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Age Determination in Belugas (*Delphinapterus leucas*): A Quest for Validation of Dentinal Layering

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Abstract

A workshop for experts in age determination of beluga whales was convened to (1) determine the number of dentinal Growth Layer Groups (GLGs) in beluga teeth and the variation therein among readers; (2) assess the deposition rate of dentinal GLGs in beluga teeth, specifically on the question of one or two GLGs per year; (3) define the appearance of dentinal GLGs in order to standardize reading methods among readers of beluga teeth; and (4) provide a consensus report with specific conclusions on deposition rate and GLG definition. Tooth specimens from ten belugas, all originally from Churchill, Manitoba, Canada, with captive histories ranging from 4 to 30 y, were the focus of the investigation. Three of these animals had medication histories of tetracycline antibiotics which "time-mark" hard tissues. Results from the inter-reader GLG comparisons, GLG counts compared with captive history, and tetracycline mark placement indicated that despite considerable problems with the inter-reader count variability, using certain assumptions, there was evidence that two GLGs per year was not possible in six of the ten specimens; however, there were some specimens for which it was clear that two GLGs per year could be feasible, and yet others where the derived estimate of age at first capture did not appear to be compatible with the most likely age for that size of animal. The conclusions were that one GLG annual deposition rate in dentine was clearly upheld in some instances but that the results were equivocal for several specimens for a variety of reasons. In light of the fact that tooth GLGs are likely to continue being the predominant method for aging in this species, the workshop members agreed on a list of seven recommendations that included, as a priority, experimental approaches that could help to standardize and validate GLG counting in age determination.

Key Words: beluga whales, *Delphinapterus leucas*, age estimation, aging, teeth, dentine, growth layer groups, GLG, age validation, tetracycline

Introduction

A Growth Layer Group (GLG) in a tooth has been defined by the International Whaling Commission (IWC) (1980) as groups of incremental growth layers which may be recognized by virtue of a cycle repetition, generally at constant or regularly changing relative spacing in the component lamina structure delineating the layers. Such a cyclic repetition of incremental growth layers must involve at least one change—that is, between translucent and opaque, dark and light, ridge and groove, more stained and less stained—and may involve more than one change. For most odontocetes, GLGs in dentine and cementum have been defined to represent one year's growth. Hohn et al. (1989), for example, demonstrated this effectively with a sample of free-ranging, known-age bottlenose dolphins (*Tursiops truncatus*). In contrast, using similar growth-layer characteristics, researchers have typically accepted that two GLGs rather than one form annually in the dentine of teeth of belugas (*Delphinapterus leucas*) as a result of the initial suggestion of Sergeant (1959) that the deposition rate in belugas could be similar to that of sperm whales. At that time, it was believed that sperm whales deposited two GLGs per year in dentine, but this has long since been revised to one per year (IWC, 1969, 1980; Best, 1970; Gambell, 1977). Further investigation of deposition rate in dentine for three captive belugas attempted to resolve any uncertainty in deposition rate (Brodie, 1982; Goren et al., 1987; Heide-Jørgensen et al., 1994). None of the results and arguments for two GLGs per year that came from these investigations are unequivocal, although the specimen used by

Heide-Jørgensen et al. (1994) is still available and allows for a possible re-examination and reinterpretation. Thus, until very recently, there has still been uncertainty whether one or two GLGs form annually in belugas, yet accurate age determination is critical to correct estimation of population parameters.

Hohn & Lockyer (1999), using information on two captive belugas of known history, one with tetracycline antibiotic marking in the teeth, presented new evidence that there is an equally likely deposition rate of one GLG per year. Tetracycline antibiotics leave a permanent deposit in hard tissues and may be observed fluorescing in thin sections viewed under reflected UV light. It is clear that the conflict surrounding GLG deposition rate should be resolved with certainty. To this end, additional specimens and data from captive, known-history belugas, including those with records of tetracycline medication administration, were acquired.

The most effective way to resolve the matter was to convene a workshop of experts who have extensive experience in age estimation of beluga teeth (IWC, 2000; North Atlantic Marine Mammal Commission [NAMMCO], 2001) to examine teeth from wild and captive beluga whales. The workshop was hosted at the National Oceanic and Atmospheric Administration's (NOAA) Beaufort Laboratory in March 2001, and it included the authors of this report. The objectives of the workshop were as follows:

- To determine the number of dentinal GLGs in beluga teeth and the variation therein among readers.
- To assess the deposition rate of dentinal GLGs in beluga teeth, specifically on the question of one or two GLGs per year.
- To define the appearance of dentinal GLGs in order to standardize reading methods among readers of beluga teeth.
- To provide a consensus report with specific conclusions on deposition rate and GLG definition.

