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RESEARCH ARTICLE

Comparative skeletochronological analysis of Kemp's ridley (*Lepidochelys kempii*) and loggerhead (*Caretta caretta*) humeri and scleral ossicles

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Abstract Skeletochronological analysis of Kemp's ridley (*Lepidochelys kempii*) and loggerhead (*Caretta caretta*) sea turtle humeri and scleral ossicles was conducted to (1) describe the characteristics of scleral ossicles in these species, (2) determine whether the scleral ossicles contain annually deposited skeletal growth marks and (3) evaluate the potential for skeletochronological analysis of ossicles to obtain age data for size classes and species of sea turtles whose humeri exhibit prohibitive amounts of growth mark resorption. Humeri, entire eyes, and/or individual scleral ossicles were collected from stranded, dead sea turtles that were found along the coasts of Florida, North Carolina, Virginia, and Texas, USA. Samples were taken from a total of 77 neritic, juvenile Kemp's ridleys ranging from 21.1 to 56.8 cm straightline carapace length (SCL), as well as two Kemp's ridley hatchlings. For loggerheads, samples were obtained from 65 neritic juvenile and adult turtles ranging from 44.7 to 103.6 cm SCL and ten hatchlings. Examination of the ossicles revealed the presence of marks similar in appearance to those found in humeri. The number of marks in the ossicles and humeri of individual juvenile Kemp's ridleys for which both structures were collected (n = 55) was equivalent, strongly indicating that the marks are annual. However, in large juvenile and adult loggerhead turtles (n = 65), some significant resorption of early growth marks was observed, suggesting that although ossicles might be useful for skeletochronological analysis of small juveniles, they may not provide a reasonable alternative to humeri for obtaining age estimates for older loggerhead sea turtles.

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Introduction

Many sea turtle populations around the world are declining or are negatively impacted by a variety of different threats, such as habitat destruction and fisheries interactions (Lutcavage et al. 1997). Advances in population modeling techniques have provided a means of predicting the long-term impacts of management actions intended to bring about the recovery of sea turtle populations (Heppell and Crowder 1998; Heppell et al. 2003a). However, accurate parameterization of these models requires adequate knowledge of demographic information such as age-specific growth and survival rates and age to maturity, which are generally lacking for many sea turtle species (Chaloupka and Musick 1997; Heppell et al. 2003a, b).

Despite the need for age-related data for sea turtles, various factors make it difficult to obtain this information. Sea turtle growth rates appear to be highly variable, both among individuals, as well as within individuals during different years (Klinger and Musick 1992; Parham and Zug 1997), so that carapace length and other somatic measures often do not strongly correlate with age estimates (Castanet 1994; Zug and Parham 1996). Furthermore, unlike other turtle species that deposit growth marks on the scutes of the plastron or carapace that can sometimes be counted to estimate age (Germano and Bury 1998; Wilson et al. 2003), sea turtle scutes are generally smooth and exhibit no external age-related characteristics. Finally, sea turtles are highly migratory throughout their lives (reviewed by

Musick and Limpus 1997; Plotkin 2003), making it extremely difficult to follow individuals not only within a given life stage, but also from one ontogenetic stage to the next, to monitor growth and to determine age. This last characteristic is particularly pronounced in the leatherback sea turtle (*Dermochelys coriacea*), which spends the majority of its life in the open ocean (Plotkin 2003). As a result, current estimates of growth rates and age at maturity for this species are tentative (Zug and Parham 1996).

Skeletochronology, the technique of analyzing skeletal growth marks to obtain life history information, is one potential means of investigating sea turtle age and growth (Zug 1990). This method is based on the premise that skeletal growth in many vertebrates is cyclic, with periods of faster bone growth alternating with periods of slower bone growth, and frequently exhibits annual periodicity (Castanet et al. 1993; Castanet 1994). These growth cycles are often visible as growth marks in sections taken of skeletal elements (Castanet 1994; Klevezal 1996); an individual skeletal growth mark consists of a wide zone of active bone deposition followed by a narrower zone representing little to no bone growth, often termed a line of arrested growth or LAG (Castanet et al. 1993; Snover and Hohn 2004).

Recent skeletochronological analyses have indicated that growth mark deposition is annual in the humeri of Kemp's ridley (*Lepidochelys kempii*) (Snover and Hohn 2004) and loggerhead (*Caretta caretta*) (Klinger and Musick 1995; Coles et al. 2001; Snover 2002; Snover and Hohn 2004) sea turtles. Analyses of skeletal growth marks in humeri, typically without validation of deposition frequency, have also been conducted for a number of hard-shelled sea turtle species to estimate ages, stage durations, and growth rates (Zug et al. 1986, 1995, 1997, 2001, 2006; Chaloupka and Zug 1997; Parham and Zug 1997; Zug and Glor 1998; Snover 2002; Bjorndal et al. 2003; Snover et al. 2007). Age determination in all of these studies has been hampered by the fact that early growth marks in the humerus are generally destroyed as the interior of the bone is reconstructed during growth, making it impossible in most cases to obtain direct age estimates (Zug et al. 1986). Also, in contrast to the bones of hard-shelled turtles, extreme levels of growth mark resorption occur in all of the major skeletal elements of the leatherback turtle, including the humerus, making skeletochronological analysis for this species problematic (Zug 1990; Castanet 1994).

