Age estimation for bowhead whales (*Balaena mysticetus*) using aspartic acid racemisation with enhanced hydrolysis and derivatisation procedures

D.L. Wetzel[1], J.E. Reynolds, III[1], P. Mercurio[1,2], G.H. Givens[3], E.L. Pulster[1,4] and J.C. George[5]

Contact e-mail: dana@mote.org

ABSTRACT

Accurate determination of the ages of individual whales is key to developing effective conservation strategies for the bowhead whale (*Balaena mysticetus*). Previous attempts to develop reliable methods of age determination for this species have included using body length and baleen length measurements, baleen carbon cycling analysis, assessments of corpora accumulation, and aspartic acid racemisation (AAR; conversion of L to D enantiomers) measurements. Each of these methods has its limitations. The primary objective of this study was to improve the AAR analysis technique for determining age in bowhead whales in order to obtain consistent, reproducible results for D/L ratios for estimated ages. Using a modified AAR method, lenses from 68 bowhead whales were analysed and ages estimated. A comparison of the results to previous ageing by corpora counting or baleen carbon cycling methods for 11 of the whales showed smaller standard errors for the AAR analyses. The modified AAR methods applied in this study increase the precision of D/L measurements and provide improved bowhead whale AAR results.

KEYWORDS: BOWHEAD WHALE; AGE DETERMINATION

INTRODUCTION

Conservation can be hindered by a lack of knowledge regarding the health of individuals, population demography and the extent to which threats are identified and managed (Reynolds *et al.,* 2005). The future long-term survival of bowhead whales (*Balaena mysticetus*) and other ice-adapted species will, in particular, be influenced by the direct effects of climate change (and resultant changes in human activities) on reproduction, longevity, and other life history parameters. The reliable age estimation of individual bowhead whales is important for the evaluation of such effects and the informing of conservation and management decisions.

For most marine mammals, age is calculated by counting growth layer groups (GLGs) in teeth (Hohn, 2009). However, GLG counting is not applicable for bowhead whales or other baleen whales (Suborder Mysticeti; Order Cetacea). Aspartic acid racemisation (AAR) of the lens nucleus provides an alternative method of ageing when no teeth are present. During gestation, two enantiomers (L and D) of aspartic acid with D/L ratios that are slightly greater than zero at the genesis of formation, are laid down in the nucleus of the lens where no metabolic activity occurs (Bada *et al.*, 1980). Thus, additional conversion of L to D aspartic acid in the lens nucleus takes place only due to racemisation over time following birth. The racemisation rate constant of aspartic acid enantiomers (K_{asp}) can be ascertained using the Arrhenius equation, which accounts for the effect of temperature on that constant and, therefore, on the reaction rate.

Although the use of AAR has shown considerable promise and led to several published analyses for age estimation of bowhead whales (Bada *et al.,* 1980; George *et al.,* 1999; Rosa *et al.,* 2004, 2013; Wetzel *et al.,* 2007), analytical problems have previously been encountered when using this method. The most troubling of these issues includes reproducibility of the D/L ratio as a consequence of the AAR analytical protocols, instrument response fluctuations, sample/standard instability and natural variability in living animals. Consequently, our primary goal was to examine and refine the amino acid analysis method used in previous studies of bowhead whales (Bada *et al.,* 1980; George *et al.,* 1999; Rosa *et al.,* 2004; 2013; Wetzel *et al.,* 2007) and other species (Olson and Sunde, 2002) to enhance precision of bowhead whale age estimates, and thus the value of those estimates to inform conservation and management decisions. Our results include the first age estimates for 57 whales not previously aged by any method.

MATERIALS AND LABORATORY METHODS

Sample acquisition

For decades, scientists with the North Slope Borough, Department of Wildlife Management (NSB-DWM), have worked closely with Alaska Native hunters to examine bowhead whales taken during subsistence hunts. During this period, a range of bowhead tissue samples were collected and archived, and basic biological data documented for each whale. Included in these collections were bowhead whale eyes from individuals spanning the breadth of age and reproductive status (fetus through adults), which had been preserved (frozen, intact). For this study, 68 archived bowhead eyes were selected from whales ranging in size and