Materials and Methods

Available Materials

Tooth sections from ten captive beluga whales, all live-captured near Churchill, Canada, that had died after 4 to 30 y in captivity were available. Two of these animals had records of tetracycline antibiotic treatments (Table 1). Generally, a single tooth or maximum of two teeth were available per animal. In addition, tooth sections from free-ranging beluga whales from Russia (8 different specimens) and Greenland (up to 20 different specimens) were available. Photographs of beluga tooth sections from eastern Canada were present for reference. Tooth sections from other species of cetaceans (including *Tursiops*, *Phocoena*, and *Kogia*), including some teeth with tetracycline marks, were also available.

Before attempting tooth GLG counts for the main sample and performing inter-reader comparisons, a brief review of tooth GLG reading methods and comparison of several different odontocete species' teeth was undertaken, with special emphasis on what was defined as a GLG. The sample teeth specimens had been prepared both as untreated thin sections and decalcified stained sections for most specimens by Lockyer and Hohn prior to the reading exercise. The methods of preparation were as follows.

Tooth Preparation

The teeth were prepared in two ways. First, whole teeth were glued temporarily to wood blocks and then positioned in a chuck of an Isomet slow-speed circular saw (Buehler) in such a way that a full section ca 100 μm thick could be made centrally through crown and root. The sections were not treated further in any way.

Second, whole teeth, glued temporarily to wood blocks, were trimmed on an Isomet saw in such a way that a central slice through crown and root about 3 to 4 mm thick remained. This slice was then fixed in 10% formalin for several hours, subsequently rinsed in water for 2 to 3 h and then decalcified in RDO, a commercial bone decalcifier from Apex Engineering (Aurora, Illinois, USA), for 2 to 24 h, depending on the thickness of the section and the relative age of the animal, with older animals requiring the longer times. The decalcified slice was rinsed in running water overnight and then sectioned on a freezing microtome to 25 μm thickness through crown, pulp cavity, and root. The loose sections were stained for about 20 min in an agitated solution of freshly prepared haematoxylin, rinsed in water, "blued" in a weak ammonia solution, and then sorted and dehydrated in a 50% glycerin/water solution then 100% glycerin before finally being mounted in pure glycerin. Several sections were available for each tooth, but only one or two central sections were selected for reading.

Digital Images of Tooth Sections

A set each of electronic digitised images and enlarged photographs of each tooth specimen were also available for every participant to facilitate the independent precise marking of which layers were counted as GLGs and why they were so identified.

Table 1. History and tooth data for captive beluga whales available for the aging workshop; all of the animals were collected from Churchill, Manitoba, Canada.

Topic	Allua	Moby	Aurora	Winston	Churchill	No-See-Um	Big Mouth	SW-DL-7903	Immiayuk	Illamar
Source	Zoo Duisburg; captured together		Mystic Aquarium	NY Aquarium	Navy	Navy	Minnesota Zoo via SeaWorld via Navy	SeaWorld	Shedd Aquarium, Chicago	National Aquarium, Baltimore
Sex	F	F	F	M	M	M	M	F	F	F
Collection date	05 Aug. 69	05 Aug. 69	17 July 84	16 July 84	15 July 77	04 Aug. 77	31 July 77	24 July 79	28 July 89	29 July 85
Collection length (cm)	(285; 06 Sept. 69)	(280; 06 Sept. 69)	246	?	304	257	11'5" (348)	262	267	9'3" (282)
Collection color	Grey	Grey	--	--	White	Grey	White	--	Med. grey	--
Date of death	26 July 84	June 99	06 Sept. 99	11 Oct. 98	08 May 85	04 April 99	16 July 90	20 Aug. 87	26 Dec. 99	10 Aug. 89
Length at death (cm)	372	355	329	380	340	402	406	329	388	ca 11' (335)
Time in captivity	14 y 11.5 mo	29 y 11 mo	15 y 2 mo	14 y 3 mo	7 y 10 mo	21 y 8 mo	13 y	7 y 11 mo	10 y 5 mo	4 y
Tetracycline?	?	?	N	N	N	N	Y	Y	Y	?
Neonatal line present	Y	N	Y	N	Y	N	Y	Y	Y	Y
Stained sections (st)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Untreated thin sections (untr)	Y (several)	Y (1)	Y (2)	N	Y (?)	Y (2)	N	Y (2)	Y (several)	Y (2)
Digital photos (untr = untreated; st = stained)	Y, untr; Y, st	Y, untr; Y, st	Y, untr; Y, st	Nil, untr ; Y, st	Y, untr; Y, st	Y, untr; Y, st	Nil, untr; Y, st	Y, untr; Y, st	Y, untr; Y, st	Y, untr; Y, st
Other comments:	Specimen shown in Figure 1 of Heide-Jørgensen et al. (1994)								Died after 1st calving	