Zug and Parham (1996) reported the presence of marks in leatherback scleral ossicles (small bones surrounding the pupil of the eye) whose appearance was consistent with skeletal growth marks and used these marks to obtain age estimates. However, the authors were unable to validate the nature of those marks and it is possible that they represented dietary or other environmental influences as opposed to annual cycles (Zug and Parham 1996). In this study, we conducted a skeletochronological analysis of scleral ossicles obtained from hatchling, neritic juvenile, and adult Kemp's ridley and loggerhead sea turtles to make further observations on ossicle structure, as well as the marks within the ossicles. In addition, as a first step toward determining the frequency of ossicle mark deposition, we compared the number of LAGs present in the ossicles and humeri of individual turtles. Finally, we evaluated skeletochronological analysis of ossicles as an option for obtaining age estimates for older, hard-shelled turtles whose humeri exhibit a great deal of resorption.

Materials and methods

Sample collection

Scleral ossicles and humeri of Kemp's ridley (*L. kempii*) and loggerhead (*C. caretta*) sea turtles to be used for skeletochronological analysis were obtained through cooperation with the National Sea Turtle Stranding and Salvage Network (STSSN), as well as through opportunistic collection of samples by researchers at the NOAA Fisheries Laboratory in Beaufort, North Carolina. All samples were taken from turtles that were either found dead or were debilitated at stranding and were later euthanized; no turtles were sacrificed to obtain samples for this study. The left front flipper and left eye were generally collected from each turtle, although in some instances the right flipper and eye or both front flippers and eyes were taken. In addition, entire heads were collected from 17 Kemp's ridleys and 11 loggerheads so that dissection of eyes to determine ossicle orientation and position for each species could be conducted using features of the head as a reference. Although typically standard straightline carapace length from the nuchal notch to the tip of the longest posterior marginal (SCL) to the nearest 0.1 cm was measured for each stranded turtle, in some cases only curved carapace length (CCL) was obtained. When this occurred, CCL was converted to SCL using the following equations, which were previously established for Kemp's ridleys and loggerheads that stranded along the east coast of the USA (Snover 2002):

Kemp's ridleys: $\mathrm{SCL} = 0.957 \times \mathrm{CCL} - 0.696$

Loggerheads: $\mathrm{SCL} = 0.923 \times \mathrm{CCL} + 0.189$

Samples were acquired from 77 neritic, juvenile Kemp's ridleys ranging from 21.1 to 56.8 cm SCL ($\overline{X}$= 32.2 ± 8.6 SD), as well as two Kemp's ridley hatchlings. For loggerheads, samples were obtained from 65 neritic juveniles and adults ranging from 44.7 to 103.6 cm SCL

($\overline{X}$= 68.8 ± 14.2 SD), and ten hatchlings. The majority of humeri and ossicles used in this study were collected in North Carolina; however, samples were also obtained from two loggerheads and three Kemp's ridleys that stranded in Florida and the two Kemp's ridley hatchlings were collected in Texas.

Sample processing

After humeri were extracted from flippers, they were flensed and boiled to remove any remaining tissue, passed through a 1:1 bleach:water solution, and allowed to dry outdoors for a minimum of 2 weeks. A low-speed isomet saw (Buehler, Lake Bluff, Illinois, USA) was then used to cut a 2–3 mm thick cross-section of each humerus just distal to the delto-pectoral crest, perpendicular to the long axis of the bone, using the distal end of the insertion scar for the deltopectoral muscle as a reference point (Fig. 1a).

In cases where ossicles were dissected from intact heads, the head was stabilized while the outer half of the eyeball was cut away and then pinned down in a dissection tray, taking care to retain the original dorsoventral and antero-posterior orientation. Using a scalpel and forceps, ossicles were then removed from the outer half of the eyeball one at a time, keeping each ossicle in its original orientation and position within the ossicle ring. To document ossicle ring characteristics, photographs were taken using a digital camera with a macro lens (Canon EOS 10D digital camera with Canon Telephoto EF 100 mm f/2.8 USM Macro Autofocus lens) before the ossicles were processed further (e.g. Fig. 1b) and then again after subsequent cleaning. When only eyeballs or parts of eyeballs/ossicle rings had been collected, ossicles were dissected out of the tissue in no particular order. Excess tissue was removed from all ossicles by alternating immersion in a 1:1 bleach:water solution and tap water, while using metal probes to scrape tissue away from the bone. Ossicles were briefly immersed in the bleach:water solution, but were transferred to water for the majority of the cleaning process, as it was found that although the bleach was very effective in removing excess tissue, ossicles (especially those obtained from younger turtles) tended to disintegrate after prolonged soaking.

