Dentinal growth layer counts of captive, known-age, mother and daughter belugas (*Delphinapterus leucas*): confirming two growth layer groups (GLG/2) per year; consequences for recovery and management

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ABSTRACT

Captive mother and daughter belugas (*Delphinapterus leucas*) of Hudson Bay origin died at the Vancouver Aquarium, in November 2016, aged 29.25 and 21.25 years. Aurora, captured at age three and captive since 1990, gave birth to Qila in 1995. Age and histories being known, the focus of this study was on total number of tooth growth layer groups (GLGs) present; one (GLG/1) or two (GLG/2) per year. Teeth from Aurora indicated two growth layers per year (GLG/2) during pre-capture and captivity. Qila deposited GLG/2 throughout her captive life. Clearly an assumption of GLG/1 over GLG/2 doubles the duration of all life stages, including lifespan and age at attainment of sexual maturity. Arguments for GLG/1 have been based on inferences (e.g. from fallout of bomb radio carbon) from adults of unknown age and history, while those for GLG/2 are based on the projection of direct observations from newborn, known-age young to 21 and 29 year-old adults from captivity. Use of radiocarbon is ingenious but such analyses have not been rigorously tested on marine mammals and the sensitivity to necessary correction factors not addressed. The new information from this study contradicts the GLG/1 hypothesis in that such an assumption would place Aurora's birth at the 1958 peak fallout of bomb radiocarbon (14C) yet she was born 29 years later in 1987, while the birth of her daughter Qila would pre-date the birth of her mother by 13 years. This paper re-assesses three studies that assigned GLG/1 based ages to older adults using GLG/2 and concludes that their supposed births would also pre-date the realistic births of their own mothers plus as many as two previous generations. Proponents of GLG/1 have overestimated the ages of previous calves-at-capture by 200–800%. Given the growth pattern in teeth of these 21 year and 29 year old female belugas, with increasingly finer dentine GLGs deposited as the pulp tissue diminishes, it is difficult to envisage the GLG/1 tooth structures of those assumed to be 60–80 years; none are as yet available. An holistic analysis using direct observations and cross-referenced parameters does not substantiate GLG/1which implies a 40% reduction of the intrinsic rate of natural increase; as a consequence, overestimating historical population size as well as recovery target population and the predicted date of recovery in Cumberland Sound.

KEYWORDS: AGE DETERMINATION; BELUGA; BOMB RADIO-CARBON ^{14}C; CAPTIVE; DENTINAL GLGS; GROWTH; KNOWN-AGE INDIVIDUALS; MANAGEMENT; MODELLING; NARWHAL; REPRODUCTION; SEXUAL MATURITY; POPULATION

INTRODUCTION

The white whale or beluga (*Delphinapterus leucas,* hereafter beluga) and the narwhal (*Monodon monoceros*) are unique amongst cetaceans, in that they inhabit the north polar region year-round. Migration and access to their habitat is contingent upon ice conditions (although less constraining for belugas in the Gulf of St. Lawrence) and both males and females have adapted to this strict seasonality through intensive herd integrity and synchrony (e.g. see Brodie *et al.,* 2013). The sequence of these evolved annual transitions is reflected in a series of growth layers in dentine and cementum of their teeth. Tooth growth layer groups (GLGs: Perrin and Myrick, 1980) are readily resolved in tooth sections of wild belugas, though less defined in those captured as calves and held in captivity. This paper updates Brodie *et al*. (2013) who reviewed thoroughly the then available evidence on the annual deposition rate of GLGs (GLG/1 is one GLG per year and GLG/2 is two GLGs per year). Those authors concluded that 'the suite of data now available from captive belugas and studies of younger known-age animals are consistent with GLG/2 and do not support GLG/1'.

Based upon GLG/2, Brodie and colleagues (Brodie, 1971; Brodie *et al.,* 1981) had earlier reached several associated conclusions on biological parameters, i.e. that female sexual maturity was attained at 5 years and at 85% of the physically mature length. Ovulation would occur 9–10 months later during the breeding season in early spring at age 5.75 years. Gestation was calculated to be 15–16 months (*ca.* 458–488 days), with births in late July-early August followed by 24 months of lactation during which the female becomes pregnant in the spring of the second year of lactation, for a reproductive cycle of 36 months. Tooth eruption began in the second year with partial eruption by the third. In males, Brodie (1971) concluded that sexual maturity was attained at 8 years at 90% of physical maturity, with the first opportunity for breeding estimated to be at 8.75 years the following spring. Physical maturity of both sexes was attained after 10 years and lifespan in the wild was estimated to be 30–35 years. Herd integrity, synchrony, cumulative under-ice experience of co-existing generations, continuous communication, massive energy reserves and insulation, intensive parental investment and rapid growth may combine to offset the higher natural mortality of both young and adults which might be presumed in such a challenging habitat (Brodie, 1969b; Brodie *et al*., 2013).

This paper considers the new information (including teeth readings) from two animals that died in Vancouver Aquarium

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in November 2016 and how this new information fits with the discussion of Brodie *et al*. (2013). It also considers additional work from other authors on this topic since 2013 in the Discussion section.

