"Allow your curiosity to lead you to the answer you seek"
- Wing's fortune cookie -

University of Alberta

Reconstructing life histories using cementum: information recorded in premolars of polar bears (*Ursus maritimus*)

by

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ABSTRACT

Annuli formed within cementum, a mineralized tissue surrounding the root of mammalian teeth, are commonly used to age wildlife. Cementogensis is influenced by a number of physiological and environmental factors and therefore cementum is a tissue capable of recording life history. Mark-recapture data from the Western Hudson Bay population of polar bears (*Ursus maritimus*) allows comparison of observed life history events to patterns in cementum. Sources of variation in cementum growth were identified and protocols developed to reduce error and permit comparisons within and between individuals. Cementum growth in years when females are accompanied by cubs-of-the-year (COY) are significantly less than the year of pregnancy (paired t- test, $\bar{x}diff = 0.054$, $t = 8.16$, df = 103, $P < 0.001$), and yearling accompaniment ($\bar{x}diff = 0.034$, $t = -3.88$, df = 31, $P < 0.001$). The predictive model correctly classified COY for 0.73 of the females with COY, while pregnancy (0.71) and yearling (0.77) were correctly classified as non-COY.

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My own research, and my participation in other Derocher student's projects, has increased my curiosity and concern for Arctic issues. The amazing opportunities to travel and work in the Arctic has shown me a simplistic beauty delicately balanced between cycles of light and dark, and cold and colder. With many unknowns for the future of polar bears, and all ecological interactions in the north, it is an exciting (if not terrifying) time to be a part of Arctic research.

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1.0 INTRODUCTION

1.1 A HISTORY OF USING TEETH FOR AGE DETERMINATION OF WILDLIFE

Knowledge of the age composition of wildlife populations is critical for understanding growth, recruitment, and mortality and to support management decisions (Caughley 1977). Many age determination techniques are intrusive and samples can only be collected post mortem: ossification, skull suture closure, changes in the lens of the eye, baculum dimensions, and examination of testes and ovaries (Larson and Taber 1980; Lønø 1970). For conservation research, destruction of the animal is often contrary to the objectives and non-lethal techniques for age determination are required (e.g. body size, mass, horn annuli; Karels et al. 2004, Larson and Taber 1980). Analysis of annuli within dental structures provide an accurate estimations of age for a variety of mammals (Klevezal 1996; Laws 1962; Sergeant 1967), while the removal of a tooth is not life threatening, and if the appropriate tooth is selected, would not compromise foraging efficiency (Childerhouse et al. 2004; Craighead et al. 1970). Additionally, teeth are highly mineralized and are not subject to post-mortem decay, or suffer the same degree of resorption as bone (Armitage 1991; Schroeder 1986). The persistence of dental tissues in life and after death has lead to the use of these structures for determining age and season of death in biology, archaeology, anthropology, and forensic sciences, and amongst a variety of taxa (Burke and Castanet 1995; Kagerer and Grupe 2001b; Klevezal and Shishlina 2001; Wedel 2007).

The roots of teeth are composed of two highly mineralized substances: dentine and cementum (Fig. 1.1). In 1932, Eidmann reported light and dark patterns that corresponded with age in the dentine of red deer (*Cervus elaphus*). The use of teeth for age determination gained more support following the publication of Scheffer (1950) and Laws (1952). Scheffer (1950) observed concentric ridges on the surface of teeth roots that could be used to estimate the age of young Alaskan fur seals (*Callorhinus ursinus*). Laws (1952), using cross sections of elephant seal (*Mirounga leonina*) canines, observed layers in dentine that could be used to estimate age. Age determination using cementum was first validated by Mansfield and Fisher (1960) from a known-aged harbour seal (*Phoca vitulina*). Following these studies, the use of dentine and cementum to estimate the age of wildlife had expanded to include many marine and terrestrial mammals (Grue and Jensen 1979; Klevezal 1996; Laws 1962; Sergeant 1967).

1.2 USING TEETH TO OBTAIN LIFE HISTORY INFORMATION

As researchers began using dental annuli to determine age, they began comparing observed patterns of cementum and dentine deposition to life history and environmental events. Most hypotheses have resulted from observations from wildlife populations and, therefore, testing is complicated by limited known histories of the animals sampled and by inseparable variables. Few controlled experiments have been performed to examine the influence of endogenous and exogenous variables on the formation of cementum and dentine.

1.2.1 Cementum

Cementum is the mineralized tissue surrounding the roots of teeth and has the functional role of maintaining anchorage within the alveolus, tooth eruption, and root repair (Bosshardt and Selvig 1997; Schroeder 1986). Cementum is deposited onto the exterior surface of the tooth root and continues to be deposited throughout an animal's lifetime (Klevezal and Kleinenberg 1967; Stewart et al. 1996). For animals in which the permanent dentition is not present by the end of its first year, the time before tooth eruption may need to be added to the number of observed annuli to accurately represent age (Condon et al. 1986; Grue and Jensen 1979; Low and Cowan 1963; Marks and Erickson 1966; Sergeant 1967).

Differences in the rate of cementogenesis results in visible layers with differing cell density and/or collagen orientation (Fig. 1.2; Cool et al. 2002; Lieberman 1994; Smith et al. 1994). Teeth sectioned and stained for transmitted light microscopy exhibit a pattern of broad light staining bands followed by narrow dark lines reflecting periods of rapid and slow cementum formation respectively (Bosshardt and Schroeder 1990). The thin, dark staining, dense cementum is commonly termed an incremental line, while the annual deposition of the wide translucent band and associated incremental line(s) is referred to as a growth layer group (GLG; terminology of Perrin and Myrick 1980).

Reproduction

Teeth collected from females have shown irregular cementum deposition during pregnancy and lactation. In humans, pregnancy was recorded by the deposition of an unusually broad incremental line (Kagerer and Grupe 2001b). Female Atlantic walrus (*Odobenus rosmarus*) in general produce a cementum that is irregular and reduced in quantity compared to males (Mansfield 1958). Reduced width of cementum GLGs when offspring are present were observed in female black bears (*Ursus americanus*) and sea otters (*Enhydra lutris*; Carrel 1994; Coy and Garshelis 1992; Rogers 1975; von Biela et al. 2008). Paired incremental lines in the cementum of mature female polar bears (*U. maritimus*) has been attributed to reproduction, however, known reproductive histories for the specimens were unavailable to test the hypothesis (Kirkegaard 2003).

The formation of "false" or "accessory" lines has been attributed to physiological and hormonal changes within the reproductive cycle (Kolb 1978; Sergeant 1967). In male ungulates, the accessory line has been called a "rutting layer" and has been attributed to reduced food intake and perhaps also hormone changes during the breeding season (Mitchell 1967; Reimers and Nordby 1968). Accessory lines in male bears give the appearance of paired annuli which are similar in appearance to the true paired annuli observed in reproductive females (Christensen-Dalsgaard 2006; Coy and Garshelis 1992; Kirkegaard 2003). One author has suggested that accessory lines may reflect mating effort (Christensen-Dalsgaard 2006) because male polar bears reduce food intake while courting females and have blood urea creatinine ratios similar to a fasting individual (Ramsay et al. 1991).

However, not all species, or individuals within a species, show evidence of reproduction in cementum GLG patterns. Mitchell (1967) failed to detect a difference in cementum patterns between lactating red deer, which are generally in poorer condition, and dry mature females. Known reproductive events of female grizzly bear (*U. arctos*), closest relative to polar bears, were not be detected in cementum (Matson et al. 1999). Additionally, other calcium demanding events such as skeletal trauma, renal disease, or cold stress may produce cementum similar in appearance to reproductive patterns (Cipriano 2002; Kagerer and Grupe 2001b).

Climate

Climate alone does not cause the formation of incremental lines. Different species from the same geographic location can have differences in the clarity and time of formation of incremental lines despite being exposed to identical climate conditions (Grue and Jensen 1979). However, distinct cementum annuli were observed in primates originally from tropical regions but held in captivity through an extremely cold winter in temperate regions (Cipriano 2002). It was concluded that cold stress, and the resulting impact on serum calcium levels, reduced the available calcium during cementogenesis (Cipriano 2002). Changes to incremental lines were not observed in red deer exposed to the same severe winter season, therefore climatic extremes outside of the species normal range may be needed to interrupt normal cementum formation (Mitchell 1967).

Growth and Sexual Maturation

A number of authors have attributed a decrease in the width of cementum, or the reduction in the number of incremental lines per year, to the onset of sexual maturity (Hewer 1964; Klevezal and Stewart 1994; Mitchell 1967; Mundy and Fuller 1964). A decrease in the width of a cementum GLG corresponds with both a reduced rate of growth and a decrease in the length of the annual growth period (Klevezal 1980). Cementum responds disproportionately to levels of circulating growth hormone (Smid et al. 2004). Additionally, alkaline phosphatase activity is positively correlated to the amount of cementum produced and is more abundant during adolescence (van den Bos and Beertsen 1999). A decrease in the circulation of hormones and enzymes related to growth when sexual maturity is reached could also result in decreased widths of cementum GLGs.

Physical Condition

Most comparisons of cementum annuli formation to body condition do not involve physical measurements of the animals but inferences on the seasonal abundance of food in relation to the time of incremental line formation (Grue and Jensen 1979). Mitchell (1967) observed that the incremental line corresponded with low resource availability and in samples collected from severely nutritionally stressed animals, the incremental line was outermost. For black and grizzly bears, the incremental line has been observed to initiate before hibernation, while the light staining layer is formed over the summer months (Craighead et al. 1970; Mundy and Fuller 1964; Stoneberg and Jonkel 1966). The amount of stored adipose tissue of bears peaks before entering their dens; contradicting the hypothesis of poor nutritional state being associated with the formation of the incremental line while supporting the deposition of the light cementum during periods of high weight gain and growth (Craighead et al. 1970).

Seasonality

Seasonality is a common explanation for the difference in cementum structure that results in light and dark staining layers (Lieberman 1994; Lockard 1972). In a widely distributed species, cementum GLGs are more distinct in regions with greater seasonality (Haug et al. 1994; Klevezal 1996). However, change of season are coupled with changes in behaviours related to reproduction, activity (e.g., hibernation, haul-out), and the abundance and quality of food. Most examples of seasonal influences on the development of cementum cannot be isolated from other factors such as growth, reproduction, and body condition (Klevezal 1980; Lieberman 1994; Mitchell 1967).

Formation of the incremental line in response to poor quality and abundance of forage has been proposed for a number of wildlife species. Waterbuck (*Kobus defassa ugandae*) live in a region with two wet and two dry seasons per year. Their cementum displays two incremental lines per year, each coinciding with the dry season (Spinage 1967). A controlled study using goats (*Capra hircus*) mimicked the changes in forage quality in different seasons by comparing cementum formation between high and low nutrient level diets, and between course and soft forage (Lieberman 1993). The course forage resulted in changes in the orientation of the collagen fibers and produced a layered appearance. When the low nutrient diet was consumed, overall growth ceased, the amount of cementum was reduced, and there was greater mineralization of cementum tissue (Lieberman 1993). Comparisons between animals reared in different environment conditions support that incremental lines are more pronounced when forage quality is low (Lieberman 1993; McCullough 1996; Mitchell 1967; Reimers and Nordby 1968).

Incremental lines will continue to form in the absence of fluctuations in both climate and food availability but their reliability as indicators of age decreases. Domestic dogs (*Canis familiaris*) kept as pets will form incremental lines, but are indistinct, irregular, and not strongly correlated with known ages; sled dogs exposed to seasonal changes in climate and fluctuations in food availability formed incremental lines similar to those observed in wild carnivores (Grue 1976). Modern humans also form incremental lines despite being buffered from seasonal changes in climate and food availability, although age is less closely correlated to the amounts of visible incremental lines. (Condon et al. 1986; Stein and Corcoran 1994). Our long lives and the reduced ability to distinguish incremental lines in older individuals may also contribute to this error (Condon et al. 1986; Kagerer and Grupe 2001a; Kvaal and Solheim, 1995; Renz and Radlanski 2006).

Fasting and inactivity related to hibernation of temperate species has been strongly linked to the timing of incremental line formation. No incremental lines were visible in the canines from a 2 year old grizzly raised in captivity that remained active throughout the year (Rausch 1969). Further, a black bear cub in captivity was fed and remained active into December and later examination of his premolar showed a much wider first GLG than wild individuals (Rogers 1978). When hamsters (*Mesocricetus brandti*) were prevented from hibernating they continued to increase their weight over the winter months and failed to produce typical incremental lines in cementum (Klevezal 1980). It was assumed that the availability of nutritional provisions throughout the winter, and a lack of hibernation, supported continuous growth and therefore the continued deposition of light-staining cementum.

Functional response of cementum

In addition to physiological and environmental influences, cementum formation responds to mechanical forces on the tooth. The thickness of cementum may, in part, reflect the functional role of this tissue to maintain tooth position and attachment in the alveolus. Cementum corrects for tooth movement by increasing cementum deposition on surfaces experiencing periodontal ligament tension, and reducing deposition, or even the resorption of cementum, where compression is occurring (Dastmalchi et al. 1990; Polson et al. 1984; Schroeder 1986; Tronstad 1988). Tension and compression forces occurring during mesial drift would explain the increase in cementum width observed in distal cementum (Hensel and Sorensen 1980; Rausch 1969). Additionally, low food quality and excessive wear on the teeth indicate that high occlusal forces on a tooth has resulted in increased cementum width (Bosshardt and Selvig 1997; Lieberman 1993; Mitchell 1967).

1.2.2 Dentine

Dentine comprises the bulk of the tooth tissue with the most recent dentine forming adjacent to the root cavity (Avery 1991). Fluctuations in the mineralization and orientation of dentine tubules creates the appearance of incremental lines and GLGs (Avery 1991; Klevezal and Kleinenberg 1967). In animals with ever-growing teeth, dentine formation is continuous and eventually old dentine is worn away at the surface of the tooth (Eidmann 1932; Klevezal and Kleinenberg 1967). When tooth development is determinate, dentine formation is limited by the space available within the pulp cavity, and age determination could be limited to younger members of the population (Klevezal and Kleinenberg 1967; Laws et al. 2002; Stewart et al. 1996).

Factors which have been shown to influence cementum patterns also effect dentine formation. Pregnancy and lactation have been recorded through the development of distinct layers (Klevezal and Myrick 1984; Klevezal et al. 1987; Laws 1958; Schour et al. 1937), and gonad activity of both males and females was recorded in the dentine of elephant seals (Carrick and Ingham 1962). Sexual maturity also coincides with a decrease in the width of annual dentine deposits (Hewer 1964; Laws 1958). While activities, such as weaning, haul-out (seals, walrus, etc.), fasting, and hibernation have been recorded as differing densities of dentine (Carrick and Ingham 1962; Goodwin and Ryckman 2006; Hewer 1964; Laws 1958).

Dentine can also record information which is either not recorded in, or inaccessible, from cementum. Dentine formation begins in the early stages of tooth development, before eruption (Avery 1991). For this reason, periods of fetal and early life development may be recorded only in the dentine because cementum is not yet formed (Carrick and Ingham 1962). Daily growth increments in the dentine of ever-growing teeth allow more precise comparisons of physiological and environmental variables (Fox 2000; Goodwin and Ryckman 2006; Rinaldi and Cole III 2004). And, the greater bulk of dentine permits stable isotope analysis to reveal dietary inputs or environmental conditions for specific periods of an individuals life (Cruwys et al. 1997; Hobson and Sease 1998). The decision to use dentine or cementum for age determination and/or life history investigations will likely depend not only on the questions being asked but on the clarity of incremental lines in either tissue of the species you are investigating. In polar bears, cementum is preferred over dentine, which produces few discernable annuli.

1.3 RELIABILITY OF AGE ESTIMATES FROM LAYERS IN DENTAL TISSUES

To evaluate the use of teeth to infer life history it is critical that GLGs are correctly assigned to age and/or calendar year. Research on the precision and accuracy of using dental tissues for age determination has been performed on a number of known-age samples, with all reporting some degree of error (Childerhouse et al. 2004; Christensen-Dalsgaard 2006; Costello et al. 2004; Evans et al. 2002; McLaughlin et al. 1990). Even multiple teeth from the same individual have generated different age estimates (Charles et al. 1986; Evans et al. 2002; Keay 1995). Costello et al. (2004) estimated corresponding ages for 74% of the 236 pairs of teeth from hunter-killed black bears; the agreement between pairs of teeth removed from living individuals after a period of time was even lower (46%). One common observations was that older individuals were more likely to be aged incorrectly because of the reduced width of the cementum deposited, merging and splitting of incremental lines, and/or the presence of accessory or "false" lines (Childerhouse et al. 2004; Lockard 1972; Matsonet al. 1993; McLaughlin et al. 1990; McLaughlin et al. 1990; Moffitt 1998; Rogers 1978).