Note: Y = yes; N= no

Tooth Reading Procedures

Once a standard procedure had been adopted for the readings, all participants independently examined tooth sections from all of the available captive specimens without access to data on the captive history, using transmitted and polarised light microscopy with variable magnification. The GLG counts were recorded for each specimen after multiple readings (reader-dependent until a consistent count was obtained), when an agreed final count was provided for each specimen by each individual reader. This approach is similar to the one used in a workshop on age estimation in harbour porpoises (Bjørge et al., 1995). Unlike the other participating readers, both Lockyer and Hohn had access to data associated with the tooth specimens prior to the reading exercise. During the reading exercise, all teeth were labelled with codes so that the precise identity was unknown during the test readings, thus reducing the possibility that their readings would be biased. The teeth with tetracycline marks were examined using a compound microscope with reflected UV light and a filter specific for tetracycline antibiotics. Tetracycline antibiotics are known "time-markers" in hard tissues and have been used with success to mark GLGs in teeth of marine mammals (Myrick et al., 1984; Lockyer, 1993) in order to validate age and check GLG deposition rate. The readers identified the position of the fluorescent time marks relative to the GLGs in the dentine.

The independent GLG counts from each reader were ultimately compiled into a spreadsheet. Using these spreadsheets and the photographs and images of the tooth sections with marked GLGs, the participants compared counts and the growth layers each had identified as GLGs. Comparison of structures was facilitated using a microscope-mounted video camera and digital image analysis system simultaneously. During these discussions, a new copy of the digital images from each tooth specimen was marked to indicate the GLGs identified by each reader. An attempt was made to reach a consensus on what constituted a GLG and the final GLG count for each specimen.

All readers' final GLG counts for each tooth specimen were compared and analysed for mean and standard deviation. The participants then compared the range of counts among readers to the known history data for each specimen and evaluated whether the counts best supported the hypotheses of one or two GLGs per year.

Results

Reader Comparability

Differences among readers generally increased with the number of GLGs in the tooth (Table 2; Figure 1). For samples from over half of the animals, the GLG counts from at least three of the readers were generally close (±1), while the counts from the other reader(s) were not (Table 2, Note 1). For the other half of the samples, the readings ranged considerably (±2 or more) (see Table 2, Note 2). In some cases, this was related to the quality of the tooth section (dependent on intrinsic clarity of GLGs but also crown wear, damage, and off-centre sections), while in other cases, the readers were counting different structures as GLGs. For all animals, at least three readers out of the five counted within ±2 GLGs for eight of the ten animals (Table 2, Note 3).

Consensus on Minimum and Maximum Ages Estimate

Although no consensus was reached on best dentinal GLG counts for most of the animals, participants did reach a consensus on the minimum and maximum counts for each animal. The minima and maxima were based on values determined after group discussion and group GLG counting on video-projected images, as well as inter-comparisons of individuals' GLG counts (Table 2). They were not the minimum and maximum counts determined individually in Table 2 but new agreed group counts for a minimum and maximum range. These minimum and maximum counts were used to test the hypothesis of one or two GLGs per year (Tables 3 and 4, using agreed minimum and maximum counts, respectively).