Background information

Structure of beluga teeth and difficulties in reading in the context of estimating GLG deposition rates

Brodie *et al.* (2013) provided a short review of the nature and physiology of beluga teeth. This section focuses on some key issues relevant to the question of deposition rate. Delayed tooth eruption (beginning in the second and third year) suggests reduced necessity for functional teeth in early years for a number of possible reasons (see discussion in Brodie, 1969b; 1971; 1985). Stewart and Stewart (2014) examined early tooth growth in greater detail and confirmed the age of tooth eruption proposed by Brodie (1971). Beluga teeth do not interdigitate, rather upper teeth abut lower teeth at an angle, resulting in the characteristic wear pattern and effectively sharpening contact areas (Brodie *et al*., 2013). This excavation into the portion of upper abutting teeth exposed above the gum can occasionally be extreme, however the diminishing pulp tissue deeply embedded in the tooth socket is not compromised. That teeth of belugas, both in the wild and in captivity, appear to reflect periods of stress through restructuring of dentine that are not apparent in cementum) suggests a lower functional priority of teeth compared to many other odontocetes.

Dentine of beluga GLGs are conical structures, the earliest being three to five-fold greater in height than later GLGs (see plate 1 in Brodie *et al*., 2013). As the width of succeeding cones is constant, early GLGs have a greater surface area. Early GLGs are thinner in cross-section than later GLGs (figs 1 and 2 in Brodie *et al*., 1990). However, with greater surface areas, the total volume of early GLGs equals or exceeds more recent mid-life GLGs, unlike sperm whales. When analysing teeth from captive animals, some authors have relied upon the dentino-cemental junction nodes (Goren *et al*., 1987; Hohn and Lockyer, 1999) as evidence of pre- and post-capture growth, since the central GLG structures and markers are sometimes obscured or redistributed by irregular growth in captivity. In examining teeth sections (Brodie *et al*., 2013), a loss of early GLGs can be identified by examining the characteristics of the remaining GLG pattern and the progressive increase in total angle of deposition of the GLGs from the tip of the tooth to the base. Beluga teeth have characteristics which are unlike sperm whales (illustrated in Scheffer and Myrick, 1980; as described in Brodie *et al*. (2013).

Brodie *et al*. (2013) noted that GLGs in longitudinal sections of wild beluga teeth from Cumberland Sound exhibit a general pattern (see Plate 1) beginning with a total acute angle at the pulp cavity of 25–30°, increasing by 1–2° increments to 40–50° after 12 GLGs, 70–80° after 18 GLGs, about 110° after 30 GLGs, and then 120° and increasing thereafter until they often appear to be compacted at 150–170° at the base of the tooth. Commensurate with the decreasing height and increasing angle in dentine layers, the conical pulp cavity within the tooth, consisting of connective tissue and dentinoblasts, diminishes in height, volume and, relative to early growth, to 10%–20% of the pulp interfacing with the dentine core (Fig. 1) resulting in the formation of fine layers of diminishing thickness and volume (170–200µ), *ca* 20% that of central growth layers. While the terms 'compacted' and 'compressed' seem appropriate descriptions for these thin layers ('varves' in geological terms) they are not actively 'compressed' – rather their diminishing thickness and altered shape define the gradual collapse of pulp volume, hence reduced capacity to produce dentine at the pulp cavity interface. Beluga males have larger, more robust teeth, with thicker layers (1000–1200µ). They are more socially demonstrative (e.g. performing jaw-claps) and this may result in increased erosion. Increasingly amongst older adults, the volume of dentine and thickness of individual growth layers begins to diminish into a series of thin, almost flat layers, deposited at the base of the tooth. Thus, as many or more GLGs are deposited in this lower 25–30%, than in the earlier and much larger portion of the tooth. This would indicate that the tooth was being extruded at a diminishing rate as the tooth volume (increasingly dominated by the continuous growth of peripheral cementum deposits) thus greater exposed tip surface (proportional to tooth radius: πr^2) experienced less reduction in height through erosion. By this stage the pulp tissue interface is reduced from a cone to a shallow disc. In the case of Aurora (described below), after depositing approximately 60 GLGs, the tooth appears to almost cease extensive growth of dentine.

Known-age or known-history animals

First-hand observations of summer calving concentrations of belugas in Cumberland Sound and the tagging of hundreds of animals in Hudson Bay (Sergeant and Brodie, 1969) provided evidence of the rapid early growth of newborn and calves (fig. 2 in Brodie, 1971 and see discussion in Brodie *et al.,* 2013). Restricted breeding and calving periods for these highly social animals results in identifiable year-classes; a pattern of social and reproductive behaviour evident in the majority of captive belugas, including those born in captivity and reflected in tooth growth. Growth curves of wild belugas (as in fig. 3 of Brodie ,1971) and of captive animals (as in fig. 6 of Robeck *et al.* 2005; and see fig. 2 in Brodie *et al.,* 2013) also demonstrate rapid early growth; thus the chance of introducing error to total age is minimal. Capture of calves for aquaria in remote areas is limited by logistics, selecting animals of manageable size (the majority estimated at 2–4 years old, although two Alaskan calves (Alex and Lugosi) could only have been *ca* 1.25 years of age as described in Brodie *et al*. (2013). Under the assumption of GLG/1, assigning excessive numbers of growth layers to calves greatly exaggerates their age at capture, such that estimated pre-capture life often exceeds that of many years in captivity (see the analysis of Hohn and Lockyer, 1999, in Brodie *et al*., 2013).

