

# Age estimation for young bowhead whales (*Balaena mysticetus*) using annual baleen growth increments

S.C. Lubetkin, J.E. Zeh, C. Rosa, and J.C. George

**Abstract:** We compiled age estimates and baleen plate  $\delta^{13}\text{C}$  data from 86 bowhead whales (*Balaena mysticetus* L., 1758). We used previous whale age estimates based on aspartic acid racemization (AAR) and corpora counts to extend the use of  $\delta^{13}\text{C}$  data for age determination from cycle counting to a modified exponential model using annual baleen growth increments. Our approach used the growth increment data from individual whales in a nonlinear mixed effects model to assess both population-level and whale-specific growth parameters. Although age estimates from baleen-based models become less precise as the whales age, and baleen growth and length near steady state, the growth increment model shows promise in estimating ages of bowhead whales 10–13.5 m long with baleen lengths <250 cm, where other techniques are less precise or the data are scarce. Ages estimated using the growth increment data from such whales ranged from 6.4 to 19.8 years.

**Résumé :** Nous avons compilé les estimations d'âge et les données de  $\delta^{13}\text{C}$  des fanons chez 86 baleines franches boréales (*Balaena mysticetus* L., 1758). Nous avons utilisé des estimations antérieures de l'âge des baleines basées sur la racémisation de l'acide aspartique (AAR) et le dénombrement des corps ovariens pour élargir l'utilisation des données de  $\delta^{13}\text{C}$  pour la détermination de l'âge, d'une énumération des cycles à un modèle exponentiel modifié utilisant les incréments annuels de croissance des fanons. Notre méthodologie emploie les données d'incrément de croissance chez des baleines individuelles dans un modèle non linéaire à effets mixtes afin d'évaluer les paramètres de croissance, tant au niveau de la population que celui de baleines particulières. Bien que les estimations de l'âge obtenues des modèles basés sur les fanons deviennent moins précises à mesure que les baleines vieillissent et que la croissance et la longueur des fanons s'approchent d'un état d'équilibre, le modèle des incréments de croissance semble offrir la possibilité d'estimer l'âge des baleines franches boréales de taille de 10–13,5 m de longueur et avec les fanons de longueur <250 cm, lorsque les autres techniques sont moins précises ou lorsque les données sont restreintes. Les estimations d'âge obtenues à partir des données d'incrément de croissance chez ces baleines vont de 6,4 à 19,8 années.

[Traduit par la Rédaction]

## Introduction

Historically, the ages of bowhead whales (*Balaena mysticetus* L., 1758) have been difficult to determine, and, as a result, many of their life-history parameters are better known in terms of length than age. Photographic identification has been limited by lack of identifying marks, especially in young whales. In addition, even when animals are marked, it is difficult to obtain repeated observations of individuals from large populations that range over large, remote areas. Current techniques allow reasonable age estimation of mature whales using aspartic acid racemization (AAR) (George et al. 1999; Rosa et al. 2004) and counts of corpora albicantia and ovulation rates in sexually mature females (George et al. 2004). While age at sexual maturity is estimated to be in the late teens to late twenties (Schell and Saupé 1993; Zeh et al. 1993; George et al. 1999, 2004), it is

more clearly known as occurring when whales reach 12–14 m long (Koski et al. 1993), with the majority of females reaching sexually maturity by 13.5 m (George et al. 2004) and males by 12.5–13 m (O'Hara et al. 2002). The smallest female known to be sexually mature was 12.6 m long, and the largest known immature female was 14.2 m (George et al. 2004). The smallest male known to be sexually mature was 12.7 m long (O'Hara et al. 2002).

Baleen length and body length are not well correlated in bowhead whales <9 m long or with baleen <200 cm (Schell et al. 1989a). For these young whales, baleen length is a better indicator of age than body length, particularly if we assume that in the first few years of life growth resources are dedicated to forming the baleen structure which will enable them to feed, with body length increases occurring later. D.M. Schell and others (Nerini et al. 1987; Schell et al. 1989a, 1989b; Withrow et al. 1992; Schell and Saupé

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1993) have investigated using baleen plate isotopic patterns as a tool for determining ages of bowhead whales. Most bowheads in the western Arctic population migrate annually from wintering areas in the Bering Sea to their spring and summer grounds in the Beaufort Sea. In simplified terms, plankton from the Bering and Chukchi seas have a more enriched (higher)  $\delta^{13}\text{C}$  than plankton from the Beaufort Sea. Baleen grows throughout the life of a bowhead and reflects the isotopic composition of the food that the whale had ingested over a lifetime. As the whales migrate from the Bering and Chukchi seas in spring to the Beaufort Sea in summer and return to the Bering and Chukchi seas in fall, they eat plankton with distinctly different isotopic signatures, and those differences are reflected in the baleen isotopic signatures. The regular  $\delta^{13}\text{C}$  peaks (winter feeding) and valleys (summer feeding) in the baleen isotopic records are thought to be indicative of feeding in locations with isotopically different food sources. If so, by counting the number of peaks (or valleys), the age of the baleen can be estimated. However, baleen age estimates can be misleading, as the growing baleen is continuously worn away. This wear may cause the number of cycles in the baleen to under-represent the ages of the whales. Also, occasionally, a bowhead may not migrate to the Beaufort Sea in spring, and thus, the baleen signature will be missing evidence of that annual migration.

The purpose of this work is to use annual baleen growth increments to estimate ages of bowhead whales for which other aging techniques are impossible or inaccurate. Specifically, we wish to close the gap in age-estimate coverage between mature whales for which AAR-based and (or) corpora-count-based estimates are reliable and the younger whales for which annual baleen growth increments can simply be counted. In a practical sense, this means we wish to use baleen-based techniques up to a maximum baleen length of about 250 cm. Whales with longer baleen will be at a point where the baleen-based techniques become less precise and where other techniques are available and preferable.

## Materials and methods

### Data used

Our data are a compilation of  $\delta^{13}\text{C}$  measurements at incremental lengths from the baleen plates of 86 whales, 40 of which (Table 1) had age estimates available for fitting the growth increment model based on  $\delta^{13}\text{C}$  cycle counts, AAR (Rosa et al. 2004), and (or) corpora counts (George et al. 2004). Portions of the isotopic data were provided by D.M. Schell (University of Alaska Fairbanks), and D. Withrow (National Oceanographic and Atmospheric Administration, NOAA). Other baleen plates were sampled by C. Rosa. S.C. Lubetkin transcribed an additional three plates (whales 66B, 68B, and 71B) from published plots of isotopic data (Nerini et al. 1987; Schell et al. 1989b).

### Baleen isotopic analysis

The collection, sampling, and isotopic analysis methodology for the data from D. Withrow and D.M. Schell are presented in their previous works (Schell et al. 1989a, 1989b; Schell 1992; Withrow et al. 1992). For the new data shown here, single baleen plates were collected from 31 bowhead

whales over an 8-year period (1990 and 1997–2003). All samples were obtained via the Inupiat bowhead whale subsistence hunt at Barrow, Alaska, and Kaktovik, Alaska. The sample collections were conducted with permission of the Barrow Whaling Captain's Association and the Alaska Eskimo Whaling Commission through the Department of Wildlife Management (North Slope Borough, Alaska) under the purview of NOAA permits (#932-1489-00 and 932-1489-03 for the Marine Mammal Health and Stranding Response Program).

For stable isotope analysis of the new baleen plates, the tissue at the base of each baleen plate was removed with a metal paint scraper. Samples were obtained from the outside ("nonhaired") edge of the baleen with an engraving tool at 1 or 2 cm intervals, starting at the base of the plate (below the gum line). The samples were then weighed in tin cups and  $\delta^{13}\text{C}$  measured via elemental analysis – isotope ratio mass spectrometry (EA-IRMS). This method utilizes a Costech Elemental Analyzer (ESC 4010) and a Finnigan MAT ConFlo III interface with a Delta+XL Mass Spectrometer. The combustion reactor consists of a reaction tube packed with chromium oxide and silver/cobalt oxide. The reduction tube was packed with reduced copper wire. Other elemental analyzer (EA) conditions are listed in Table 2.

