AGE ESTIMATION, LIFE-HISTORY TRAITS, AND FEEDING ECOLOGY OF MODERN AND FOSSIL MYSTICETES FROM SOUTH AFRICA

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Project Background Information and Literature Review

Histology of mammalian bone and teeth

The microstructure of bones and dental tissues often provides information regarding growth during different periods of an animal's life (Chinsamy-Turan, 2005; Köhler et al., 2012). Fluctuations in bone growth create a series of bands with zones of fast-growing, vascularised bone, alternating with slow-growing lines of arrested growth (LAGs; annuli). LAGs indicate a pause in osteogenesis (bone formation) (Chinsamy-Turan, 2005). These sets of fast-growing bone and accompanying LAGs are known as growth layer groups (GLGs) and can be counted to estimate age - a method called skeletochronology (Castanet et al., 1993; Klevezal & Kleinenberg, 1969; Peabody, 1961).

It is widely accepted that LAGs occur in the bones of all groups of mammals and are deposited annually (Chinsamy-Turan, 2012; Frylestam & von Schantz, 1977; Köhler et al., 2012; Kolb et al., 2015). Köhler et al. (2012) noted that cyclical growth is a trait of homoeothermic endotherms and that GLGs are detectable in the bones of animals in all climates. Therefore, the presence of LAGs are not entirely impacted by external factors (Huttenlocker, Woodward & Hall, 2013; Köhler et al., 2012), and environmental factors such as climate, availability of food and water and other stress factors (Suarez-Bregua, Guerreiro & Rotllant, 2018) can significantly impact the bone microstructure (Chinsamy-Turan, 2005; Köhler et al., 2012; Angst et al., 2017).

Some mammals may not form GLGs until they are several years of age (Frylestam & von Schantz, 1977; Chinsamy-Turan, 2005). Only when the animal reaches an age where slow incremental growth occurs, will GLGs be visible (Chinsamy-Turan, 2005; Klevezal, 2017). This presents challenges in skeletochronology. Other challenges also occur as GLGs are sometimes difficult to count if LAGs are numerous and tightly packed (Sensor et al., 2018), or if they split thus causing one LAG to appear as two (Klevezal, 2017). Furthermore, in older animals, the earlier laid GLGs can become resorbed and are therefore no longer detectable (Chinsamy-Turan, 2005).

These challenges need to be taken into consideration and it is important to have a good understanding of the ecology of the animal you are looking at; the area(s) in which the animals live, and to study growth series (animals from the same species at different ontogenetic stages) to interpret the histological patterns correctly (Angst et al., 2017). However, hard tissue histology is still a useful tool for deciphering various aspects of the lives of vertebrates. In addition to age estimation, assessment of GLGs and the last deposited bone type can provide an estimate of the season in which the animal died e.g. if the last deposited bone type was fast-growing bone, it is likely that the animal died during its favourable season, and if it was slow-growing bone/LAG, it is more likely to have died during its unfavourable season (Chinsamy-Turan, 2005). Furthermore, in some populations, one can detect extra-long periods of growth in the bone, which may suggest extra-long time spent in their feeding grounds, for instance (Sensor et al., 2018). Conversely, when feeding is limited the GLGs tend to be narrower and the incremental layers thinner (Sensor et al., 2018). Histology can also provide additional information about life events such as calving events in dolphins (Klevezal & Myrick, 1984), moulting in birds (Angst et al., 2017), pathologies and trauma such as healed fractures (e.g. Chinsamy & Tumarkin-Deratzian, 2009; Tschopp et al., 2014; Woolley et al., 2019) that may have contributed to the animals' death.

Mysticete whale bone histology and age estimation

Klevezal and Mitchell (1971) investigated the histology of skeletal elements of fin, sei, and humpback whales. They found that the main bones of mysticete skeletons are composed of loose intensively vascularized bone tissue, without resting lines (Klevezal, 1972). It was concluded that in large, fast-growing mammals such as whales, the recording structures are present in bones growing with negative allometry (growing at a slower rate than the rest of the skeleton), such as the TB (Klevezal, 1972; Klevezal, 2017). The TB is a 'conch'-shape pachyosteosclerotic bone, which functions to improve the reception of sound underwater (Groves, Peredo & Pyenson, 2021) and is the only bone in baleen whales that contains GLGs that are correlated with age (Klevezal, 1972).

