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Sighting history of a naturally marked humpback whale (*Megaptera novaeangliae*) suggests ear plug growth layer groups are deposited annually

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Since the mid-twentieth century, biologists have used counts of baleen whale ear plug growth layer groups (GLGs) to estimate age in studies of commercially hunted whale populations. The deposition rate of GLGs was uncertain until Roe (1968) established that fin whale ear plugs form an annual GLG composed of one light- and one dark-colored *lamina*. Subsequently, annual rates of GLG deposition were found for minke whales (*Balaenoptera acutorostrata*), Bryde's whales (*B. brydei*), gray whales (*Eschrichtius robustus*), pygmy blue whales (*B. musculus brevicauda*), and sei whales (*B. borealis*) (Lockyer 1984*a*). Although annual GLG deposition is accepted for these species, there are no existing data that independently confirm the deposition rate of GLGs for any baleen whale (Lockyer 1984*a*). For humpback whales, uncertainty about whether ear plug GLGs accumulate annually or biannually remained the subject of considerable debate (Bannister *et al*. 2000, Best in press). The question remained open for many years because usable ear plugs are extremely difficult to obtain. Here we provide evidence that humpback whale ear plugs accumulate one GLG annually, based on the long sighting history of a single humpback whale of unknown age whose ear plugs were collected after death. An earlier study concluding that humpback whale ear plugs accumulate two GLGs per year relied heavily on corroboration from counts of baleen tracings (Chittleborough 1959), a technique that was never adequately calibrated (Best in press). The biannual GLG deposition rate was thought to be confirmed by examining the ear plug of a male humpback whale that was killed after being tagged at sea as "a yearling approximately 30 ft in length" (Chittleborough 1960) but difficulties in accurate whale size determination at sea (Dawbin 1959, Best 1984) cast doubt on assumptions about the whale's size and age at marking (Ohsumi 1964, Roe 1968).

An ear plug is a two-part keratin and lipid structure in the auditory *meatus* of baleen whales (Turner 1913, Lillie 1915, Purves 1955) that measures approximately 15 cm long and 2–3 cm wide at the base in humpback whales (Turner 1913) and varies in size among other mysticetes. The ear plug's outer covering is secreted by epithelial cells in the auditory *meatus*, while the ear plug core, comprising concentric light and dark colored *laminae*, is secreted by *papillae* on the surface of the glove finger (Purves 1955). The literature on ear plug ageing has often used vague and inconsistent terminology. In this article, a GLG is a pair of light and dark *laminae*, synonymous with "lamina" in most previous publications, while *lamina* here refers to a light or dark layer within a GLG. The "neonatal line" (Ichihara 1964) composed of a dark *lamina* and a lighter "fatty layer" defines the start of the ear plug core when counting GLGs. A "transition zone" in the ear plug, where widely spaced GLGs abruptly become much closer together, indicates the age at sexual maturity and is believed to be linked with changes in the skull's growth rate after sexual maturity (Lockyer 1972, Kato 1983).

Purves (1955) originally proposed that dark *laminae* formed during biannual migrations during which no skull growth occurred, and light *laminae* formed during active growth periods. Roe (1968) clearly established the link between mysticete migration patterns and *lamina* formation by examining the most recently formed *lamina* in fin whale ear plugs collected in all months of the year, finding that light *laminae* with a high fat content form in the summer feeding season and nonfatty dark *laminae* composed of keratinized epithelial cells form during the seasonal fast in winter. Narrow, uneven, poorly defined, minor *laminae* of both the light and the dark type termed "accessory lines" (Roe 1968) were confusing to early investigators (Ichihara 1966), but determined to be insignificant in age determination by others (Roe 1968, Ohsumi 1964). One investigator noted that odd numbers of GLGs were observed frequently in humpbacks on the Australian breeding grounds although if GLGs accrued at a rate of two per year, then all whales at their breeding grounds should have an even number of GLGs (Robins 1960). Ohsumi (1964) ascertained that fin whale ear plugs could not deposit two GLGs per year by dividing the elapsed years between marking and recapture of numerous fin whales by the number of GLGs; moreover, no whales accumulated less than one GLG per year. Although an annual GLG accretion rate was the simplest alternative hypothesis, some investigators (IWC 1962, Ichihara 1966) concluded that young whales may accrue between one and two GLGs per year. However, later investigators concluded that accessory lines in the ear plugs of young whales were the source of the confusion, and that these could be attributed to juveniles' irregular nutritional and migratory habits (Roe 1968; Lockyer 1972, 1984*b*).

Table 1. Counts of ear plug growth layer groups (GLG) of whale #68.