Both the 2–3 mm thick humerus cross-sections and entire ossicles were fixed in 10% neutral, buffered formalin (Fisher Scientific, Fairlawn, New Jersey, USA), rinsed thoroughly in water, and decalcified in RDO, a commercially prepared decalcifying solution (Apex Engineering Corporation, Plainfield, Illinois, USA). Decalcified bones were soaked overnight in water to remove any residual RDO, after which thin sections (25 μm thick) were obtained using a freezing-stage microtome (Leica Microsystems, Inc., Bannockburn, Illinois, USA) (see Fig. 1c for plane of ossicle sectioning). Thin sections were stained using a 1:1 solution of either Ehrlich's (humeri) or modified Mayer's (ossicles) (Myrick et al. 1983) hematoxylin stain and distilled water. Stained sections were mounted on microscope slides in 100% glycerin for further examination.

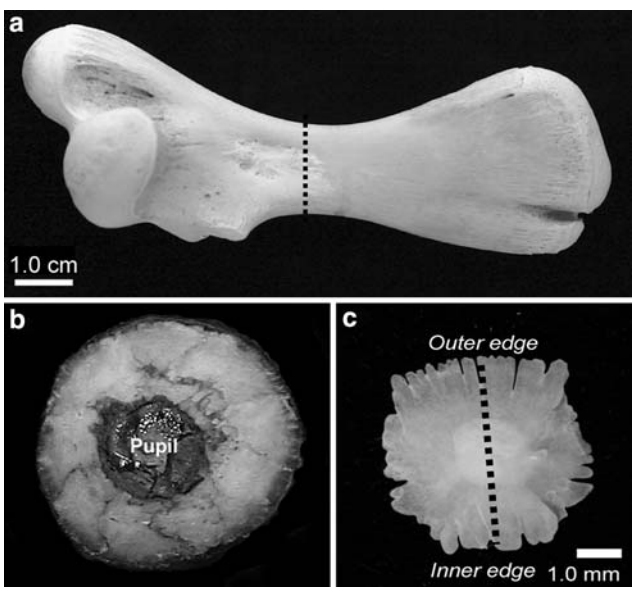

Fig. 1 **a** Ventral view of juvenile Kemp's ridley (*Lepidochelys kempii*) humerus. *Dotted line* denotes site of sectioning, perpendicular to long axis of humerus and just distal to insertion scar of the delto-pectoral muscle; **b** Intact loggerhead (*Caretta caretta*) scleral ossicle ring showing relative position and overlap of ossicles; **c** individual juvenile Kemp's ridley ossicle. *Dotted line* denotes plane of sectioning

Analysis

Mounted humerus and ossicle sections were viewed and measured using an Olympus BX41 trinocular compound microscope in conjunction with a Colorcube-12 Color CCD, 1.4 megapixel digital camera and Microsuite Basic image analysis software (OPELCO, Dulles, Virginia, USA). An individual observer (LA) conducted independent LAG counts for both humeri and ossicles at a minimum of 5 day intervals, until a consensus count was reached for each specimen. Although consensus was generally reached in 2–3 counts, in a small number of cases where LAGs were compressed or unclear, 4–5 counts were necessary. After digital ossicle images were acquired, measurements of ossicle section features were obtained using the polygon measurement tool in the image analysis software package. For those Kemp's ridleys for which entire ossicle rings were obtained, lengths of cleaned, whole ossicles were measured using digital calipers (Fowler Co., Inc., Newton, Massachusetts, USA).

Because data were not normally distributed, non-parametric statistics were used for analysis (Zar 1996). Sets of ossicle measurements and LAG counts were compared using a Wilcoxon paired sample test when sample sizes in the groups being compared were equal and a Mann–Whitney test was used when sample sizes were not equal. A Kruskal–Wallis test was applied to investigate differences in the ranges of LAG counts and measurements relative to SCL for individual turtles. In addition, correlation analyses were performed to determine if any general relationships existed between ossicle and LAG characteristics and SCL, as well as between LAG counts obtained from ossicles and humeri. Where necessary, a Student's *T* test was applied to ascertain whether differences existed between correlation coefficients.