There are several other methods used to estimate the age of mysticetes. Methods involving soft tissues include counting growth lines in whale ear plugs; counting corpora lutea, c. albicantia and c. atretica in whale ovaries (Olsen et al., 2003), and aspartic acid racemization with whale eye balls (Olsen & Sunde, 2002). However, these methods are inappropriate for long-stranded, decomposing animals, and for skeletal remains or fossil remains.

Keratinous tissues such as baleen can be used for age estimates by statistically modelling baleen and body length at age for whale species (Lubetkin et al., 2012); however, this is a lengthy process that requires a large sample size and is not appropriate for fossil remains as baleen rarely becomes fossilised (Marx et al., 2017) and large fossil samples can be quite rare. Isotope analysis along the length of a baleen plate provides a reasonably accurate estimate of the age of the whale (Sensor et al., 2018) by recording isotopic oscillations (spikes and dips in the nitrogen and carbon isotope values) created

during annual migrations and feeding events. This method is most useful until a certain age whereby the distal end of the baleen plate begins to wear down (Bentaleb et al., 2011), thus removing initial isotopic oscillations.

GLG count have been shown to be a reliable method for ageing mysticete (Sensor et al., 2018), and additional information about the individual can also be determined from TB histology e.g. feeding ecology and migrations (Sensor et al., 2018). Different species of mysticete have different life history traits such as growth rate, migration patterns, seasonal changes in fasting and feeding, and reproduction rates. The TB document some record of the life history events for mysticete whales, and because of this one would expect that these interspecies differences may be reflected in the bone microstructure.

Most of the publications to date that deal with TB histology aim to provide an estimate of age, but qualitative descriptions of the histology of the TB as they are related to different species are scarce. A qualitative description and a set of anatomical and histological characteristics for different species of mysticete such as size of TB, thickness of involucrum, thickness of each of the three regions, bone types in the three regions, level of vascularisation, and level of bone remodelling would be beneficial in understanding how histology relates to life history patterns. This could be presented in the form of a key for different species of baleen whale from Southern Africa as well as from other publications where the information is present. This key will be beneficial when histologically examining the fragmentary fossil TB from Langebaanweg (LBW) as it would assist in understanding the life history traits as well as possibly the taxonomic identification of the fossil whales. Studies using histology as a method for understanding the palaeobiology of fossil mysticete whales are scarce. Several authors have investigated archaeocetes and Miocene whales' biology using bone histology (De Buffrénil et al., 1990; Gray et al., 2007; Houssaye et al., 2015; Uhen, 1999), but none have examined the histology of fossilised TB to assess the palaeobiology of extinct whales.

Stable isotope analyses of marine mammals

Stable isotope analyses of marine mammals have been used in four key research areas as described by Newsome, Clementz & Koch (2010) namely, (1) physiology and fractionation, (2) foraging ecology and habitat use, (3) ecotoxicology, and (4) historic ecology and paleoecology. Isotopes are forms of the same element that contain different numbers of neutrons within the nucleus, and all elements have multiple isotopes (Fry, 2006). In the field of ecology, isotopes offer a way to directly trace details of element cycling. Those that are particularly useful for studying current ecology are those that cycle closely with organic matter namely, hydrogen, carbon, nitrogen, oxygen, and sulphur (Clementz, 2012; Fry, 2006). These stable isotopes provide a useful tool for obtaining ecological and physiological information about both modern and fossil mammals (Bocherens, Fizet & Mariotti, 1994; Clementz, 2012; Martin et al., 2015).

Sensor et al. (2018) was able to match yearly migration patterns from carbon and nitrogen isotopes in bowhead whale baleen to the laminations in the TB, which provided an accurate chronology of the life. They found that the δ^{13}C and δ^{15}N values start oscillating clearly along the length of the baleen plate once weaning occurs (see Figure 2A) and the individuals are independently feeding, this is because the isotope signals are dampened due to the buffering effect of the mother's milk during nursing (Newsome, Clementz & Koch, 2010). For each oscillation there is a GLG boundary that can be noted within Region 3 of the tympanic bulla thin section (Figure 2). Figure 2 indicates that this individual was a minimum of 4 years old when it was caught. Region 3 of the TB was only found in bowhead whales older than 1 year (Sensor et al., 2018), which makes sense given that this species is estimated to nurse for approximately 9 months (George et al., 2016).