Dried, homogenous samples were placed in tin capsules. The tin capsules were then closed and placed in the EA autosampler. The samples were then dropped into the EA, where they were combusted. The  $\text{CO}_2$  combustion gas was separated chromatographically and then transferred to the IRMS, where the isotopes were measured.  $\delta^{13}\text{C}_{\text{PDB}}$  values were reported in reference to international isotope standards.

The quality assurance and quality-control scheme involved analyzing tin-capsule blanks and laboratory working standards. Blanks were analyzed every 20 samples and working standards (Peptone: No. P-7750 meat-based protein; Sigma Chemical Company lot #76f-0300) were analyzed every 10 samples. Twice a year the laboratory working standards are compared with the National Institute of Standards and Technology standards to confirm quality assurance.

### $\delta^{13}\text{C}$ cycle sampling idiosyncrasies

The baleen isotopic data had sampling inconsistencies. First, not all of the plates were sampled along their entire lengths. Second, the sampling interval varied both by and within component data sets. Some plates were sampled as finely as every 1 cm for the entire plate length, others every 5 cm. The plates prepared by C. Rosa were the most finely sampled on a consistent basis, with intervals of 1 or 2 cm for all whales. The data from D.M. Schell also contained some plates sampled every 1 or 2 cm, but usually only for a short section of the baleen. Most of the plates from D.M. Schell were sampled every 2.5 cm, although some sampling was at 5 cm intervals. The plates from D. Withrow were sampled every 2.5 or 5 cm. Older plates were often sampled at coarser intervals. However, it became apparent that 5 cm sampling increments were too large for accurately characterizing the  $\delta^{13}\text{C}$  patterns, especially if the stable isotope data were also to be used to study feeding or nutrition. When possible the baleen plates sampled were the longest ones for each whale. Samples from neighboring plates and plates across from one another in the mouth confirm that

**Table 1.** Age estimates of bowhead whales (*Balaena mysticetus*) used in fitting the growth increment model.

Growth increment model	<i>n</i>	Length range (m)		Estimated age range (years)
		Body	Baleen	
Cycle counting				
Males	14	7.6–10.4	1.18–1.90	4–11
Females	10	7.5–10.3	1.30–1.92	3.5–9
AAR				
Males	3	14.5–17.4	2.89–3.84	57–172
Cycle counting and AAR				
Males	2	10.5–10.9	1.74–1.92	9.6–9.8
Females	1	9.7	1.75	5.2
Corpora counts				
Females	8	13.6–17.7	2.60–3.56	27.5–52
Corpora counts and AAR				
Females	2	14.5–17.5	2.73–3.31	31–73.2
Total	40			

Note: AAR, aspartic acid racemization.

**Table 2.** Elemental analyzer conditions (ESC 4010).

	ESC 4010
Combustion tube temperature (°C)	1020
Reduction tube temperature (°C)	700
Helium flow rate (mL/min)	120
GC column (length (m); sieve (Å mol))	3; 5
GC oven temperature (°C)	70
Water trap	Magnesium perchlorate

isotopic patterns are internally consistent within each whale (data not shown).

### Modeling overview

We aged the smallest whales by examining the  $\delta^{13}\text{C}$  patterns in the baleen, counting the number of cycles present, and adjusting this value by the number of early growth cycles that appeared to be missing or partial. Slightly older whales were aged by fitting a growth model to the baleen growth increments, using the youngest whales to characterize the initial decrease in baleen growth rate and older whales with age estimates from AAR or corpora counts to estimate the eventual asymptotic baleen growth rate.

We looked at the changing baleen growth increments to estimate the parameters governing baleen growth. Once we had estimates of those values, we were able to use them with growth increments from whales with up to 250 cm baleen to arrive at age estimates and associated standard errors (SEs). All modeling and statistical calculations were performed using S-PLUS.

### Young whales — age estimates and age-specific baleen growth increments

It is critical to eliminate as much subjectivity and reader bias as possible from counting the number of cycles in the baleen (Lawson et al. 1992; Hohn and Fernandez 1999). S.C. Lubetkin and C. Rosa independently used patterns in the  $\delta^{13}\text{C}$  from 24 (12 male and 12 female) of the smallest (and presumably youngest) whales to estimate their ages (Table 3). S.C. Lubetkin estimated the ages of the remaining

35 young whales (20 male and 15 female). The subset of whales with two age estimates allowed us to evaluate the consistency of ages estimated by different examiners working independently and to calculate SEs of the estimated ages.

We also measured the annual baleen growth increments in the youngest whales. First we established “landmarks” in the baleen patterns of the very smallest of these whales, which often did not have baleen lengths >120 cm and few of the regular cycles that are common in older whales. Following the methodology of Schell and Saupe (1993), we aligned growth increments, measured valley to valley (i.e., from the end of one summer to the end of the next summer), of similar lengths across whales. After aligning growth increments across whales, we calculated mean increment lengths of the complete annual cycles. Age estimates in these young whales were the result of the number of cycles present in the baleen and how the pattern of baleen cycles and increments aligned with other young whales. The distance from the last  $\delta^{13}\text{C}$  valley to the proximal terminal end of the baleen was always counted as an incomplete baleen cycle, regardless of its length. Assuming that all whales were born in spring, we adjusted the age estimate of fall-caught whales by 0.5 years (Fig. 1). If that last partial (by definition) cycle was over half the length of its preceding cycles, we interpreted the truncated cycle as a portion of the next full  $\delta^{13}\text{C}$  cycle and added 0.5 years to the counted cycles to arrive at the age estimate. If the last partial cycle was short, then we interpreted that last partial cycle as noise around the true  $\delta^{13}\text{C}$  valley, indicating that the cycle count was too high, and we subtracted 0.5 years from the age estimate (Whale 99B22, Fig. 1a). In general the fall–fall increments were easier to measure unambiguously, with the spring  $\delta^{13}\text{C}$  peaks being softer than the fall valleys. However, the number of cycles counted remained the same regardless of which counting method was used.

### Growth increment model

We based our growth increment model on annual growth increments from young whales aged 3 years or older based

**Table 3.** A subset of 24 youngbowhead whales (*Balaena mysticetus*) for which S.C. Lubetkin (SL) and C. Rosa (CR) independently estimated ages from  $\delta^{13}\text{C}$ .

Whale	Sex	Body length (m)	Baleen length (m)	Age estimates (years)			
				SL	CR	Mean	Difference
99B19	F	8.1	0.74	1.5	1.0	1.25	0.5
98B14	M	8.2	0.74	1.5	1.5	1.5	0
01B23	F	8.0	0.84	1.5	1.5	1.5	0
01B16	M	8.6	0.94	1.0	2–2.5 <sup>a</sup>	1.63	–1.25
01B22	F	8.2	0.87	2.0	1.5	1.75	0.5
99KK1	F	7.7	0.85	1.5	2.5	2.0	–1.0
01B13	F	8.2	0.94	2.0	2.0	2.0	0
99B20	F	9.0	0.98	2.5	2.0	2.25	0.5
00KK3	F	8.8	1.12	2.5	2.5–3 <sup>a</sup>	2.63	–0.25
03B3	F	9.0	1.18	3.0	2.5–3 <sup>a</sup>	2.88	0.25
01B10	M	8.1	1.26	3.0	3.5	3.25	–0.5
01B12	M	9.1	1.41	3.0	3.5	3.25	–0.5
98B13	M	9.3	1.38	3.5	3.0	3.25	0.5
01KK1	F	13.2	1.48	3.5	4.5	4.0	–1.0
01B21	M	8.9	1.41	4.5	4.5	4.5	0
99KK3	M	8.3	1.30	3.5	7.0	5.25	–3.5
99B22	F	9.7	1.42	5.5	5.0	5.25	0.5
98B24	F	10.3	1.75	7.5	5.5	6.5	2.0
02B19	M	9.4	1.63	7.5	6.5	7.0	1.0
00B13	M	9.4	1.76	6.5	8.0	7.25	–1.5
02B17	F	9.3	1.85	8.5	8.0	8.25	0.5
02B10	M	9.5	1.76	8.5	8.0	8.25	0.5
99B23	M	10.9	1.60	10.5	8.0	9.25	2.5
99B21	M	10.5	1.74	10.5	8.5	9.5	2.0

**Note:** Whales are ordered by increasing mean age.

<sup>a</sup>In cases where C. Rosa left the estimate as an age range, we used the midpoint of the range to compute means and differences in the paired *t* test and *F* test.

on wear-adjusted  $\delta^{13}\text{C}$  cycle counts and from older whales for which we had both isotopic data and an independent estimate of age from AAR and (or) corpora counts. These large, old whales were critical for achieving a reasonable estimate of the intrinsic asymptotic baleen growth rate parameter. Whales 86KK2 and 87B6 had large sections of their baleen sampled every 5 cm rather than every 2.5 cm. We only used the youngest baleen (grown when the whale was oldest) that was sampled every 2.5 cm from these whales in these analyses. Even with this restriction on the baleen used, we had nine complete cycle lengths from whale 86KK2 and six from 87B6.