Results

Within the entire eyeballs that were dissected, we found between 9 and 16 ossicles in individual ossicle rings ($\overline{X}$ = 11.5 ± 0.71 SD, n = 33) for Kemp's ridleys measuring 22.9–54.0 cm SCL ($\overline{X}$ = 34.4 ± 9.3 SD, n = 19) and 11–13 ossicles ($\overline{X}$ = 12 ± 0.83 SD, n = 11) for loggerheads ranging from 53.7 to 98.0 cm SCL ($\overline{X}$ = 68.8 ± 12.5 SD, n = 11). There was no significant relationship between SCL and the number of ossicles per eye for either Kemp's ridleys ($P > 0.50$, $r^2 = 0.01$, n = 33 eyes) or loggerheads ($0.50 > P > 0.20$, $r^2 = 0.29$, n = 11 eyes). Examination of ossicle rings dissected from the intact heads of Kemp's ridleys revealed no consistent pattern in the size distribution of ossicles in rings obtained from different individuals (Fig. 2a, b). While in some cases it appeared that the smaller ossicles were positioned ventrally (Fig. 2a), quite often smaller ossicles were scattered throughout the ring (Fig. 2b). In contrast, loggerhead ossicle rings displayed a recurrent, marked asymmetry in ossicle size with the smallest ossicles positioned anteroventrally and the largest ossicles positioned dorsoposteriorly (Fig. 2c). In both Kemp's ridleys and loggerheads, ossicle lengths for turtles of different sizes were significantly different from one another ($P < 0.001$, Kruskal–Wallis test for both species; Kemp's ridleys n = 19; loggerheads n = 11). However, despite this overall difference, in each species ossicle lengths overlapped to a great extent, irrespective of turtle size (Fig. 3a, b). Overall, in both species, SCL and ossicle lengths (all ossicles from each turtle) were found to be positively correlated (Kemp's ridleys $P < 0.001$, $r^2 = 0.41$, n = 426; loggerheads $P < 0.01$, $r^2 = 0.23$, n = 132). However, for loggerheads, the relationship did not remain significant when data from the 98-cm SCL individual were removed from the analysis ($P > 0.25$, $r^2 = 0.06$, n = 121).

Examination of hematoxylin-stained ossicle thin sections demonstrated that marks consistent in appearance with the LAGs previously observed in humeri were apparent in ossicles processed using our histological techniques (Fig. 4a, b). In those ossicles that exhibited little to no resorption, a distinct mark at the center of the ossicle section was visible that was subsequently termed the "core mark" (Fig. 5a). Comparison of core mark and hatchling ossicle lengths for both Kemp's ridleys and loggerheads demonstrated that within each species the lengths were not significantly different (e.g. Fig. 5a, b; $P > 0.20$, Mann–Whitney U^2 Test for both species; Kemp's ridleys n = 18 core mark lengths, two hatchling ossicle lengths; loggerheads n = 3 core mark lengths, ten hatchling ossicle lengths).

In the larger Kemp's ridleys we examined, the number of LAGs visible at the tips of the ossicle sections was greater than the number visible at the lateral edges (Fig. 4b, c). However, the majority of Kemp's ridleys included in this study were relatively small individuals whose ossicles did not contain a large number of LAGs, and as a result we lacked the sample size to examine the disparity further in this species. Instead, we made the comparison using a subset of loggerhead ossicle sections obtained from turtles 44.7 to 103.6 cm SCL ($\overline{X}$ = 67.2 ± 16.0 SD, n = 33).

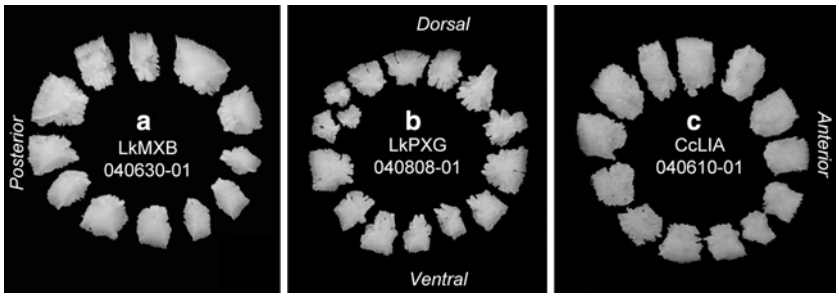

Fig. 2 Cleaned and separated ossicle rings obtained from: **a** juvenile Kemp's ridley (*Lepidochelys kempii*) 41.5 cm standard straightline carapace length (SCL) showing smaller ossicles positioned anteroventrally; **b** juvenile Kemp's ridley 30.8 cm SCL showing smaller ossicles scattered throughout; **c** juvenile loggerhead (*Caretta caretta*) 62.4 cm SCL demonstrating characteristic asymmetry of ossicle size with smallest ossicles positioned anteroventrally