Within Ursidae, polar bears have some of the most difficult teeth from which to determine age (Grue and Jensen 1979; Hensel and Sorensen 1980; Klevezal 1996; Lønø 1970; Rausch 1969). Typically, the ages of young bears are overestimated while the age of older bears are underestimated (Calvert and Ramsay 1998; Christensen-Dalsgaard 2006; Hensel and Sorensen 1980). There appears to be regional differences in clarity, with cementum layers more easily discernable from bears in the southern ranges of their distribution (Calvert and Ramsay 1998; Christensen-Dalsgaard 2006). For polar bears, permanent first premolars may appear as early as 2-3 months of age, and are fully present by 9 months of age (Hensel and Sorensen 1980; Lønø 1970). Therefore the number of fully formed, true incremental lines represents the age of the bear in years. False incremental lines have been detected in both adult and juvenile bears, and have complicated age determination in some populations (Christensen-Dalsgaard 2006; Hensel and Sorensen 1980; Lønø 1970; Rausch 1969).

Evaluation of age determination using teeth sampled from known-aged polar bears produced 75% accuracy to the exact age, and 93% accuracy to within

1 year, indicating that cementum was reliable for monitoring age demographics in this species (Calvert and Ramsay 1998). Age estimate errors of one year have little impact on population demographics of long-lived animals with low rates of reproduction. However, in short-lived species, or those with short reproductive cycles, inaccuracies in age estimations can greatly influence estimates of population parameters. Regardless of life history strategy, incorrect age assignment would lead to inaccurate measurement of GLG width, and the assignment of cementum layers to certain life history events or years.

1.4 POLAR BEARS OF WESTERN HUDSON BAY

Polar bears exist in the northern circumpolar region with the most southern populations extending into Hudson Bay, James Bay and along the east coast of Labrador, Canada (DeMaster and Stirling 1981). Although its body form is more suited to a terrestrial lifestyle, polar bears are dependent on the marine ecosystem for their primary prey: ringed seal (*Phoca hispida*; Iverson et al. 2006; Stirling and Archibald 1977). Sea ice is critical for polar bears to access this prey, but also has an important role in dispersal and the location of mates (Ramsay and Stirling 1986).

Polar bears are currently managed as 19 distinct subpopulations (Derocher et al. 1998). The Western Hudson Bay population extends eastwards to 88°30'W, north to 63°10'N, and to the south and west by the bears tendency to remain close to marine habitat (Fig. 1.3). In western Hudson Bay the sea ice melts entirely during the summer (July – November; Gagnon and Gough 2005). During the ice-free season, polar bears in western Hudson Bay return to land but do not exploit this habitat for significant amounts of food (Derocher et al. 1993; Derocher and Stirling 1990; Knudsen 1978; Ramsay and Hobson 1991; Ramsay and Stirling 1982; Russel 1975). Activities during the ice-free season depends on the age, gender, and reproductive status of the bears (Derocher and Stirling 1990; Latour 1981). Pregnant females move farther inland in late September and enter maternity dens where they will remain until late February-March (Derocher and Stirling 1990; Ramsay and Stirling 1988). Pregnant females experience nearly 8 months of fasting along with the additional demands of pregnancy, birth, and lactation (Ramsay and Stirling 1988; Watts and Hansen 1987). Females accompanied by cubs-of-the-year (COY) will also move inland after sea-ice breakup, however, females with COY or yearling cubs will return to the sea following freeze-up to resume hunting (Derocher and Stirling 1990). Most subadult bears, solitary females, and adult males remain close to the coast, eventually congregating in areas where freeze-up occurs earliest (Derocher and Stirling 1990; Latour 1981). The activity of this southern population of polar bears differs from those of northern populations where the possibility of persistent multiyear pack ice provides feeding opportunities throughout the year (Ferguson et al. 2000; Garner et al. 1990).

Polar bears in western Hudson Bay have been involved in long-term population monitoring, on-going since 1965. This has enabled the detection of a number of trends for this population. Prior to 1984, female polar bears in western Hudson Bay frequently produced cubs every second year; now cubs are more typically produced every third year, a strategy observed in polar bears from more northern regions (Derocher and Stirling 1995; Ramsay and Stirling 1986). The number of yearling cubs that were weaned at 1.5 years of age has decreased from 81% prior to 1980 to 15-20% in since 1991; with more bears weaning at 2.5 years (Derocher and Stirling 1995; Stirling et al. 1999). Body mass of bears has also declined over the period of population monitoring (Derocher and Stirling 1995). The western Hudson Bay polar bear population has transitioned from one of the most productive populations to one currently experiencing low survival of cubs, subadult, and senescent-adult individuals (Regehr et al. 2007).

Between 1971 and 2003 the duration of the ice-free season in western Hudson Bay has increased (Gagnon and Gough 2005). Correlations between an earlier breakup of sea ice and a decrease in body condition and survival of bears have been observed (Regehr et al. 2007; Stirling et al. 1999). Consequences of the poor body condition of female bears is reflected in the decreased fecundity and cub recruitment (Atkinson and Ramsay 1995; Derocher and Stirling 1996; Regehr et al. 2007; Stirling et al. 1999; Stirling and Parkinson 2006). After experiencing a period of population growth in the 1960s and 1970s the Western Hudson Bay polar bear population has declined. The most current population estimate is 935 bears (2004); a 22% decrease from 1,194 bears in 1987 (Regehr et al. 2007).

1.5 AIM OF STUDY

Cementum is recognized as a potential recording structure; preserving life history and environmental events within its structure and pattern. The factors regulating cementogenesis and incremental line formation are still poorly understood and/or appear variable between species. Teeth have been collected for age determination of polar bears during the monitoring of the Western Hudson Bay population. The result is an extensive archive of teeth with accompanying partial known reproductive histories and morphometrics. This population is therefore ideal for evaluation of cementum as a recording structure for polar bear life history as well as improving our understanding of cementogenesis.

This thesis describes the preliminary work towards extracting life histories and records of environmental events from the longitudinal sections of polar bear first premolars. Chapter 2 examines the development of methods to assess precision and accuracy of measurements, reduce bias by identifying sources of error, and standardize estimates through the creation of indices. Chapter 3 applies these methods to teeth from female polar bears to evaluate the potential of cementum to record successful cub rearing and to evaluate the probability of predicting reproductive events.

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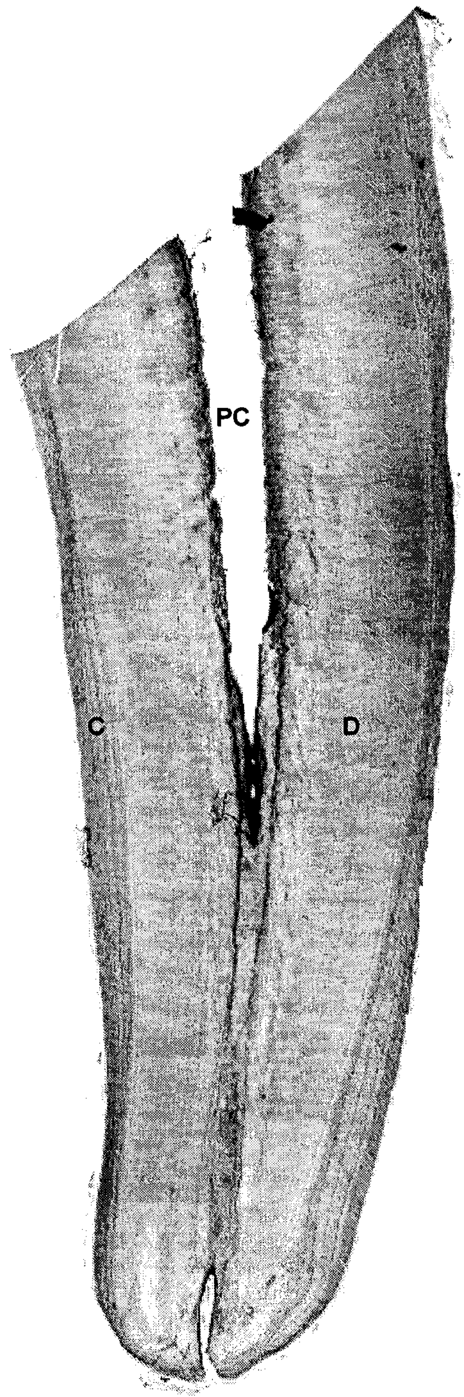

FIG 1.1: Longitudinal section of polar bear (*Ursus maritimus*) lower first premolar, decalcified, and stained (Toluidine Blue), and viewed using transmitted light. C – cementum, D – dentine, PC – pulp cavity

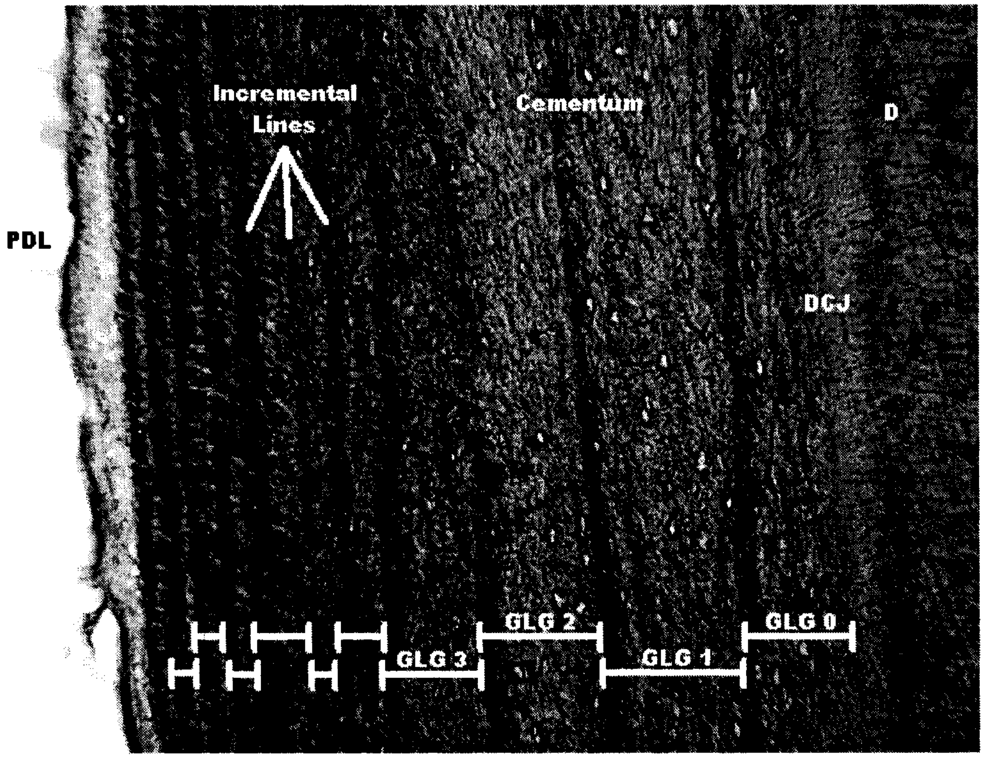

FIG 1.2: Cementum of polar bear (*Ursus maritimus*) in its 10th year of life; 10 fully formed growth layer groups (GLG) indicated, 11th year forming. PDL – periodontal ligament, D – dentine, DCJ – dentinocemental junction

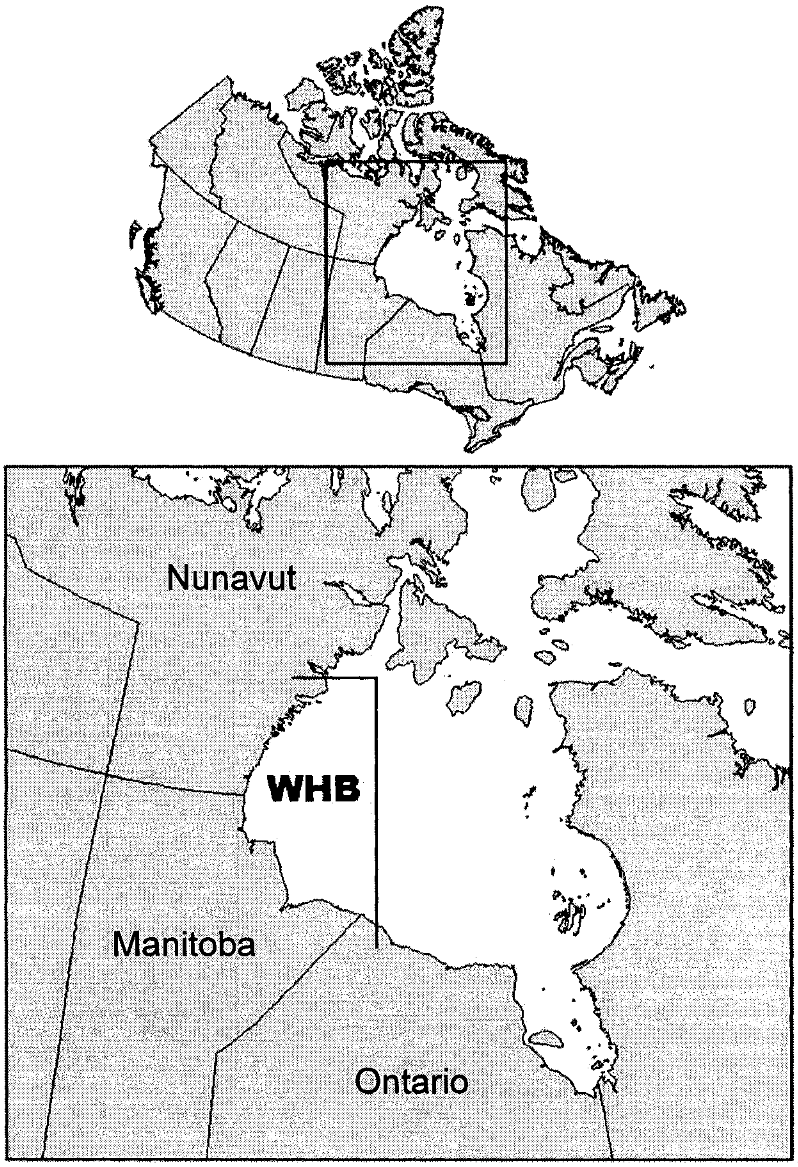

FIG 1.3: Western Hudson Bay (WHB) population of polar bears (*Ursus maritimus*), Hudson Bay, Canada.

2.0 ESTIMATING CEMENTUM ANNULI WIDTH IN POLAR BEARS: IDENTIFYING SOURCES OF VARIATION AND ERROR[1]

2.1 INTRODUCTION

Cementum is the mineralized tissue surrounding the tooth root and assists in anchoring the tooth within the alveolus, tooth eruption, and root repair (Bosshardt and Selvig 1997, Schroeder 1986). Cementum is deposited throughout an animal's life but the rate of formation fluctuates, resulting in visible layers with different cell density, and/or collagen orientation (Fig. 2.1; Cool et al. 2002; Lieberman 1994; Smith et al. 1994). Teeth sectioned and stained for light microscopy exhibit a pattern of broad, light staining bands followed by narrow, dark lines reflecting periods of rapid and slow cementum formation, respectively (Bosshardt and Schroeder 1990). The thin, dark staining cementum is often termed an incremental line while the annual deposition of the wide translucent band and successive incremental line is commonly referred to as a growth layer group (GLG). Counts of distinct layers of cementum in teeth are used to estimate age in a variety of mammalian species (reviews in: Grue and Jensen 1979; Klevezal and Kleinenberg 1967; Sergeant 1967; Spinage 1973).

A number of endogenous and exogenous factors may influence cementum production. Annual layers have been correlated with both the rate and the duration of the growth period in each year of an individual's life (Grue and Jensen 1979;

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Klevezal 1980; Klevezal 1996). Cementum may respond directly to levels of growth hormone (Smid et al. 2004) or as a functional response to secure the tooth's position within the growing alveolus (Schroeder 1986). In addition to growth, cementum can respond to mechanical stresses against the tooth with increased cementum production to maintain tooth apposition, root attachment, and continued eruption (Dastmalchi et al. 1990; Lieberman 1993; Mitchell 1967; Schroeder 1986). The formation of additional dark staining lines within an annual GLG may result from hormonal changes occurring within the reproductive cycle. However, these effects may be confounded by changes in behaviour and/or seasonal changes in resources (Kolb 1978; Sergeant 1967).

A greater body of evidence supports the appearance of incremental lines as the result of exogenous forces such as seasonal changes in prey/forage abundance and quality, or to periods of prolonged fasting (Grue et al. 1979; Lieberman 1994; McCullough 1996; Mitchell 1967; Sergeant 1967; Spinage 1967). Incremental lines are formed in the winter for a number of species including those which are inactive during resource poor seasons (review in Grue and Jensen 1979; Sauer et al. 1966; Stoneberg and Jonkel 1966). Male cervids may produce a "rutting line"; an additional dark staining line coinciding with the breeding season, and this has been attributed to reduced food intake and perhaps also a direct hormonal influence on cementogenesis (Low and Cowan 1963; Mitchell 1967; Reimers and Nordby 1968). Seasonal effects on incremental line formation is supported by the reduced distinction of cementum annuli in both tropical regions and extreme polar regions where seasonality is less pronounced, or when inhabitants of one region are translocated to environments with different seasonal timings or are buffered from seasonal variations in climate and food availability (Grue 1976; Grue and Jensen 1979; Klevezal 1980; Klevezal 1996; Mitchell 1967; Spinage 1973).