Using the agreed minimum counts in Table 3, it is clear that the hypothesis for two GLGs per annum is unrealistic for six of the ten test animals as the expected age at capture would then be negative years. Using the agreed maximum counts in Table 4, two of the ten test animals still have unrealistic negative ages at first capture with the hypothesis of two GLGs per annum. Additionally, it is clear that the hypothesis for one GLG per annum is unrealistic for several of the animals. For example, in Table 3, Aurora and No-See-Um had estimated GLG counts at capture (17 and 20+, respectively) that were higher than is likely. Both of these animals were around 250 cm at capture, which would indicate a probable age of 5 y or less based on recent growth data for captive belugas originating from Churchill (Robeck et al., 2005). Further discussion on the results are provided below, considering other factors related to age such as colouration and length at capture, and also the potential influence of crown wear resulting in GLG loss on total observed GLGs.

Validation of Age Using Tetracycline Time Marks

Three beluga whales received clinical treatments of tetracycline (i.e., they were not treated for

Table 2. Best estimates of number of GLGs by each reader and the average and standard deviation of GLGs for each beluga whale; biases are given as the difference between an individual estimate and the average for that animal. A positive bias means that average individual readings are above the average count.

Reader	Allua	Moby	Aurora	Winston	Churchill	No-See-Um	Big Mouth	SW-DL-7903	Immiayuk	Illamar	
Reader A	27	38	--	27	32	46	25	16	20	13	
Reader B	32	30	27	27	30	42	30	21	28	14	
Reader C	25	41	32	25	24	45	25	19	21	11	
Reader D	28	43	32	26	24	46	34	19	21	11	
Reader E	27	38	35	19	29	35	24	16	23	10	
Average	27.80	38.00	31.50	24.80	27.80	42.80	27.60	18.20	22.60	11.80	
SD	2.59	4.95	3.32	3.35	3.63	4.66	4.28	2.17	3.21	1.64	
Biases (mean deviation)											Total
Reader A	-0.80	0.00	--	2.20	4.20	3.20	-2.60	-2.20	-2.60	1.20	2.60
Reader B	4.20	-8.00	-4.50	2.20	2.20	-0.80	2.40	2.80	5.40	2.20	8.10
Reader C	-2.80	3.00	0.50	0.20	-3.80	2.20	-2.60	0.80	-1.60	-0.80	-4.90
Reader D	0.20	5.00	0.50	1.20	-3.80	3.20	6.40	0.80	-1.60	-0.80	11.10
Reader E	-0.80	0.00	3.50	-5.80	1.20	-7.80	-3.60	-2.20	0.40	-1.80	-16.90

Notes:

1. The GLG counts from at least three of the readers were generally close (±1), while the counts from the other reader(s) were not for Allua, Winston, No-See-Um, Big Mouth, Immiayuk, and Illamar.
2. For four of the samples, the readings ranged considerably (±2 or more) for Moby, Aurora, Churchill, and SW-DL-7903.
3. For all animals, at least three readers out of the five counted within ±2 GLGs for eight of the ten animals: Allua, Winston, Churchill, No-See-Um, Big Mouth, SW-DL-7903, Immiayuk, and Illamar.

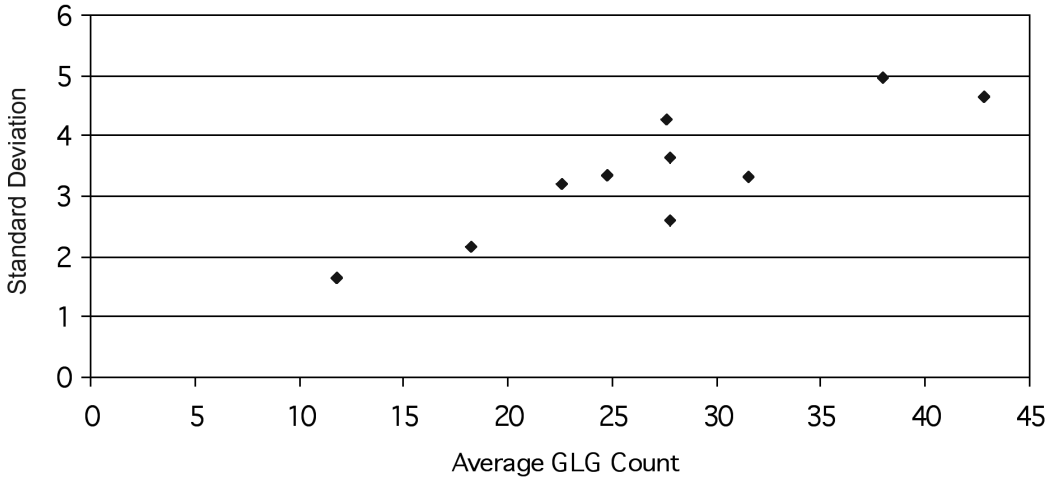

Figure 1. The standard deviation in number of GLGs counted among readers (n = 5) increased with the average number of GLGs