The case histories for the animals that died in 2016 are summarised below.

AURORA

In November 2016 Aurora, an adult female, died at estimated age 29.25 years, one week following the death of her adult daughter Qila, aged 21.25 years, at the Vancouver Aquarium. The earlier history of both females was described in Brodie *et al.* (2013). Aurora was captured in Churchill, Manitoba,

Fig. 1. Tooth sections from female belugas: 29.25 year old Aurora (A, left) and her 21.25 year old daughter Qila (B, right), using transmitted flash on unstained sections. A: Aurora has retained her prenatal tooth and neonatal line, with six GLGs representing her 3.25 years prior to capture. Post-capture GLGs are less defined and may also reflect changes in tooth metabolism during reproductive cycles. The enlarged 15mm segment at the tooth base (34% of tooth length) contains 39 GLGs of diminishing thickness with increasing dentine angles (60–170 deg.) Total GLGs estimated in two sections: 55+ and 60+/–. B: Qila had lost most of her prenatal tooth and neonatal line with all layers present. The dentine core approximates Aurora's in diameter, however the cementum layers are thicker on Qila. While slightly obscure at the very tip, there are identifiable GLGs for most of the length, some less well-defined, possibly related to her reproductive cycle. The 10mm segment within the enlarged image near the tooth base (25% of tooth length) contains 21 GLGs of diminishing thickness with increasing dentine angles (80–170 deg). Total GLGs estimated 40–42. The changing structure of GLGs in both of the enlarged images defines the change in shape and the gradual transition or 'collapse' of the tooth pulp from a cone to a flat disc approximating *ca*. 10–20% of the earlier interface with the dentine core, eventually depositing growth layers *ca*. 20% the thickness and of greatly reduced tissue volume relative to earlier layers. The figure Illustrates the reduction of the pulp tissue volume and interface, beginning after physical maturity. Given that these teeth are from 29.25 and 21.25 year old females, it would be challenging to resolve (or envisage) a 200–267% increase in GLGs added to this structure, based on the 60–80+ year life-span as proposed by some proponents of GLG/1. [See higher definition tooth sections and close ups for Aurora and Qila in Online Supplementary Material for this paper.]

on western Hudson Bay, Canada on 15 August 1990 and was approximately 250cm on 30 August 1990. The growth curve for known-age females (Robeck *et al.*, 2005) indicated that she would have been a minimum of 2.25 years. and a maximum of 3.25 years (thus, born in the wild in June 1987 or 1988). In September 1992 she was 325cm and on 23 July 1995, at a now-estimated age of 8 years, she gave birth to her first calf, Qila. Conception would therefore have occurred *ca.* 15 months prior, at age 6.75 years. Thus, after an estimated 3.25 years in the wild, she conceived and gave birth within the timeframe estimated for wild belugas by Brodie (1971) and directly observed for captive females in the presence of a known breeding male, although she may have been capable of conceiving one year earlier (Robeck *et al.*, 2005; Brodie *et al*., 2013). On 7 June 2009, Aurora gave birth to a female, Nala who was substantially larger than Tiqa at birth and who died on 21 June 2010 at one year, larger than 'usual' (length 243cm, max. girth 172cm and calculated weight 260–270kg). Last live length taken for Aurora was 343cm with a post-mortem weight of 645kg.

QILA

Qila conceived in early 2001 at 5.75 years but lost the 200mm foetus in September 2001. At just under 13 years Qila gave birth to a female calf (Tiqa) on 10 June 2008 who died at 3.25 years (length 298cm and axillary girth 189cm). Aurora therefore became a grandmother at age 21 years, although this could have been as early as 15 years had Qila not miscarried. Qila was 356cm, axillary girth 211cm, post-mortem weight 560kg. Calves Nala and Tiqa were large for their age, suggesting that Aurora may have been as well.

MATERIALS AND METHODS

Additional age data of captive animals

Methods for preparation of tooth sections

At post-mortem, teeth were extracted from each animal and sent on to PB. Teeth were hand-held and initially ground against a vertical sanding belt from both sides of the tooth, reducing the mid-length section to approximately 1.5mm thickness. This procedure allowed the section to be reduced while following tooth curvature, thus reducing the mid-layer distortion resulting from standard embedded thin sections and, in the case of Aurora, retaining a remnant of the partially eroded prenatal tooth. Sections were then hand-held against a horizontal fine-grit, water-lubricated rotary stone and further reduced to a thickness of 200–300µ; appropriate for examination by transmitted light, yet sufficiently robust to allow the section to be manipulated by hand to detect layering. The use of thicker tooth sections can be manipulated under varying light to reveal growth layers while retaining sufficient definition which may be lost near the base in thinner sections, resulting in underestimation of GLGs. Sections were initially stored in glycerine, however they began to slightly curl upon drying, distorting photography. To maintain a flat section, they were soaked in hot water, then placed between glass slides and held together with elastic bands, further immersed in hot water (to avoid fracturing) until they were gradually drawn flat. Sections were stored in this manner and could be removed for examination under a *Wild* binocular dissecting microscope using transmitted light at 12 to 25 magnifications. For long-term storage the elastics were replaced by tape. This procedure is suggested as an alternative to thin sectioning of embedded or curved teeth when proper laboratory equipment is not available. Images were taken with an *Apple iPad* mini with additional 4X magnification using a *Kenko Real Pro* clip lens. GLG counts of dentinal layers were done by PB (see Fig. 1); cementum layers were not readily resolved in these thicker sections. High resolution images of complete sections using transmitted flash were later provided by K. Bentham of Bedford Institute of Oceanography.