We chose a model structure using nonlinear regression and compared the residuals and residual standard errors (RSEs) of several growth models. After considering Gompertz, von Bertalanffy, and exponential models, we chose a modified exponential decay equation to model the decreasing baleen growth increments. The modification allowed for an intrinsic baleen growth rate that persists after the initial burst of baleen growth. This growth model does not account for any potential nursing effects that would increase baleen growth in the years 1 and 2. Therefore, for ages  $t \geq 3$ ,

$$[1] \quad \text{Growth (cm/year)} = G \times [1 + \exp(-k \times t)]$$

This intrinsic growth rate  $G$  is necessary to preserve baleen length, since the distal end is worn away as the

whale ages. The parameter  $k$  controls the rate at which the exponential function decays. Since the growth rate declines with age,  $k > 0$ .

#### Nonlinear mixed effects models with fixed (sex) and random effects

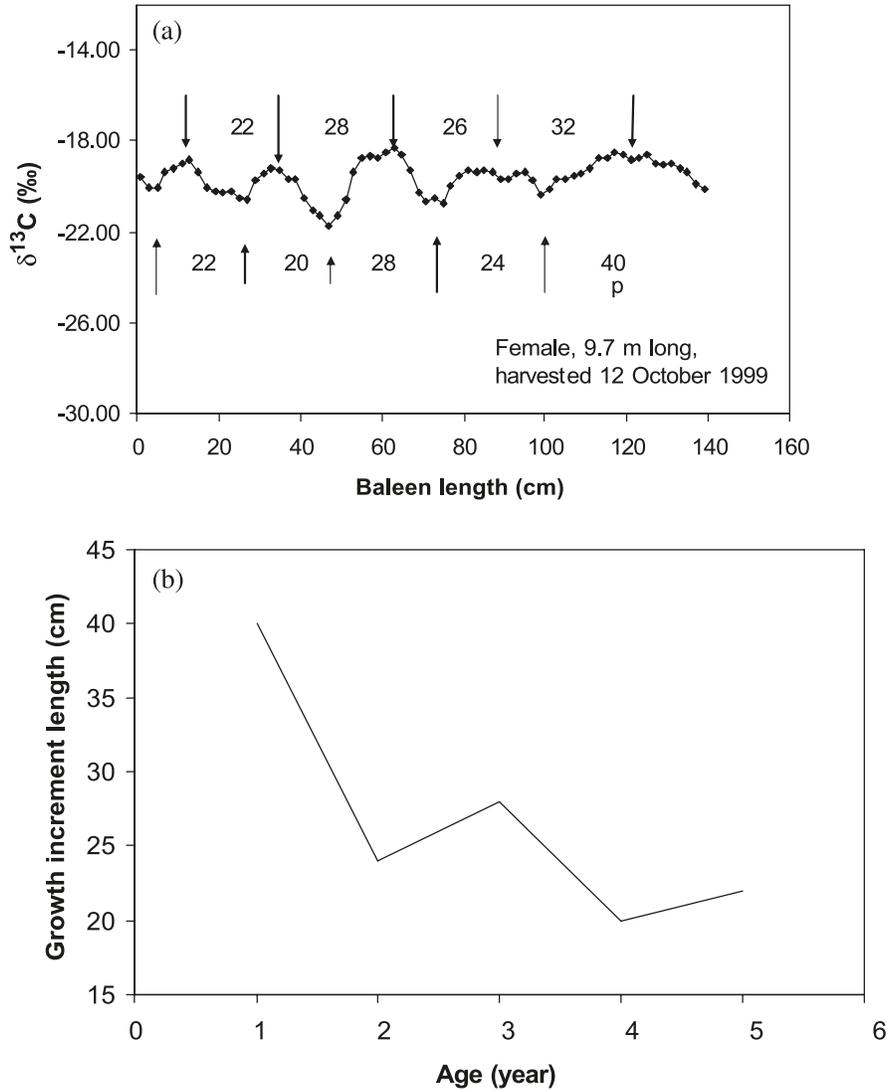
We used nonlinear mixed effects (NLME) models to estimate parameter values, which allowed us to fit each whale's growth curve specifically and see how much variation there was around the population parameters (Pinheiro and Bates 2000). NLME models allowed us to examine how much variability there was around the predicted growth curve by whale, by group (males vs. females), and within the entire population. We were also able to test if there were fixed (i.e., sex) or random effects (whale-to-whale variability) on  $G$  and (or)  $k$ .

We examined models with sex effects on  $G$  and (or)  $k$  and random effects on  $G$  and (or)  $k$  together, and chose the best model fit using the Bayesian information criterion (BIC), a measure which takes into account the likelihood of the data given the parameter estimates and the complexity of the model, using

$$[2] \quad \text{BIC} = -2 \log\text{Lik} + n_{\text{par}} \log(N)$$

where  $n_{\text{par}}$  is the number of parameters in the model,  $N$  is the number of observations, and  $\log\text{Lik}$  is the log of the

**Fig. 1.** (a) The  $\delta^{13}\text{C}$  trace for young bowhead whale (*Balaena mysticetus*) 99B22, a 9.7 m long female harvested 12 October 1999, which had a cycle count-based estimated age of 5.5 years (by S.C. Lubetkin). The distal end of the baleen is at the right end of the graph. This is the oldest baleen, grown when the whale was youngest. The upper arrows show where we could measure annual spring-to-spring growth increments, with growth increment lengths (in cm) shown between them. The lower arrows show where we could measure annual fall-to-fall growth increments, with growth increment lengths (in cm) shown between them. Partial growth increments are denoted with a “p”. (b) The age-specific growth increments for whale 99B22. Ages shown are the ages in the spring, at the points shown by the upper arrows in Fig. 1a.



likelihood (Pinheiro and Bates 2000). Smaller BIC values correspond to simpler models with better fits to the data.

We first used a jackknife procedure to determine whether particular whales had undue influence on the fit that resulted in biologically implausible parameter estimates. We eliminated one whale that did from all subsequent analyses. We fit the growth increment model using a NLME model and the multiple observations from each of the remaining whales for which we had age estimates and cycle-length data to arrive at estimates of  $G$  and  $k$ .