[1] *Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, Florida, 34236.*
[2] *National Research Centre for Environmental Toxicology (Entox), The University of Queensland, Queensland Health Precinct, 39 Kessels Road, Coopers Plains, Qld 4108, Australia.*
[3] *Givens Statistical Solutions, LLC, 4913 Hinsdale Drive, Fort Collins, CO 80526.*
[4] *The University of South Florida, St. Petersburg, FL.*
[5] *Department of Wildlife Management, North Slope Borough, Barrow, AK 99734.*

possible ages for AAR analysis. All available fetal eyes were analysed for this study. While a range of age classes was selected as specimens for this study, we do not consider the dataset to be representative of the population age structure (i.e. it is not a random sample). The primary objectives of the paper were to investigate improvements of the AAR method for estimating the age of bowhead whales over a range of size classes and to estimate ages for a large number of whales not previously aged.

Eye lens removal and analysis of extracts

The methods employed for acquisition of lens nuclei were similar to those described by George *et al.* (1999), Olson and Sunde (2002) and Rosa *et al.* (2004; 2013). For each eye, the lens nucleus was removed and trimmed. One half of the nucleus was retained frozen in a clean glass vial, and the other half was analysed.

Sample extracts were hydrolysed and derivatised using methods modified from those previous studies. Analyses for D and L isomers of aspartic acid were done in triplicate on a HyperClone reverse phase C18 column (120A, 250 × 4mm, 5micron; Phenomenex, Torrance, CA, USA) using a high performance liquid chromatograph (HPLC; Agilent Technologies, Santa Clara, CA, USA), equipped with an autosampler and scanning fluorescence detector (λ_{ex} = 230nm; λ_{em} = 445nm). The HPLC flow rate was 1.5 ml/min, the column temperature was set to 30ºC and methanol (A), acetonitrile (B) and N-acetyl-L-cysteine (NAC) buffer (C) were used as eluants.

Hydrolysis

George *et al* (1999) and others have followed the methodological lead of J.L. Bada, to estimate age for large whales (e.g. Bada *et al.,* 1980). In general, previous work on bowhead amino acid racemisation employed a lens hydrolysis protocol using 6M HCL at 100ºC for 6 hours of hydrolysis. For this study, we evaluated a range of hydrolysis times and temperatures to optimise this step for bowhead lens amino acid preparation. Specifically, our study found that stable hydrolysis occurred using 6M HCl at 80ºC for 8 hours. With shorter hydrolysis time periods and/or higher temperatures, inconsistent hydrolysis was observed. Neither 80ºC nor 100ºC are temperatures of hydrolysis that could affect the aspartic acid D/L ratio (Goodfriend, 1997; Goodfriend and Myer, 1991).

Standards

The calibration of the D/L ratios measured by HPLC included eight different ratios of D and L isomer standards of aspartic acid which were analysed in triplicate for each set of 10 sample analyses. The standard ratios (D:L) used included: 0.5:99.5, 1:99, 2:98, 5:95, 10:90, 20:80, 30:70, and 50:50. We paid particular attention to designing a robust initial calibration followed by bracketing each set of three samples (all samples were run in triplicate) with a 5:95 D/L standard to confirm instrument and sample stability. The ability to generate stable and consistent calibration curves eliminates the need for daily adjustments or modelling instrument calibration responses (George *et al.*, 1999; Rosa *et al.*, 2004)

All standards in this study were corrected for trace cross contamination of the D isomer in the L isomer aspartic acid standard and vice versa. For each standard calibration curve analysis, we required the regression coefficient of determination (R^2) to be at least 0.99; otherwise a new standard calibration mixture was made and analysed until an acceptable R^2 value was achieved.

Derivatisation

Previous studies (e.g. George *et al.*, 1999; Olsen and Sunde, 2002; Rosa *et al.*, 2004) conducted derivatisations in which the amino acid sample extract was diluted 1:1000 with distilled water, and a subsample of the dilution was placed in a centrifuge with 10µl of OPA-NAC (ortho-phthaldialdehyde and N-acetyl-L-cysteine). This mixture was shaken for 20 seconds, and centrifuged for 15 minutes, at which time 475 µl of 0.05 M sodium acetate buffer was added. Finally, 200 µl of this solution was analysed by HPLC using methanol and sodium acetate for mobile phase at 1ml/min.