Measurement of cementum annuli has been used to identify season of death (Burke and Castanet 1995; Wedel 2007), sexual maturation (Laws et al. 2002), and reproductive success (Carrel 1994; von Biela 2008). Quantitative evaluation of cementum deposition allows more rigorous evaluation of life history correlations and provides greater statistical power in hypothesis testing; however the majority of support for detecting life history variables from cementum remains qualitative and anecdotal (Cipriano 2002; Coy and Garshelis 1992; Kagerer and Grupe 2001a; Klevezal and Shishlina 2001; Klevezal and Stewart 1994). Largely, authors have failed to address the complications that may arise from the variation found in cementum annuli.

Quantitative cementum evaluation is complicated by the variation in patterns of cementum deposition around the surface of the root which may not be comparable between or within individuals (Fig. 2.1; Childerhouse et al. 2004; Solheim 1990). This variation requires evaluation of whether the various aspects of the root surface can be pooled for analysis. Other possible sources of variation include the section (location of longitudinal section within the tooth) which is being sampled, and which tooth (e.g. first upper Left Premolar = LP^1, first lower left premolar = LP_1, first upper right premolar = RP^1, first lower right premolar = RP_1) is being evaluated. For cementum to be a reliable indicator of physiological or environmental states, patterns of cementum deposition must be reflected equally in multiple teeth sampled from the same individual. GLGs of the same age from different teeth of an individual are formed under identical physiological and environmental conditions and cementum, once mineralized, is not usually modified. Large amounts of variation within aspect, section, and tooth may obscure the differences in cementum widths making it important to understand how these factors contribute to the overall variance and identify sampling methods to reduce this source of statistical error.

Precise estimates of GLG width are required to further reduce erroneous conclusions. Too few measurements will result in poor estimates of GLG width. Additionally, direct comparisons of GLG widths are impaired by the unequal rate of cementum deposition throughout an individual's life and by individual differences in cementum formation (Carrel 1994; Laws et al. 2002; von Biela 2008; Zander and Hürzeler 1958). Indices have been developed that simplify comparisons of cementum width, both within and among individuals. The index values can be used in future investigations to identify specific climate or year effects or the influence of physiological conditions (e.g., body condition, reproduction) on cementum deposition.

A large archive of premolar teeth has been collected for age determination from an ongoing, long-term study of the population ecology of polar bears (*Ursus maritimus*) in western Hudson Bay, Canada. Approximately 80% of the adult bears are uniquely identifiable and have been captured on more than one occasion. This has resulted in many bears being of known age, having had multiple first premolar teeth collected, and having provided detailed life history information (Stirling et al. 1977b). This collection is invaluable for evaluating sources of statistical error and establishing sampling protocols. This paper describes methods to identify and reduce sources of variation in cementum measurements from longitudinal tooth sections and to produce representative indices of cementum width against which further comparisons of life history and environmental conditions can be made.

2.2 MATERIALS and METHODS

Between 1965 and 2006, > 2600 teeth were collected from > 1800 individual bears from western Hudson Bay for age determination and/or to confirm age estimates from cementum annuli of known-age bears (first observed and marked as cubs-of-the-year). Bears were chemically immobilized during tooth extraction which involved loosening the tooth with a dental elevator and removal with dental pliers (Stirling et al. 1977b; Stirling et al. 1989). Most teeth were fixed in 10% neutral buffered formalin shortly after collection, some teeth were dried for several weeks with no obvious negative effects on the fixation of GLGs (Calvert and Ramsay 1998). Following fixation (minimum 72h), whole teeth were decalcified in 25% formic acid solution (end point determined by solution test for calcium ions), and the tooth crown removed. After post-decalcification washing, roots were mounted with an optimal cutting temperature compound for cryostat sectioning (Stirling et al. 1977a). Roots were sectioned into 10μm-thick distal-mesial longitudinal sections at different levels passing through the root canal. For each tooth, eight sections in order of approaching center, center, and receding from the center were mounted on a glass slide (Calvert and Ramsay 1998). Sections were stained with Toluidine Blue 0 in alkaline water (pH 8-9).

Longitudinal sections were photographed using a Leica DFC480 camera (Leica Microsystems Limited, Switzerland) mounted to a transmitted light microscope (Leitz Ortholux II). Images were captured at 63X magnification. GLG widths were obtained from single images from the distal and mesial aspect of the root surface. Within these two locations images were taken where the cementum was widest and/or where all GLGs could be identified. Measurements were made perpendicular to the incremental lines, starting at the dentinocemental junction, using calibrated image analysis software (Rincon™, IMT i-Solution Inc, Goleta, CA, USA). When possible, teeth from bears of known age (first captured as cub-of-the-year) were used; otherwise age was determined by the agreement of two technicians experienced in age determination (Calvert and Ramsay 1998). Teeth were excluded from this investigation if the age assigned by the experienced readers did not correspond with visible GLGs, teeth were damaged during processing, or there were too few previous captures of the same individual to facilitate life history comparisons.

2.2.1 Sampling intensity

To determine the appropriate sample size required for a precise estimation of annual cementum growth, 50 measurements were obtained for each GLG from a digital image of distal cementum from 15 bears chosen for clarity of cementum annuli. The order of the 50 measurements of GLG width was randomized 20 times, creating 20 blocks of equally probable estimates of width for any given sampling effort (i.e. 1-50). For example, a sample of 10 measurements consisted of 20 different estimates of the GLG width created by taking the average of the first 10 measurements (of the original 50) randomly assigned to each of the 20 blocks. This procedure resulted in a range of estimates of width for each sample measurement size. The coefficient of variation (CV) for the range of possible estimates was calculated for each sample size and compared between bears to determine how many measurements were required to obtain a precise estimate of GLG width. A CV between 5 and 6 indicate precision for linear morphometrics (Simpson et al. 1960).

2.2.2 Identifying sources of variation and reducing error

To identify how the variables, 'bear' (individual), 'aspect' (of tooth, e.g. distal or mesial), 'tooth' (LP^1, LP_1, RP^1, RP_1), and 'section' (location of longitudinal section within the tooth) contribute to experimental error, a series of nested ANOVAs, and subsequent variance component analysis, were performed (Table 2.1). Male bears from which two premolar teeth had been collected when the bears were between the ages of 5 and 15 years of age, and which showed distinct cementum annuli, were selected for this analysis (n=16). GLG data up to 5 years was available from all 16 bears and up to 6 years of age for 13 bears. Although eight sections from each tooth were mounted for age determination, only the first, third, fifth, and seventh were included in this analysis as each pair of sections (1&2, 3&4, 5&6, 7&8) are obtained close to one another (Calvert and

Ramsay 1998). Each of the four tooth sections had two aspects, distal and mesial, photographed and measured (Fig. 2.1). Two estimates of GLG width were obtained from each aspect from the mean of ten measurements. The estimated widths were $\log_{10}$ transformed to normalize data for parametric analysis. A four-level nested ANOVA, (aspect[section[tooth[bear]]]) was performed for each GLG using SYSTAT 11.0 (Systat Software Inc., San Jose, CA, USA) and was followed by a variance component analysis (Bailey and Byrnes 1990; Blackwell et al. 2006). Negative contributors to the variance component analysis were removed (set to equal 0) and the partitioning re-executed (Brown and Mosteller 1991; Quinn and Keough 2002). A 3-level nested ANOVA was then performed controlling for the factor with the greatest contribution to variation in the 4-level nested ANOVA to observe how the variation was distributed within the remaining variables.

Intra-bear variation in GLG widths was assessed using individuals from which multiple teeth had been collected. Paired t-tests were used to compare age and region-specific GLG width estimates obtained from the LP_1 and RP_1 of an individual to detect potential bilateral asymmetry (n = 30). A difference in cementum deposition between upper and lower first premolars was investigated by comparing an individual's LP^1 to LP_1, or RP^1 to RP_1 (n = 22). Both actual GLG width and proportional GLG width (PW) were calculated for distal and mesial regions.

$$\mathrm{PW}_i = \frac{x_i}{\sum_0^i x_i} \quad \text{or} \quad \mathrm{PW}_0 = \frac{x_0}{\sum_0^1 x_i}$$

PW was calculated by dividing the width of the GLG at a particular age (x_i) by the sum of all GLGs widths up to and including that age. The first year of an animal's life is a critical time for growth, with cementum potentially recording valuable growth information. A meaningful representation of the first year's growth can be achieved by using the actual width of GLG_0 divided by the sum of GLG_0 and GLG_1. This gives an estimation of PW_0 in relation to a standard period of subsequent growth. Proportional width is a better representation of cementum patterns because it accounts for possible size differences between teeth.

2.2.3 Intra- and inter-bear comparisons using indices

Indices permit the testing of statistical hypotheses by representing GLG width as a parameter with a normal distribution, removing the age effect (i.e., decrease in GLG width with age), and sources of bias stemming from the individuals sampled. Indices of GLG width were developed considering the results for appropriate sampling intensity and protocols to reduce error introduced by inappropriate pooling of measurements. GLG widths from the distal aspect of first lower premolars collected from 67 known-aged bears (n = 36 females, n = 31 males) were estimated using the average of 20 measurements per GLG. GLG widths were transformed into an index created using the PW.

The Proportional Width Index (PWI; similar to Carrel 1994) can be used to test hypotheses related to changes in the pattern of cementum deposition within individuals, or between individuals of different ages. PWI can be used to test whether a GLG is greater or less than the sampled mean after accounting for individual patterns in cementum deposition, rather than assuming that all bears at all ages have the potential to deposit the same amount of cementum regardless of individual differences. PW_i values are calculated for each GLG. PWI values are created by dividing an individuals PW_i by the sex specific mean PW_i for age *i*. However, as this value can never be negative but theoretically has no upper limit, and the mean PWI of any GLG will always equal one, the resulting data is inherently skewed. Log transformation removes the skew of data when the result is less than one, and better meets assumptions of normality and homoscedasticity for future parametric tests of hypotheses.

$$\mathrm{PWI}_i = Log10\left(\frac{PW_i}{\overline{PW}_i} + 1\right)$$

2.3 RESULTS

2.3.1 Sampling intensity

Variation in cementum width were greater at younger ages (Fig. 2.2), therefore only the first six complete years of cementum deposition were evaluated. The range of GLG estimates decreases with an increase of the number of sample measurements, which was supported by a decrease in the CV (Fig. 2.3). To determine a suitable sample size to reduce measurement error and to get an accurate approximation of GLG width, I focused on the number of sample measurements where change in the value of the CV with an increasing number of sample measurements transitioned from a rapid decrease to a steady decline attributable to sampling with replacement of the same 50 measurements. For most bears, and at most ages, this occurs between 8 and 14 sample measurements.

A sample $\geq$ 10 would result in the desired CV < 5.0 based on observations of 95% confidence intervals. At all ages observed (0-5 years), a maximum CV < 6.0 was first observed with 12 measurements from a GLG. For a maximum CV < 5.0, 18 sample measurements were required. The maximum CVs observed differed between GLGs. For Ages 0, 1, and 2 years maximum CVs did not exceed 17.8. At Age 3 years of age, the maximum CV was 24.6, and at 4 years, the maximum CV was 21.5. For the last age investigated, 5 years of age, the maximum CV was 16.9.

2.3.2 Identifying sources of variation and reducing error

'Aspect' ($\bar{x}$ = 54.7%) was identified as the strongest factor contributing to variation in the 4-level nested ANOVA (aspect[section[tooth[bear]]]; Table 2.2). This was followed by the variables 'bear' ($\bar{x}$ = 28.6%) and 'tooth' ($\bar{x}$ = 14.3%). The variation introduced by sampling different sections was negligible, resulting in negative values in the initial partitioning of the variance. The negative values were removed and the partitioning of variance recalculated. For the 4-level ANOVA all factors were significant (P-values < 0.001 in all cases).

Controlling for aspect in the 3-level ANOVA, the variation was partitioned differently between the mesial and distal aspects (Table 2.3). Within the mesial aspect, the highest source of variation was observed between 'bears' ($\bar{x}$ = 50.6%) followed by variation between 'tooth', 'section', and finally 'error'. In the distal aspect the variable 'tooth' contributed the most variance ($\bar{x}$ = 49.1%), followed by 'bear', 'section', and 'error'. For the 3-level ANOVA controlling for 'aspect', all factors were significant ($P < 0.001$).

Comparisons between LP_1 and RP_1 of an individual showed that there were no significant differences (after Bonferroni adjustment) in actual or proportional GLG width in the distal or mesial aspect (Table 2.4). When comparing actual GLG width from LP^1 to LP_1 or RP^1 to RP_1 of an individual, there were several significant differences in the distal aspect (at age 0, 2, 3, 4, and 5) but none in the mesial aspect (Table 2.5). There were fewer significant differences (at age 0, 1, and 3) in the pattern of cementum deposition, as indicated by PW values, and one of these occurred at a different age than the significant differences observed using actual width.

Bear X05905 is a known-aged bear from the Western Hudson Bay polar bear population. First premolars were collected during 4 different captures allowing comparison of cementum annuli between multiple teeth from the same individual. Fig. 2.4 shows cementum from the distal aspect of each first premolar. The significant difference in cementum width between upper and lower premolars is distinct, while the similarity between left and right premolars is also apparent. Additionally, all four teeth show a similar pattern of cementum deposition with the first and second incremental line being formed close to one another, with the second growth layer group reduced compared to the first and third. After the 4^{th} incremental line the cementum narrows and deposition becomes more regular in all of the teeth.

2.3.3 Intra- and inter-bear comparisons using indices

PWIs removed the age effect and the individual differences in growth (Fig. 2.5). The PW value represents how cementum width compares in one GLG to previous cementum deposition for an individual while PWI values relate how cementum deposition compares to other individuals in the sampled population. The variation that remains within the PWI values may then be correlated to the physiological history of individuals or with environmental factors.

2.4 DISCUSSION

Quantification of cementum GLG widths from longitudinal sections of premolars is complicated by the variation of cementum growth within and between teeth. In most morphometric studies, there is a definable target measurement (e.g., condylobasal length or maxillary tooth row). However, measurement of cementum annuli are complicated by the deposition of a tissue that fluctuates in width and clarity over the surface of the root (Childerhouse et al. 2004; Craighead et al. 1970; Laws et al. 2002; Lockard 1972). I identified three stages at which measurement error and variation can be reduced: obtaining precise estimates of GLG width, identifying and controlling for introduced error and, the development of a width index to remove bias from age and/or individual differences in cementum growth. These procedures could be applied to a collection of teeth from any taxa to aid in the development of sampling protocols before attempting to extract life history information from cementum. However, such evaluations on teeth from different taxa, or geographic areas, may not yield the same results and therefore species- or geographic-specific interpretation and sampling protocols may be necessary.

2.4.1 Sampling intensity

The width of GLGs along the root surface fluctuates and it would be unrealistic to assume that one or two lines of measure would reflect a representative GLG width. Variation in the estimate of mean GLG width decreases with an increase in the number of sample measurements. Sampling an aspect ≥ 10 times for the width of a polar bear GLG produced a mean with an acceptable variance of the estimate. At 18 measurements, all ages (0-5) showed CV maximum < 5. Higher numbers of measurements may improve the estimate but would be impractical if large numbers of teeth are examined. The purpose of this data exploration is to understand sampling issues at practical sample sizes. Using the same 50 lines, 20 times in a random order, did not provide a clear indication of the true variance for higher numbers of sample measurements; for low numbers of sample measurements the likelihood that the same lines would be included in different randomized blocks is reduced.

2.4.2 Identifying sources of variation and reducing error

Considerable error may be introduced by pooling different aspects (distal and mesial) without accounting for their differences. The simplest alternative would be to restrict sampling to only one aspect or to use multiple aspects as independent variables. There is a high likelihood that not all cementum from teeth would be distinct in two or more locations, it may be preferable to focus effort on the most consistent aspect. The distal aspect of the tooth deposits the widest cementum layers, which may be attributed partially to increased tensile forces (Bellucci and Perrini 2002; Polson et al. 1984; Schroeder 1986). Wider layers of cementum generally have better GLG definition than more compact layers and are less prone to resorption due to compression (Chan and Darendeliler 2006; Hensel and Sorensen 1980; Rausch 1969). Additionally, the partitioning of variance when only the distal aspect was evaluated indicated that controlling for which tooth was sampled will further reduce introduced variation. If the mesial was used, controlling for tooth would not have as great an effect on reducing error because more variation was observed between bears; choosing the distal aspect, in this case, was the more conservative approach. The variance component analysis showed little difference between sections. Choosing the section that lies closest to the median longitudinal plane, discernable as the greatest amount of pulp cavity would provide the best representation of GLG width but deviations from center will have little impact on overall results.