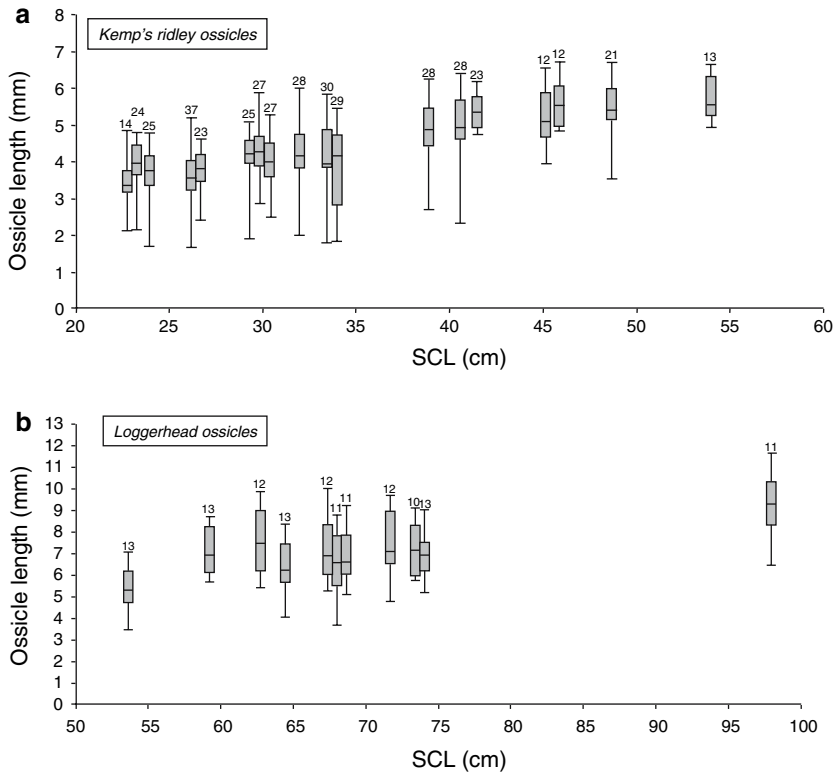

Fig. 3 Box and whisker plots of standard straightline carapace length (SCL) and ossicle lengths for **a** Kemp's ridleys (*Lepidochelys kempii*) (n = 32 individual ossicle rings) and **b** loggerheads (*Caretta caretta*) (n = 11 individual ossicle rings). *Shaded bars* represent first and third quartiles of ossicle lengths for different carapace lengths (Kemp's ridleys) or individual turtles (loggerheads). *Lines* within *shaded bars* represent median ossicle length and whiskers denote range of lengths. *Numbers* above each top whisker denote number of ossicles analyzed for that particular SCL

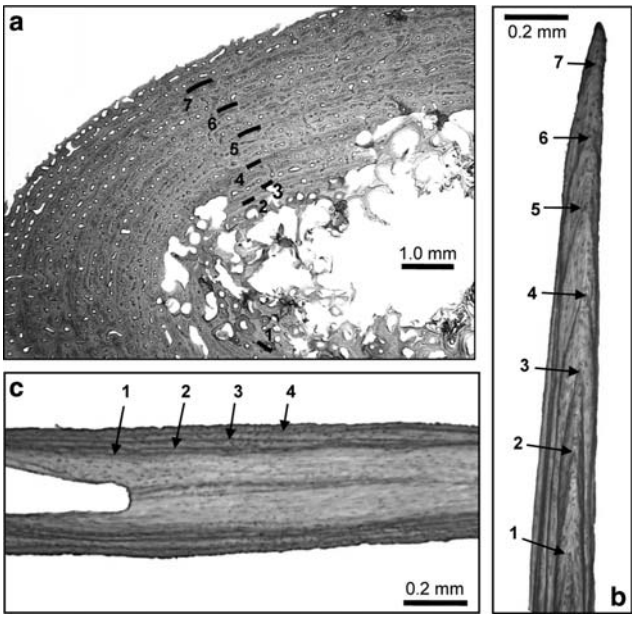

Fig. 4 Stained humerus and ossicle thin sections taken from juvenile Kemp's ridley (*Lepidochelys kempii*) 50.5 cm SCL. **a** Humerus section containing seven lines of arrested growth (LAGs); **b** ossicle section showing seven LAGs at tip; **c** center of same ossicle section depicted in **b**, showing lateral edges with four LAGs apparent at upper edge

Without exception, the number of growth marks visible at the section tips was equal to or greater than the number visible laterally ($P < 0.0005$, Wilcoxon paired sample test, n = 33) and this discrepancy increased with size ($P < 0.001$, $r^2 = 0.30$, n = 33). In addition, we found that the number of marks visible at the two lateral edges of a section was significantly different ($P < 0.001$, Wilcoxon paired-sample test, n = 33). As a result of these findings, all LAG counts to be used for further analyses were obtained from section tips and not from the lateral edges.

Many of the Kemp's ridley humeri examined in this study (n = 55) exhibited little to no resorption, with retention of either all or part of the annulus (diffuse LAG) marking the end of the first year (Snover 2002), allowing us to obtain direct age estimates based on humerus LAG counts. For turtles ranging from 21.1 to 50.6 cm SCL, age estimates ranged from 1 to 8 years. Ossicles obtained from these same turtles also exhibited little to no resorption and comparison of the LAG counts for ossicles and humeri of individual turtles revealed that the number of LAGs in the two structures was not significantly different ($P > 0.50$, Wilcoxon paired-sample test; n = 55). Correlation between LAG counts in the two structures was quite strong, deviating very little from a 1:1 relationship (Fig. 6; $P > 0.50$, Student's t test, n = 55).