RESULTS

Fig.1 shows that Aurora's 44 and 45mm tooth structures remained complete after 29.25 years, with both prenatal tooth and neonatal line evident, whilst the more robust 38 and 37mm teeth of 21.25 year-old Qila were slightly worn with much of the prenatal tooth missing. This is in keeping with the pattern of growth in older adults and the diminishing thickness and increasing angle of GLGs as they are deposited near the base (Brodie *et al.,* 2013). Earlier GLGs in the smaller teeth of females are 800–1000µ in thickness, while those compacted as they approach the base diminish from 500µ to 300µ, approaching 200µ, the result of greatly reduced pulp; often difficult to resolve *ca.* 2mm from the pulp cavity. Lockyer *et al*., (2007) in their Table 4 refer to these as 'uncounted (uncountable?) compacted [layers] GLGs', however these *ca.*170µ growth increments (5–6 per mm) can often be resolved by the process described, and are significant, representing several years of additional deposition. Pregnancies and lactation may result in more diffuse GLGs, a consequence of resorption and restructuring of the dentine; in the wild, this may be evidence of a response to massive trauma such as formation of scars resulting from large bullet wounds (Brodie *et al*., 1990).

Aurora

Aurora's tooth shows 6 clear GLGs immediate to the prenatal tooth and neonatal line; assuming GLG/2 this is in accord with Aurora being some 3.25 years prior to capture (Brodie *et al.,* 2013); four of these GLGs would have been deposited before the tooth erupted and was exposed to wear. Assuming GLG/1, these 6 layers would represent six years. This would be inconsistent with the estimated 2.5m at capture. It would also imply that the animal was sexually maturity at capture. The 15mm segment at the base of the tooth (34% of total length) contains *ca.* 39 GLGs of diminishing thickness and with increasing angles from 60° to 180° (Brodie *et al.,* 2013). This small section, including the fine layers near the pulp cavity, contained more GLGs than required to satisfy arguments for GLG/1 for a 29.25 year-old beluga. The middle section is more diffuse, however the nodes of the dentine-cementum juncture could be resolved and when added to the post-natal layers, gave an estimated a total of 55+ GLGs. A second tooth section was better defined with 60+/– GLGs (see Fig. 1A) indicating GLG/2 for Aurora.

Qila's two tooth sections, while more obscure near the tip, showed readily identified GLGs for much of the length. Similar to Aurora, 10mm at the base of the tooth (25% of total length) contained 21 GLGs of diminishing thickness and with increasing angles from 80° to 170–180°. As with Aurora, this small section contained GLGs sufficient to satisfy arguments for GLG/1 for a 21.25 year old. Total GLGs were estimated at 40–42 (Fig. 1B), indicating GLG/2 for Qila.

DISCUSSION

Both belugas were of known-age (or almost known age) and growth layers were clearly correlated with two, rather than one, layers per year, i.e. GLG/2. Aurora spent 26 of her estimated 29.25 years (90%) in captivity, while Qila spent her entire 21.25 years captive. After an estimated 3.25 years in the wild, depositing six GLGs, Aurora continued depositing layers consistent with GLG/2. The observed change in tooth structure attributed to capture may not be immediate, rather a post-captive lag response; the calf being in good condition and well adapted to establishing abundant reserves in a wild existence, sufficient to continue the lamination pattern for weeks or months in captivity. Despite being held at a different latitude, without experiencing pack-ice and migrations, while being hand-fed year-round, both retained a GLG/2 sequence. However, laminae were generally not as distinct as those in the wild; the more diffuse sections of the teeth possibly reflect periods related to reproductive cycles of both females. The teeth of Aurora and Qila indicate that both the diminishing pulp volume and rate of dentine deposition began at about physical maturity (Fig. 1). The structural progression observed in teeth from these known-age belugas, with increasingly finer GLGs deposited as the pulp tissue diminishes in function and dentine growth almost ceases, is difficult to reconcile with the tooth structures of belugas deemed according to GLG/1 as 60–80 years old, i.e. 200–267% older, as recorded in AMMPA: Alliance of Marine Mammal Parks and Aquariums); however, tooth specimens of that purported age have not been examined by PB.

The tooth structures of these known-age females allows comparison with those presumed to have known histories. In Hohn and Lockyer (1999) and Lockyer *et al.* (2007), the female beluga (SW-DL-7903) was assessed to have 18.2 (SD 2.17: 16.03–20.37) layers; therefore, based on their assumption of GLG/1, she would have been 18 years old, i.e. just younger than known-age (21.25 years) Qila. This is not consistent with the image of the tooth section in their fig.1, which clearly shows a tooth representative of a young adult, with an open pulp cavity of 70–90°, less complex and with no evidence of the compressed laminae observed in the lower tooth segment of Qila. The lack of evidence of tooth resorption suggests that she probably did not experience any stages of pregnancy in captivity (had she been held captive with a known breeding male, she had the potential for two reproductive cycles). Their assumption that she was similar to 356cm Qila in age is also inconsistent with the length data – based on 262cm at capture, 7.92 years in captivity and 329cm at death, beluga SW-DL-7903 was more likely around 11 years old i.e. 52% the age of 21.25 year old Qila and 38% the age of Aurora (Brodie *et al.,* 2013).