In contrast to the multi-step process described above, our study followed a different approach (Kaufman and Manley, 1998). We used OPA-IBLC (ortho-phthaldialdehyde and N-isobutyryl-l-cysteine) instead of OPA-NAC; our amino acid extract was rehydrated with 0.01M HCL and sodium azide (antibacterial) at 0.04 ml/mg lens and was subsequently was placed on the HPLC where derivatisation was performed in a single step within the autosampler syringe. Conducting the derivatisation in a single step within the syringe eliminates the possibility of inconsistencies or errors being introduced at each step of a separate derivatisation processes due to contamination and technician error. This in-needle derivatisation followed by immediate HPLC analysis greatly decreases chemical stability problems that have been observed with previous methods (George *et al.*, 1999; Olsen and Sunde, 2002; Rosa *et al.*, 2004).

STATISTICAL METHODS

Age estimation

Estimates of $(D/L)_i$ for the *i*th whale and $(D/L)_0$ are used to estimate age according to the equation

$$\mathrm{Age}_i = \frac{\log\{(1+(D/L)_i)/(1-(D/L)_i)\} - \log\{(1+(D/L)_0)/(1-(D/L)_0)\}}{2K_{asp}}$$

(Masters *et al.*, 1977; Bada *et al.*, 1980; George *et al.*, 1999). The $(D/L)_0$ value was estimated using an inverse variance weighted average of five values. The first value is 0.0250 (SE = 0.0013) from Rosa *et al.* (2013). This is estimated from a regression model using D/L data mostly for young whales of known ages (using corpora counts, baleen growth increments and fetal data). The remaining values are means of triplicate D/L measurements for four fetuses included in the present dataset. These values ranged from 0.0256 to 0.0293 with standard errors ranging from 0.0001 to 0.0005. The final estimated $(D/L)_0$ value used in the age equation is 0.0286 (SE = 0.0000629). For the *i*th whale, the observed data value of $(D/L)_i$ is taken to be the average of our three replicated measurements. We used K_{asp} = 0.000145 (SE 0.000145) from Rosa *et al.* (2013).

Variance estimation used a hybrid parametric and non-parametric bootstrap approach (Davison and Hinkley, 1997). The variance and 95% confidence interval were estimated

separately for each whale. Also, separately, for each whale, we re-sampled the three independent D/L measurements uniformly with replacement. Within each bootstrap iteration we also employed parametric re-sampling of $(D/L)_0$ and K_{asp}. The approximate correlation between the estimates of $(D/L)_0$ and K_{asp} is minimal (i.e. 2×10^{-10}), so this was ignored during re-sampling. Together, these bootstrap sampled quantities were used to generate one bootstrap pseudo-estimate of Age_i. We used 10,000 bootstrap replications for each whale. Confidence intervals were generated using the percentile method.

Growth curve estimation

We used age estimates from 238 whales to estimate growth curves. Of these, 182 were previously aged using AAR, corpora counts, and baleen ageing methods (Lubetkin *et al.*, 2008; George *et al.*, 2011; IWC data[6]). We fit the two-stage von Bertalanffy II (1938) model to estimate sex-specific growth curves. We included 68 whales with ages estimated in the current study, which includes 11 whales aged here that had also previously been aged. Altogether, these amount to all bowhead ages we know to exist, except 4 cases excluded as obvious outliers (96B1, 98B25, 00B16, 01KK1), one case with reported standard deviation equal to 0 (95B8F), one whale of unknown sex (00B8) and one pseudohermaphrodite (81WW2). Lubetkin *et al.* (2012) also removed outliers. Like those authors, when more than one estimated age was available for a whale we used the inverse variance weighted mean. We also adopted the same modelling approach used by Lubetkin *et al.* (2012) except that we did not account for the growth spurt they modelled. Although the growth spurt is biologically plausible, we found that the simpler model had superior Bayes Information Criterion and seem to yield as good a fit with less complexity.

[6] IWC Datasets 'AARAges06.txt' and 'ages.060927.csv' available from the IWC Secretariat, 135 Station Road, Impington, Cambridge, CB24 9NP, United Kingdom [*http://www.iwc.int*].

