Validation and interpretation of annual skeletal marks in loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles

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Abstract—Numerous studies have applied skeletochronology to sea turtle species. Because many of the studies have lacked validation, the application of this technique to sea turtle age estimation has been called into question. To address this concern, we obtained humeri from 13 known-age Kemp's ridley (*Lepidochelys kempii*) and two loggerhead (*Caretta caretta*) sea turtles for the purposes of examining the growth marks and comparing growth mark counts to actual age. We found evidence for annual deposition of growth marks in both these species. Corroborative results were found in Kemp's ridley sea turtles from a comparison of death date and amount of bone growth following the completion of the last growth mark (n=76). Formation of the lines of arrested growth in Kemp's ridley sea turtles consistently occurred in the spring for animals that strand dead along the mid- and south U.S. Atlantic coast. For both Kemp's ridley and loggerhead sea turtles, we also found a proportional allometry between bone growth (humerus dimensions) and somatic growth (straight carapace length), indicating that size-at-age and growth rates can be estimated from dimensions of early growth marks. These results validate skeletochronology as a method for estimating age in Kemp's ridley and loggerhead sea turtles from the southeast United States.

Manuscript submitted 15 August 2003 to the Scientific Editor's Office.

Manuscript approved for publication 3 June 2004 by the Scientific Editor.

Fish. Bull. 102:682–692 (2004).

The basic tenet of skeletochronology is that bone growth is cyclic and has an annual periodicity in which bone formation ceases or slows before new, relatively rapid bone formation resumes (Simmons, 1992; Castanet et al., 1993; Klevezal, 1996). This interruption of bone formation is evidenced within the primary periosteal compacta by histological features, which take two forms in decalcified and stained thin-sections. The most common form is a thin line that appears darker than the surrounding tissue, termed the "line of arrested growth" (LAG) (Castanet et al., 1977). The second, less-common form is a broader and less distinct line that also stains darker, referred to as an annulus (Castanet et al., 1977). Alternating with LAGs or annuli are broad zones that stain homogeneously light, and represent areas of active bone formation. Together, a broad zone followed by either a LAG or an annulus represents a skeletal growth mark (GM) (Castanet et al., 1993). To apply skeletochronology to a species, the annual periodicity of the GM must be validated.

Validation studies are necessary not only to confirm the annual nature of the GM but also to identify and interpret anomalous LAGs. Anomalous LAGs that are a common problem in skeletochronology studies of reptiles and amphibians include double (Chinsamy et al., 1995; El Mouden et al., 1997; Guarino et al., 1998), splitting (Guarino et al., 1995; 1998; Coles et al., 2001), and supplemental (Guarino et al., 1995; Lima et al., 2000; Trenham et al., 2000) lines. In addition to anomalous LAGs, there are two other difficulties typical in skeletochronology studies; compression of LAGs at the periphery of the bone and resorption of the innermost LAGs. In older animals the GMs are compressed at the outer periphery of the bone as a result of decreased growth. Francillon-Vieillot et al. (1990) term this phenomenon "rapprochement" and it is a problem when the LAGs become too close together to be differentiated —usually in the small phalangeal bones used in amphibian studies (Eggert and Guyetant, 1999; Lima et al., 2000; Leclair et al., 2000).

In addition to anomalous and compressed LAGs, the loss of early GMs through endosteal resorption is another problem with skeletochronology. Although this does not present a problem with most amphibian species (Kusano et al., 1995; Castanet et al., 1996; Sagor et al., 1998), the problem is extreme in skeletochronology studies of loggerhead (*Caretta caretta*; Klinger and Musick, 1995; Zug et al., 1995; Parham and Zug, 1997), green

Table 1
Species and history of known-age sea turtles analyzed in this study.

Sample	Species	History during captivity	Age (yr)
LK-1	*Lepidochelys kempii*	Captive for first year, then released	5.0
LK-2	*L. kempii*	Captive for first year, then released	6.5
LK-3	*L. kempii*	Captive for first year, then released	4.5
LK-4	*L. kempii*	Tagged and released after hatching	1.27
LK-5	*L. kempii*	Tagged and released after hatching	1.70
LK-6	*L. kempii*	Tagged and released after hatching	1.72
LK-7	*L. kempii*	Tagged and released after hatching	2.37
LK-8	*L. kempii*	Tagged and released after hatching	2.37
LK-9	*L. kempii*	Tagged and released after hatching	3.25
LK-10	*L. kempii*	Tagged and released after hatching	2.0
LK-11	*L. kempii*	Tagged and released after hatching	2.75
LK-12	*L. kempii*	Tagged and released after hatching	3.0
LK-13	*L. kempii*	Tagged and released after hatching	4.25
CC-1	*Caretta caretta*	Captive during entire life	29.4
CC-2	*C. caretta*	Captive for first two years, then released	8.0