Similar arguments apply to the conclusion of the same authors that the male (Churchill) was 27.8 years (SD 2.17: 25.6–30 years), based on GLG/1, i.e. supposedly near the age (29.25 years) of Aurora. Again, the tooth section (fig. 3 in Hohn and Lockyer, 1999) does not exhibit the structure of an older adult when compared to Qila and Aurora. Churchill would have been sexually mature by age nine (Brodie, 1971; Robeck *et al.,* 2005). The age is also inconsistent with the length data (304cm at capture, 7.83 years in captivity and 340cm at death) suggests that Churchill was around 13 years old; 45% of the age of Aurora and 61% of the age of Qila (Brodie *et al.,* 2013).

As observed in Brodie *et al.* (2013) with reference to Hohn and Lockyer (1999): ‘the appearance and interpretation of the tooth sections for the female (figs. 1 and 2 in Hohn and Locker, 1999) implies that *ca.* 65%–75% of the volume of tooth deposition occurred before capture; this has not been observed in calves and sub-adults of that size.’ The images of teeth from Aurora and Qila would seem to confirm this, for example: had their histories not been known (other than being captive for 21 and 26 years) then the previous 65% – 75% of their total tooth volume and excess GLGs (half of the total) would have been assigned to pre-capture growth (equal to the time in captivity) overestimating Aurora’s age as a calf by 900%, yet it is known that Aurora must have been captured at three years and would have been in the early stages of tooth eruption (Brodie, 1971). Qila was born in captivity, yet the same assumptions would have incorrectly assigned her a pre-capture life of 21 years.

Applying GLG/1 to animals captured as calves and held in captivity for extensive periods results in the assignment of (GLG/2 based) two-fold excess growth layers deposited before and during captivity, to the pre-capture period of unknown history, for example:

(1) In Hohn and Lockyer (1999) the pre-capture ages are overestimated by as much as 400% (Brodie *et al.,* 2013); and

(2) In fig. 4 of Lockyer *et al.* (2007) a calf (No-See-Um) was 257cm at capture (thus of maximum age 3.2 years) (Robeck *et al.*, 2005 and Brodie *et al.*, 2013) however in their table 4 the age at capture ranged 24.3 years + (column K) to 2.6 years + (column L) a 900% discrepancy. With maximum age 24.9 years (3.2 years + 21.7 years captive) and maximum GLGs (column H) at 46+ (+ indicating tooth wear) provides evidence for GLG/2.

Re-examination of the Lockyer *et al.* (2007) data set (Brodie *et al*., 2013) concluded that GLG/2 was appropriate for all 10 specimens. Clearly, the history of these animals was not known during their analysis. Heide-Jøgensen *et al.* (1994), also documented in Hohn and Lockyer (1999) and Lockyer *et al.* (2007), examined GLGs for a captive female beluga Allua. Her history was examined in greater detail (Brodie *et al.,* 2013) concluding there was no evidence from wild or captive belugas supporting Hohn and Lockyer (1999) or Lockyer *et al.* (2007) with reference to Allua, all evidence being in the direction of GLG/2, as originally determined by Heide-Jørgensen *et al.* (1994).

Stewart *et al.* (2006) and Campana and Stewart (2014) used bomb radiocarbon 14 dating (^{14}C) from atmospheric fallout in 1958 to calibrate age estimates based on growth layers in teeth of wild belugas. In the 2006 study, five females (of unknown length and history) were selected so that 'back-calculation from their year of death would place their birth date either before the period of atmospheric atomic testing if age = GLG/1 or after bomb testing if age = GLG/2'. Although no information on body length was provided, an examination of the tooth structure suggested that the females were older adults and the male was younger but still sexually and physically mature. Brodie *et al.* (2013) pointed out the uncertainties in the technique and the correction factors used in the analysis used (see quote from B. Buchholz, in Brodie *et al.,* 2013) as well as the need to take into account the effect on ^{14}C burden of intensive feeding during pregnancy and transfer from mother to calf; these issues remained in the 2014 paper of Campana and Stewart. Given the conclusion of GLG/1 by Stewart *et al.* (2006) despite these difficulties, Brodie *et al.* (2013) had examined the histories of five additional animals either captured as calves or young adults (ages 1–6 years), with evidence sufficient to consider them of known age, having achieved adulthood during the late 1960s to the 1980s i.e. after the peak ^{14}C fallout; again the evidence was inconsistent with GLG/1 for these animals. With the additional evidence presented here for GLG/2 regarding Aurora and Qila, the ages of six belugas in table 1 of Stewart *et al*. (2006) are reassessed below; halving their age estimates as determined by the authors, from GLG/1 to GLG/2, as well as a revised date of birth and suggested reproductive status:

(1) Female (B92–105) hunted in Iqaluit, Baffin Island 1992, estimated at 55 years old assuming GLG/1, i.e. born about 1937. Their fig. 1 provides a good example of a female tooth structure from an animal in the wild (distinct GLGs, few if any missing at the tip, phasing to 10 compacted layers at the base of the tooth). However, greater tip erosion would be expected in an animal of that age; based on the tooth structure illustrated in the present paper, we estimate that this animal was about the same age or younger than Aurora i.e. born about 1964 with first-birth *ca.*1971 and a potential for seven reproductive cycles;