RESULTS

Age estimation

Table 1 provides estimated ages, bootstrap standard errors and bootstrap 95% confidence intervals for the age data for 68 bowhead whales. Negative age estimates can occur because the D/L values include measurement uncertainty and there may be minor model misfit and/or underestimation of uncertainty at the extreme lower range of our data. Such estimates should be interpreted as 'very young'. Three of the negative estimates are for foetuses and the fourth whale is probably a yearling (8.4m).

The most striking aspect of these results is that there is further evidence that some bowhead lifetimes may extend nearly 200 years or beyond. This is consistent with previous findings from other researchers (George *et al.,* 1999). Past recoveries of harpoons and bomb lances in landed

Table 1

Whale ID, length in meters (L), sex, age estimates (years), 95% confidence interval limits (%), standard error (SE).

Whale	L	Sex	Age	2.5	97.5	SE	Whale	L	Sex	Age	2.5	97.5	SE
81WW2	17.7	P*	73.3	55.6	100.1	11.6	05B21	8.8	F	12.9	9.8	17.9	2.1
96B5	14.9	F	121.5	91.7	167.9	19.5	05B25	13.2	F	14.8	11.2	20.5	2.4
97B5	10.1	F	4.9	3.7	6.7	0.8	05S5	16.5	F	47.3	36.0	64.6	7.4
97B7	13.2	F	13.0	9.9	18.0	2.1	05S7	18.0	F	81.5	61.7	112.2	13.1
97B8	13.6	F	18.5	14.0	25.7	3.0	06B6	13.3	M	28.0	21.0	38.7	4.5
97B10	16.7	F	58.2	43.9	80.4	9.3	06B10	6.3	F	1.3	1.0	1.9	0.2
97B12	15.3	M	67.4	51.1	92.7	10.7	06B18	14.4	M	54.6	41.4	75.3	8.7
98B4	13.1	F	22.0	16.7	30.1	3.5	07B8	14.9	M	87.3	66.1	120.7	14.0
98B5	15.1	M	95.4	72.1	130.9	15.2	07B9	14.3	F	31.4	23.7	43.3	5.1
98B10	13.0	F	13.7	10.4	19.0	2.2	07B9F*	4.1	F	–2.2	–3.1	–1.6	0.4
98B20	11.8	F	16.2	12.2	22.3	2.6	07B10	16.1	F	37.8	28.5	51.9	6.1
98B21	15.2	M	48.5	36.6	66.6	7.7	07B11	15.0	M	77.9	58.8	107.4	12.6
98WW2	14.1	F	20.9	15.7	28.8	3.3	07B12	14.8	F	32.1	24.4	44.2	5.1
02B2	16.7	F	51.9	39.4	71.8	8.3	07B13	16.6	M	88.5	66.9	121.3	14.2
02B3	19.2	F	106.3	80.5	145.8	16.9	07B16	14.4	F	28.4	21.5	38.9	4.5
02B5	8.5	F	4.7	3.6	6.5	0.8	07G3	15.3	F	39.8	29.7	55.6	6.6
02B17	9.3	F	7.4	5.6	10.1	1.2	07G4	15.2	F	29.1	21.9	40.3	4.7
02B21	10.0	F	12.5	9.4	17.3	2.0	07S1	10.0	M	9.4	7.1	12.8	1.5
02B22	8.1	F	1.6	1.2	2.3	0.3	07S2	8.3	F	7.4	5.5	10.3	1.2
03B6	13.9	F	19.3	14.5	26.6	3.2	07S3	10.7	M	17.7	13.3	24.5	2.9
03B9	16.4	F	68.3	51.6	93.4	11.0	07S4	15.2	F	34.8	26.3	47.7	5.5
04B4	14.2	F	22.1	16.8	30.3	3.5	08B14	13.6	F	27.3	20.8	37.7	4.4
04B5	16.8	F	80.0	60.3	110.9	12.9	08S3	19.1	F	187.6	141.7	258.1	30.2
04B5F*	4.1	F	0.7	0.5	1.1	0.1	09KK1F*	1.6	F	–3.2	–4.5	–2.4	0.5
04B8	13.6	F	23.9	18.2	32.8	3.8	10B15	12.5	M	20.0	15.1	27.5	3.2
04B9	14.9	F	18.5	14.0	25.6	3.0	11B3	17.5	F	55.9	42.4	77.1	9.0
04G2	8.7	F	4.3	3.2	5.9	0.7	11B4	7.8	F	2.5	1.2	4.1	0.7
04KK1	15.8	M	123.2	93.1	169.2	19.9	11B5	16.0	F	37.0	27.9	50.5	5.9
04KK2	6.7	M	0.0	–0.2	0.2	0.1	11B6	16.9	F	71.0	53.4	98.6	11.5
04KK3	8.4	F	7.0	5.2	9.8	1.2	11B7	15.4	F	157.2	119.5	217.2	25.1
04WW4	16.8	F	68.3	51.8	94.3	11.0	12B15	8.4	F	–0.6	–1.3	–0.2	0.3
05B8	8.2	M	1.7	1.1	2.5	0.4	12S2	13.6	M	23.2	17.6	32.3	3.8
05B11	12.1	F	18.6	13.9	25.6	3.0	12S2F*	3.8	F	–1.6	–2.4	–1.1	0.4
05B12	14.2	F	28.1	21.2	39.1	4.5	12S3	8.1	F	1.1	0.5	1.8	0.3