In contrast, the loggerhead samples used for this study were obtained from neritic juveniles and adults 44.7–103.6 cm SCL that were likely a minimum of 7 years of age (Bjorndal et al. 2003) and therefore were at least as old, if not considerably older, than the Kemp's ridleys. While resorption levels in the loggerhead humeri were generally

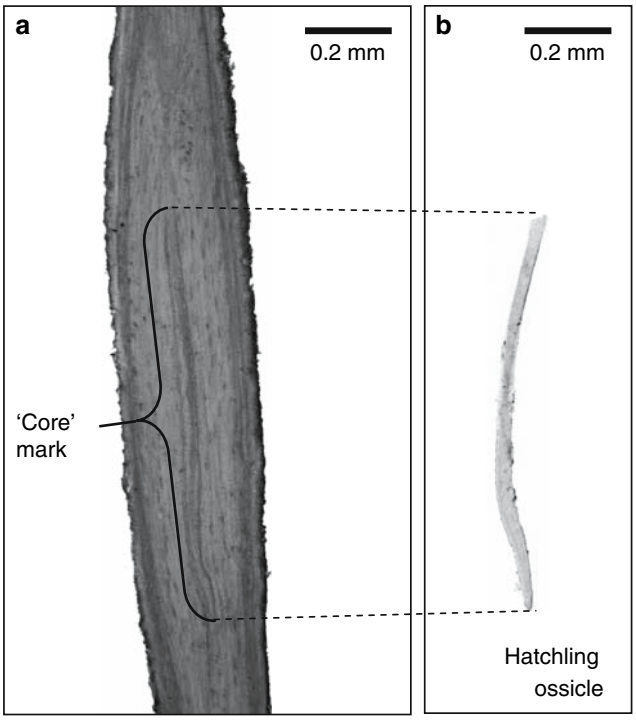

Fig. 5 Sample comparison of **a** core mark length in an ossicle from a large juvenile Kemp's ridley (*Lepidochelys kempii*) 50.5 cm SCL and **b** hatchling ossicle length

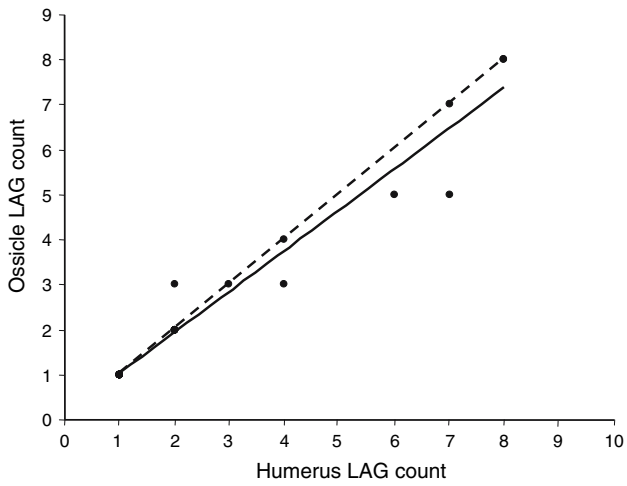

Fig. 6 Correlation between number of LAGs in humerus and ossicles of individual Kemp's ridleys (*Lepidochelys kempii*) (*solid line*: $P < 0.001$, $r^2 = 0.96$; $n = 55$). Correlation coefficient of *solid line* is not significantly different from 1, represented by *dashed line* ($P > 0.50$; Student's t test). *Solid dots* represent data points from pair-wise comparisons of humerus and ossicle LAG counts for 55 individual turtles; due to overlap of data points only ten dots are visible

high, as found in previous studies (Zug et al. 1986; Klinger and Musick 1992; Snover 2002), resorption levels in the ossicles (and therefore the number of visible LAGs remaining that could be counted) were highly variable and significantly different among individuals ($P < 0.001$, Kruskal–Wallis test, $n = 11$), even those of similar sizes ($P < 0.001$, Kruskal–Wallis test, $n = 10$ without 98-cm SCL individual) (Fig. 7). In addition, within individual turtles, the number of observed LAGs varied greatly, depending on which ossicle from a given ossicle ring was examined (Fig. 7). There was no significant correlation between ossicle length and the number of LAGs retained ($0.20 < P < 0.50$, $r^2 = 0.0088$, $n = 132$). Despite the extensive range of LAG retention in the ossicles, comparison of maximum LAG counts for those loggerheads for which both ossicles and humeri were available demonstrated that a greater number of marks generally remained visible in the humeri ($P < 0.005$, Wilcoxon paired-sample test; $n = 54$), particularly in larger, older turtles (Fig. 8). For example, whereas the maximum number of LAGs observed for all of the loggerhead ossicles examined was only 16, the maximum present in the humeri was 45.

Discussion

Ossicles or humeri?