The archived collection of teeth of polar bears over the past few decades contains a mixture of LP^1, LP_1, RP^1, RP_1. I failed to find any significant differences between GLG widths and proportional widths from LP_1 and RP_1 and conclude that data from these teeth could be pooled. The lack of significant differences in cementum widths between contralateral teeth has been noted by others (Fig. 2.4; Rausch 1969; Solheim 1990; von Biela 2008). This observation supports the theories that physiological or environmental influences are recorded in cementum deposition, and that life histories could be inferred. Comparison of LP^1 to LP_1, and RP^1 to RP_1 from individuals indicated that the upper and lower teeth may have different rates and patterns of cementum in the distal aspect. This lack of agreement between patterns of cementum deposition would not necessarily affect age determination, apart from issues of clarity that may result from crowded incremental lines, but may interfere with comparisons of life history information. If the distal aspect is used for analysis then data from upper and lower premolars should not be pooled. The archived collection of polar bear teeth has more lower first premolars, and future study will likely focus predominantly on lower premolars, at least until more comparative studies have been completed. It follows that researchers should note which premolar is collected from sampled animals.

2.4.3 Intra- and inter-bear comparisons using indices

Proportional width indices (PWIs) account for the decrease in GLG width with age and an individual's pattern of growth; removing the risk of considering a small growth layer for a large bear equal to a large growth layer for a small bear. The PWI reduces the noise in GLG width data so that the remaining variation can be compared to life history information. PWIs are more appropriate than actual values of GLG width for addressing temporal questions within an individual's lifetime such as reproduction, physiology, or annual measurements of environment characteristics.

Carrel's (1994) relative width index was created using proportions based on previous growth but ignored the loss of data for GLG_0 and used a 7^{th} order polynomial line fitted to the proportional width of all bears to establish an estimated proportional width. Carrel (1994) investigated reproduction in female black bears (*Ursus americanus*) and was not concerned with the loss of data by defaulting $RWI_0 = 1$. The first year of cementum deposition could be one of the most interesting because litter size, cub growth, and reproductive success change with environmental conditions (Derocher and Stirling 1995; Stirling et al. 1999). It was unclear why Carrel (1994) chose a 7th order polynomial when it inaccurately estimates proportional width at older ages because the sample size decreases (fewer older animals exist, teeth are not pulled, or measurements are excluded due to difficulties distinguishing GLGs). Using the average value for each GLG provides a more robust and independent estimate for each age group and can more simply address whether the observed values are greater or less than the average of the sampled population. This approach differed from Carrel (1994) which only used two measurements from transverse sections when quantifying cementum deposition. Further, his relative width index did not require log transformation likely due to smaller sample size (n = 17 bears) and all the first year relative width indices equaled one. Longitudinal sections are often preferred for age determination because they allow observers to follow the merging and splitting of incremental lines around the tooth surface and improving the accuracy of an age estimate (Laws 1962). The methods described here for calculating PWIs are based on a more precise estimate of GLG width (20 vs. 2 sample measurements) and are more robust for parametric tests of hypotheses by meeting the assumptions of normality.

Initially, software developed for dendrology was used to evaluate the option of automating the delineation of GLGs. I found that the software incorrectly identified dark staining cementoblast lacunae as incremental lines, and greater time was spent checking and adjusting outputs than required for manual identification of GLG boundaries. Czermak et al. (2006) applied automated GLG identification to digital images taken from transverse sections of premolars from medieval human remains using bright field microscopy. Results from automated GLG identification differed from both manual GLG counts and estimated ages based on morphology, and no known-aged specimens were used to confirm accuracy (Czermak et al. 2006).

An important consideration before attempting to correlate life history events to cementum GLG is the correct assignment of age, or calendar year, to specific GLGs. Identifying GLGs can be difficult because of indistinct incremental lines, accessory lines that inflate age estimates, crowding of lines that may decrease age estimates, and/or damage during tooth processing (Hensel and Sorensen 1980; Hewer 1964; Klevezal and Kleinenberg 1967; Rogers 1978). Age estimation requires the identification and counting of GLGs; however both intra- and inter-observer differences in age estimates for a tooth may occur (Calvert and Ramsay 1998; Charles et al. 1986; Costello et al. 2004; Hensel and Sorenson 1980; Stewart et al. 1996). Attempts at quantitative cementum evaluation are complicated by the possibility of incorrect age assignment. To avoid this bias, teeth from known-aged individuals should be used when initially evaluating cementum patterns as a recording structure for life history.

The ability to extrapolate life history information from the cementum of polar bears, and other mammals, could improve monitoring and population projections. Teeth are one of the few biological samples collected from the 500+ polar bears taken annually by hunters in Canada (Lee and Taylor 1994). In many cases these specimens and information from hunters are the only sources of data for infrequently monitored populations. Teeth may be collected from both living and deceased individuals and the highly mineralized nature of the tissue allows it to persist for long periods of time exposed to the elements. Additionally, correlations between GLG indices and the life history and physiological data obtained from individuals during population monitoring and mark-recapture surveys will further the understanding of physiological or environmental influences on cementum patterns.

Quantifying cementum GLG width is complicated by the differences in deposition both within and between individuals. Before testing hypotheses on cementum as a recording structure of life history, errant sources of variation need to be minimized. For longitudinal sections of polar bear premolars, minimums of 10 measurements were needed to avoid overly imprecise estimates of GLG width for a single image. To reduce error, observations should be limited to lower premolars and restricted to cementum from the distal aspect of the tooth. Using the most central section will provide the most accurate GLG width, but slight deviations from the center of the tooth did not influence measurement error. Once GLG widths are determined, the PWI corrects for age or individual biases on cementum patterns, which permits comparisons between different years, ages, or individuals. These protocols were derived from the results of evaluating cementum GLG widths within a tooth, partitioning of variance between factors (individual, tooth, aspect, and section), and observation of variation between multiple teeth from a single individual for the subpopulation of Western Hudson Bay polar bears. Similar analyses on the precision of width estimates and sources of variation in teeth collected from other taxa may produce different results; therefore the sampling protocol may be species-specific or dependent at the population level.

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Table 2.1: Variance components for model II four-factor nested ANOVA used to calculate intra- and interfactor percentage measurement error for the western Hudson Bay population of polar bears. df = degree of freedom, MS = mean square, b = number of bears, t = number of teeth, s = number of sections, a = number of aspects, n = number of samples

Source	df	Variance Component	Estimated MS	Estimated Variance Component	F-ratio
Bear (B)	b-1	S_B^2	$\sigma^2_\varepsilon + nast\sigma^2_B + nas\sigma^2_{T\vert B} + na\sigma^2_{S\vert T\vert B} + n\sigma^2_{A\vert S\vert T\vert B}$	$\frac{MS_B - MS_{T\vert B}}{nrst}$	$MS_B / MS_{T\vert B}$
Tooth|Bear (T|B)	b(t-1)	$S_{T\vert B}^2$	$\sigma^2_\varepsilon + nas\sigma^2_{T\vert B} + na\sigma^2_{S\vert T\vert B} + n\sigma^2_{A\vert S\vert T\vert B}$	$\frac{MS_{T\vert B} - MS_{S\vert T\vert B}}{nrs}$	$MS_{T\vert B} / MS_{S\vert T\vert B}$
Section|Tooth|Bear (S|T|B)	bt(s-1)	$S_{S\vert T\vert B}^2$	$\sigma^2_\varepsilon + na\sigma^2_{S\vert T\vert B} + n\sigma^2_{A\vert S\vert T\vert B}$	$\frac{MS_{S\vert T\vert B} - MS_{A\vert S\vert T\vert B}}{nr}$	$MS_{S\vert T\vert B} / MS_{A\vert S\vert T\vert B}$
Aspect|Section|Tooth|Bear (A|S|T|B)	bts(a-1)	$S_{A\vert S\vert T\vert B}^2$	$\sigma^2_\varepsilon + n\sigma^2_{A\vert S\vert T\vert B}$	$\frac{MS_{A\vert S\vert T\vert B} - MS_{within}}{n}$	$MS_{A\vert S\vert T\vert B} / MS_{within}$
n|Aspect|Section|Tooth|Bear (Residual Error)	btsa(n-1)	S_{within}^2	σ^2_ε	MS_{within}	

Table 2.2: Variance components analysis following a 4-level nested ANOVA, Aspect[Section[Tooth[Bear]]], for cementum growth layer group (GLG) width from longitudinal sections of first premolars (Sample sizes: Bear = 16, Tooth = 2, Section = 4, Aspect = 2, GLG estimate = 2). All factors are significant $P < 0.001$. Variance component calculations resulting in a negative value were set to equal 0 and percentage recalculated.

	Estimated %Variance Components [df]				
GLG	Bear [15]	Tooth [16]	Section [96]	Aspect [128]	Error [256]
0	19.2	13.0	0*	66.4	1.4
1	38.0	20.4	0*	37.9	3.7
2	27.6	9.4	0*	60.3	2.7
3	20.2	15.0	0*	62.9	2.0
4	27.5	15.5	0*	54.8	2.3
5†	39.1 [12]	12.5 [13]	0* [78]	46.2 [104]	2.2 [208]
Average	28.59	14.30	0	54.74	2.37

*originally negative
†only 13 bears

Table 2.3: Variance component analysis following a 3-level nested ANOVA controlling for aspect, section[tooth[bear]], for cementum growth layer group (GLG) width from longitudinal sections of polar bear premolars (Sample sizes: Bear = 16, Tooth = 2, Section = 4, GLG estimate = 2). All factors from ANOVA are significant $P < 0.001$.

	Estimated % Variance Component [df]							
	Distal				Mesial			
GLG	Bear [15]	Tooth [16]	Section [96]	Error [128]	Bear [15]	Tooth [16]	Section [96]	Error [128]
0	31.8	58.1	7.7	2.5	58.2	26.4	12.7	2.7
1	52.4	37.6	6.0	4.0	51.6	33.5	9.7	5.2
2	59.8	31.9	4.2	4.1	45.0	35.1	14.0	6.0
3	28.8	58.2	9.3	3.8	49.6	39.6	7.2	3.6
4	33.0	58.5	5.6	2.9	45.0	45.1	5.2	4.6
5*	42.0 [12]	50.2 [13]	4.9 [78]	3.0 [104]	54.2 [12]	38.8 [13]	4.2 [78]	2.8 [104]
Average	41.31	49.06	6.26	3.36	50.57	36.42	8.85	4.16

- only 13 bears

Table 2.4: Results of paired t-tests between cementum growth layer group (GLG) widths (actual width and proportional width) between left and right lower first premolars from polar bears (*Ursus maritimus*). Bonferroni adjusted level of significance $P < 0.008$.

		Distal				Mesial			
	GLG	$\bar{x}diff$ (R-L)	df	t	P-value	$\bar{x}diff$ (R-L)	df	t	P-value
ACTUAL	0	-6.40	30	-1.07	0.293	8.39	29	2.51	0.018
	1	1.35	30	0.31	0.756	-1.19	29	-0.23	0.822
	2	-3.27	30	-0.47	0.640	-2.98	29	-0.91	0.369
	3	-1.97	30	-0.30	0.769	-0.39	29	-0.14	0.890
	4	-2.34	26	-0.48	0.633	-3.70	25	-1.47	0.155
	5	0.28	19	0.09	0.933	-1.31	18	-0.42	0.678
PROPORTIONAL	0	-0.01	30	-0.48	0.633	0.04	29	2.015	0.053
	1	0.01	30	0.48	0.633	-0.02	29	-1.08	0.291
	2	-0.001	30	-0.07	0.948	-0.03	29	-1.88	0.070
	3	-0.001	30	-0.10	0.922	-0.01	29	-0.98	0.338
	4	0.002	26	0.26	0.793	-0.02	25	-2.12	0.044
	5	<0.000	19	-0.07	0.942	-0.002	18	-0.26	0.799

Table 2.5: Results of paired t-tests between cementum growth layer group widths (actual width and proportional width) between upper and lower first premolars from polar bears (*Ursus maritimus*). Bonferroni adjusted level of significance P < 0.008.

		Distal				Mesial			
	GLG	$\bar{x}diff$	df	t	P-value	$\bar{x}diff$	df	t	P-value
ACTUAL	0	-29.21	21	-3.36	0.003*	8.04	21	1.33	0.198
	1	8.62	21	1.41	0.174	-4.02	21	-0.84	0.412
	2	-17.65	21	-2.38	0.027	1.62	21	0.35	0.730
	3	-34.79	19	-4.21	0.001*	0.65	19	0.15	0.885
	4	-23.34	15	-3.70	0.002*	1.98	15	0.43	0.676
	5	-17.56	11	-3.37	0.006*	8.44	11	1.53	0.155
PROPORTIONAL	0	-0.10	21	-3.74	0.001*	0.03	21	1.30	0.208
	1	0.10	21	3.74	0.001*	-0.03	21	-1.30	0.208
	2	-0.02	21	-1.27	0.217	0.01	21	0.73	0.476
	3	-0.06	19	-4.25	0.0004*	-0.01	19	-0.60	0.559
	4	-0.03	15	-1.93	0.073	-0.004	15	-0.31	0.762
	5	-0.01	11	-0.89	0.395	0.02	11	2.15	0.054

- *significant at Bonferroni adjusted level of significance ($P < 0.008$)

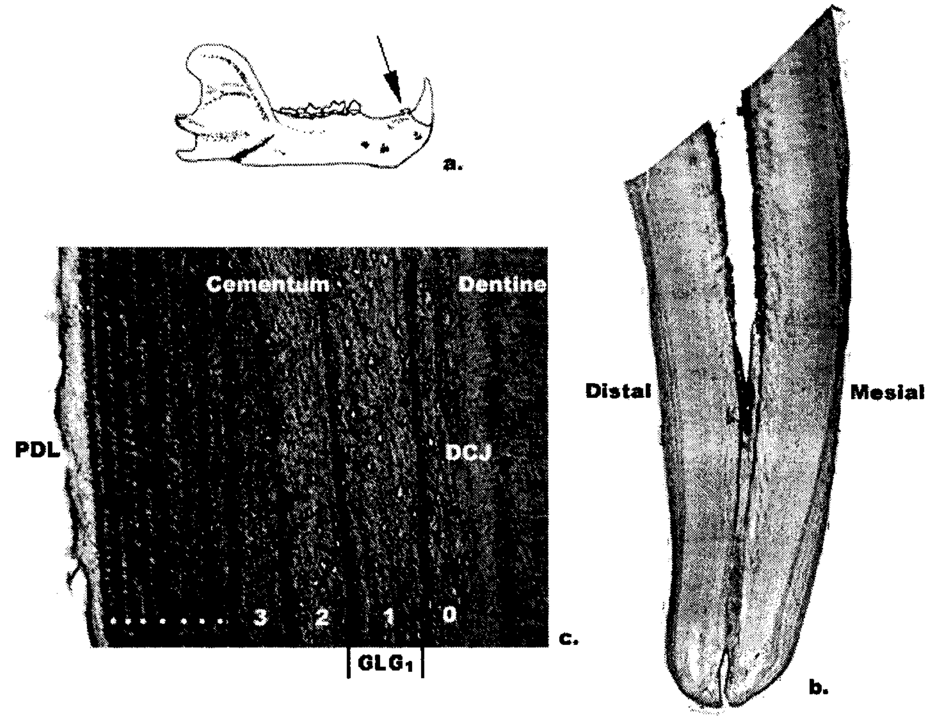

FIG 2.1:Right mandible of polar bear with arrow indicating location of first premolar (a.) longitudinal section of polar bear first premolar root indicating the distal and mesial aspects (b.), and distinct cementum growth layer groups (e.g., GLG_1; c.). Note the decrease in width of cementum layers as the animal ages in c. and variation in width around the root surface in b. GLG – Growth Layer Group, DCJ – Dentinocemental Junction, PDL – Periodontal Ligament

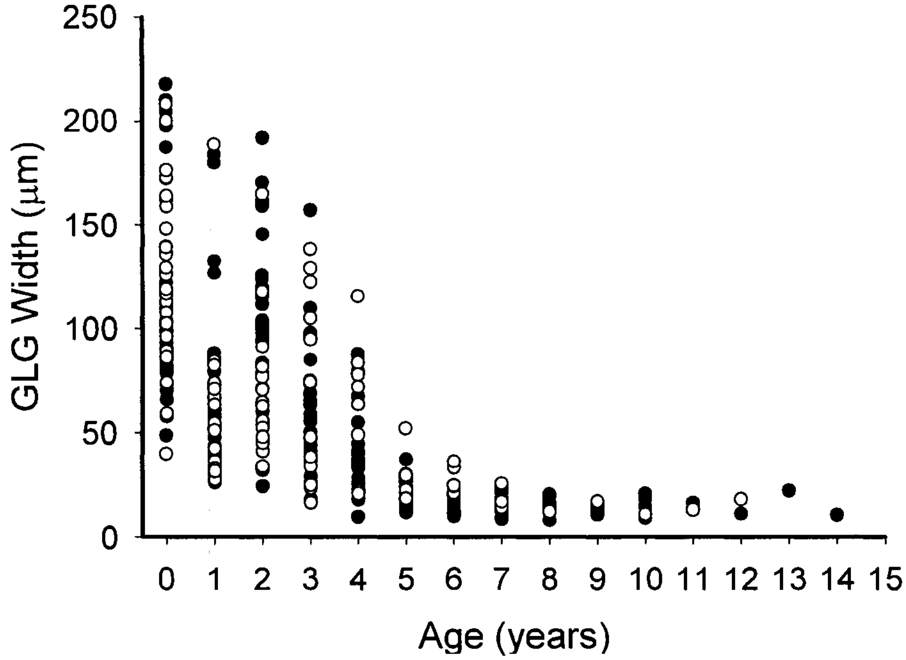

FIG 2.2: Width of cementum growth layer groups for male (○) and female (●) known-aged polar bears from western Hudson Bay, Canada.