*F=foetus; P=pseudohermaphrodite.

whales also point to very long lifespans (George and Bockstoce, 2008).

The ages of 11 of the 68 whales assessed in this study have previously been estimated by other researchers using corpora counting (n = 9) and baleen isotope cycle analysis (n = 2) ageing techniques. Table 2 compares this study's estimates to other ageing results with the same whales. Although sample sizes for comparisons of age estimates using these different techniques are very small, it appears that the AAR estimate generated from this study is consistent with the two whales for which a baleen cycle age estimate had been determined (Lubetkin *et al.,* 2008). However, the corpora counting age estimates of George *et al.* (2011) for the same whales are generally higher than the age estimates determined from this AAR study.

Growth curve estimation

Fig. 1 shows a plot of the age estimates for the whales in this study, and 164 additional whales previously aged by other researchers. Each whale is represented by a circle (the point estimate) and a horizontal bar (spanning the 95% confidence interval for the corresponding age estimate). Red bars correspond to females, and males are represented by blue. The whales aged in our study are shown with heavier lines than for the whale ages from other researchers. The black lines in Fig. 1 show the fitted sex-specific von Bertalanffy II (1938) growth curves with female whales being larger than males of the same age.

It is worth noting that the confidence intervals in Fig. 1 are not used in the curve fitting, nor are they an output of it. They are merely to provide additional information about the age estimates using the best available information. Although they are broadly comparable, there may be some differences between methods, as examined in Table 2.

DISCUSSION

Considerable effort has gone into development and application of methods to age bowhead whales. Several approaches have emerged; each has some limitations. From the simplest to the most sophisticated, these methods assess body length, baleen length, baleen carbon cycling, number of corpora lutea and corpora albicantia, and AAR.

Measuring body length is not an effective method for estimating age. The correlation between body length and age in bowheads is poor, especially for older whales, and the relationship is sex-specific (Rosa *et al.,* 2011; George *et al.,* 1999). This phenomenon is well illustrated by the growth curves shown in Fig. 1.

Baleen length has also been used to estimate age. However, baleen is continuously worn down as bowheads grow older, and wear rates need to be estimated to apply the technique; hence, baleen length correlates best with age for whales under 10 years old (Lubetkin *et al.,* 2012) and is much less reliable for older individuals. Similarly, baleen carbon cycling analysis can only be reliably used for young whales (Lubetkin *et al*., 2008).

Corpora counting cannot be used for immature animals and is obviously not applicable for males. Resulting age estimates in mature female whales can have high standard errors similar to those from the earlier AAR age studies for bowheads (e.g. Olsen and Sunde, 2002; George *et al.,* 1999; 2011). To appropriately apply corpora ageing techniques, it is necessary to know life history and other parameters including age of sexual maturity, age of onset of senescence (or even whether there is senescence), ovulation rate (and potential changes thereof), and whether corpora albicantia persist through the life of the animal (Olsen and Sunde, 2002). Even so, the correlation of age estimates between corpora counting and AAR methods is surprisingly high for