(2) Female (LH91–27) hunted in Kimmirut, Baffin Island 1991, 51 years old assuming GLG/1 i.e. born about 1940. Using GLG/2 the age is 25–26, thus born in 1965–66 with first-birth in 1972–73 and a potential for six reproductive cycles;

(3) Female (B92–34) hunted in Pangnirtung, Baffin Island 1992, 60 years old assuming GLG /1, i.e. born in 1932. Using GLG/2 the age is 30, thus born about 1962 with first-birth in 1969 and a potential for seven reproductive cycles;

(4) Female (B92–108) hunted in Pangnirtung, Baffin Island 1992, 59 years old assuming GLG/1, i.e. born in 1933. Using GLG/2 the age is 30, thus born in 1962–63 with first-birth in 1969–70 and a potential for seven reproductive cycles;

(5) Female (B97–037) hunted in Kimmirut, Baffin Island 1997, 59 years old assuming GLG/1, thus born in 1938. Using GLG/2 the age is 30, thus born in 1967 with first-birth in 1974 and a potential for seven reproductive cycles; and

(6) Male (ARLHxx1003) hunted in Kimmirut, Baffin Island 2001, 22 years old assuming GLG/1, i.e. born in 1979. Using GLG/2 the age is 11, thus born in 1990, sexually mature in 1998–99 and, depending on social status, capable of breeding.

The reassignment of ages using GLG/2 would mean that the five oldest female belugas listed in the group above were born after peak fallout of ^{14}C, as were the group of five adults of similar ages and profile examined in Brodie *et al.* (2013). It seems unlikely that two groups of adult belugas would have two-fold differences in age, the wild group living twice as long as the (known-age) captive group, until they were eventually taken by hunters. It is more plausible that both groups would have received their major burden of ^{14}C through generational transfer from their mothers, augmenting their own burdens acquired later from their food base. Belugas (1), (2) and (5) could have been from second reproductive cycles following peak fallout, further diluting any acquired ^{14}C overburden. Beluga females appear capable of at least seven complete reproductive cycles in their lifetime, and potentially four generations by age 28 years (Brodie *et al.,* 2013); assuming GLG/2, the females above would have had similar potential. Given that, the 1930–1940 birth dates assigned by Stewart *et al*. (2006) to the five older belugas would, based on GLG/2, pre-date, not only the births of their mothers, but the births of two previous generations as well. Assuming GLG/2 seems a more plausible explanation of the information presented.

Tables 1 and 4 of Lockyer *et al.* (2007) provide collection dates and lengths of calves that can be used to estimate age at capture for nine of the ten specimens (Fig. 2); Brodie *et al.* (2013) provided information indicating that the remaining male (Winston) was probably captured as a calf. With capture dates ranging from 1969 to 1989 and using realistic ages assigned at capture, all specimens were consistent with GLG/2, with births 6–17 years after peak ^{14}C fallout in 1958. Given the interval since 1958, burdens of ^{14}C in these animals would have indicated background levels acquired during their lifetime of feeding, augmented by some level of ^{14}C generational transfer from their mothers, who would have experienced dilution of their ^{14}C burden during 2–5 previous reproductive cycles before births of the calves in question. These post ^{14}C peak fallout animals share similar profiles to the six animals examined by Stewart *et al*. (2006). The most parsimonious evaluation of the available information, considering the uncertainties already noted for the Stewart *et al*. (2006) study, is the hypothesis of GLG/2.

This is confirmed by evidence from 29.25-year-old Aurora; if GLG/1 is assumed she would have been born near the peak ^{14}C fallout in 1958, yet she was born 29–30 years later in 1987, implying that she would predate the actual births of her mother and at least two previous generations. Similarly, Qila would pre-date the birth of her mother Aurora by 13 years.

A recent workshop on age determination (Lockyer *et al.,* 2016) addressed techniques for age determination of belugas using tooth growth layer counts. Apart from one captive specimen (Tiqa, a 3.25-year-old born in captivity) all tooth samples were from wild populations with typically clear GLG definition, but of unverified age and length. Fig. 2