Although numerous structures containing growth marks have been used to study age and growth in vertebrates, some retain a record of age more effectively than others. Fish otoliths are quite suitable for these types of analyses, as they continue to grow even after cessation of somatic growth in mature individuals (Beamish and McFarlane 1987) and are not subject to resorption (e.g. Pereira et al. 1995). Similarly, in the ever-growing teeth of some mammals, annually-deposited layers of dentin provide a continuous record of age (Klevezal 1996). In contrast, whereas periosteal bone of the type found in load-bearing skeletal elements such as limb bones often retains a number of skeletal growth marks, skeletochronological analysis of this type of bone is frequently complicated (Klevezal 1996). Because of the extensive, but varying, levels of bone remodeling typical of periosteal bone, to use limb bones such as sea turtle humeri to obtain age estimates, it is first necessary to establish a reliable method for estimating the number of resorbed growth marks, which can be quite challenging (Hutton 1986; Parham and Zug 1997).

Despite these difficulties, many skeletochronology studies have documented and attempted to compensate for the high levels of resorption observed in sea turtle humeri using a variety of different analytical techniques (Zug et al. 1986, 1995, 2001, 2006; Klinger and Musick 1992, 1995; Parham and Zug 1997; Zug and Glor 1998). However, because resorption in humeri has been such a confounding factor, the possibility that scleral ossicles might exhibit lower levels of resorption was intriguing, as it suggested that they could

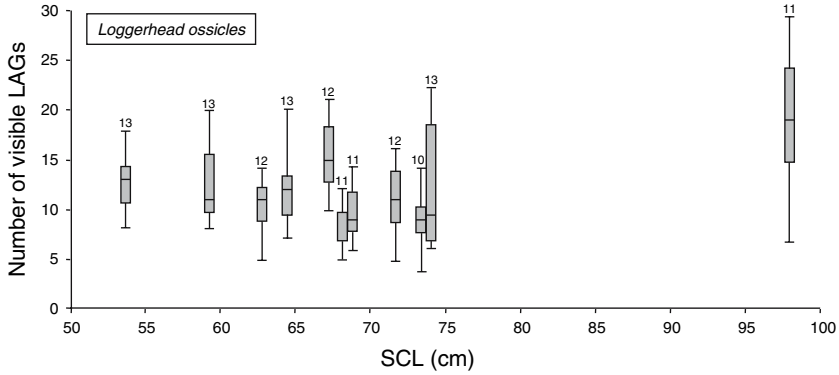

Fig. 7 Box and whisker plot of loggerhead (*Caretta caretta*) standard straightline carapace length (SCL) and number of visible LAGs within ossicles. Data are included only from those turtles for which entire eyes were available. *Shaded bars* represent first and third quartiles of number of visible LAGs for individual turtles of a range of carapace lengths, with each bar representing one turtle (n = 11 individual ossicle rings). *Lines* within *shaded bars* represent median number of visible LAGs and *whiskers* denote range. *Number* above each top whisker denotes number of ossicles analyzed for that particular SCL

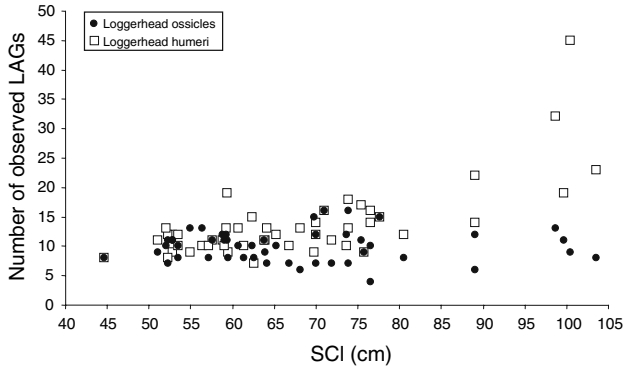

Fig. 8 Graph of number of LAGs observed in humeri and ossicles obtained from individual loggerheads (*Caretta caretta*), showing increasing disparity between counts in these two structures with increasing SCL (n = 48). Data are included only from individuals for which both eyes and humeri were available

potentially serve as alternative structures for sea turtle age and growth analyses (Zug 1990; Zug and Parham 1996). In this study, we verified that the humeri and ossicles of Kemp's ridleys 1–8 years of age can be equally useful for age estimation (Fig. 6).

In contrast to the Kemp's ridleys, however, the respective utility of ossicles and humeri was not nearly as straightforward for older, neritic juvenile and adult loggerheads. For a small number of loggerheads, every LAG deposited throughout the turtle's life could be observed in the ossicles, whereas extensive resorption precluded this possibility in the humeri taken from these same individuals. However, in many other loggerheads, particularly older, larger individuals, resorption levels in the ossicles were extremely high and the number of growth marks retained at the ossicle tips for a given turtle was far less than the number that remained in its humerus (Fig. 8). Furthermore, highly variable resorption in the loggerhead ossicles (Fig. 7) and the lack of correlation between ossicle size (and therefore position within the ossicle ring) and the number of retained LAGs for individual turtles did not allow us to predict which ossicles might retain the greatest number of growth marks. Unfortunately, this uncertainty necessitates the processing and analysis of all ossicles for each turtle to ensure that the one with the least resorption is found. As a result, it appears that although ossicles can sometimes provide useful age data for both Kemp's ridleys and loggerheads, the humerus generally remains the better structure for skeletochronological analysis in these hard-shelled sea turtle species.