**Age estimation**

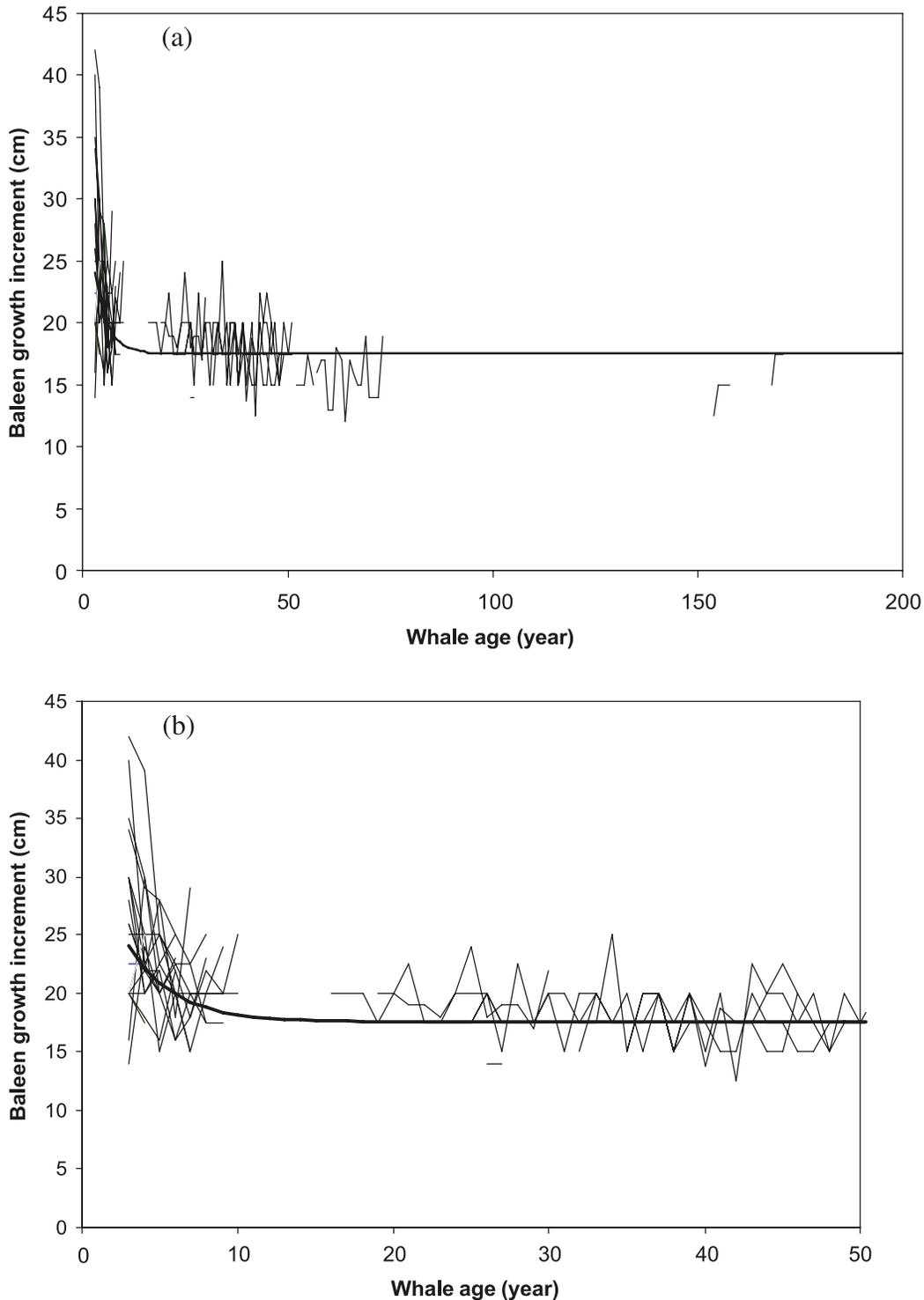
After fitting the growth increment model, we used nonlinear least squares analysis to estimate the ages of whales lacking age estimates obtained by other methods. We used two variants of the NLME model to estimate whale age.

The first of these used nonlinear least squares on annual growth increments without a random effect on  $G$ :

$$[3] \quad \text{Growth (cm/year)} = G \times \{1 + \exp[-k \times (t - \text{CycleDiff})]\}$$

where CycleDiff was the difference between the position of a given baleen cycle and the last baleen cycle grown. For the newest baleen cycle (produced when the whale was oldest), there is no difference between the whale’s age  $t$  and the cycle number. None of the cycle lengths was old or long enough to be 1st- or 2nd-year baleen. (Had 1st- or 2nd-year baleen been present, we would have been able to estimate age via the cycle-counting technique.) Our second age estimate used nonlinear least squares analysis on the annual growth increments with a random effect on  $G$ , and yielded estimates of whale age and  $G_{\text{random}}$ :

**Fig. 2.** (a) Age-specific growth increments for bowhead whales (*Balaena mysticetus*). The heavy solid line is the growth increment model fit by NLME, where  $G = 17.55 \text{ cm}\cdot\text{year}^{-1}$  and  $k = 0.332 \text{ year}^{-1}$ . (b) A detail of the growth increments, focusing on growth to age 50.



$$[4] \quad \text{Growth (cm/year)} = (G + G_{\text{random}}) \times \{1 + \exp[-k \times (t - \text{CycleDiff})]\}$$

We limited our age estimation using eqs. 3 and 4 to whales  $\leq 13.5 \text{ m}$  long with  $< 250 \text{ cm}$  of baleen. Whales exceeding those body and baleen lengths had cycle lengths that were too uniform to get reasonable age estimates, as

the baleen growth curve flattens out very quickly (Fig. 2, Table 4). With the exception of whales 98KK1, 98KK2, and 98KK3 which had only part of their baleen plates sampled, whales with ages estimated using these models had between 6 and 11 complete baleen growth increments. Our purpose in using two methods in estimating the ages of the whales was twofold: first, to investigate the effects of

**Table 4.** Annual  $\delta^{13}\text{C}$  valley to valley (autumn to autumn) baleen growth increments in young bowhead whales (*Balaena mysticetus*).

Year	Males			Females			Schell and Saupe (1993)
	Baleen length (cm)	SD	<i>n</i>	Baleen length (cm)	SD	<i>n</i>	
0	25.92	8.00	13	21.00	6.90	12	
1	53.71	10.84	12	53.47	5.37	15	>45
2	35.45	7.23	11	34.96	7.13	14	35–45
3	24.89	7.55	14	24.08	7.39	12	27.5–35
4	24.13	3.87	12	23.72	6.22	9	<27.5
5	21.45	3.85	11	23.75	3.50	4	
6	20.25	2.97	10	23.17	1.61	3	
7	20.00	4.35	9	20.00	—	1	
8	21.07	2.46	7	17.50	—	1	
9	20.30	2.33	5				
10	22.50	3.54	3				
≥50	15.63	1.19	3 (13) <sup>a</sup>	17.32	1.33	4 (25) <sup>a</sup>	

**Note:** Schell and Saupe (1993) also measured growth increments as the distance between  $\delta^{13}\text{C}$  valleys. The year-0 increment includes all baleen grown until the whale's first autumn, including fetal baleen. The year-1 growth increment begins at the whale's first autumn.

<sup>a</sup>There were three males with growth increment measurements occurring at ages ≥50 years. There were 5, 4, and 4 cycles observed in those males for a total of 13 observations in older males. There were four females with growth increment measurements occurring at ages ≥50 years. There were 15, 4, 4, and 2 cycles observed in those females for a total of 25 observations from older females.

including  $G_{\text{random}}$  in the age-estimation process; and second, to have an alternative method for estimating the SEs of the age estimates.

We used a bootstrapping procedure to estimate whale ages and their SEs. We created our bootstrap samples from the known age whales used in fitting the NLME model. The jackknife procedure to check for individual whales with undue influence on the NLME model fit identified one outlier whale that was removed from subsequent analyses. The presence of that whale led to an estimate of  $G$  around 4  $\text{cm}\cdot\text{year}^{-1}$  smaller than any measured growth increment, as well as an unrealistically small estimate of  $k$ .

We drew a sample of 39 whales from the remaining 39 whales of known age (sampling with replacement). We re-fit the NLME model using the bootstrap sample to estimate  $G$ ,  $k$ , and  $\text{SD}(G_{\text{random}})$ , which is a measure of individual variation among whales' asymptotic growth rates. We created 100 bootstrap samples for each whale we wished to estimate the age of. The bootstrap parameter values were used in two methods, first with a  $G_{\text{random}}$  value drawn from the characterization of the overall random effects in the population (method 1; Fig. 3), and second with a whale-specific  $G_{\text{random}}$  drawn from solving eq. 4 (method 2; Fig. 3). In method 1, we used the estimated  $k$  in eq. 3. However, instead of using the estimated  $G$  alone, we drew a  $G_{\text{random}}$  from  $N(0, \text{SD}(G_{\text{random}}))$  and added it to the estimated  $G$ . Using the resulting sum for  $G$  in eq. 3 gave greater variation in the asymptotic baleen growth rates, instead of just whatever that particular iteration's population mean was. (With many iterations the  $G_{\text{random}}$  should still be symmetrically distributed around 0.) Then we used nonlinear least squares (NLS) to solve eq. 3 for  $t$  using the parameter estimates from the bootstrap NLME model fit. We calculated the mean and SD of the resultant estimates of  $t$ . We estimated age as the mean and its SE as the SD over the 100 bootstrap iterations.