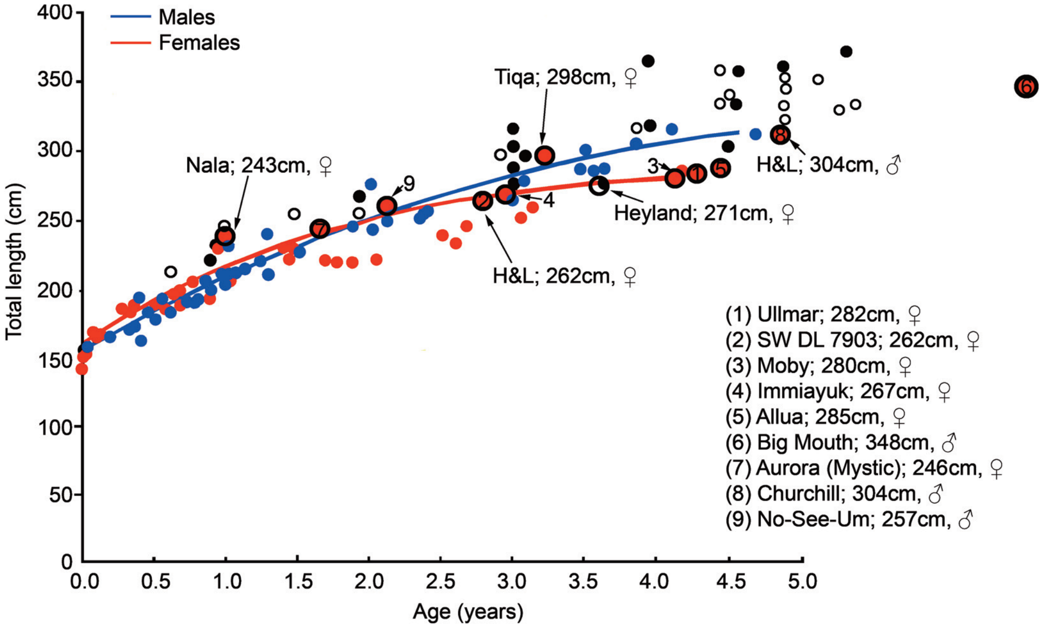

Fig. 2. Based on known-age captive belugas (from fig. 6 in Robeck *et al.,* 2005), augmented with known-age wild belugas (open ♀ and black circles ♂) from Cumberland Sound (Brodie 1971: fig.2 in Brodie *et al.,* 2013). Belugas in Cumberland Sound are larger, as is evident. Captive-born, known age: one year old Nala (243cm F) and 3.25 year old Tiqa (298cm F) are included (described in Brodie *et al.,* 2013). Nine specimens from Lockyer et al (2007), Table 4., are plotted based on lengths at capture (column C). Ages from the above (125 data points, including newborn) graph of known-age captive and wild calves are compared with their assigned age at capture based on GLG/1 (their fig.4 column K) indicating errors in age estimates ranging from 200–800%. Ages of all specimens in their fig. 4 were found to be consistent with GLG/2, both here and previously in Brodie *et al.* (2013).

shows 125+ data points correlating length and age of newborn, calves and adolescents. Lockyer *et al.* (2016) confirm the age of Tiqa but do not provide the length; it was previously given in Brodie *et al.* (2013) as 298cm at death and is included in Fig. 2. The GLGs in stained and unstained tooth sections in fig. 12 of Lockyer *et al.* (2016) were described as 'ill-defined' as is typical of captive specimens. They stated that the consensus was 'that there were 3 fully formed GLGs in the dentine and a partial fourth' – this seems a minimal estimate to these authors based upon the figure. Judging from the image of the unstained section, this seems a minimal estimate. It is unclear (a) why this tooth section was not subject to the high quality imagery applied to the others and (b) why the uncertainty reflected in the discussion in the report and in a previous workshop (Hohn *et al*., 2016) it is stated that 'interpreting one GLG as an annual record is irrefutable'. When stating that a hypothesis is 'irrefutable', it is essential to examine the implications of the hypothesis with additional cross-referencing. For example, the length of Tiqa at death (298 cm) and age (3.25 years) is consistent with Brodie *et al.* (2013), but not with with Hohn and Lockyer (1999) and Lockyer *et al.* (2007) without assuming unrealistically large numbers of GLGs pre-capture. For example (table 4 of Lockyer *et al.* (2007), the animal No-See-Um was 257cm at capture, substantially less than 298 cm 3.25 year old Tiqa and thus unlikely to be more than 3 years old. Assuming GLG/1 requires him to have been 24.3+ years old at capture – this is slightly older than know-age 24-year-old Alex who died at length 405cm and *ca* fourfold greater weight of 1,200kg (Goren *et al.*, 1987; Brodie *et al.,* 2013) and clearly implausible. Similarly (same table), the female Aurora (Mystic) was 246cm in length at capture, and spent 15.2 years in captivity and had an estimated maximum 37 GLGs at death. The 21.8 excess GLGs from the 15.2 years in captivity were assigned to the 246cm calf; again implausible.

Evidence presented in Brodie *et al.* (2013) is not properly addressed by Lockyer *et al.* (2016) or Hohn *et al*. (2016) and the new data presented in this paper provide additional evidence that as a minimum, evidence for GLG/1 cannot be described as 'irrefutable'; indeed, the evidence for GLG/2 is far more convincing. The tooth sections of Qila and Aurora in Fig.1 demonstrate the diminishing size deposition of dentine GLGs, similar to those in Lockyer *et al.* (2016); i.e. well established in animals known to be 21.25 and 29.25 years old.

Implications for management

Brodie *et al.* (2013), noting Stewart *et al.*'s (2006) comment that incorporation of vital parameters based on GLG/1 in models estimating population growth results in a 40% reduction of the intrinsic rate of natural increase, commented that with respect to the Cumberland Sound population: 'A more sophisticated population modelling exercise would be valuable to further explore the implications of life history parameters for specific populations for which good abundance data over time are available.' The same comment is relevant to Matthews and Ferguson (2014) and a recent paper by Waugh *et al.* (2018), see below.