Ear plug	Reader	GLG count	
		Total	At transition
A	Kato	47	7
A	Lockyer	44	7
B	Kato	45	7
B	Lockyer	42	7

In July 2001, a ship struck a 13.9 m (45.5 ft) adult female humpback whale that was later found dead at the mouth of Glacier Bay in southeastern Alaska. Both ear plugs were collected during a necropsy that was performed a few days *post mortem* to determine the cause of death. Counts of ovarian *corpora* that correlate with age in mysticetes were not possible because the ovaries were too decomposed to be identified at the necropsy (although bones from a fetus were found, indicating pregnancy). The ear plugs were preserved in 10% buffered formalin. We bisected the ear plugs longitudinally, shaving them with a razor blade until GLGs were visible from end to end, such that the maximal number of GLGs was evident in the core. After polishing the ear plug surfaces with a fine grindstone (Symons and Weston 1958), two highly experienced ear plug readers (Kato and Lockyer) without knowledge of the whale's sighting history independently counted GLGs in both ear plugs (Table 1) with the aid of a magnifying lens. Efforts to count GLGs in high-resolution photographs of the ear plugs were unsatisfactory. The mean GLG count from independent readings of both right and left ear plugs conducted on May 5, 2007 was 44.5 (SD = 2.08, 95% CI 42.46–46.54; Table 1, Fig. 1). The transition zone was consistent at seven GLG (Table 1). All epiphyseal discs in the spine were fused, indicating physical maturity as would be expected for a 13.9 m whale with 45 GLGs (Chittleborough 1959, 1965; Nishiwaki 1959), but this fact does not help distinguish between the annual vs. biannual GLG accretion hypotheses.

Individual identification using unique markings on the ventral flukes of the dead whale indicated that it was catalog #68 (Straley and Gabriele 1997), sighted many times in Alaska and Hawaii since her first sighting in Glacier Bay in 1975 (Table 2). Whale #68 was not a calf when first sighted in 1975, because she appeared to be the same size as other adult whales.[1] We assume that she was at least 1 yr old in 1975; therefore its minimum age in 2001 was 27 yr. If humpback whale ear plugs accumulate two GLGs annually, then #68's ear plugs should contain at least 54 GLGs, considerably more than the number of GLGs we counted in her ear plugs. Although counting ear plug GLGs can be difficult in older whales because the *laminae* are very close together at the base (Doroshenko 2000), 54 GLGs is well outside the 95% confidence interval for these counts. Counts of large balenopterid ear plugs by different readers tend to agree closely (IWC 1962, 1969) such that any experienced ear plug reader would almost certainly obtain counts very similar to those reported

[1]Personal communication from V. Palmer, Sea Search, Ltd.; current address: 1990 Hughes Way, Juneau, AK 9980115, August 2008.

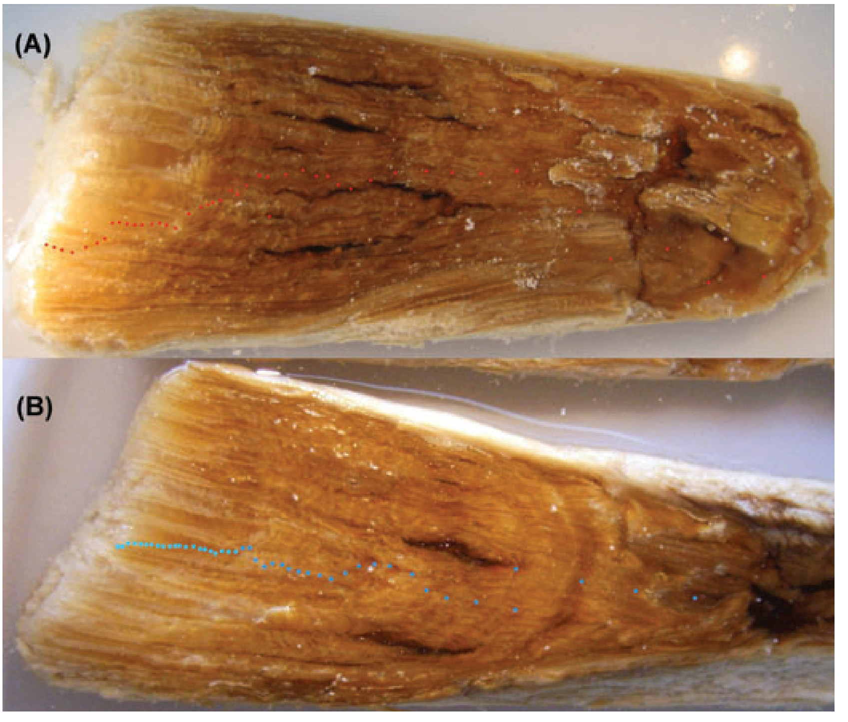

Figure 1. Digital images of ear plugs with markings (A) Lockyer's count of ear plug A showing 44 GLGs and (B) Kato's count of ear plug B showing 45 GLGs.

Table 2. Sighting history of whale #68, 1975–2001.