Method 2 parallels method 1, but has an important preliminary step (Fig. 3). We began by using NLS to estimate  $t$  and  $G_{\text{random}}$  using  $G$  and  $k$  from the NLME model fit. Then  $G_{\text{random}}$  in eq. 4 was drawn from a normal distribution with mean being the NLS estimate of  $G_{\text{random}}$  and SD being the estimated SE of this estimate. Thus,  $G$  and  $k$  in eq. 4 were obtained from the NLME model fit to the bootstrap sample, but  $G_{\text{random}}$  was drawn from an estimated distribution specific to the whale whose age was being estimated. Then  $t$  was the only parameter remaining to be estimated from eq. 4, and estimated age and its SE were obtained as when eq. 3 was used.

## Results and discussion

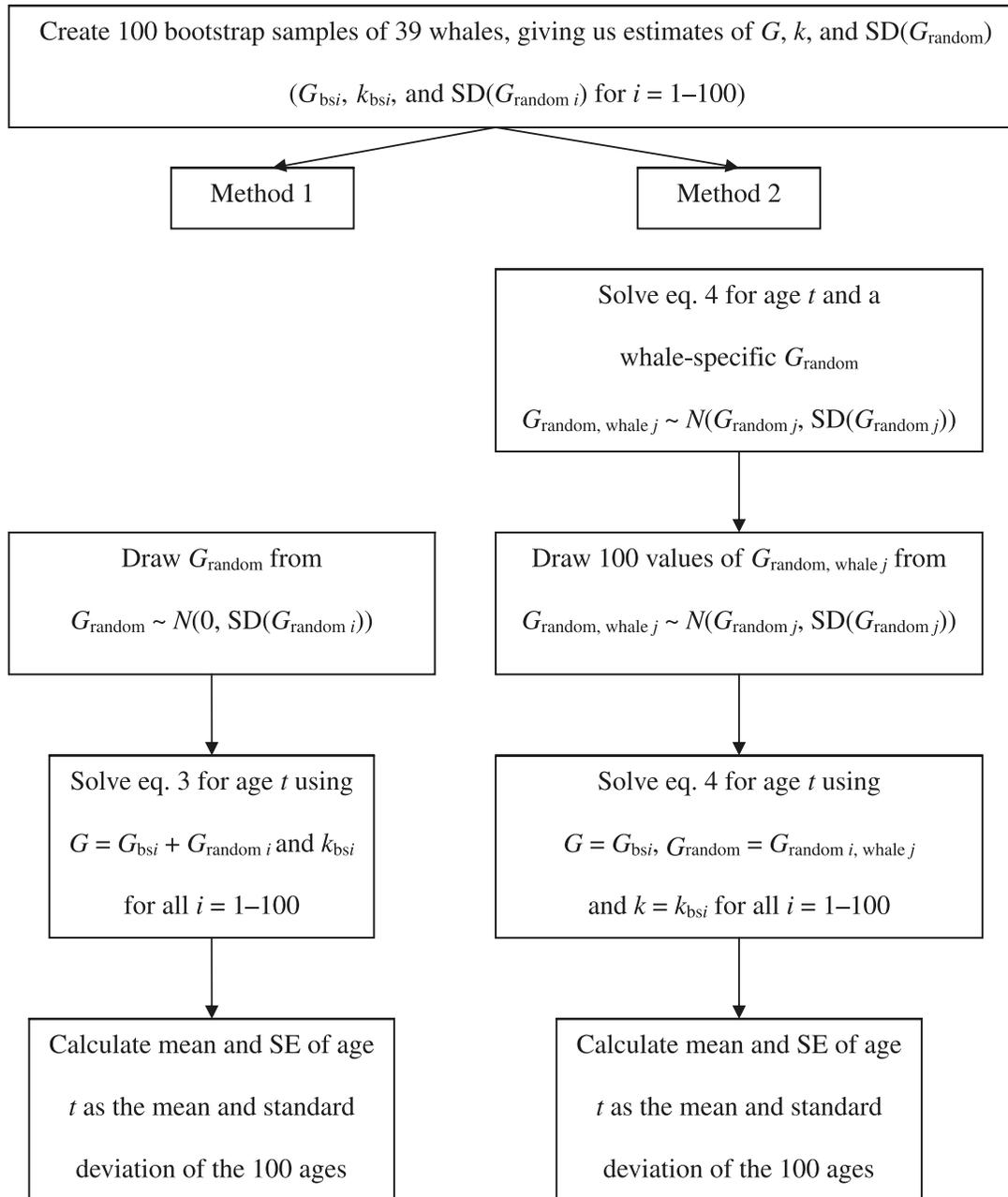
### Young whales — age estimates and age-specific baleen growth increments

Using a paired  $t$  test, we found that the age estimates by S.C. Lubetkin and C. Rosa (Table 3) were not significantly different ( $p = 0.85$ ). An  $F$  test on the variances of those whales with mean age estimates <5 years and those with mean age estimates >5 years showed a significant difference ( $p < 0.001$ ). Whales with mean estimated ages <5 years had a residual SE of 0.42 years from an analysis of variance, and whales with mean estimated ages >5 years had a residual SE of 1.51 years. These values provide rough SEs for age estimates based on cycle counting in the ranges 1–4.5 and 5–10.5 years, respectively.

Whale 01KK1 is listed in the database as being 13.2 m long with 1.48 m baleen. This pair of baleen and body length is an outlier from the trend shown by the other young whales (Table 3). It is possible that the baleen plate assigned to 01KK1 was mislabeled. We have shown the two independent age estimates for that whale based on the  $\delta^{13}\text{C}$  patterns in the plate but excluded it from further analyses.

Based on the mean lengths of complete cycles, we charac-

**Fig. 3.** A flow chart depicting the process of estimating bowhead whale (*Balaena mysticetus*) ages based on their growth increment lengths.



terized early growth for males and females (Table 4). First-year baleen is distinct from other baleen cycles in two respects: it is significantly longer (often around 50 cm; Table 4), reflecting the greater baleen growth rate of very young whales, and its  $\delta^{13}\text{C}$  cycle is smoother (Fig. 1a). This smoothness is partly a result of its greater length and partly owing to the isotopic averaging that occurs in the production of the mother's milk. After the 1st year, the baleen isotopic cycles have a fairly uniform pattern regardless of the number of cycles present. The 2nd-year baleen serves as a transition between the shallow (almost nonexistent) cycles of the 1st-year baleen growth and the regular cycles that are present subsequently. It is also distinct on the basis of its length (Table 4).

#### Growth increment model fitting

The jackknife procedure highlighted one whale, a 9.7 m long male caught in 1966 with 180 cm baleen sampled every 5 cm, that was an outlier from the other whales' baleen growth increment patterns. The growth increments of this whale were difficult to assign to specific ages, at least partly because of the coarse 5 cm sampling. S.C. Lubetkin initially listed them as 30, 25, 25, 30, 35, and 30 cm for ages 3 through 8 years, a series inconsistent with the pattern shown in Table 4. However, the  $\delta^{13}\text{C}$  valleys preceding the start of year 3 and between ages 4 and 5 years are shallower than the others. An alternate interpretation would be that the first 30 cm is only part of a growth increment, the next two should be combined, and then leave the rest as they were,

**Table 5.** Parameter estimates and model fit statistics for NLME models using the growth increment model for bowhead whales (*Balaena mysticetus*) with fixed (sex) and random effects on  $G$ ,  $k$ , or both indicate that the most parsimonious model with random effects on  $G$  was the best.