Choice of GLG/1 or GLG/2 and its effect on biological parameters has a major effect on back calculations to determine population status (e.g. Mitchell and Reeves, 1981; Richard, 2013; Marcoux and Hammill, 2016: Marcoux *et al.*, 2016); Brodie *et al.* (2013) provided arguments as to why the use of 4% to model population growth is probably an underestimate. A recent paper investigating the status of

Cumberland Sound belugas and how density-dependent relationships affects their growth and recovery, indicates uncertainty of vital parameters based upon GLG/1 (DFO., 2016) e.g. ‘sexual maturity might fall between 8 and 14 years of age, and longevity may be 60+ years.’ The report noted that ‘the estimated starting population … in 1960 was estimated to be 3,400 animals…’. Aerial survey estimates of about 800 animals were obtained from 1967 and 1977 (Brodie, 1971; Brodie *et al.,* 1981) and later confirmed in 1978 (Brodie *et al*., 2013). Assuming that there indeed was a (pre-hunt) starting population of 3,400 in 1960, we are left to address how the isolated stock had been so rapidly reduced over six intervening years to well under 1,000 in 1966. With an intrinsic rate of increase (under GLG/1), annual removals would have to have equalled some 500 animals until 1966; such removals were not recorded in catch statistics:’ A period of low apparent catches lasted from 1962 to 1975’ (see also table 1 of Brodie *et al.,* 1981) and seem implausibly high even though the numbers do not include unreported kills, hunting losses or removal of young (Brodie *et al*., 2013). Furthermore, DFO (2016) states that ‘the current (2015) population is 1,000 animals…’, which is 49 years after the supposed rapid decline to 1966 and, despite continued hunting removals (under quota since 1980) and 36 years after the excessive catches of 120, 178, 82 and 70 from 1976–79 (Table 1. Brodie *et al.,* 1981). Reassessment of surveys must address how population numbers were sustained, even indicating recovery. The DFO (2016) population estimate for 1960 would appear to be implausible.

CONCLUSIONS

Growth and age determination of belugas has been controversial for decades (e.g. Sergeant, 1959; Brodie, 1969; 1971; 1982; Brodie *et al*., 1990; Stewart *et al*., 2006; Lockyer *et al*., 2007; 2016; Willis 2012; 2013). While it is important to define and count GLGs, it is equally important to understand their interpretation in terms of the whole animal. This study adds to the body of evidence based on an holistic approach using cross-referenced parameters and direct observations of known-age animals (Brodie *et al.,* 2013), that support the hypothesis that belugas deposit two rather than one GLG annually. Ramifications are substantial; female belugas becoming sexually mature and conceiving at 5.75 years, producing a calf by age seven years (Brodie, 1971) with the potential to be a grandmother by age 14 years when, based on GLG/1 (Stewart *et al*. 2006), a female would be experiencing her first birth at that age. Under GLG/2 and on the assumption of the first-born being female, a beluga is capable of at least seven reproductive cycles in her lifetime, and potentially four generations by age 28 years (Brodie *et al.,* 2013). Females the ages of Qila and Aurora (Vancouver), in a large breeding population, could have the potential of being a great-grandmother and a great-great-grandmother respectively by those ages.

This paper provides additional evidence that the GLG/2 hypothesis is consistent with the data available from known age animals from captivity. The GLG/1 hypothesis is not consistent with length data (Fig. 2) and relies on (a) assignment of an implausibly large number of excess growth layers to pre-capture history, (b) lack of consideration of uncertainties in the ^{14}C data and the effects of pregnancy and transfer from mother to calves; (c) difficulties in reconciling the implications of biological parameters based upon GLG/1 in population dynamics and the trajectories of populations in the face of exploitation such as the reduced Cumberland Sound population that has been exposed to intensive hunting for many generations. If GLG/1 is true then one should expect even greater evidence of cumulative bullet scarring (described earlier) amongst animals deemed to be 50–60 years old, rather than half that age.

A very recent study (Waugh *et al.,* 2018), argues that daily incremental growth lines support GLG/1; yet known-age Aurora and Qila demonstrate GLG/2 (Fig.1). Their problems related to distortion when scanning the mid-axis of the curved tooth section might be reduced using the technique described above.

As noted by Brodie *et al.* (2013), it is essential that collaborative, holistic research between field scientists and those maintaining captive animals is used to determine the most plausible hypothesis for GLG accumulation. This paper attempts to do that unlike recent work by Lockyer *et al.* (2016) and we conclude that the available data support the hypothesis of GLG/2; there are many inconsistencies when examining the hypothesis of GLG/1 that the proponents have yet to fully consider. Hohn and Lockyer (1999) with reference to Grue and Jensen (1979) and Klevezal and Kleinenberg (1969) state ‘that the deposition rate of two layers per year makes belugas unique among odontocetes, and generally other mammalian species, as well’. While it may seem counterintuitive that more than one growth layer is laid down per annum, this must be seen in the context of a complex, highly social, totally aquatic, surface breathing homeotherm fully adapted to the extreme challenges of Arctic pack-ice conditions and seasonal extremes of sunlight (Brodie, 1969a). Application of GLG/1 is neither compatible with the known histories of known-age belugas, nor has there been evidence or direct observation to support the hypothesis. Mother and daughter adult belugas Aurora and Qila, had contributed substantially to an understanding of the dynamics of this unique polar species over the years and have provided invaluable evidence for age determination and growth rates following their loss. The two species of the family *Monodontidae* (belugas and narwhals) exist year-round in an Arctic habitat and may share much of the same life-history and vital parameters. Whether this growth pattern is unique to *Monodontidae* is an interesting question.

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