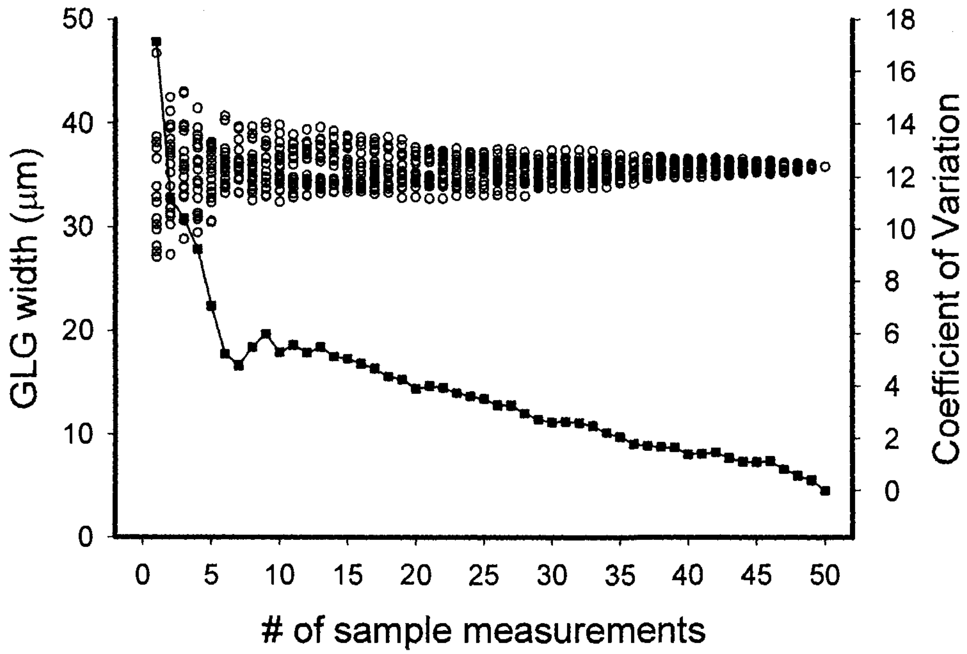

FIG 2.3: Width of cementum for growth layer group at age 2 years (i.e., GLG_2) for one polar bear as predicted by the number of sample measurements (1-50) randomly assigned into 20 blocks (○) and associated Coefficient of Variation (■) calculated from the average and standard deviation of the 20 estimates of GLG width for each sample size.

FIG 2.4: Distal cementum from first premolars collected from known-aged male polar bear (ID = X05905). a.) LP^1 age 5, b.) RP^1 age 5, c.) LP_1 age 6, d.) RP_1 age 7. All teeth were collected during autumn capture, all images are the same magnification.

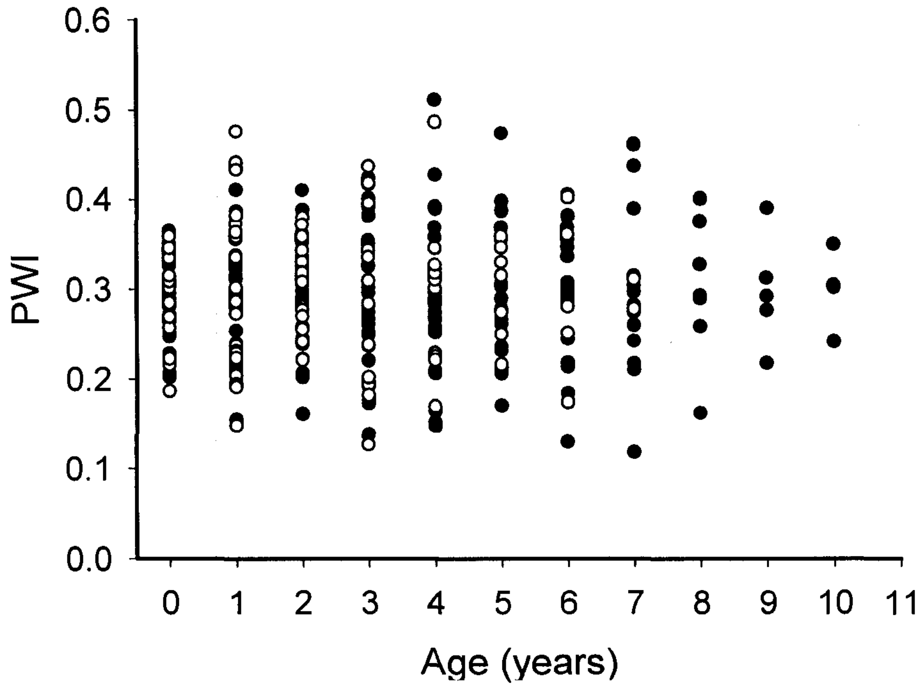

FIG 2.5: Distribution of cementum Growth Layer Group (GLG) width represented as Proportional Width Indices (PWI) for male (○) and female (●) known-aged polar bears from western Hudson Bay, Canada.

3.0 OBTAINING REPRODUCTIVE HISTORIES OF FEMALE POLAR BEARS (*URSUS MARITIMUS*) USING CEMENTUM PATTERNS OF PREMOLAR TEETH

3.1 INTRODUCTION

Shortly after biologists began to use layers in dental tissues to determine the age of wildlife (Eidmann 1932; Laws 1952; Scheffer 1950), researchers hypothesized that additional information about individuals or populations might be recorded in teeth (Grue and Jensen 1979; Klevezal 1996; Klevezal and Kleinenberg 1967; Laws 1953). Distinct patterns of deposition of cementum and dentine have been attributed to a number of physiological, environmental, and life history events: growth, inactivity, fasting, physical condition, climate, tooth movement, food quality, and reproduction (Carrel 1994; Carrick and Ingham 1962; Cipriano 2002; Coy and Garshelis 1992; Craighead et al. 1970; Grue and Jensen 1979; Klevezal 1980; Klevezal 1996; Lieberman 1994; Polson et al. 1984; von Biela et al. 2008). Because dental tissues are highly mineralized, once formed, the patterns generally remain unaltered post-mortem (Armitage 1991; Lieberman and Meadow 1992; Schroeder 1986; Wedel 2007). Thus, if it were possible to use the deposition of dentine and cementum to determine the past reproductive history of captured or harvested female polar bears (*Ursus maritimus*), it would greatly enhance the availability of information on population demographics.

Cementum formation begins at tooth eruption and functions, along with the periodontal ligament, to keep the tooth secure in the alveolus (Bosshardt and Selvig 1997; Schroeder 1986). Unlike space-limited dentine, new cementum is formed on the tooth's exterior surface and it is deposited throughout life (Laws et al. 2002; Stewart et al. 1996). Variations in the formation rate of cementum produces visible layers with differing cell density and collagen orientation (Bosshardt and Schroeder 1990; Cool et al. 2002; Lieberman 1994; Smith et al. 1994). Teeth sectioned and stained for transmitted light microscopy exhibit a pattern of broad light staining bands followed by narrow dark lines reflecting periods of rapid and slow cementum formation respectively (Fig. 3.1; Bosshardt and Schroeder 1990). In age determination literature, the thin dark-staining cementum is termed an incremental line, while the deposition of the wide translucent band and associated incremental line(s) (including accessory or "false" incremental lines) which form over the course of a year are referred to as a growth layer group (GLG; terminology of Perrin and Myrick 1980).

Reproduction and lactation are energetically and nutritionally costly processes (Bauman and Currie 1980; Linzell 1972; Oftedal 1984; Zera and Harshman 2001). Changes in the widths of cementum GLGs may be caused by the indirect effects of the physiological demands of reproduction and lactation, and/or the possible direct influences from hormones (Kolb 1978; Rogers 1978; Sergeant 1967). Disruption to regular cementum deposition during pregnancy and lactation has been detected in humans, walrus (*Odobenus rosmarus*), and ringed seal (*Phoca hispida*; Kagerer and Grupe 2001; Mansfield 1958; Stewart et al.

1996). Reduced width of cementum GLGs signify the production of offspring and lactation in sea otter (*Enhydra lutris*), and black bears (*U. americanus*; Carrel 1994; Coy and Garshelis 1992; Hristienko et al. 2004; Rogers 1975; von Biela et al. 2008). In the cementum of brown bears (*U. arctos*) known reproductive events could not be identified (Matson et al. 1999). Decreased width of cementum GLGs in female polar bears was suggested to reflect a record of reproduction, but had not been verified with field observations of reproductive state (Kirkegaard 2003).

Polar bears in several populations, such as that in western Hudson Bay, fast for several months during summer and autumn (July to November) when the sea ice completely melts and bears are forced ashore to subsist on stored fat reserves (Ramsay et al. 1991; Ramsay and Hobson 1991). While fasting, bears with sufficient fat reserves metabolize adipose tissue and little muscle protein (Atkinson et al. 1996; Polischuk et al. 2001). However, pregnant females experience the longest fast because, instead of returning to the sea-ice when it forms in the autumn, they enter maternity dens where implantation, gestation, birth, and initial lactation occur without access to food and water (Nelson 1987; Ramsay and Dunbrack 1986; Watts and Hansen 1987). During this extended fast, females typically use their fat reserve and may initiate the catabolism of protein reserves (Atkinson and Ramsay 1995; Oftedal 1993). Between March and April, after close to eight months of fasting, females accompanied by cubs (offspring less than 1 year old) return to hunting seals on the sea-ice. As spring progresses, the quantity and energy content of the milk decreases and cubs begin feeding on seals (Polischuk et al. 2001). The following ice-free season, females nursing cubs experience significantly more loss of mass compared to solitary females or females caring for yearlings (1 year old offspring) or two-year olds (Arnould and Ramsay 1994). Females caring for cubs transfer nearly 4 times more milk energy per day than those accompanied by yearlings (Arnould and Ramsay 1994).

Long-term research of the western Hudson Bay polar bears has recorded changing trends in reproductive parameters. In the 1970s and early 1980s, females often had a two-year interbirth interval and weaned offspring at 1.5 years of age (Ramsay and Stirling 1982; Ramsay and Stirling 1988). Between 1985 and 1992 fewer independent yearlings were observed, and offspring were weaned at 2.5 years of age resulting in the interbirth interval increasing to 3 years (Derocher and Stirling 1995). From 1993, observations of decreased body condition, litter production, and independent yearlings continued (Stirling et al. 1999). Currently, the polar bear population of western Hudson Bay is declining, with increased mortality of juvenile, subadult, and senescent-adult bears attributed to earlier sea ice breakup (Regehr et al. 2007). The ability to detect reproductive events from the cementum of females would allow closer monitoring of these trends through additional information acquired for periods when field observations are unavailable.

This paper examines the value of cementum as a recording structure of reproductive histories in female polar bear. Multiple recaptures of polar bears in western Hudson Bay permits the assignment of known reproductive, physiological, and environmental conditions to GLGs from teeth collected and prepared for age determination. Previous research on recording of reproduction in cementum lacked measurements of predictive success (Carrel 1994; Coy and Garshelis 1992). I build a predictive model using known reproductive conditions and evaluate the success of these models in the correct classification of reproductive status. Reproductive parameters (litter production rate, age of primiparity, litter mortality) predicted from cementum characteristics, are compared to estimates based on field observations. The introduction of additional physiological and environmental variables allows more in-depth investigation of factors that influence cementum deposition and what additional information could be retrieved by examination of cementum patterns.

3.2 METHODS

Observations of reproductive status of free-living female polar bears were collected during the long-term research of the western Hudson Bay population (1965-2006). The Western Hudson Bay polar bear population is delineated by 63°10'N and 88°30'W, and to the west and south by the limited inland travel by polar bears with most specimens collected from the denning area located between the Churchill and Nelson rivers (Fig. 3.2). First premolars were originally collected and prepared for age determination (Calvert and Ramsay 1998; Stirling et al. 1977; Stirling et al. 1977; Stirling et al. 1989). Investigation into sources of bias identified that upper and lower first premolars were not comparable (Medill et al. *in review*). Therefore, because lower first premolars were preferentially sampled in the field, these were used in analyses. Only teeth in which cementum annuli were clear and readily matched the ages of either known (bear was observed and marked as cub), or the ages estimated by experienced technicians, were included. The estimates of width for a GLG was the mean of 20 sample measurements obtained from the distal aspect of the root (Medill et al. *in review*).

Standardizing GLG width estimates into Proportional Width Indices (PWI) removes the age-effect of decreased cementum width with increase in age, and allows comparison between individuals and different ages (Medill et al. *in review*). PWIs are calculated by first determining the proportional width (PW) for a GLG:

$$\mathrm{PW}_i = \frac{x_i}{\sum_{0}^{i} x_i}$$

Where x = width (μm) and i = specific GLG or age. Then by calculating the gender-specific mean PW for each GLG_i of all bears in the sample, and calculate individual PWI:

$$\mathrm{PWI}_i = Log10\left(\frac{PW_i}{\overline{PW}_i} + 1\right)$$

GLGs were categorized into four known reproductive statuses: "Cub" if they were known to have given birth during that year, "Pregnant" for the year immediately before birth (Cub), "Yearling" if survival of the cub(s) was confirmed to exceed 12 months, and "Unknown" where observations were unavailable and could therefore be either Pregnant, Cub, Yearling, or unproductive (neither pregnant nor accompanied by offspring). In instances where females produced cubs every second year, the yearling-year GLG was also the pregnancy year for the subsequent reproductive effort.

GLGs coinciding with the year of capture could not be used in analyses because it would not have been completely formed. For females observed with yearlings in the spring or autumn the "yearling" GLGs were not included in analyses, however "Cub" and "Pregnant" were assigned to the last and second last fully formed GLGs respectively. For females accompanied by cubs in the spring or autumn the "Cub" GLGs were not examined, however "Pregnant" was confirmed for the last fully formed GLG. For the above reasons, there are fewer observations of "Cub" GLG widths to compare with "Pregnancy" GLG widths, and an even fewer number of GLG assigned to "Yearling".

3.2.1 Comparison of GLG widths between observed reproductive status

GLG widths, represented by PWI values, were compared between observed Pregnant, Cub, and Yearling GLGs. Known-aged females were evaluated independent from those aged by counts of cementum annuli to confirm observations without the potential bias from inaccurate age assignments. Cub survival was confirmed either from autumn sightings of the female with her offspring or from recapture as a yearling or adult. Mortality of cubs was confirmed by recapture of the female and the absence of her cubs, or from the presence of new cubs the following year. Confirmation of yearling survival was based on observation of the yearling with its mother or a subsequent observation as an independent bear.

3.2.2 Predicting years females are accompanied by cubs

To be a useful tool for evaluating past reproductive history, GLG widths need to reliably reflect a female's prior reproductive history without requiring additional data. Because only age and GLG width can be attributed to cementum annuli, only these variables are used here to construct models of reproduction. A binary variable COY (cubs-of-the-year) or NON-COY (no cubs-of-the-year) was assigned to the GLGs of years females were either accompanied by Cub(s) or Pregnant, respectively. From other studies, the amount of change in the GLG widths of an individual were most indicative of the presence of offspring (Carrel 1994; Coy and Garshelis 1992; von Biela et al. 2008). Therefore, the difference [$\Delta PWI = PWI_{(i)} - PWI_{(i-1)}$] was calculated for each GLG. Logistic regression between COY and NON-COY was used to determine the probability of COY based on the value of ΔPWI.

Probabilities for the presence of COY, as calculated from the logistic regression model, were applied to the remaining GLG differences where reproductive status was "Unknown" and to the additional observations of Pregnant, Cub, and Yearling GLGs from females included in the model. The probability threshold where sensitivity (correct prediction of COY) and specificity (correct prediction of Non-COY) were both maximized without compromising the other prediction (Fielding and Bell 1997). Predictions were limited to females $\geq$ 4 years old, the minimum age females have been observed with cubs in western Hudson Bay (Derocher and Stirling 1995; Ramsay and Stirling 1988). To assess whether patterns of cementum deposition were similar between males and females, and the possibility of using cementum to determine gender, probabilities were calculated for the ΔPWI for male bears ≥ 4 years old. The expected proportion predicted as COY would be 0 unless males experience similar periods of nutritional stress and energy expenditure.

3.2.3 Evaluation of estimated reproductive parameters

The assignment of COY and NON-COY, based on the probabilities determined from logistic regression, were used to predict age at first parturition, litter production rate, and interbirth intervals from GLGs of all sampled females of reproductive age (≥ 4 years). Consecutive predictions of COY were considered an indication of total litter mortality; in field observations litter production in consecutive years is observed when total litter loss occurs before the end of the breeding season making the female available for breeding. The first of consecutive predictions of COY within an individual were not considered in interbirth intervals, however, were included as a first parturition. Observations were grouped into 4 periods, 1965-1979, 1980-1984, 1985-1992, and 1993-2006, to allow comparison to published data on litter production rates for the western Hudson Bay polar bears (Derocher and Stirling 1995).