Parameter	Value	SE	$t$	$p$	$SD(G_{random})$	RSE	BIC	df
<b>Model: no sex effects, <math>G_{random}</math> (correlation of <math>G</math> and <math>k = 0.767</math>)</b>								
$G$	17.55	0.53	33.39	<0.0001	1.73	2.80	1295.07	210
$k$	0.332	0.047	7.06	<0.0001				
<b>Model: sex effect on <math>G</math>, <math>G_{random}</math></b>								
$G$	18.01	0.75	23.96	<0.0001	1.75	2.79	1299.63	209
$k$	0.352	0.052	6.80	<0.0001				
$G_{sex}$	-0.50	0.73	-0.69	0.49				
<b>Model: sex effect on <math>k</math>, <math>G_{random}</math></b>								
$G$	17.55	0.51	34.15	<0.0001	1.65	2.80	1298.77	209
$k$	0.295	0.047	6.30	<0.0001				
$k_{sex}$	0.091	0.064	1.43	0.15				
<b>Model: sex effects on <math>G</math> and <math>k</math>, <math>G_{random}</math></b>								
$G$	14.21	0.78	18.26	<0.0001	1.33	2.83	1302.29	208
$k$	0.109	0.031	3.53	0.0005				
$G_{sex}$	3.71	0.92	4.02	0.0001				
$k_{sex}$	0.304	0.074	4.13	0.0001				

**Note:** For each model type (where sex effects are included or not), we show the model with random effects on  $G$ ,  $k$ , or both that had the lowest Bayesian information criterion (BIC).  $G$  is the asymptotic baleen growth rate in  $cm\cdot year^{-1}$  and  $k$  is the rate of decline of the growth curve. The whale harvested in 1966 was removed from the analysis, leaving a sample size of 39 whales of known age. RSE, residual standard error.

**Table 6.** The age estimates of bowhead whales (*Balaena mysticetus*) based on growth increment model using  $G = 17.55\text{ cm}\cdot year^{-1}$  and  $k = 0.332\text{ year}^{-1}$  show a rough increase with body and baleen lengths.

Whale	Sex	Body length (m)	Baleen length (cm)	Number of complete cycles	Sampling interval (cm)	Growth increment model					
						Method 1		Method 2		$G_{random}$	SD
Age	SE	Age	SE								
98KK2	<i>F</i>	8.9	144	4	2	5.7	1.1	—	—	—	—
98KK3	M	9.2	170	3	2	5.3	1.6	—	—	—	—
02B21	<i>F</i>	10.0	192	8	1	11.7	2.3	14.2	3.4	2.0	1.7
98KK1	M	10.4	197	4	2	7.6	2.5	—	—	—	—
79KK2	<i>F</i>	10.5	190	7	5	9.6	1.6	12.9	3.0	2.1	1.4
86B7	M	10.7	200	8	2.5, 5	9.9	1.8	11.0	1.6	0.9	1.2
79KK5	M	10.7	200	6	5	6.3	0.9	6.4	2.8	1.6	4.8
87B3	M	11.0	195	9	2.5	12.4	2.1	11.6	1.5	-0.5	1.2
75B8	<i>F</i>	11.1	194	10	5	18.1	3.7	17.9	4.4	-0.3	1.3
89KK3	M	11.2	220	9	2.5	12.1	2.1	12.7	1.7	0.7	1.1
76H4	M	11.2	225	9	5	11.4	1.7	13.4	2.5	2.3	1.8
86B6	<i>F</i>	12.3	230	11	2.5, 5	14.2	3.4	19.8	3.7	2.3	0.7
02B20	M	12.3	233	10	1	15.7	4.5	11.8	2.2	-2.4	2.1
90B8	M	12.9	243	11	2.5	12.5	1.3	15.9	2.0	2.6	0.9
88WW3	M	13.1	210	10	2.5	14.9	2.7	14.0	2.0	-0.6	1.2
87WW2	M	13.5	215	10	2.5	14.5	2.5	14.5	2.2	0.1	1.1

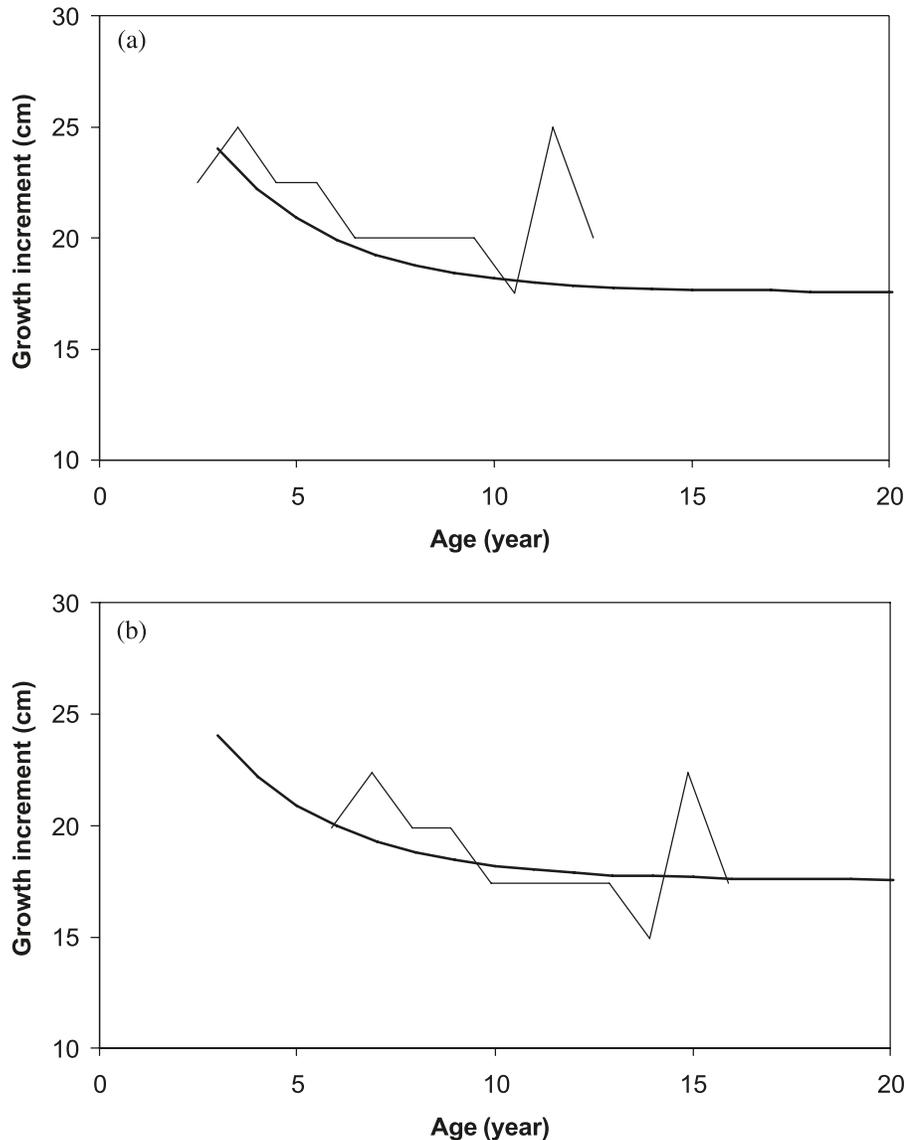
**Note:** Data for females are italicized. For whales where there were <6 complete growth increments present because their baleen plates were only partially sampled, we did not use method 2.

resulting in age-specific growth increments of 50, 30, 35, 30 for ages 1 through 4 years. This growth increment series is a better fit to the overall pattern, but results in an age estimate of 4–5 years, quite different from other males with similar body and baleen lengths, which are estimated to be about 8 years (Table 3). Based on the difficulty in assigning an age to this whale and the strong influence it had on the NLME model fitting, the whale from 1966 was removed

from the final model-fitting procedure and bootstrapping procedures.

