | A    | 8.9        | F   | NA          | 0.03185 | Barrow   | 4.1  | 10.8 |
| 00B15    | B    | 8.9        | F   | NA          | 0.03805 | Barrow   | 9.3  | 10.9 |
| 00B16    | R    | 10         | F   | NA          | 0.07938 | Barrow   | 44.6 | 13.0 |
| 00B17    | L    | 9.5        | M   | NA          | 0.03697 | Barrow   | 8.4  | 10.8 |
| 00B17    | R    | 9.5        | M   | NA          | 0.03785 | Barrow   | 9.2  | 10.9 |
| 00B18    | R    | 8.9        | F   | NA          | 0.03888 | Barrow   | 10.1 | 10.9 |
| 00B2     | L    | 14.5       | F   | 273         | 0.06028 | Barrow   | 28.3 | 11.7 |
| 00B2     | R    | 14.5       | F   | 273         | 0.05397 | Barrow   | 22.9 | 11.4 |
| 00B3     | R    | 14.6       | F   | NA          | 0.07478 | Barrow   | 40.7 | 12.7 |
| 00B4     | A    | 15.4       | F   | NA          | 0.08757 | Barrow   | 51.7 | 13.7 |
| 00B5     | R    | 18.9       | F   | 331         | 0.10366 | Barrow   | 65.5 | 15.2 |
| 00B6     | L    | 14.7       | M   | 232         | 0.04655 | Barrow   | 16.6 | 11.1 |
| 00B8     | R    | 8.6        | F   | NA          | 0.03005 | Barrow   | 2.5  | 10.8 |
| 00B9     | L    | 7.9        | F   | 70          | 0.03467 | Barrow   | 6.5  | 10.8 |
| 00B9     | R    | 7.9        | F   | 70          | 0.03238 | Barrow   | 4.5  | 10.8 |
| 00KK1    | L    | 9.2        | F   | 150         | 0.03563 | Kaktovik | 7.3  | 10.8 |
| 00KK2    | R    | 12.1       | M   | 214         | 0.06717 | Kaktovik | 34.2 | 12.1 |
| 00KK3    | L    | 8.8        | F   | 112         | 0.02788 | Kaktovik | 0.7  | 10.8 |