the purpose of marking their teeth). Tetracycline antibiotic marks were visible in teeth from one of the beluga whales and extremely faint in the tooth of another marked whale. A third animal, Big Mouth, gave no trace of a visible mark in the dentine, despite records of tetracycline treatment. The clarity of a tetracycline mark is dependent on dosage, both in terms of administered concentration and duration (Myrick et al., 1984; Lockyer, 1993), and all these marks were used opportunistically without control at the administration stage. Thus, it is not surprising that some marks were more visible than others. Using information collected prior to the workshop, it was possible to determine where the mark was located in the tooth relative to GLGs. For SW-DL-7903 (see Figure 2), tetracycline was administered approximately 4 y prior to death, and the mark occurred at a position consistent with a deposition rate of one GLG/y (Table 5). For Immiayuk, the location of the mark was inconclusive with regard to deposition rate (Table 5).

Discussion and Conclusions

General Observations on GLG Clarity and Readability

Clarity of the growth layers in tooth sections from the captive animals was less than that seen in the free-ranging populations from West Greenland and Russia examined by participants during the workshop. Two explanations are readily apparent: (1) GLGs in beluga teeth from Churchill are not well-defined relative to populations from other geographical areas or (2) captivity has affected growth layer deposition. The first possibility has parallels in other species, for example, the harbour porpoise (*Phocoena phocoena*) (Lockyer, 1995, 1999). The first explanation can be investigated by comparing teeth of wild belugas from Churchill, the location from where all the captive animals were taken, with captive animals and with teeth from animals of other stocks. Captivity affecting growth layer patterns would be a different scenario from effects seen in other species (Hohn, 1990; Lockyer, 1993); however, if the light and dark zones comprising the GLGs are influenced by the migration (and feeding) patterns of these whales, then it would be reasonable that captivity diminishes the contrast between those two types of layers. If that, in essence, changes the GLG appearance significantly, it may preclude using captive beluga whales to calibrate GLG deposition in free-ranging belugas.

Participants agreed that reduced clarity of growth layers could have resulted in increased variation in GLG counts in this sample relative to what might occur in free-ranging samples. Even when growth layers were distinct, however, the various readers often disagreed on what constituted a GLG versus what constituted an accessory layer.

The GLG pattern in the tooth of SW-DL-7903 (Figure 2) shows a clear change in contrast between the GLGs 0 to 8 and those > 8. This change in patterns was also observed in some other tooth specimens from other animals, including wild ones. The change is not always present or as clear as this, but, rather than being a phenomenon associated with captivity (as noted by Heide-Jørgensen et al., 1994), it may be linked to juvenile growth characteristics. Parallels may be seen in the GLG transition phase in ear plugs from fin whales, for example (Lockyer, 1972). We

Table 3. Age estimates calculated using two hypotheses: (1) one GLG/y and (2) two GLGs/y, based on the minimum agreed GLGs; all animals are from Churchill region, Manitoba, Canada. A "+" indicates lack of a neonatal line and therefore a possibly worn tooth; a ">" indicates that possibly uncounted (uncountable?) compacted GLGs were present.

A	B	C	D	E	F	G	H	I	J	K	L
								Hypothesis 1	Hypothesis 2	Hypothesis 1	Hypothesis 2
Animal	Sex	Length at capture (cm)	Colour at capture	Length at death (cm)	Years in captivity	Presence of neonatal line	Total GLGs (minimum of range)	Age if one GLG/y	Age if two GLGs/y	Expected GLGs at capture if one GLG/y	Expected GLGs at capture if two GLGs/y
Allua	F	285	Grey	372	15.0	Y	**27**	27	13.5	12	-3
Moby	F	280	Grey	355	29.9	N	**38+**	38+	19+	8.1+	-21.8+
Aurora	F	246	--	329	15.2	Y	**32**	32	16	16.8	1.6
Winston	M	--	--	380	14.25	N	**25+**	25+	12.5+	10.75+	-3.5+
Churchill	M	304	White	340	7.8	Y	**23**	23	11.5	15.2	7.4
No-See-Um	M	257	Grey	402	21.7	N	**42+**	42+	21+	20.3+	-1.4+
Big Mouth	M	348	White	406	13.0	Y	**24**	24	12	11	-2+
SW-DL-7903	F	262	--	329	7.9	Y	**16**	16	8	8.1	0.2
Immiayuk	F	267	Medium Grey	388	10.4	Y	**20**	20	10	9.6	-0.8
Illamar	F	282	--	335	4.0	Y	**12**	12 = column H	6 = column H / 2	8 = column I - column F	4 = (J - F) * 2