3.2.4 Factors influencing GLG width of females with cubs

Additional information about the individuals from which these teeth were collected permitted investigation into other factors that may influence cementum

GLG widths. Litter size, and the nutritional demands of lactation for multiple offspring, may result in differences in cementum width. Additionally, variables not necessarily related to reproduction may influence cementum width, particularly amongst females who are already taxed with the additional burdens of reproduction and lactation. A body condition index was created using estimated mass from axillary girth measurements (Kolenosky et al. 1989) subtracted from the expected mass determined by a von Bertalanffy growth curve for polar bears from the Western Hudson Bay population (Derocher 1991). Calendar year was included as a variable because it could record changes in cementum that reflect observed declines in size of females or other changing trends in the environment (Atkinson et al. 1996; Derocher and Stirling 1998a). The duration of the ice-free period in western Hudson Bay represents an environmental stress component (Stirling et al. 1999; Stirling and Parkinson 2006). Earlier sea-ice break-up shortens the amount of time bears can hunt young-of the-year ringed seals (*Phoca hispida*), an important component of the annual diet (Iverson et al. 2006; Smith 1980; Stirling and Derocher 1993). Duration of the ice-free period was defined as the number of consecutive days when the sea ice covered $\leq 50\%$ of the surface in western Hudson Bay based on information accumulated from satellite images, ship and aircraft observations, observations from shore and climatic information (Gagnon and Gough 2005). Both the ice-free duration during pregnancy and when females were accompanied by cubs was compared to PWIs. Because PWIs were created using the average from all females of a given age regardless of reproductive state, age may still be a significant variable within the sub-selection of reproductive females. The influence of these variables on explaining the variation in GLG width of females with cubs was examined using multiple regression techniques (Systat 12©, Systat Software, Inc. San Jose, CA). Stepwise model selection used include/exclude variables at P = 0.15.

3.3 RESULTS

3.3.1 Comparison of GLG width between observed reproductive status

PWIs were calculated for 220 females ≥ 4 years of age (mean age ± se = 9.4 ± 0.06 years, range = 4 to 21 years). Observations of 104 females with cubs (mean age ± se = 9.8 ± 0.11 years, range = 5 to 19 years) contained 135 GLGs which corresponded to observations of cubs. For statistical testing, a single reproductive effort was randomly selected from females with observations of multiple litters. PWIs of cementum GLGs when "Pregnant" were larger than the subsequent "Cub" GLG when offspring survived > 8 months (paired t- test, t = 8.16, df = 103, $P < 0.001$). GLGs from "Cub" years, when offspring survived ≥ 21 months, were narrower than the following "Yearling" year (paired t-test, t = -3.88, df = 31, $P < 0.001$). Width of "Pregnant" GLGs were not significantly different from widths of corresponding "Yearling" GLGs (paired t-test, t = 1.31, df = 31, P = 0.20).

Known-aged females showed reduced cementum width when accompanied by cubs. GLG widths (PWI) when accompanied by cubs, versus the preceding year, when pregnant, were significantly different (paired t-test, t = 3.73, df = 7, P = 0.007). All PWIs from known-aged bears accompanied by yearlings were larger than PWIs from when accompanied by cubs, however, the sample size was insufficient for statistical testing (n=2).

There were five recorded instances where parturition had occurred but all cubs died within 8 months (Table 3.1). Comparing PWI from females who experienced litter mortality against females who successfully reared cubs past 8 months, were not statistically different (t-test, $t_{2, \alpha=0.05} = -0.68$, df = 4, P = 0.53).

3.3.2 Predicting years females are accompanied by offspring

Parameters for the logistic regression model were calculated using single observations of successful reproductive events from 104 females. There was a significant relationship between ΔPWI and the presence or absence of cubs (logistic regression, $\beta_0 = -0.229$, $\beta_1 = -13.465$, $G^2 = 46.55$, df = 1, P < 0.001; Fig. 3.3). Sensitivity (71.8%) and specificity (72.0%) crossed-over at probability threshold of 0.50, while the maximum correct classification was achieved at probability threshold of 0.60 (Fig. 3.4). Predictive success was higher for females in which cub survival beyond 8 months was confirmed (Table 3.2). GLG of only three of the five litters lost before 8 months of age (n = 5) were correctly classified as COY. Litters in which survival could not be confirmed also had lower COY classifications (65%, n = 17). Reduced GLG widths observed in the cementum are associated with reduced PWI values and lead to predictions of accompanying COY (Fig. 3.5 and 3.6). When applied to male bears ≥ 4 years of age (mean age ± se = 8.6 ± 0.14 years, range = 4 to 20 years), 284 out of 647 GLGs (43.9 %) had ΔPWI indicating similar patterns are formed in male bears.

3.3.3 Evaluation of estimated reproductive parameters

A probability threshold of 0.50 was used to predict the presence or absence of cubs. From 220 females of reproductive age, 594 litters were predicted within 1,387 GLGs (42.8%). Age of first parturition, predicted from cementum annuli, occurred at 4 years of age (mode; range = 4-9 years, n = 195). For five and six year-old females that were observed to successfully raise cubs, only 38% were predicted as first parturition. A first parturition event was not predicted for 25 of the females; these were mostly young individuals which may not have given birth before tooth removal (age 4 n = 6, age 5 n = 9, age 6 n = 5, age 7 n = 3, age 8 n = 1, age 13 n = 1). There was a significant increase in the proportion of first parturition occurring at age 6 for the period 1993-2006 (Tukey; $q = 3.373$, $P < 0.05$; Table 3.3).

Consecutive predictions of COY occurred 127 times (21% of predictions). Consecutive predictions of COY were more common in young females, aged 4-9 years, than females 10 years and older ($\chi^2_{c'} = 8.17$, df = 1, n = 127, $P = 0.004$; Table 3.4). Fifty-two percent of the predictions of consecutive COY followed the predicted first parturition. Proportion of consecutive predictions of COY were unequal across the four periods with 1965-1979 (0.31) greater than 1993-2006 (0.17; Tukey test, $q = 3.653$, $P < 0.05$). There was no detectable difference in the proportion of consecutive COY predicted between 1965-1979, 1980-1984, and 1985-1992 nor from 1980-1984, 1985-1992, and 1993-2006.

The mean litter production rate (lp_x) across all age groups and periods was 0.43 litters/female/year. No difference in litter production rates between sampling periods was predicted using cementum (Table 3.5). Predicted interbirth interval ($1/lp_x$) for young adults (age 4-9 years) was 2.3 years, and for adults in their prime (age 10-15 years) 2.4 years.

3.3.4 Factors influencing GLG width of females with cubs

Litter size did not influence the width of cementum annuli and no difference in PWI was observed between females accompanied by 1 or 2 cubs (t = -0.252, df = 134, P = 0.801) There were insufficient observations to compare litter sizes of 3 (n = 5).

Univariate comparisons between PWI of females with cubs and physiological or environmental factors found only body condition (PWI = 0.00073(body condition) + 0.274; $F_{1, 53}$ = 7.54, P = 0.008, r^2 = 0.12) and ice-free duration when accompanied by cubs (PWI = 0.0014(ice-free days with cubs) + 0.071; $F_{1, 113}$ = 5.01, P = 0.027, r^2 = 0.04) to be significant. Multiple regressions were limited to the 55 females from which body condition information was available. Collinearity was not detected between body condition, year, ice-free duration, and age (Table 3.6). Both forward and backward model selection eliminated ice-free duration when pregnant (P = 0.915), ice-free duration when accompanied by cubs (P = 0.211), year (P = 0.314), and age (P = 0.890), as influencing cementum width on their own (Table 3.7). Stronger explanation of the variation in GLG width is obtained by considering interactions between body condition, year, and ice-free duration. Forward and backward selections both identified a significant interaction between year and body condition [PWI = 0.269

$+ 0.134$(body condition) $- 0.00007$(body condition·year), $r^2 = 0.21$, $F_{2,52} = 6.736$, $P = 0.003$)].

3.4 DISCUSSION

Teeth collected from western Hudson Bay polar bears were evaluated to see whether patterns in cementum annuli record reproductive histories of females. A sharp decrease in the width of a cementum GLG in female polar bears can be considered an indication of parturition and the production of offspring to a minimum age. The occurrence of cub rearing, as determined by comparisons using females with known reproductive history, can be predicted from GLG measurements with approximately 73% accuracy. Estimates of reproductive parameters are also possible, with the predicted values of litter production rates and recruitment similar to observations made during autumn captures when cub survival is high. However, when litter survival is low, cementum may record late litter loss as cubs produced, while early litter loss may not be represented in GLG width.

Incremental lines are characteristically formed when activity, feeding, and growth are reduced, while the wide light-staining cementum is produced during rapid gains in mass and structural growth (Craighead et al. 1970; Grue and Jensen 1979; Klevezal 1980). Regional variation in the timing of incremental line formation has been observed for polar bears (Grue and Jensen 1979; Hensel and Sorensen 1980). For polar bears in western Hudson Bay, the incremental line is formed between late summer and autumn, coinciding with periods of fasting, weight loss, and reduced activity (Arnould and Ramsay 1994; Atkinson et al. 1996; Ramsay et al. 1991; Ramsay and Stirling 1988; Ramsay and Stirling 1988; Stirling and Archibald 1977). Polar bears at higher latitudes can remain active and access prey on the sea ice year-round, with females entering maternity dens for shorter periods of time (Amstrup et al. 2000; Ferguson et al. 2000). This likely contributes to the differences in the timing of incremental line formation, and the reduced clarity of cementum, in teeth collected from more northern populations (Grue and Jensen 1979; Hensel and Sorensen 1980; Klevezal 1996; Rausch 1969).

The long period of fasting experienced by pregnant females in western Hudson Bay may enhance the record of reproduction in cementum. The period of time pregnant females remain inactive during late winter and early spring, in addition to the demands of gestation and lactation, may delay the apposition of light cementum and produce a narrow GLG. Inactivity, hibernation, and associated fasting were reported as aiding the formation of incremental lines and producing reduced GLG widths (Klevezal 1980; Rausch 1969; Rogers 1978; Sauer et al. 1966). Additionally, female polar bears increase bone mass during their year of pregnancy to buffer against loss to disuse, fetal development, and lactation while fasting in the maternity den (Lennox and Goodship 2008). Similarities in the hormones controlling bone and cementum formation (Ikezawa et al. 1997), suggest a connection between the increase in bone mass and cementum deposition during pregnancy, that could influence the magnitude of ΔPWI.

Research on black bears indicated that a reduction in the GLG width in female cementum occurs only when cubs are nursed for a prolonged period (Carrel 1994; Coy and Garshelis 1992; Hristienko et al. 2004). In black bears, cub mortality at ≤ 4 months of age resulted in Non-COY cementum patterns in females (Coy and Garshelis 1992). Female polar bears nursing cubs expend greater energy through lactation and experience a significantly greater loss of mass than pregnant females or females with yearlings (Arnould and Ramsay 1994). Additionally, females may wean offspring as early as 1.4 years, further reducing the additional demands of lactation during the "yearling" year (Arnould and Ramsay 1994; Ramsay and Stirling 1988). Our data also provides evidence that a minimum period of cub rearing is required before female polar bears develop a detectable difference in GLG width. Because litter size was not significantly correlated with cementum GLG patterns, ΔPWI indicates the presence or absence of whole litters. PWI of female polar bears, when cubs were confirmed lost between 3 and 8 months of age, were not significantly different from females where offspring survived > 8 months. However, the ΔPWI for two of three females that lost litters early enough for the female to rebreed the same season were classified as Non-COY, and the third was not a strong probability of COY (Table 3.1). Additionally, there were 17 litters for which survival could not be confirmed (i.e., never recaptured female in fall, no recapture of weaned offspring). Litter mortality could explain some of the missing individuals, and early litter loss would correspond to the lower percentage of the litters with unconfirmed survival being classified as COY. ΔPWI would then appear more likely to classify early litter loss as Non-COY, but GLGs when litter loss occurs after the breeding season has passed, are classified as COY.

Pregnant females typically have higher fat reserves than females accompanied by cubs or yearlings and litter loss has been associated with poor maternal body condition (Atkinson and Ramsay 1995; Derocher and Stirling 1995; Derocher et al. 1992). There was a small but significant correlation between cementum PWI and body condition. Four of the 5 females that lost litters were observed in poor condition during spring capture (Table 3.1). If females were unable to increase their body condition sufficiently after litter loss it may be possible that cementum width would remain diminished and result in prediction of COY despite early litter loss.

The slight increase in cementum GLG width with increases in the ice-free duration coinciding with when females were with cubs, is opposite to what was expected since the ice-free duration is negatively correlated to body condition (Derocher and Stirling 1995; Stirling et al. 1999). Addition of the interaction term between body condition and year improved the regression fit. However, it is unclear exactly what aspect of "year" is influencing cementum widths. It is possible, due to the large amounts of variability in cementum patterns between and within individuals, that a significant percentage of the variation in both male and female cementum can not be explained by physiological or environmental factors (Childerhouse et al. 2004; Craighead et al. 1970; Laws et al. 2002; Lockard 1972; Medill et al. *in review* ; Solheim 1990).

The amount of variation in cementum width explained by the ice-free duration when the female is with cubs, and the year is very low, but may indicate that the response of cementum to environmental influences may not be explained entirely how much they are eating but also by what is being eaten. Increases in occlusal forces and/or tension leads to increased cementum deposition to maintain tooth position (Bosshardt and Selvig 1997; Chan and Darendeliler 2006; Lieberman 1993; Polson et al. 1984). When seals are abundant and/or remain available because of late sea-ice break-up, females may choose to eat only the high fat components (Stirling and Archibald 1977; Stirling and McEwan 1975). With low seal availability, females may increase occlusal forces by scavenging carcasses, crushing bones, and tearing and chewing harder tissues (Derocher et al. 2004). They could also switch from hunting ringed seal to predating harbour seal (*Phoca vitulina*), or the larger bearded seal (*Erignathus barbatus*) which may require greater mechanical force to kill and manipulate (Iverson et al. 2006; Rosing-Asvid 2006).

The prediction of female parturition from ΔPWI values provided reasonable approximation of 0.43 litters/female/year (1965-2006). Predicted litter production rates for all ages between 1966-1979 and 1980-1984 were equal to those determined from field observations (Table 3.5). The decline in litter production rate observed in the Western Hudson Bay population (1985-1992) was not detected in the prediction of litter production using cementum widths (Derocher and Stirling 1995). Derocher and Stirling (1995) calculated litter production based on the observations of litters present during autumn captures and thus would have missed incidents of whole litter loss occurring prior to capture. Whole litter loss would increase the interbirth interval based on autumn capture observations. Whereas, predictions based on cementum would record the presence of litters when mortality occurred after the breeding season but before autumn capture. Therefore, the shorter interbirth intervals predicted in cementum do not contradict the observations of increased litter loss in the last two decades and agree with observations of stable pregnancy rates (1982-1990; measured from serum progesterone levels; Derocher and Stirling 1995; Derocher et al. 1992; Regehr et al. 2007; Stirling et al. 1999).

Whole litter loss and age at first parturition were calculated for the predicted presence of COY. Consecutive predictions of COY, in which the female was predicted as having had cubs two or even three years in a row, occurred in 21% of predicted litters. One interpretation of consecutive predictions of COY is that it represents litter mortality and rebreeding by the female. Predictions of consecutive COY were most frequent in young adult females where body condition is typically lower and may reduce cub survival (Table 3.4; Atkinson and Ramsay 1995; Derocher 1991; Derocher and Stirling 1996; Derocher et al. 1992; Polischuk et al. 2001). However, the prediction of higher litter loss occurring in 1965-1979 than in 1993-2006 is contrary to observed trends of increasing cub mortality (Derocher and Stirling 1995; Regehr et al. 2007). Width of cementum layers has been used to detect the age at first reproduction in sea otters (von Biela et al. 2008). The methods used on polar bears predicted first parturition at four years old. Observations exist of four year old females rearing cubs in western

Hudson Bay, however, most females are pregnant at four and have their first litter at five years old (Derocher and Stirling 1995; Derocher and Stirling 1998b; Derocher et al. 1992; Ramsay and Stirling 1982; Ramsay and Stirling 1988).

Several studies warn of the risk of mistaking false or accessory incremental lines, which can be present in both males and females, to be true incremental lines (Christensen-Dalsgaard 2006; Coy and Garshelis 1992; Grue and Jensen 1979; Kirkegaard 2003; Klevezal 1996; Matson et al. 1993; Rogers 1978). In research involving the measurement of GLGs, mistaking false annuli as true incremental lines would result in incorrect assignment of age and/or year to a GLG and a decreased estimate of width. Many false incremental lines are fainter than primary incremental lines, inconsistent around the surface of the root, and/or merge and fade (Calvert and Ramsay 1998; Coy and Garshelis 1992; Klevezal 1996). For these reasons, false incremental lines are easier to detect in the longitudinal sections of teeth. In this study, the person measuring was alert for false incremental lines, and when determined as such, were not considered a termination of GLG and should not have influenced GLG width. In addition, only bears with clear, unambiguous cementum patterns were evaluated, and age was pre-determined by experienced technicians.