By assessing the isotope profiles of the baleen alongside the GLGs in the tympanic bulla, Sensor et al. (2018) could confirm that the use of either of these methods are appropriate for accurately estimating the age of bowheads up to about 20 years old, somewhat appropriate for whales 20-30 years old, but not reliable for older whales. Thus, confirming that despite problems that can occur through human error (see section on *Histology of mammalian bone and teeth*), GLG counts are a useful tool for estimating the age of bowheads (and likely for other mysticete whales) in addition to the other available methods. Unfortunately, this method cannot work on the fossil mysticete TB from LBW because the isotopic signatures for C and N are affected by diagenesis and therefore are not reliable for fossil bones older than 100 000 years (Clementz, 2012), whereas the material from LBW is approximately 5 million years old (Hendey, 1982).
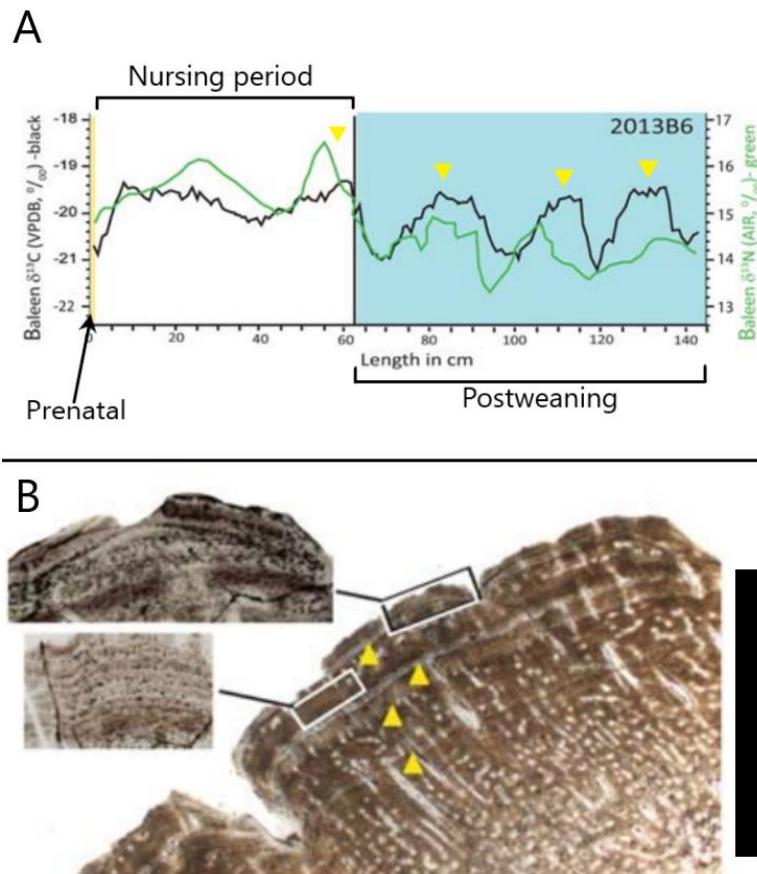
Figure 2: (A) $\delta^{13}C$ (black) and $\delta^{15}N$ (green) values from baleen keratin taken along the total length of the plate from distal (left) to proximal (right). Yellow zone indicates isotope values of prenatally formed baleen (as indicated by the neonatal notch), white indicates the nursing period, blue indicates postweaning period. Yellow arrowheads indicating oscillations in the isotope profiles suggested to correspond to feeding events. (B) The tympanic bulla microstructure showing the three regions described by Sensor et al. (2018) with yellow arrowheads indicating the position of the boundaries between GLGs. Scale bar = 1mm. Adapted from Sensor et al. (2018).

Calcium isotopes

Other isotopes such as calcium have a broader age range (<10 million years) over which different organic and mineralized tissues in mammal remains are likely to be preserved (Clementz, 2012, Figure 1). Skulan, DePaolo & Owens (1997) identified the biological fractionation of stable calcium isotopes through metabolic processes, thus $\delta^{44}Ca/\delta^{40}Ca$ values decrease with increasing trophic levels (Clementz, Holden & Koch, 2003). During fossilisation elements in bone can become degraded and the isotopic signature may be altered (Skulan, Joseph, DePaolo & Owens, 1997).