Table 4. Age estimates calculated using two hypotheses: (1) one GLG/y and (2) two GLGs/y, based on the maximum agreed GLGs; all animals are from Churchill region, Manitoba, Canada. A "+" indicates lack of a neonatal line and therefore a possibly worn tooth; a ">" indicates that possibly uncounted (uncountable?) compacted GLGs were present.

A	B	C	D	E	F	G	H	I	J	K	L
								Hypothesis 1	Hypothesis 2	Hypothesis 1	Hypothesis 2
Animal	Sex	Length at capture (cm)	Colour at capture	Length at death (cm)	Years in captivity	Presence of neonatal line	Total GLGs (maximum of range)	Age if one GLG/y	Age if two GLGs/y	Expected GLGs at capture if one GLG/y	Expected GLGs at capture if two GLGs/y
Allua	F	285	Grey	372	15.0	Y	≥ **32**	≥ 32	≥ 16	≥ 17	≥ 2
Moby	F	280	Grey	355	29.9	N	**42+**	42+	21+	12.1+	-17.8+
Aurora	F	246	--	329	15.2	Y	**37**	37	18.5	21.8	6.6
Winston	M	--	--	380	14.3	N	**27+**	27+	13.5+	12.8+	-1.5+
Churchill	M	304	White	340	7.8	Y	**32**	32	16	24.2	16.4
No-See-Um	M	257	Grey	402	21.7	N	**46+**	46+	23+	24.3+	2.6+
Big Mouth	M	348	White	406	13.0	Y	**32**	32	16	19	6
SW-DL-7903	F	262	--	329	7.9	Y	**18**	18	9	10.1	2.2
Immiayuk	F	267	Medium grey	388	10.4	Y	**27**	27	13.5	16.6	6.2
Illamar	F	282	--	335	4.0	Y	**14**	14 = column H	7 = column H / 2	10 = column I - column F	6 = (J - F) * 2

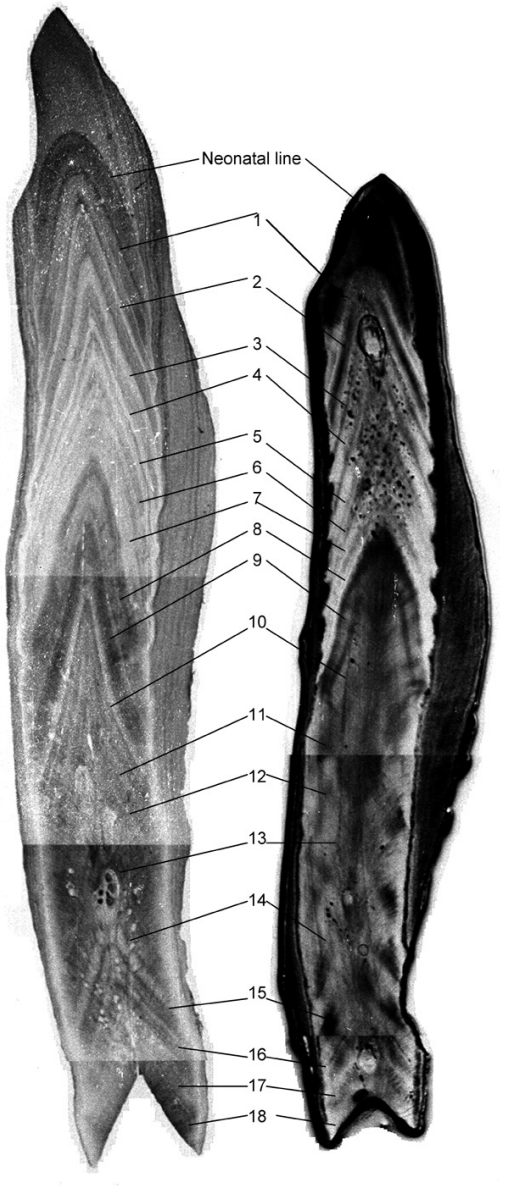

Figure 2. To the left is the decalcified and stained section of the tooth of SW-DL-7903, and to the right is the untreated section from the same tooth. There are 18 GLGs marked up in the dentine, and the neonatal line is intact. This animal was in captivity almost 8 y. Presence of a fluorescent time-mark in the dentine around GLG 14 originates from a tetracycline treatment 4 y and 2 mo before death. The conclusion is that a one GLG per year deposition rate is validated for this animal.

have no additional insight into this matter, however, although such juvenile phase GLG patterns could be worth investigating.