Male ursids produce false incremental lines in the cementum more commonly than females; these false annuli range from being faint to well defined, but should not be considered as two separate GLGs (Coy and Garshelis 1992; Kirkegaard 2003; Klevezal 1996). False incremental lines have been hypothesized to reflect mating effort in male polar bears, similar to a rutting line observed in ungulates (Christensen-Dalsgaard 2006; Coy and Garshelis 1992; Low and Cowan 1963; Mitchell 1967; Reimers and Nordby 1968). During the mating season, male polar bears may reduce hunting and food intake while courting females and develop blood urea creatinine ratios similar to a fasting individual (Ramsay et al. 1991). The mating season for polar bears can extend from mid-February into July, with peak activity occurring March-May (Rosing-Asvid et al. 2002; Spady et al. 2007), If feeding by male bears is compromised by mate seeking, courting and defending females from other males, the appearance of false incremental lines and the reduced width in GLGs (detected in this study), could reflect prolonged stress experienced by these individuals. However, data regarding mating activity of male polar bears has not been collected to allow comparisons against cementum GLG width. Additionally, false incremental lines are observed in some juvenile bears (Hensel and Sorensen 1980; Lønø 1970), which can not be explained by reproductive stress but does not rule out nutritional stress caused by periods of low food availability during intense periods of growth.

Polar bears are more closely related to brown bears than to black bears and yet reproduction was not recorded in the cementum of brown bears (Matson et al. 1999; Yu et al. 2007). One explanation for this was the differences in methodology; Matson et al. (1999) purposely sampled where the cementum layers were most regular. Had they sampled consistently in one location and chosen where incremental lines were clear, but allowed for variation in GLG width to be present, they may have observed that the variation they avoided in their sampling corresponded with cub production.

3.5 CONCLUSION

The production and rearing of cubs by female polar bears is associated with reduced cementum deposition. Similar to black bears, and dolphins (Genus *Stenella*), prolonged lactation is likely necessary to have cub rearing recorded in the dental tissues of polar bear teeth (Carrel 1994; Coy and Garshelis 1992; Klevezal and Myrick 1984). Predictions of litter production rates from cementum can provide an estimate of recruitment of minimum aged offspring (between 3-8 months old), producing litter production rates similar to those calculated from autumn field observations. Predicting the presence and absence of COY from changes in cementum width is possible, but not error free. Finer parameters, such as age of primiparity and whole litter loss, may not be adequately reflected in cementum patterns. For this reason, the use of cementum annuli in predicting reproductive parameters of the population should be used cautiously; while understanding the limitations of these methods, and the risks of false negative and false positive predictions of COY.

Teeth are collected from hundreds of polar bears harvested by native hunters to monitor harvest structure (Lee and Taylor 1994; Schliebe et al. 1999). Many specimens are from regions outside those regularly sampled by researchers. Improvements in technology and software, for both micro-imaging and measurement, means these techniques can be easily applied to teeth already prepared for age determination. Although polar bear harvests are male biased (Lee and Taylor 1994; Schliebe et al. 1999), the ability to use cementum to infer past reproduction would be a cost-effective means of obtaining additional information for the monitoring of population trends.

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Table 3.1: Year, age, and body condition of female polar bears when loss of litter was confirmed. Probability of COY (cub-of-the-year present) determined from logistic regression of known COY and NON-COY observations against ΔPWI .

Bear	Year	Age	Body Condition Index (spring)	Female Rebred	Probability of COY (from predictive model)
X05569	1991	10	7.72	?*	0.800
X09293	1988	6	-44.28	Y	0.591
X10688	1988	6	-58.97	Y	0.490
X10750	1988	7	-72.16	?*	0.889
X09034	1989	8	-77.03	Y	0.478

* no cub present at autumn capture, not known if female rebred

Table 3.2: Correct prediction of COY (cubs-of-the-year) or Non-COY of cementum GLGs of female polar bears. Classification based on probability threshold 0.50 for classifying reproductive condition from ΔPWI.

Reproductive Condition	n	Proportion Correctly Classified	False positive or False negative Error Rate
COY (cubs present)	117	0.72	0.28
Non-COY (pregnant & yearling)	264	0.72	0.28
COY lost	5	0.60	0.40
COY (survival not confirmed)	17	0.65	0.35
Yearling (survival not confirmed)	24	0.83	0.17

Table 3.3: Proportion (by age) of first parturition events predicted using cementum annuli width (ΔPWI) from female polar bears of western Hudson Bay (1965-2006).

Age (year)	1965-1979	1980-1984	1985-1992	1993-2006	χ^2 df = 3	
	n = 58	n = 35	n = 70	n = 32	χ^2	*P*
4	0.62	0.54	0.43	0.41	6.19	0.10
5	0.21	0.14	0.27	0.16	3.10	0.38
6	0.10	0.11	0.16	0.38*	12.20	0.01
7	0.05	0.17	0.09	0.06	4.29	0.23
8+	0.02	0.03	0.06	0.00	-	-

* 1985-1992 ≠ 1993-2006 (Tukey; $q = 3.373$, $P < 0.05$)

Table 3.4: Predictions, using cementum annuli width, of consecutive years of parturition by female polar bears in western Hudson Bay. Production of litters in consecutive years could only occur if there was early litter mortality in the first of two consecutive years.

Age	# of GLGs	Proportion of GLGs with consecutive predictions of COY
4	220	0.16
5	214	0.10
6	188	0.10
7	171	0.09
8	138	0.09
9	113	0.05
10	89	0.04
11	69	0.06
12	55	0.07
13	42	0.05
14	26	0.08
15	19	-
16	13	0.08
17	10	-
18	10	0.10
19	6	-
20	3	-
21	1	-

Table 3.1: Litter production rate (lp_x; litters per female each year) of female polar bears by age-class and sampling period for western Hudson Bay. Source: GLG = predictions based on cementum growth layer group widths (this study). Capture = field observations (reported in Derocher and Stirling 1995).

Age (years)	Source	1966-1979 lp_x	1966-1979 *n*	1980 – 1984 lp_x	1980 – 1984 *n*	1985 – 1992 lp_x	1985 – 1992 *n*	1993-2006 lp_x	1993-2006 *n*	G test *G*	G test *P*
4-9	GLG	0.48	65	0.41	45	0.42	75	0.44	35	3.16	0.37
	capture	0.43	56	0.34	79	0.26	264			7.10	0.03
10-15	GLG	0.47	16	0.47	24	0.35	34	0.38	15	3.70	0.30
	capture	0.54	26	0.54	56	0.46	164			1.38	0.50
16-21	GLG	1	1	0.50	2	0.47	7	0.35	4	-	-
	capture	0.71	7	0.51	37	0.40	139			3.74	0.16
Overall	GLG	0.48	82	0.43	71	0.40	116	0.42	54	3.88	0.27
	capture	0.48	90	0.43	174	0.34	622			9.29	0.01

Table 3.5: Correlation matrix between variables Age, Year, Body Condition, and duration of Ice-Free period for females accompanied with cubs and while pregnant western Hudson Bay (all Pearson correlations).

	Age	Year	Body Condition	Ice-Free Days (Cub)	Ice-Free Days (Pregnant)
Age	1.00				
Year	0.221	1.00			
Body Condition	0.194	-0.158	1.00		
Ice-Free Days (Cub)	-0.002	0.231	-0.038	1.00	
Ice-Free Days (Pregnant)	-0.067	-0.135	0.020	0.305	1.00

Table 3.6: Results for additive linear regression model selection for explaining variance in GLG width using Proportional Width Index values for female polar bear accompanied by cubs (n=55). Bold indicates best model fit for statistic (BCI — body condition index; Year — calendar year; Age — age of female when accompanied with cubs; Ice-free C — Duration of ice-free period when female was accompanied with cubs; Ice-free P — Duration of ice-free period when female was pregnant).

Model	# of var.	r^2	*P*	AIC Corrected	BIC
BCI	1	0.125	**0.008**	**-136.27**	**-130.72**
Year	1	0.005	0.595	-129.25	-123.70
Age	1	0.007	0.532	-129.37	-123.82
Ice-free C	1	0.022	0.279	-130.19	-124.63
Ice-free P	1	0.000	0.879	-128.98	-123.43
BCI + Year	2	0.142	0.019	-135.03	-127.80
BCI + Ice-free C	2	0.151	0.014	-135.62	-128.39
BCI + Age	2	0.125	0.031	-133.97	-126.74
BCI + Ice-free P	2	0.125	0.031	-133.96	-126.73
Year + Ice-Free C	2	0.023	0.536	-127.95	-120.72
Year + Age	2	0.011	0.760	-127.21	-119.98
Year + Ice-free P	2	0.006	0.848	-126.98	-119.75
Ice-free + Age	2	0.030	0.459	-128.28	-121.05
Ice-free C + Ice-free P	2	0.023	0.550	-127.89	-120.66
Age + Ice-free P	2	0.008	0.809	-127.08	-119.85
BCI + Year + Ice-free C	3	**0.160**	0.030	-133.80	-124.98
BCI + Year + Age	3	0.142	0.049	-132.62	-123.81
BCI + Ice-free C + Age	3	0.151	0.038	-133.21	-124.40
BCI + Ice-free P + Age	3	0.125	0.076	-131.56	-122.74
BCI + Ice-free P + Year	3	0.143	0.048	-132.66	-123.85
BCI + Ice-free C + Ice-free P	3	0.152	0.037	-133.28	-124.47
Year + Ice-free C + Age	3	0.030	0.667	-125.88	-117.07
Year + Age + Ice-free P	3	0.012	0.896	-124.85	-116.04
Year + Ice-free C + Ice-free P	3	0.024	0.741	-125.54	-116.73
Age + Ice-free C + Ice-free P	3	0.030	0.668	-125.87	-117.06
BCI + Year + Ice-free C + Age	4	0.160	0.064	-131.27	-120.98
BCI + Age + Ice-free C + Ice-free P	4	0.152	0.077	-130.77	-120.48
BCI + Year + Ice-free C + Ice-free P	4	0.160	0.063	-131.29	-120.99
BCI + Year + Age + Ice-free P	4	0.143	0.097	-130.15	-119.86
Year + Age + Ice-free C + Ice-free P	4	0.030	0.815	-123.37	-113.07
BCI + Age + Year + Ice-free C + Ice-free P	5	0.160	0.116	-128.66	-116.99

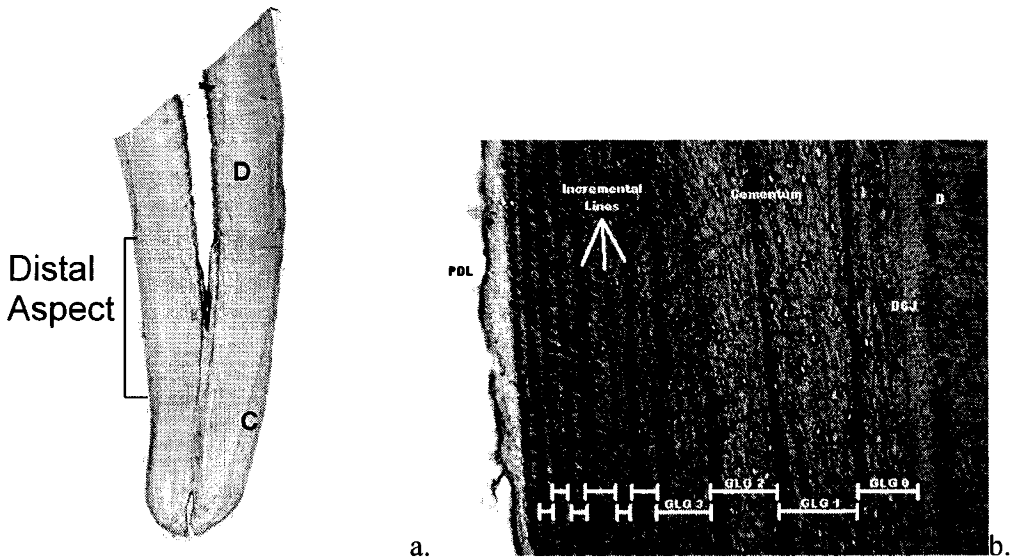

FIG 3.1: Longitudinal section of polar bear (*Ursus maritimus*) lower first premolar, decalcified, and stained (Toluidine Blue), and viewed using transmitted light (a).Cementum growth layer groups (GLG), representing annual deposition (b). C – cementum, D – dentine. PDL – periodontal ligament, DCJ – dentinocemental junction

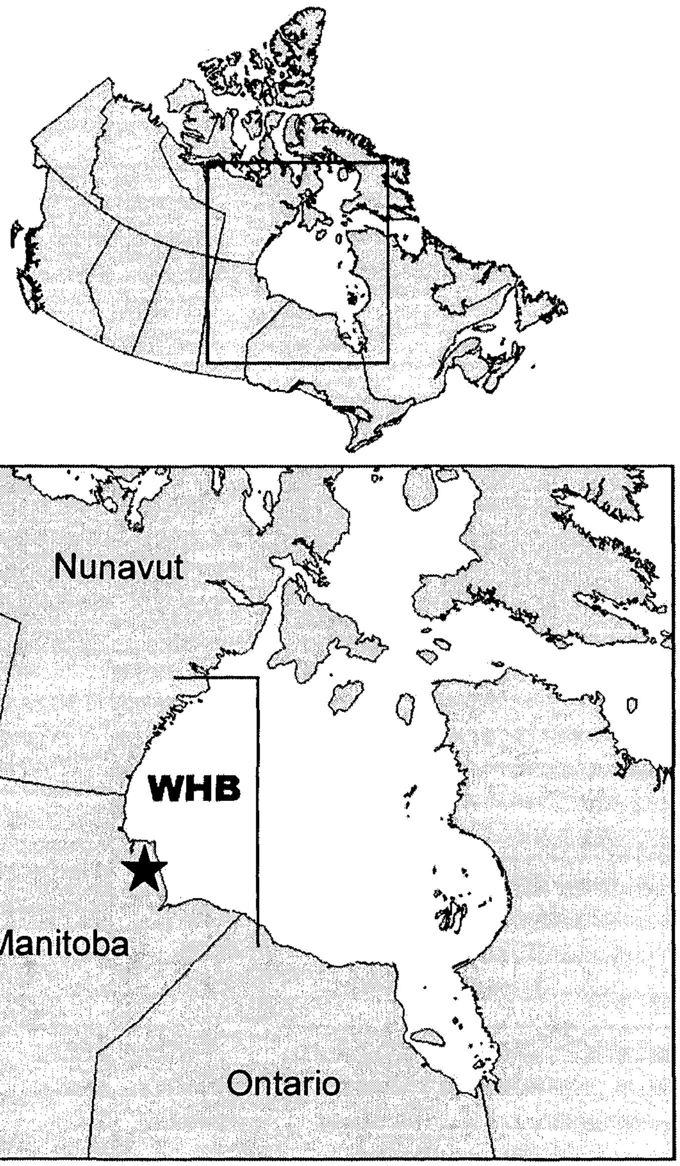

FIG 3.2: Western Hudson Bay (WHB) population of polar bears (*Ursus maritimus*), Hudson Bay, Canada. Most samples were collected from the maternity denning area between the Churchill and Nelson rivers (star).

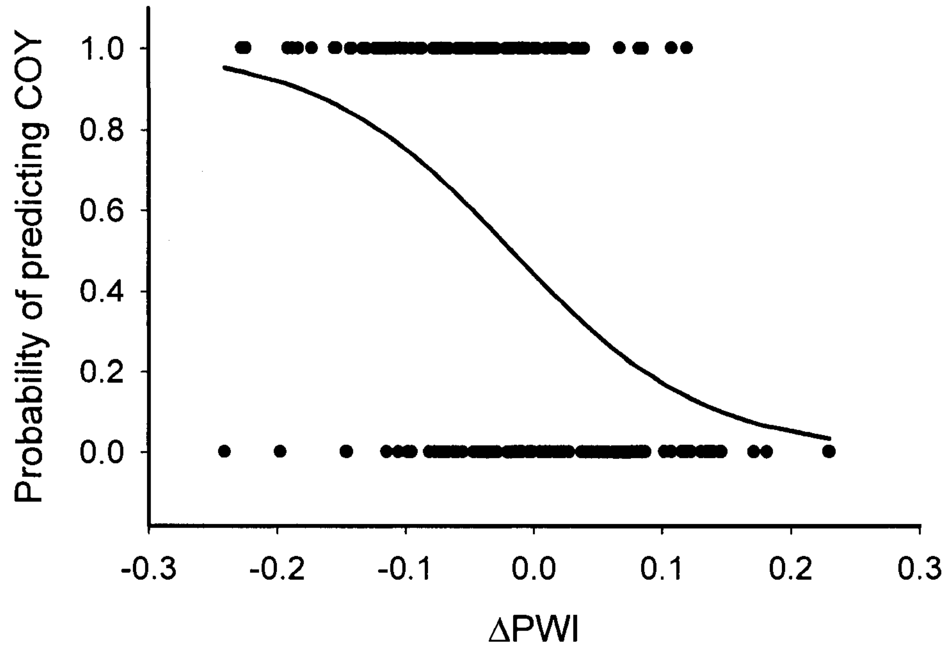

FIG 3.3: Distribution of ΔPWI for cementum growth layer groups classified as COY (1) and NON-COY (0) and the probability of predicting COY based on logistic regression on 104 female polar bears from western Hudson Bay.