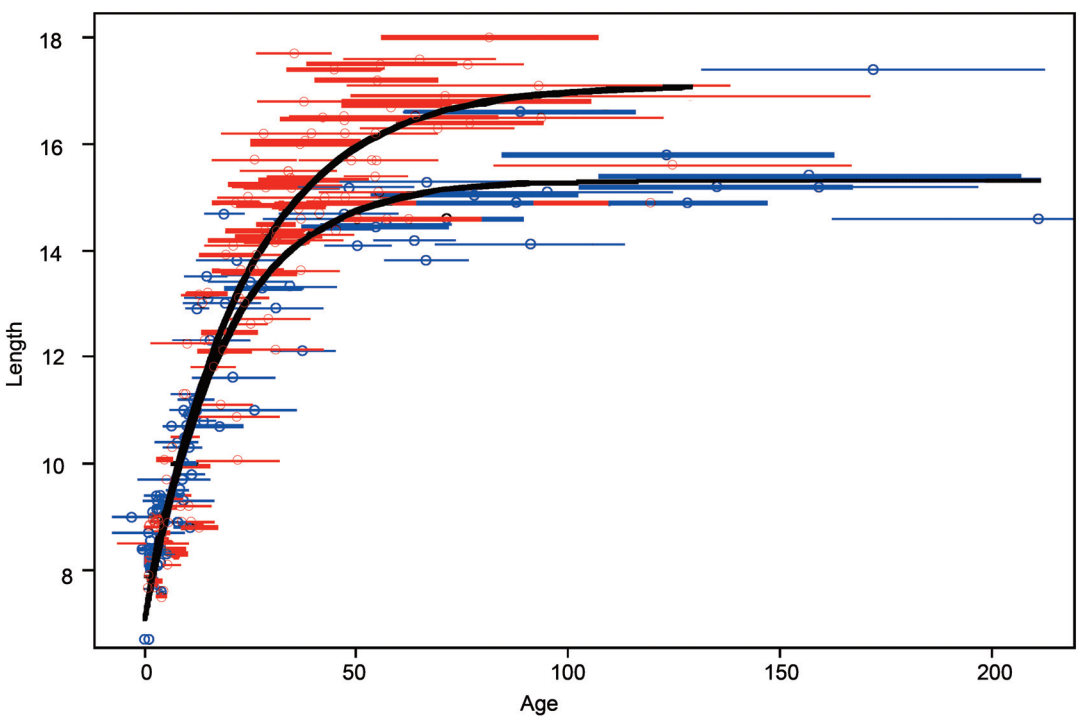

Fig. 1. Fitted von Bertalanffy growth curves. Each whale is represented by a circle (the point estimate) and a horizontal bar (spanning the 95% confidence interval). Red bars correspond to females and blue bars to males. The whales aged in this study are represented by thicker lines.

Table 2

Comparison of age estimates from other studies using alternative aging methods. L08 refers to Lubetkin *et al.* (2008). G11 refers to George *et al.* (2011). DAA refers to data available from the International Whaling Commission under its Data Availability Agreement (DAA).

Whale	Age	SE	Method	Citation
02B17	7.4	1.2	AAR	Here
	6.8	1.1	Baleen	DAA
02B2	51.9	8.3	AAR	Here
	79	18	Corpora	G11
	65.9	12.0	Corpora	DAA
02B21	12.5	2.0	AAR	Here
	11.7	2.3	Baleen	L08
02B3	106.3	16.9	AAR	Here
	139	38	Corpora	G11
	114.1	23.5	Corpora	DAA
03B9	68.3	11.0	AAR	Here
	102	26	Corpora	G11
	85.0	15.9	Corpora	DAA
04B8	23.9	3.9	AAR	Here
	31	6	Corpora	G11
04B9	18.5	3.0	AAR	Here
	43	8	Corpora	G11
05B12	28.2	4.6	AAR	Here
	38	7	Corpora	G11
96B5	121.4	19.5	AAR	Here
	125	38	Corpora	G11
	114.1	23.5	Corpora	DAA
97B10	58.2	9.3	AAR	Here
	65	14	Corpora	G11
	55.2	9.3	Corpora	DAA
97B8	18.5	3.0	AAR	Here
	31	6	Corpora	G11
	27.5	5.1	Corpora	DAA

mature female bowhead whales and CVs are sometimes comparable (George *et al.,* 2011).

In summary, alternative methods to AAR for age determination all have noteworthy drawbacks when applied to whole populations of bowhead whales. AAR appears to be the most promising method for population level assessments of age-specific life history parameters.