Another problem that cannot easily be resolved is the matter of wear at the crown of the tooth. Table 1 indicates that three tooth specimens had crown wear resulting in the loss of the neonatal line. In effect, this means that it is impossible to know how many post-natally deposited GLGs have been lost through wear, and only a minimum age can be assigned. Unfortunately, the teeth in this category were from Moby, Winston, and No-See-Um—all animals that were among those with the longest record of captivity and thus known-history and those with the greatest potential for resolving the hypothesis options. In Tables 3 and 4, these animals are also in the group where expected age at capture using the two GLGs/y hypothesis resulted in an unrealistic negative age. Loss of early-deposited GLGs negatively biases the calculated ages using the two GLGs/y hypothesis. So, the negative age estimates for these animals have two explanations: (1) an incorrect assumption of deposition rate and (2) loss of GLGs due to wear.

With the wide variation in counts and interpretation of GLG components, regular tetracycline treatment of captive animals may be required to resolve the question of what comprises one GLG and how many of those are deposited each year. Because obtaining those samples may require an extended period of time, participants discussed the merits of investigating other means of aging beluga whales from the use of other techniques to taking a probabilistic approach to assigning age. Furthermore, as long as there remains ambiguity in the deposition rate of GLGs, population modellers should take into account both interpretations of deposition rate because these would have implications with respect to longevity and also reproductive output, which would affect population productivity and, in turn, management decisions.

Other Considerations

Table 1 also provides ancillary data on the animals at capture such as body colouration and length. It has often been considered that grey colouration in belugas indicates juvenility; however, longer-term studies in captive facilities suggest that using colouration as a relative age diagnostic is unreliable as many animals remain grey into adulthood, and colour change is very individualistic to timing when it occurs (Heide-Jørgensen & Lockyer, 2001). Similarly, body length can be used as a rough guide to age, but again, there is wide individual variation in growth rates, and apart from knowing the likely birth size and size in newly mature females, both easily defined life stages, we cannot be certain of the age that should be assigned to a certain body size. The argument becomes circular because average growth curves have been predicted using combined data

Table 5. Tetracycline marking record for beluga tooth samples used during the workshop

	SW-DL-7903	Immiayuk
Source	SeaWorld	Shedd Aquarium
Geographic origin	Churchill	Churchill
Sex	F	F
Collection date	24 July 79	28 July 89
Collection length (cm)	262	267
Collection color	--	Medium grey
Date of death	20 Aug. 87	26 Dec. 99
Length at death (cm)	329	388
Time in captivity	7 y 11 mo (7.9)	10 y 5 mo (10.4)
Neonatal line present	Yes	Yes
Estimated age range	16 to 18	20 to 27
Tetracycline:		
Dates	17-24 June 83	26-31 Aug. 89
Dose	9 g orally 2x/day	4 g bid
Location of tetracycline Mark in tooth	1 mark at GLG 14 in dentine and cement	1 mark close to GLG 7, 8, or 9, depending on reader, in dentine and cement
Other comments	Consistent with hypothesis 1 GLG per year	Inconclusive—could be 1 or 2 GLGs/y; died after 1st calving

from many animals, using the assumption that two GLGs form annually. Since the work of this group, there has been publication of early growth of captive belugas (Robeck et al., 2005), but this study is still in its early stages and cannot yet provide a definitive answer as to anticipated length at age, although the referenced study also examined animals from Churchill. Robeck et al. do provide at least a rough estimate of length at age for very young animals up to age 5 y; yet, it is not clear how to interpret the apparent contradiction between their monitored lengths at age for captive individuals and the much older estimated ages relative to body length for Aurora and No-See-Um, assuming an annual GLG deposition rate.

One or Two GLGs per Annum?