Clements, Holden & Koch (2003) measured the $\delta^{44}Ca$ composition of tooth enamel and bone from modern marine mammals including sirenians, pinnipeds, and cetaceans (e.g. harbour porpoise, Dal's porpoise, pilot whale, false killer whale and sperm whale, but no baleen whales were assessed) (Clementz, Holden & Koch, 2003). Thereafter they measured the $\delta^{44}Ca$ composition of tooth enamel and bone from 15 Ma fossil marine mammals from southern California. They found that the mean $\delta^{44}Ca$ values were significantly different from modern $\delta^{44}Ca$ values for mammals of similar trophic level relationship, but the $\delta^{44}Ca$ values of specimens followed the same pattern as observed in modern faunas i.e. a decrease in $\delta^{44}Ca$ values with an increase in trophic level. Therefore they concluded that the relative spacing of $\delta^{44}Ca$ values amongst fossil taxa can serve as a valuable tool for defining trophic levels of fossil organisms and ancient food webs.

Martin et al. (2015) assessed the calcium isotope composition ($\delta^{44}Ca/\delta^{42}Ca$) of elasmobranch teeth, fish teeth and marine mammal bone. Their results showed that zooplanktivore and primary consumer elasmobranchs showed the most positive values (average value of −0.44‰ ± 0.08 1SD; n = 7, 7 sp.). Thereafter, the second group comprised of secondary consumer elasmobranchs that feed on pelagic cephalopods and fishes (average value of −0.57‰ ± 0.05 1SD; n = 8, 8 sp.), and the third group, which feed on fishes, turtles and mammals had the most negative $\delta^{44}/^{42}Ca$ values (average value of −0.72‰ ± 0.05 1SD; n = 4, 2 sp.) (Martin et al., 2015).

The marine mammals that were tested (dugong, walrus, and dolphin) showed the same decreasing $\delta^{44}/^{42}Ca$ value pattern with increasing trophic level as was found by Clements, Holden & Koch (2003). Both bone and teeth were tested from the same individuals of dugong, walrus, and dolphin, and in all three, the teeth showed a slightly more positive $\delta^{44}/^{42}Ca$ value

than the bone. The whale was considered an outlier by Martin et al. (2015). The authors indicated that the enriched $\delta^{44}/^{42}$Ca value of the whale bone was likely due to it primarily feeding on krill, which have a similar isotope composition to that of seawater. The whale bone $\delta^{44}/^{42}$Ca values were more positive than the zooplanktivorous elasmobranchs (Table 1). However, different species of mysticetes have different diets that can include a wide variety of prey from tiny plankton to small crustaceans, copepods and amphipods, and small pelagic fish, in varying proportions (Best & Folkens, 2007). Some mysticetes also have temporal changes in their diets.

Interestingly, Martin et al. (2015) assessed both modern (n =2) and fossil white shark (n = 1) teeth enameloid, of which all showed very similar δ^{44}Ca/ δ^{42}Ca values of -0.74 ± 0.07‰, -0.78± 0.12‰ and -0.72± 0.09‰ respectively (Table 1). The fossil tooth was from the early Pliocene, which is similar in age to the fossil whales from LBW. They indicated that the calcium isotope data for elasmobranchs and marine mammals is not comparable and the -0.14‰ stepwise decrease between feeding groups cannot be applied to marine mammals due to physiological differences behind calcium cycling. The authors suggested that future research to develop data on marine mammals will be useful in refining this hypothesis.

Overview of the biology of the study animals

The following section provides an overview of the modern and fossil mysticetes that will be assessed in this study. All three modern mysticete families are present in the Southern African subregion, namely Balaenidae, Neobalaenidae (*Caperea marginata)* and Balaenopteridae, and all are represented in this study by at least one species.

Modern species

Most of the whales from the Southern African subregion follow the usual baleen whale paradigm with regards to migratory behaviour, which involves movement between the summer feeding grounds in higher latitudes and winter breeding and nursing grounds in the lower latitudes (Best & Folkens, 2007). Furthermore, they are all filter feeders with apparently slightly different prey preferences and feeding strategies. Table 1 provides a summary of the modern whales assessed in this study along with details of their migration and distribution and feeding ecology taken predominantly from Best & Folkens (2007) species summaries.

Table 1: Summary of the modern whale species assessed in this study in terms of their representation in the scientific literature since 1900, migration and feeding patterns as described and summarised by Best & Folken (2007).