The alternative methods of age estimation mentioned above are fundamentally based on biological processes of individual whales (which can vary across a population based on health status, nutrition, and other factors). In contrast, AAR analysis is based on the rate of a chemical reaction governed only by physical-chemical processes, and as such, there is less inherent variation in response among individuals due to biotic factors (Bada *et al.,* 1980; George *et al.,* 1999; Rosa *et al.,* 2004; 2013; Wetzel *et al.,* 2007).

Although earlier studies using AAR provided valuable contributions to the development of a chemical analysis method for ascertaining ages of mysticetes, the modifications to the previously used AAR method offer improvement of an important tool for determining age. The specific AAR method modifications identified and applied in this study include (a) species specific hydrolysis time and temperature that optimises the preparation of the bowhead lens proteins for further AAR analysis; (b) using alternate amino acid derivatising reagents and (c) elimination of the separate amino acid derivatisation steps to reduce technician error and sample contamination, which helps to ensure stable and consistent results for greatly improved reproducibility.

The methodological changes provided D/L data that are remarkably consistent, and hence provide age estimates with greater precision than has been reported for other studies (Table 3). The average coefficients of variation (CVs) and ranges of CVs in this study were notably lower than those generated by four other AAR studies for estimating age of bowhead or minke whales (*Balaenoptera acutorostrata*). The use of this AAR modified method to provide estimates that have improved precision can enhance the value of ageing data and may help scientists to establish reliable age-specific vital rates (e.g. age at sexual maturation; reproductive rates; age-specific survival; longevity) of both sexes and all ages of bowhead whales. In turn, this may help resource managers consider possible changes in bowhead life history parameters in a changing Arctic environment. Moreover, having an accurate and precise ageing technique and a fitted growth curve for both sexes can help scientists and managers better understand whether particular demographic groups are most vulnerable to ship strikes and entanglements; assess changes over time in the extent to which fishing, shipping and other threats (e.g. contaminants) affect particular age groups; and proactively inform effective mitigation actions before consequences of threats become critical.

The benefits of using AAR assays to estimate age do not end with bowhead whales. The approach can be applied to other hard-to-age homeotherms (e.g. other mysticetes, birds, deep sea invertebrates) of ecological or economic importance to help provide improved life history information for better-informed management and conservation decisions.

ACKNOWLEDGEMENTS

This study could not have been done without funding provided by the North Slope Borough, Department of Wildlife Management, and the National Marine Fisheries Service. In addition to funding, the Borough provided incredible logistic support in the field as well as generous use of office space and computers. We are especially grateful to Cyd Hanns, Dr. Robert Suydam and Gay Sheffield for their support and specimen collection. We also thank the whaling captains and crews for allowing us to work with them during spring and fall hunts to acquire the specimens that were used in this study. Finally, we thank Drs. Judith Zeh and Susan Lubetkin (University of Washington) for

Table 3

Comparison of the CVs of estimated average ages, CVs of ranges of ages, and method differences from five AAR whale studies.

	George *et al*. (1999)	Olsen and Sunde (2002)	Rosa *et al.* (2004)	Rosa *et al.* (2012)	Wetzel *et al.* (this study)
Hydrolysis time and temperature	6 hrs @ 100°C	6 hrs @ 90°C	6 hrs @ 100°C	6 hrs @ 100°C	8 hrs @ 80°C
Derivatisation reagents	OPA-NAC	OPA-NAC	OPA-NAC	OPA-NAC	OPA-IBLC
Multi-step derivatisation	Yes	Yes	Yes	Yes	No
Average age estimate CVs	55%	78%	90%	34%	17%
Range of age estimate CVs	17–600%	22–245%	17–432%	16–143%	15–24%

sharing their expertise on bowheads. The bowhead samples were collected under NMFS Permit #17350 to the NSB Department of Wildlife Management.

REFERENCES

Bada, J.L., Brown, S. and Masters, P.M. 1980. Age determination of marine mammals based on aspartic acid racemization in the teeth and lens nucleus. *Rep. int. Whal. Commn* (Special Issue 3): 113–118.

Davison, A.C. and Hinkley, D.V. 1997. *Bootstrap Methods and Their Applications.* Cambridge University Press, Cambridge, England. 582pp.