We compared variations of the growth increment model with no sex effects, with sex effects on  $G$  alone or  $k$  alone, and with sex effects on both  $G$  and  $k$ . For each variation, we considered models with random effects on just  $G$ , just  $k$ , and on both  $G$  and  $k$ . We found that the best model for baleen growth rate (as determined using BIC) did not have sex ef-

**Fig. 4.** An example of fitting the growth increments of bowhead whale (*Balaena mysticetus*) 90B8 (a 12.9 m long male with 243 cm baleen) of unknown age to the growth curve. (a) When only the whale's age is estimated, the growth increment can only be moved "horizontally" (along the x axis) to find the best match with the mean population growth increment model, in this case at  $t = 12.5$  year. (b) When  $t$  and  $G_{\text{random}}$  are estimated, the baleen growth increment series can be moved "horizontally" ( $t$ ) and "vertically" ( $G_{\text{random}}$ ) to find the best fit to the curve ( $t = 15.9$  year when  $G_{\text{random}} \sim N(2.6 \text{ cm}\cdot\text{year}^{-1}, 0.9 \text{ cm}\cdot\text{year}^{-1})$ ) based on the NLS fit to eq. 4.



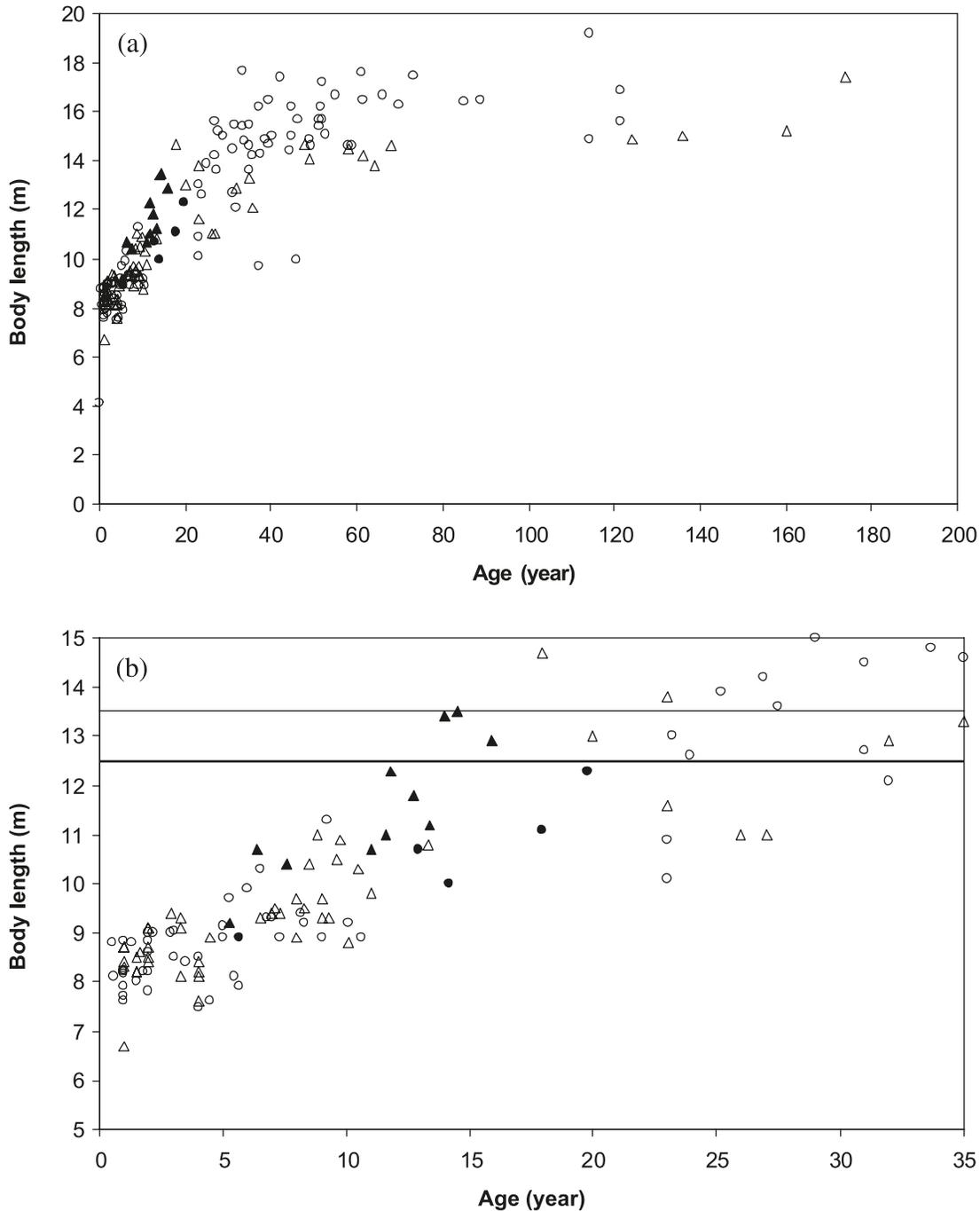
fects on  $G$  or  $k$ , and included random effects on  $G$ , but not on  $k$ , indicating that while the shape of the decline in baleen growth with time remains basically the same across whales, there is significant variability in the asymptotic baleen growth rate (Table 5). We found that when we included sex effects on just  $G$  or  $k$ , they were not statistically significant. Interestingly, although sex effects were statistically significant when present on both  $G$  and  $k$ , the best overall model was the one which had no sex effects (Table 5). The oldest males and females used in fitting the growth increment model did not have significantly different mean annual baleen growth rates. A two-sample  $t$  test based on Table 4 results in  $G_{\text{female}} = 17.3 \text{ cm}\cdot\text{year}^{-1}$  ( $n = 4$ ) and  $G_{\text{male}} = 15.6 \text{ cm}\cdot\text{year}^{-1}$  ( $n = 3$ ), and had  $t = 1.7375$  and  $p = 0.1428$ . While it appears that females may have slightly greater an-

nual baleen growth as adults, we have too few observations to merit including sex effects in the model. Using the baleen growth increment model without sex effects, we found  $(G, k) = (17.55 \text{ cm}\cdot\text{year}^{-1}, 0.332 \text{ year}^{-1})$ . At first glance, the estimated values for  $G$  and  $k$  appear to be a poor fit for the oldest whales (Table 4, Fig. 2a), but individual whales can have different asymptotic growth rates ( $\text{SD}(G_{\text{random}}) = 1.73$ ; Table 5) and the estimated parameter values are good descriptors of the growth increments of most whales up through age 50 (Fig. 2b). The Fig. 2b. range includes the young whales whose ages can be estimated using the growth increment model.