Year	Area	Region	Age-sex class	Researcher	Number of sightings
1975	Glacier Bay	Alaska	Adult	Jurasz	2
1979	Seymour Canal	Alaska	Adult	Straley	1
1981	Maui, Niihau	Hawaii	Adult	Baker *et al*. 1985	2
1982	Glacier Bay	Alaska	Mother	Baker *et al*. 1985	1
1984	Frederick Sound	Alaska	Unknown	McSweeney	1
1985	Seymour Canal	Alaska	Adult	Straley	2
1986	Maui	Hawaii	Adult	Gabriele 1992	1
1986	Frederick Sound	Alaska	Mother	GBNP,[a] Straley	3
1993	Frederick Sound	Alaska	Mother	Sharpe	1
1994	Peril Strait, Frederick Sound	Alaska	Adult	Straley	2
1996	Frederick Sound	Alaska	Adult	Darling, Sharpe	3
1997	Frederick Sound	Alaska	Mother	Darling	1
2000	Sitka Sound	Alaska	Mother	Straley	2
2001	Icy Strait	Alaska	Adult	GBNP	1
2001	Glacier Bay	Alaska	dead pregnant	GBNP	1

[a]GBNP = Glacier Bay National Park.

here. Therefore, we conclude that one GLG was deposited annually and that #68 was 44.5 yr old when she died.

Sexual maturity at the seven GLG transition phase in #68's ear plug is also more consistent with an annual GLG deposition rate than the biannual rate. Female humpback whales in southeastern Alaska bear their first calves at age 8–16 yr (Gabriele *et al*. 2007) with the age at sexual maturity at least a year earlier. If GLGs accumulate biannually, this would mean that #68 was sexually mature at age 3.5 yr, making her an extreme outlier for southeastern Alaska. By contrast, if GLGs accumulate annually, then #68 reached maturity at 7 yr of age, within the age distribution of *primiparous* females in her natal population (Gabriele *et al*. 2007).

We compared our age estimate of 45 yr for #68 to maximal GLG counts and distributions in historic commercial whaling data. On average, fewer than 9% of the humpback whale ear plugs studied had 45 GLGs or more, but the oldest whales had 57–96 GLGs (Symons and Weston 1958, Chittleborough 1959, Nishiwaki 1959, Robins 1960, Doroshenko 2000). Therefore, #68's GLG count is within the documented range for this species, but this whale was older than the vast majority of mid-twentieth century whales examined during commercial whaling in the North Pacific, Indian, and Southern oceans.

Several other whales with long sighting histories in the Glacier Bay area provide evidence in favor of an annual GLG deposition rate. Ten of 13 whales (76%) photo-documented in the earliest years of the Glacier Bay study 33–35 yr ago (Vequist and Baker 1987 unpublished data) were still sighted in 2008. If two GLGs are deposited per year, these whales would be expected to have 66 or more GLGs, although such whales were extremely rare in earlier studies (Symons and Weston 1958, Chittleborough 1959, Nishiwaki 1959, Robins 1960, Doroshenko 2000). The true ages of most of these Glacier Bay whales are unknown but their longevity is far more consistent with one GLG per year.

Additionally, a biannual GLG deposition rate has previously been noted to be inconsistent with other humpback whale life-history data, including reconciling female ovulation rates with pregnancy rates. Chittleborough (1965) concluded that successful pregnancies occurred only after an average of 2.5–3 ovulations (Best in press), a suspiciously high figure because polyoestry is very unusual in mysticetes (Lockyer 1984*a*), and histological evidence of this phenomenon in humpback whales is lacking. Similarly (Best in press), natural mortality rates that assume two GLGs per year are high (8.6%–8.7%) (Chittleborough 1965) in comparison with the 3.7%–4.9% mortality rates derived from mark-recapture histories of naturally marked whales (Mizroch *et al*. 2004). In both cases, assuming one GLG per year provides parameter estimates that are much closer to what is now known about humpback whale biology.

While definitive evidence could only come from reading the ear plugs from several whales of known age, the mean count of 44.5 GLGs in light of the 27 yr minimum age of #68 supports annual GLG deposition. Collateral evidence regarding discrepancies in the life history parameters found during commercial whaling and in longitudinal studies also suggests annual GLG deposition. Moreover, a newly developed age determination method using blubber fatty acid ratios (Herman *et al*. 2008) corroborates our age estimate for #68 (Herman *et al.* in press). The parsimonious approach would be to add these findings to the weight of existing evidence for other mysticetes and accept a rate of one GLG per year for humpback whale ear plugs. To do otherwise requires postulating an entirely different mechanism for GLG deposition in humpback whales than for other baleen whales.

Reinterpreting historical data using an annual GLG deposition rate will allow more accurate assessments of humpback whale demographic parameters. For example, uncertainty over humpback whale ear plug GLG deposition created prolonged confusion over females' age at sexual maturity (Gabriele *et al.* 2007, Best in press) and thus hindered resolution of the plausibility of rates of increase greater than 10% found for Southern Hemisphere populations (Clapham *et al.* 2006). Reanalysis of historical data has the potential to contribute greatly to our understanding of humpback whale life histories, and is especially important for populations that have been or may become subject to commercial hunting or other anthropogenic disturbances.

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