Frequency of LAG deposition

Skeletochronology is an appealing method for rapidly collecting age-related data; however, it is essential that certain assumptions be met before the technique can be applied with confidence. In particular, it is important to verify that growth marks are deposited annually for each species in each skeletal structure being studied (Beamish and McFarlane 1983; Klinger and Musick 1992; Castanet et al. 1993). Although mark deposition generally appears to be driven by endogenous factors that may be reinforced by seasonal cycles, other influences such as environmental or physiological stressors can also affect deposition frequency (Zug et al. 1986; Castanet et al. 1993).

Typically, the frequency of growth mark deposition is verified by examining the number of LAGs deposited over a known period of time in samples obtained from either known-age wild or captive animals, or from those whose bones have been previously marked with fluorescent compounds or vital dyes (Frazier 1982, 1985; Klinger and Musick 1992; Castanet et al. 1993; McFarlane and Beamish 1995; Snover and Hohn 2004). However, given the

challenges inherent in mark-recapture studies involving highly migratory marine animals, it is quite rare to recover samples from sea turtles whose bones have been marked or that are of known age (Parham and Zug 1997; Snover and Hohn 2004). Furthermore, growth patterns in captive animals must be interpreted with caution (Frazier 1982).

In light of these logistical difficulties, we approached the issue of determining the frequency of mark deposition in ossicles indirectly. During this study, we observed the presence of a distinct mark at the core of those Kemp's ridley and loggerhead ossicle sections that exhibited little to no resorption. Previous skeletochronological analyses have revealed that the juveniles of some reptile species occasionally deposit a hatch mark separate from a first year mark (Castanet et al. 1993), which suggested that further analysis was necessary to characterize this "core" mark (Fig. 5). Subsequent comparison of the lengths of stained hatchling ossicle sections to core mark lengths revealed that they were not significantly different from one another. This result strongly indicates that the core mark is laid down at hatching and should not be incorporated into counts of annual LAGs used to estimate age.

Annual deposition of growth marks has previously been documented in Kemp's ridley humeri (Snover 2002; Snover and Hohn 2004). Therefore, the next step in our approach involved comparison of the number of marks present in the ossicles to the number present in humeri taken from the same turtles to determine if they were equivalent. Although it might still be interesting to verify this result through traditional means described above, the outcome of this analysis indicates that, with the exception of the core mark, the marks visible in the ossicles are deposited annually and can be used for skeletochronological studies.

Location of LAG counts

During their skeletochronological analysis of leatherback scleral ossicles, Zug and Parham (1996) counted and analyzed LAGs discernible only at the lateral edges of ossicle sections to obtain age estimates. Our results demonstrate that while the number of marks observed at the lateral edges and the tips of ossicle sections was comparable for small juvenile Kemp's ridleys, the number of marks present at the tips was far greater for large neritic juvenile and adult loggerheads. The discrepancy between the smaller and larger turtles could have been caused by several factors, either operating separately or in conjunction with one another. The pattern of ossicle LAG deposition observed during this study indicates that ossicles grow more rapidly in length than in thickness and consequently the growth marks at the lateral edges are spaced more closely together than at the tips. When growth marks become compressed in this manner, compaction can cause multiple LAGs to fuse together, making it difficult to distinguish between individual marks (Zug et al. 1986). Also, resorption appears to occur to a greater extent at the center of the ossicles than at the tips, making it more likely that LAGs associated with the lateral edges will be resorbed. Regardless of the cause of this phenomenon, it is better to obtain growth mark counts from the tips of ossicles sections, otherwise the number of LAGs present and, ultimately, the ages calculated from LAG counts may be underestimated.

Application to other species

Although scleral ossicles might not be the preferred alternative to humeri for skeletochronological studies of Kemp's ridleys and loggerheads, it is still possible that ossicles could be useful for other sea turtle species. Obtaining age and growth data for leatherbacks (*D. coriacea*) has proven particularly problematic, as they exhibit an exceptionally oceanic lifestyle; after leaving their natal beaches as hatchlings, leatherbacks are rarely sighted again until they have reached sub-adult size (Eckert 2002). As a result, although growth rate and age-related information is essential for the management of this endangered species (NMFS SEFSC 2001), estimates of these demographic parameters for leatherbacks remain somewhat speculative (Zug and Parham 1996). Preliminary examination of leatherback ossicles processed using the techniques described here has revealed the presence of growth marks similar in appearance to those observed in loggerhead and Kemp's ridley ossicles (Avens and Goshe unpublished data). Further application of the analytical methods described herein to leatherback ossicles, particularly evaluation of potential discrepancies between lateral and tip LAG counts, may facilitate collection of age-related data for this poorly understood species.

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