The results of the dentinal GLG investigations from known-history animals in this study throw great doubt on the two GLGs/y hypothesis and, on balance, favour the one GLG/y hypothesis. The greatest problem appears to be the frequent difference in GLG counts among readers as discussed above. The most compelling current evidence in support of the hypothesis of one GLG/y deposition rate is from a recent study on an analysis of radiocarbon isotopes from atomic bomb fallout in the dentinal GLGs of belugas (Stewart et al., 2006), where all the individual GLGs in the dentine were analyzed for isotope content.

There appears to be good evidence that one GLG is formed annually with respect to tetracycline time-marking (Table 5: SW-DL-7903), and also by default from the fact that the two GLGs/y hypothesis is not feasible for six of the ten specimens in Table 3 (and for two of these in Table 4) because of the calculated negative ages at capture. A clear-cut conclusion on deposition rate is not possible, however, because, unfortunately, what seems to be most apparent from this investigation is that full standardization of GLG counting among readers is not yet attainable, and further effort will be required to interpret GLGs with confidence. This is an important finding from this workshop.

The results from this study clearly indicated that an annual deposition rate was most likely. Notwithstanding the recent new evidence in support of the hypothesis of one GLG/y deposition rate by Stewart et al. (2006), there are a number of recommendations that would still be valid in furthering the work of age validation and standardization in this species. Dentinal GLGs will continue to be one of the most used methods for age determination of belugas taken from free-ranging populations.

Recommendations

Although it was not possible to reach a consensus in the identification of GLGs or their deposition rate, participants did agree on a number of ways to proceed in resolving the controversy over the hypotheses of either one or two GLGs/y. They include seven recommendations, listed in the following sections, comprising two main two-part specific recommendations and five general ones.

Specific Recommendations on Determining GLG Deposition Rate—Given the uncertainty that remains in interpreting GLGs in beluga whale teeth, there is a high priority on using tetracycline marks to map growth-layer patterns. Captive animals provide the most efficient approach for obtaining sufficient sample sizes and allowing for multiple treatments. To this end, we recommend that

- captive animals be treated at a minimum of 1-y intervals throughout their life, with treatments occurring either on their birthday (when known) or at least on the same date from year to year.
- treatment dosages be sufficiently high or continued for a sufficient period of time to allow for an unambiguously visible mark.

In the event that growth-layer patterns in beluga teeth are affected by captivity and would, thereby, potentially affect interpretation of growth layers in teeth from free-ranging animals,

- tetracycline also should be administered to live-caught (and released), free-ranging belugas (although it is recognised that this is potentially problematic in areas where animals are hunted for food).
- growth layers in teeth from free-ranging animals from Hudson Bay should be compared to those from captive animals originally from Hudson Bay.

General Recommendations on Beluga Age Determination—Teeth should be acquired from captive-born belugas that have died, whether or not their teeth contained tetracycline marks, since these are known-age animals.

Certain requisite data, such as life history events, health records, and reproductive history, from captive animals will be required to adequately evaluate growth layers and tetracycline marks. A list of the data needs should be provided to each captive facility.

Because of the highly contrasting pattern in beluga teeth from some geographic locations and the potential differences in migration patterns (large-scale vs relatively minor movements), beluga whales might be a good candidate for understanding what factors influence the deposition of growth layers. Growth layers should be compared among populations with more or less pronounced migrations.

Other means of age determinations of beluga whales need to be investigated for the purpose of validating GLGs or to otherwise age beluga whales, particularly for older animals when the early-deposited layers, including the neonatal line, have worn away—for example, additional work on radio-isotopes, and also aspartic acid racemisation of eye lens (NAMMCO, 2006), which is a method comparing the change in the ratio of two enantiomers of aspartic acid over time (Garde et al., 2007). The rate of change may be species specific.

Given the possible range in ages when counting GLGs, taking a probabilistic approach to age estimation may provide a more robust means of using GLG counts for population models.

In providing these recommendations, the authors are aware that to put them into effect requires funding. There are several Arctic communities that exploit beluga populations and require the best information possible for sustainable management. It is hoped that in order to further this work, some of the recommendations may be supported nationally and by governmental organisations. Additional workshop(s) in the future to address standard protocols for GLG counting in beluga teeth would be valuable to try to eliminate the problems of reader variability and the production of a guide for GLG readers, such as that for the harbour porpoise (Bjørge et al., 1995; Hohn & Lockyer, 1995), would be valuable as well.

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