#### Age estimates for whales up to 13.5 m long

Although strongly correlated, age estimates from the

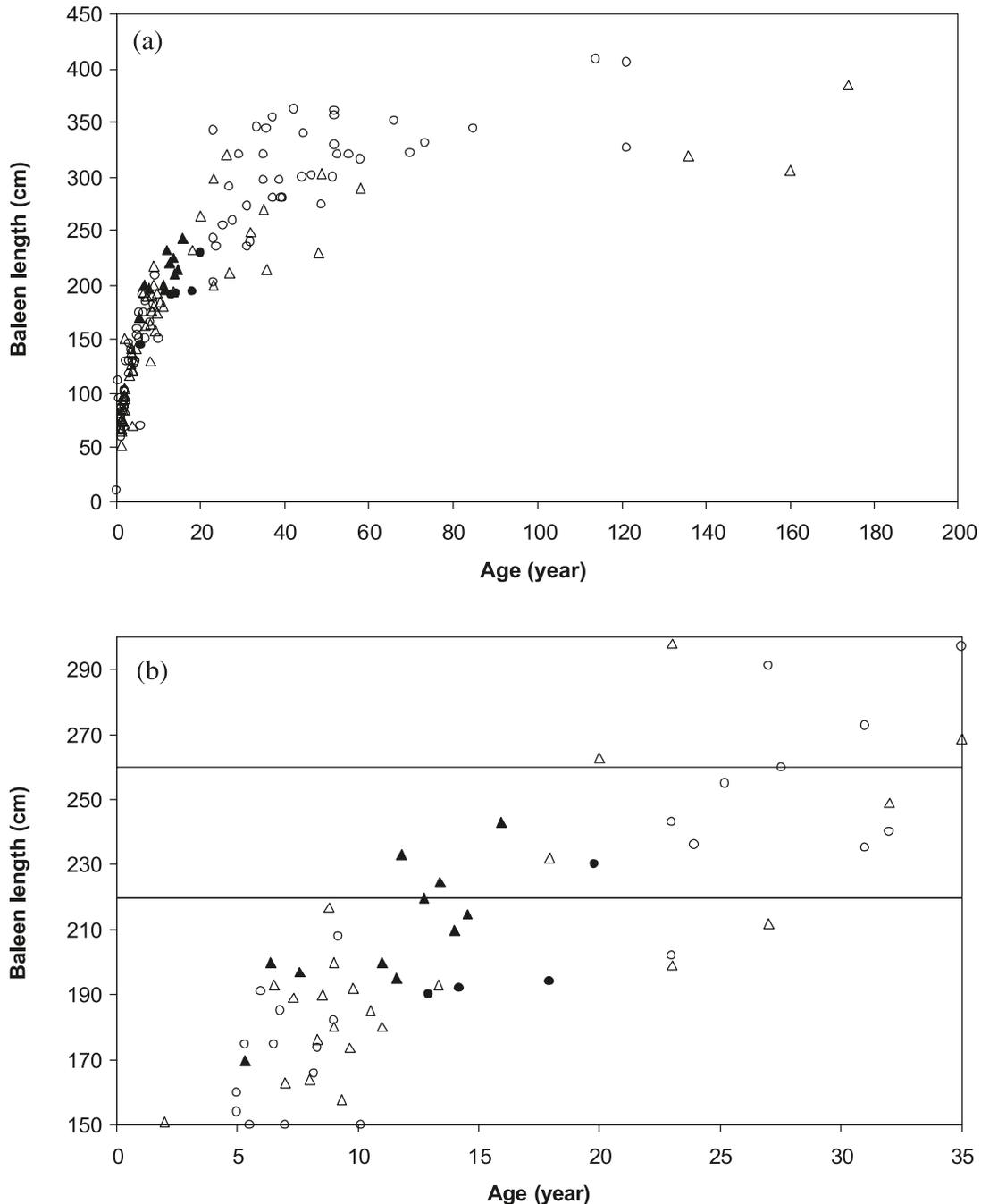
**Fig. 5.** (a) Age estimates and body lengths for male and female bowhead whales (*Balaena mysticetus*). Open symbols represent age estimates that came from aspartic acid racemization, corpora counts, and baleen cycle-counting methods. Closed symbols are age estimates from growth increment models (Table 6) made using method 2 if possible, method 1 otherwise. Triangles represent males and circles represent females. (b) A detail of bowhead body length at age up to age 35. The horizontal lines bound the body length range where most male and female bowheads reach sexual maturity (between 12.5 and 13.5 m).



baleen growth increments did not increase monotonically with body or baleen length (Table 6). Of whales with their complete baleen lengths sampled, individual age estimates using method 2 ranged from 6.4 (whale 79KK5, a 10.7 m long male with 200 cm baleen containing 6 complete growth increments) to 19.8 years (whale 86B6, a 12.3 m long female with 230 cm baleen containing 11 complete growth

increments). SEs from the bootstrap procedures were fairly small, reaching only as high as 4.4 years for whale 75B8. There was no significant increase in SE with increasing age estimate, in part because older whales with longer baleen plates have more growth increments to use in estimating whale age. Whales 98KK1, 98KK2, and 98KK3, with only partially sampled baleen plates, had method-1 age estimates

**Fig. 6.** (a) Age estimates and baleen lengths for male and female bowhead whales (*Balaena mysticetus*). Symbols are the same as in Fig. 5. (b) A detail of bowhead baleen length at age up to age 35. The horizontal lines bound the baleen length range where most male and female bowheads reach sexual maturity (between 220 and 260 cm).



that were consistent with other similarly sized whales shown in Table 3, which had the entire lengths of their baleen sampled.

The differences in ages estimated using eq. 3 (method 1) and those estimated using a whale-specific  $G_{\text{random}}$  (method 2) behaved in predictable ways. Those whales with positive  $G_{\text{random}}$  also had higher age estimates when both age and  $G_{\text{random}}$  were estimated. Also, when  $G_{\text{random}}$  could not be well estimated for a given whale (for example, whale 79KK5), the method-2 SEs of the ages were higher. Method-2 SEs of the estimated ages also tended to be higher when the sampling

interval was 5 cm for at least part of the baleen plate, reflecting that the rather subtle decrease in growth increment lengths was not captured when the plate was sampled that coarsely (whales 79KK2, 79KK5, 75B8, 76H4, and 86B6). The other larger age SEs occurred when the growth increments did not follow the overall trend very cleanly, and were distinctly not monotonically decreasing with time (i.e., whale 02B21, with growth increments of 20, 22, 18, 21.5, 21.5, 24, 16, and 18 cm from tip to gum). Because method 2 can adjust for whale-to-whale variation in baleen growth rate, including how well that difference is characterized, whereas method 1 cannot (Fig. 4), we

prefer the age estimates and SEs from method 2 when there are sufficient growth increments to use it.

When we estimated whale age and  $G_{\text{random}}$  with the growth increment model, the data suggested that females may have larger asymptotic baleen growth rates. Three of the four females had a difference between the population mean  $G$  and their asymptotic growth rate of at least 2.0 cm·year<sup>-1</sup> (Table 6). Of the nine males, only two had random effects that were 2.0 cm·year<sup>-1</sup> or more. With the exception of whale 79KK5, the precision with which the random effects were estimated was remarkably consistent across all the whales we estimated ages for, with all the  $G_{\text{random}}$  having SDs ranging from 0.7 to 2.1 cm·year<sup>-1</sup>. However, the results from Table 6 echo those from the model with sex effects on  $k$  and  $G$  (Table 5), suggesting that there may be sex effects in bowhead baleen growth, but that those differences are too subtle to warrant using a sex-specific baleen growth model at this time. Both Tables 4 and 5 suggest that the  $G$  value of 17.55 cm·year<sup>-1</sup> from the model chosen using BIC may be more representative of females than males. If the mean asymptotic baleen growth rate for males is lower than that for females, the ages estimated for males could be negatively biased.

### Age at sexual maturity

Baleen growth increments are only useful for age estimation until those increments reach their asymptotic values, and, thus, these techniques have limited use in making inferences about bowhead age at sexual maturity. The immature females in Table 6 were smaller and had shorter baleen lengths than males estimated to be of similar ages, a trend which does not hold true for other immature whales (Table 3) or sexually mature whales (Figs. 5, 6). Negative bias of the male ages in Table 6 might explain this.

We considered males in Table 6 that were >12.5 m long as possibly sexually mature. These individuals had age estimates ranging from 14.0 to 15.9 years (Table 6, Figs. 5b, 6b). We did not have any females >12.5 m long with growth increment data and baleen length <250 cm for whom ages could be estimated. At 12.3 m long, whale 86B6 was the largest female in Table 6 and almost certainly sexually immature. She was estimated to be 19.8 years old, so our only indication from the growth increment model is that females attain sexual maturity in their 20s or later (Table 6, Figs. 5b, 6b). This is consistent with the results of George et al. (1999, 2004).

### Recommendations for the use of these models

For whales <10 m long, cycle counting appears to be the best method for determining age. The 1st year of baleen growth is quite distinctive and reader bias appears low. Baleen growth increment modeling is effective until the whales reach a maximum body length of about 13.5 m or maximum baleen length of 250 cm. Sampling of  $\delta^{13}\text{C}$  at intervals  $\leq 2.5$  cm along the baleen provides an effective way of estimating bowhead whale ages in individuals that are too young (<20 year) to have other techniques, such as AAR or corpora counts, applied effectively. These techniques fill a large gap in bowhead age estimation methods.

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