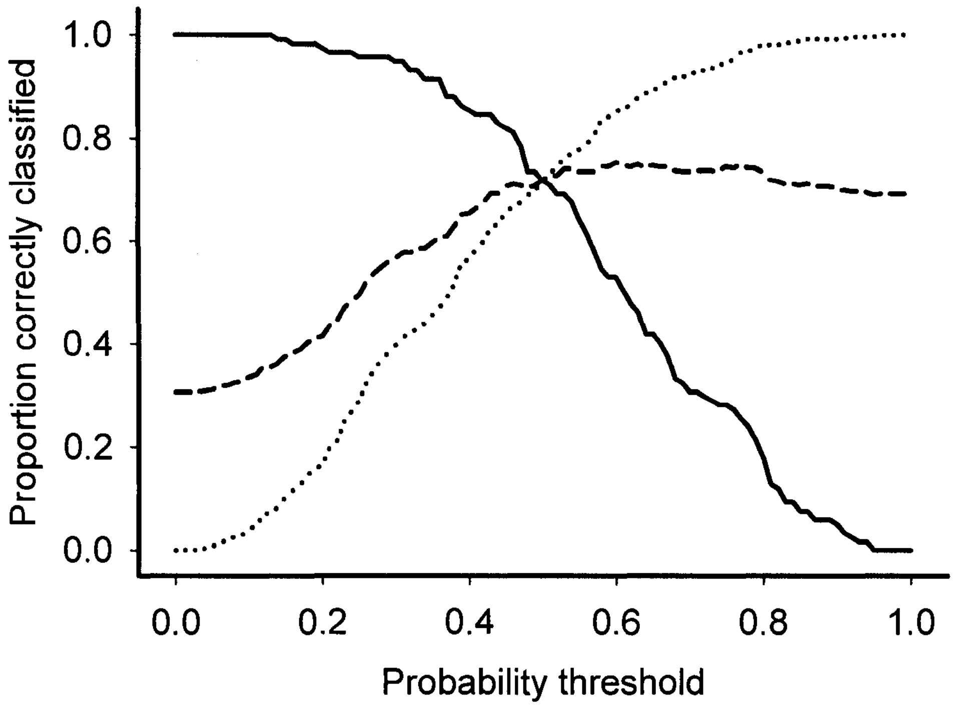

FIG 3.4: Sensitivity (solid line), specificity (dotted line), and total proportion of correctly classified growth layer groups (dashed line) for probability threshold, increasing at 0.01 intervals, to classify presence or absence of cubs based on the difference in cementum width (ΔPWI) of female polar bears from western Hudson Bay, Canada.

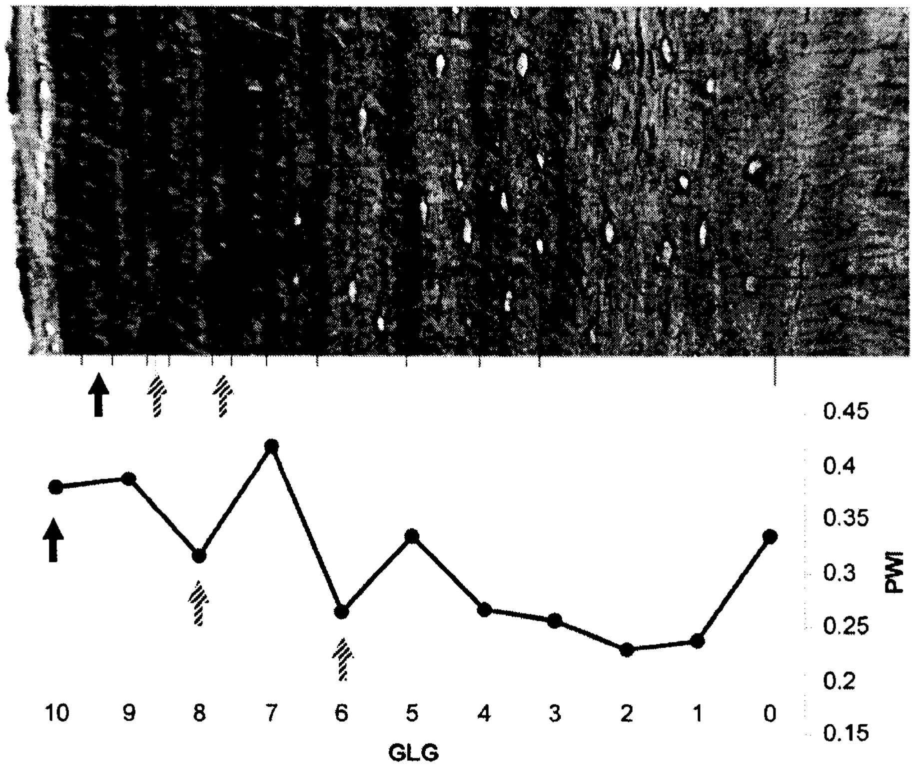

FIG 3.5: Distal cementum of known-aged female polar bear X12393 (11 years old). The female was observed with cubs-of-the-year when growth layer group (GLG) 6th, 8th, and 10th was formed, cub presence predicted when GLG 6th and 10th were formed. PWI – proportional width index, Observed cubs – black arrow, Predicted and Observed cubs – hatched arrow.

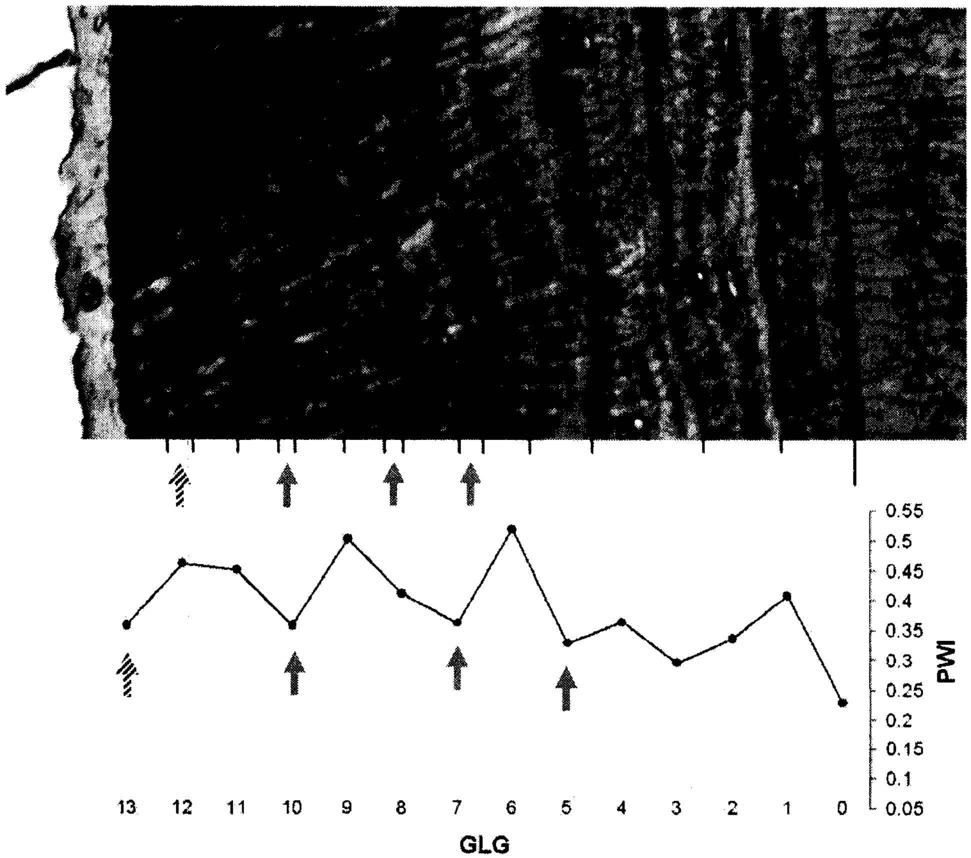

FIG 3.6: Distal cementum of female polar bear X32424 (14 years old). This female was observed with cubs-of-the-year when growth layer group (GLG) 13 was formed. Cub presence predicted when GLG 5th, 7th, 10th, and 13th were formed. PWI – proportional width index, Predicted cubs– grey arrow, Predicted and Observed cubs – hatched arrow.

4.0 GENERAL DISCUSSION AND CONCLUSIONS

4.1 SUMMARY

Polar bears (*Ursus maritimus*) are listed as vulnerable on IUCN red list, species of special concern by COSEWIC, and as threatened under the United States Endangered Species Program (IUCN 2008, COSEWIC 2008, United States Fish & Wildlife Service 2008). Current research is intense as we try to understand how polar bear populations will respond to predicted changes in climate (Derocher et al. 2004; Ferguson et al. 2005; Gagnon and Gough 2005; Regehr et al. 2007; Stirling and Derocher 1993). Teeth collected from captured and harvested bears for the purpose of aging individuals and estimating population demographics may also be used to observe responses to changing environmental trends.

Quantitative analysis of cementum is rare in the literature and evaluation of sources of error when attempting to measure cementum width have not been adequately addressed (Burke and Castanet 1995; Carrel 1994; Laws et al. 2002; von Biela et al. 2008; Wedel 2007). Cementum can be difficult to interpret quantitatively because its clarity and width varies around the surface of the root, and distinct patterns are sometimes indiscernible at the same root surface location between teeth of different individuals (Childerhouse et al. 2004; Hensel and Sorensen 1980; Hewer 1964; Solheim 1990; von Biela et al. 2008). For these reasons, it was necessary to examine the potential to introduce error from pooling samples from multiple locations on the surface of the root, and sampling different premolars (upper, lower, left, or right). In Chapter 2, variance component analyses indicated that limiting sampling to one location on the root surface would reduce error. The distal region was selected as the most accessible amongst polar bears, having wide cementum deposits and distinct incremental lines. Additionally, comparing sets of multiple teeth from the same individual revealed that upper and lower premolars were not readily comparable. The collection of archived polar bear premolars contain almost twice as many lower premolars than upper so further sampling was restricted to left and right lower premolars. The level of sectioning did not contribute significantly to the error estimate. I feel confident that as long as the most central section possible is selected for analysis, slight deviations from this will not significantly influence statistical testing of hypotheses.

The width of cementum deposited annually decreases with age, requiring standardization to remove this age effect to allow comparisons between different ages within an individual. Individuals will also vary in the size of tooth and amount of cementum deposited meaning that the use of direct measurements could be biased. Chapter 2 describes the conversion of actual cementum width measurements into Proportional Width Indices (PWIs) that account for allometric variation between individuals and allows for comparison of multiple individuals at different ages. PWIs are used in Chapter 3 to evaluate whether cementum patterns are influenced by reproductive condition along with physiological and environmental factors.

In female polar bears, reduced cementum widths (i.e., reduced PWI) indicated females were accompanied by cubs during that year (Chapter 3). Ecological and physiological conditions experienced by female polar bears, particularly those forced onto land during ice-free periods, may aid the formation of reproductive patterns in cementum. Females in western Hudson Bay experience large increases in body mass and bone density during pregnancy, and the extended period of fasting and inactivity while in maternal dens (Arnould and Ramsay 1994; Craighead et al. 1970; Lennox and Goodship 2008; Ramsay and Stirling 1982; Rausch 1969; Sauer et al. 1966). Prolonged lactation by females with cubs appears to be the strongest factor influencing cementum width, as females divert large amounts of energy and nutrients into the rearing of offspring (Arnould and Ramsay 1994; Oftedal 1993). These findings were similar to what was observed in black bears (*U. americanus*); where cub survival $\geq$ 4 months is required in order for cub production to be recorded in cementum (Carrel 1994; Coy and Garshelis 1992; Hristienko et al. 2004). The period of lactation required to reduce the cementum width in female polar bears is likely between three to eight months, and is probably dependent on the maternal body condition. A female in good condition may have to have her litter present longer for cementum to record the reproductive event, whereas, a female in poor condition may record early litter loss as cubs present.

4.2 FUTURE RESEARCH & APPLICATIONS

The use of cementum to infer life history may require the standardization of field protocols for the collection of teeth. This includes careful recording of which tooth is pulled and from what location, and the gender of the individual. An accurate age is necessary to correctly assign both age and years to annuli. Teeth from younger animals are generally more accurately for age determination than teeth from older individuals (Calvert and Ramsay 1998; Christensen-Dalsgaard 2006; Hensel and Sorensen 1980; McLaughlin et al. 1990). However, the younger the individual is when teeth are collected the less information recorded within the cementum. If multiple captures are anticipated, protocols should state which tooth/teeth will used for quantitative cementum analysis and these should not be pulled from younger, sexually immature individuals.

The importance of known-aged individuals, for both evaluating the success of age determination and for the comparison of cementum annuli width against life history information, needs to be stressed. Population demographics for long-lived species are generally robust against errors of ± 1 or 2 years, particularly in older individuals. However, when measuring cementum widths it is critical to know exact ages. If the age of the bear is overestimated the measurements of two or more growth layer groups (GLGs) will be reduced, and year and age assignments will be incorrect. If the age of the bear is underestimated then the width of GLGs will be inflated, and again, birth year and age will be assigned incorrectly. In this study, many of the known-aged females did not have a tooth removed after sexual maturity. This is partly because the age of the bear was known and young teeth were only collected to be used to verify reader accuracy, or from a policy in which once an agreement in age estimate between two teeth was obtained from an individual, no more teeth were collected.

Future investigations comparing PWIs to physiological and environmental variables can include GLGs from sexually immature bears and the teeth collected from younger known-aged bears will be invaluable in addressing bias introduced by estimating ages. Additional investigations using teeth collected from male bears will hopefully reduce the variation introduced by reproductive activities; however, males may also be prone to changes in cementum width depending on the mating effort and duration of the breeding season. Additionally, certain events in an individual's life (e.g., weaning, sexual maturity, serious injury) may influence cementum deposition or even contribute to the resorption of this tissue. Resorption patterns in wildlife have received little attention, though one study linked increased root resorption to radioactive contamination (Klevezal et al. 2001).

Differences in tooth ultrastructure, GLG patterns, and dental morphology have been proposed as a method for identifying sub-populations of harbour propoise (*Phocoena phocoena*; Lockyer 1993). Regional differences in the clarity and time of formation of incremental lines suggest that ecological differences are reflected in polar bear cementum (Grue and Jensen 1979; Hensel and Sorensen 1980). Currently, the Southern Beaufort Sea population of polar bears are experiencing changing sea-ice dynamics, with more bears appearing on land during the open-water period (Fischbach et al. 2007; Schliebe et al. 2008). Comparing archived teeth with recently collected samples from this region may reveal a transition towards the more distinct GLGs observed in western Hudson Bay.

No information has been published on the effect of premolar removal on polar bears; the tooth is vestigial and apparently non-functional. Bears with premolars removed have often been recaptured with no apparent long term effects. Grizzly bears have shown a 2-week recovery period after the removal of the larger fourth premolar (Craighead et al. 1970). However, short-term effects of tooth removal on feeding or other behaviours are not known. In a culture of increasing concerns on the ethics of wildlife research, knowledge of the duration of healing or apparent discomfort would be valuable in deciding whether to incorporate cementum analysis into a research program.

Improvements in software technology have simplified the acquisition and measurement of digital images of cementum from teeth that already collected and prepared for age determination. Automation of cementum annuli detection has been attempted but results were inconsistent with other age determination methods and could not be verified with known-aged specimen (Czermak et al. 2006). Attempts made during this research to use software designed to identify and measure tree rings (Windendro, CDendro 5.3.46, Lignovision 1.36). Dark staining cementocyte lacunae were regularly misidentified as incremental lines and more time was required to correct automated measurements than to manually delineate annuli. However, I feel that automation has the potential to be reliable and facilitative, but may require different imaging techniques and therefore different preparation of teeth.

Frequent observations of marked individuals have made the comparison of physiological and environmental conditions against cementum patterns possible.

Without this information, teeth would remain exclusively indicators of age. The results presented in this thesis are specific to the polar bears of western Hudson Bay, however, these methods can be applied to other populations of bears, or other taxa. Dental tissues are particularly desirable for life history analysis because of their persistence after death. Once it is established how cementum functions as a recording structure in a species, it would become possible to evaluate teeth from live or harvested individuals, found remains, and/or museum or research archives. The previous two chapters describe important steps towards a better understanding of how cementum functions as a recording structure and how information, such as reproductive histories, can be obtained from collections of teeth prepared for age determination.

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