Species	Common name	Number of papers referring to species in SA (1900 to 2011 or in press)[1]	Summary of migration patterns	Primary prey inferred from stomach contents and feeding behaviour
Eubalaena australis	Southern right whale	48	Nursery grounds on the coast of southern continents during winter, and offshore feeding grounds usually south of the nursery grounds in the warmer months.	Copepods, euphausiids and small crustacea Skim-feeder[2]
Caperea marginata	Pygmy right whale	5	Feeding and breeding grounds are unknown, but feeding is likely to be in highly productive regions of higher latitudes (e.g. STC zone) as with other baleen whales, as well as areas with coastal upwelling (e.g. Benguela).	Calanoid copepods, amphipods and euphausiids Most likely skim-feeders
Megaptera novaengliae	Humpback whale	29	Migrations follow the usual baleen whale paradigm. Two major winter-feeding stations in the region, west coast (Angola, Congo, Gabon, and offshore islands such as Soa Tome Principe and Annobón, also called Pagalu) and on the east coast (Mozambique, Tanzania, Comoros, Madagascar). There are several proposed migratory routes around SA (Barendse et al., 2010).	Clupeoid fish, euphausiids, amphipods. Lunge-feeders
Balaenoptera acutorostrata subsp.	Dwarf minke whale	1	Undescribed migration pattern. May range as far south as 65°S, but are common around 60°S during summer, and were caught off Durban during autumn/winter	Myctophid fish, euphausiids Most likely lunge-feeders
Balaenoptera borealis	Sei whale	11	In summer, most are found around the STC and Antarctic Convergence. They tend to avoid the coldest waters, with even the largest individuals rarely sited in latitudes higher than 60°S. In SA waters, whales from south of 40°S and 50-85°E were associated with the east coast and one whale from around Tristan da Cunha (12°W) was associated with the west coast of SA.	Copepods, euphausiids, amphipods Feeding behaviours include "swallowing"[3] and "skimming"[4]. Sei whales within the Antarctic appear to have a diel pattern of feeding, whereby feeding largely occurs in the early mornings.
Balaenoptera edeni	Bryde's whale	9	No migration to the Antarctic, but populations appear to have smaller seasonal movements (Best, 2001) Inshore population is resident over Agulhas Bank and makes small movements up east and west coast in winter. The (presumed) offshore population migratory information comes from catches, where whales are furthest north in the winter (around 5°S) and are at found higher latitudes in the summer (25-28°S)	Inshore population – predominantly pelagic fish, but shoaling squid has been identified in faeces. Offshore population on the west coast – mainly of euphausiids, then fish and small crustacea Whales south of Madagascar – euphausiids Feeding behaviour includes horizontal and vertical lunge feeding[5]

[1] Taken from Elwen et al. (2011), Table 1

[2] The whales swim through the water column with their mouths open and capturing planktonic organisms in their baleen (Best & Folkens, 2007)

[3] Whales lunge at the prey and take in discrete mouthfuls of food.

[4] Whales swim through aggregations of prey with the mouth open for prolonged periods of time.

[5] In horizontal lunge feeding the whale lunges on its side at the surface with its mouth open, whereas in vertical lunge feeding, the whale comes from the bottom and lunges at a shoal of fish trapped at the surface (Best & Folken, 2007).

The fossil whale assemblage from Langebaanweg

LBW is a Mio-Pliocene locality located on the southern west coast of South Africa, famous for its abundant and diverse vertebrate fauna and juxtaposition of fossil terrestrial and marine fossils found within the bonebeds (Brumfitt, Chinsamy & Compton, 2013; Govender, Bisconti & Chinsamy, 2016; Hendey, 1982). The stratigraphic column of LBW encompasses a wide range of depositional environments from a meandering fluvial valley and estuary during middle Miocene, to a high energy marine environment during late Miocene, to estuarine, marine, or fluvial environment during the early Pliocene, to coastal aeolian/aeolian[6] through the Pleistocene/Holocene (Roberts et al., 2011). There are also several microhabitats present (Govender, Bisconti & Chinsamy, 2016).

Govender, Bisconti & Chinsamy (2016) described the late Miocene–early Pliocene baleen whale assemblage from LBW, which were recovered from 'E' Quarry. There are 13 specimens in total, 12 of which include TB and one which comprises two mandibular rami with skull fragments (Govender, Bisconti & Chinsamy, 2016, Table 1). Three of the specimens (SAM-PQL-20482; SAM-PQL-55001; SAM-PQL-21230) could be assigned to *Plesiobalaenoptera*-like taxa (Bisconti, 2010). The rest of the LBW specimens were assigned to Balaenopteridae gen. et sp. indet. due to their fragmentary nature (Govender, Bisconti & Chinsamy, 2016).

To date, no calcium isotope research on any material from LBW has been done and no histological assessments have been published on the whale assemblage.

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[6] Environments that undergo erosion, transportation, and deposition of sediment by the wind, as opposed to fluvial environments, which pertain to those arising from the action of water.

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