George, J.C. 2009. Growth, morphology and energetics of bowhead whales (*Balaena mysticetus*). Doctoral Dissertation, University of Alaska Fairbanks. 168pp.

George, J.C., Bada, J., Zeh, J., Scott, L., Brown, S.E., O'Hara, T. and Suydam, R. 1999. Age and growth estimates of bowhead whales (*Balaena mysticetus*) using aspartic acid racemization. *Can. J. Zool.* 77: 571–80.

George, J.C. and Bockstoce, J.R. 2008. Two historical weapon fragments as an aid to estimating the longevity and movements of bowhead whales. *Polar Biology* (31):751–4.

George, J.C., Follmann, E., Zeh, J., Sousa, M., Tarpley, R. and Suydam, R. 2011. A new way to estimate whale age using ovarian corpora counts. *Can. J. Zool.* 89: 840–52.

Goodfriend, G.A. 1997. Aspartic acid racemization and amino acid composition of the organic endoskeleton of the deep-water colonial anemone *Gerardia*: determination of longevity from kinetic experiments. *Geochimica et Cosmochimica Acta*. 61:1,931–9.

Goodfriend, G.A. and Meyer, V.R. 1991. A comparative study of the kinetics of amino acid racemization/epimerization in fossil and modern mollusk shells. *Geochimica et Cosmochimica Acta*. 55:3,355–67.

Hohn, A.A. 2009. Age estimation. pp.11–17. *In:* W.F. Perrin, B. Würsig and J.G.M. Thewissen (eds.) *Encyclopedia of Marine Mammals*. Elsevier Inc., San Diego, CA. 1,352pp.

Kaufman, D.S. and Manley, W.F. 1998. A new procedure for determining DL amino acid ratios in fossils using reverse phase liquid chromatography. *Quat. Geochronology*. 17:987–1,000.

Lubetkin, S.C., Zeh, J.E., Rosa, C. and George, J.C. 2008. Age estimation for young bowhead whales (*Balaena mysticetus*) using annual baleen growth increments. *Can. J. Zool*. 86:525–38.

Lubetkin, S.C., Zeh, J. and George, J. 2012. Statistical modeling of baleen and body length at age in bowhead whales. *Can. J. Zool*. 90(8):915–31.

Masters, P.M., Bada, J.L. and Zigler, J.S. 1997. Aspartic acid racemization in the human lens during ageing and in cataract formation. *Nature.* 268:71–3.

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

Reynolds, J.E., Perrin, W.F., Reeves, R.R., Ragen, T.J. and Montgomery, S. (eds.) 2005. *Marine Mammal Research: Conservation Beyond Crisis.* Johns Hopkins University Press, Baltimore, Maryland. 223pp.

Rosa, C., George, J. C., Zeh, J., Botta, O., Zauscher, M., Bada, J. and O'Hara, T. M. 2004. Update on age estimation of bowhead whales (*Balaena mysticetus*) using aspartic acid racemization. Paper SC/56/BRG6 presented to the IWC Scientific Committee, June 2004 Sorrento, Italy (unpublished). 15pp. [Paper available from the Office of this Journal]

Rosa, C., Zeh, J., George, J.C., Botta, O., Zauscher, M., Bada, J. and O'Hara, T.M. 2011. Age estimates based on aspartic acid racemization for bowhead whales (*Balaena mysticetus*) harvested in 1998–2000. Paper SC/63/BRG5 presented to the IWC Scientific Committee, June 2011, Tromsø, Norway (unpublished). 14pp. [Paper available from the Office of this Journal].

Rosa, C., Zeh, J., George, J. C., Botta, O., Zauscher, M., Bada, J. and O'Hara, T.M. 2013. 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. *Mar. Mamm. Sci.* 29:424–45.

Von Bertalanffy, L. 1938. A quantitative theory of organic growth (Inquiries on growth laws, II). *Hum. Biol.* 10(2):181–213.

Wetzel, D.L., Mercurio, P., Reynolds, J.E. and George, J.C. 2007. The pursuit of precise and accurate methods to determine ages of bowhead whales (*Balaena mysticetus*). Paper presented at 17th Biennial Conference on the Biology of Marine Mammals, Cape Town, South Africa, November–December 2007.