(*Chelonia mydas*; Zug and Glor, 1998; Zug et al., 2002) and Kemp's ridley (*Lepidochelys kempii*; Zug et al., 1997) sea turtles. In each of these studies, the authors used various protocols to estimate the number of layers lost. Any protocol estimating the number of layers lost to resorption relies on the concept that the spatial pattern of the LAGs is representative of the growth of the animal. To confirm this assumption, researchers must establish a correlation between bone dimensions and body size (Hutton, 1986; Klinger and Musick, 1992; Leclair and Laurin, 1996).

Two of the studies that have applied skeletochronology to sea turtles have demonstrated annual GMs in both juvenile (Klinger and Musick, 1992) and adult (Coles et al., 2001) loggerhead sea turtles. Numerous additional studies have applied skeletochronology to sea turtles. To date, the technique has been applied to loggerhead (Zug et al., 1986; Zug et al., 1995; Bjorndal et al., 2003), green (Bjorndal et al., 1998; Zug and Glor, 1998; Zug et al., 2002), Kemp's ridleys (Zug et al., 1997), and leatherback (*Dermochelys coriacea*) (Zug and Parham, 1996) sea turtles. What is needed for the appropriate application of skeletochronology to sea turtle species is additional validation of annual GMs and a guide to their interpretation. Furthermore, because resorption is a problem in sea turtle bones, the validation of a proportional allometry between bone and somatic growth is necessary to enable back-calculation.

In this study, we address each of these issues for Kemp's ridley and loggerhead sea turtles by examining humeri from known-age animals. We analyzed each humerus without prior knowledge of the animal's age and we present the results of our analyses, including reinterpretations of bones for which we were incorrect in our age assessments. The purpose of this study was to use known-age samples both to validate the likelihood that GMs are annual and as a learning tool for the best guide to interpreting GM in wild animals.

Materials and methods

We obtained samples from two known-age loggerhead and 13 known-age Kemp's ridley sea turtles (Table 1). In addition, we collected samples from 240 wild loggerhead and 262 wild Kemp's ridley sea turtles. With the exception of one loggerhead, CC-1, all of the sea turtles died in the wild and samples were retrieved from the carcasses. Sample CC-1 died in captivity.

Sample preparation

Zug et al. (1986) analyzed skeletal elements of the cranium and right forelimb of loggerhead sea turtles and determined that the humerus was most suited to skeletochronology studies. Therefore, we also used the humerus. Specimens arrived as either dried bones or whole flippers. For flippers, we dissected out the humerus, which was then flensed, boiled, and air-dried for at least two weeks. We cross-sectioned each humerus at a site just distal to the deltopectoral crest. At this site, the ratio of cortical to cancellous bone is highest (Zug et al., 1986), and the region immediately distal to the insertion scar of the deltopectoral muscle on the ventral side of the bone maximizes that ratio (see Zug et al., 1986 for diagrams of the loggerhead sea turtle humerus). This site also provided a landmark that allowed us to section at equivalent sites on every humerus.

We removed 2–3 mm thick sections at that site using a Buehler® isomet low speed saw. This section was

fixed in 10% formalin then decalcified by using a commercial decalcifying agent (RDO, Apex Engineering Products Corporation, Calvert City, Kentucky). Time to decalcification varied with the size of the bone and the strength of the solution, usually between 12 and 36 hours. Following decalcification, 25-μm thick cross-sections were made by using a freezing-stage microtome. Sections were stained in Erlich's hematoxylin diluted 1:1 with distilled water (Klevezal, 1996) and mounted on slides in 100% glycerin.

Known-age sea turtles

We received the humeri from each of two captive, known-age loggerhead sea turtles after they died (Table 1). The first specimen, CC-1, was held in an outdoor tank during the summer months and inside a greenhouse during the winter months (this turtle was the same captive female noted in Swartz, 1997). The second, CC-2, was raised in captivity for two years then released from Panama City, Florida, into the Gulf of Mexico.

For the Kemp's ridley sea turtles, we received humeri from 13 dead known-age animals (Table 1). The head-start Kemp's ridleys were raised in captivity for one year, then released as part of a binational program operated jointly by state and federal U.S. agencies and the Instituto Nacional de la Pesca (INP) of Mexico (Klima and McVey, 1995). The coded-wire–tagged (CWT) Kemp's ridley sea turtles were tagged and released as hatchlings. This tagging program is operated jointly by the U.S. National Marine Fisheries Service (NMFS) Galveston Laboratory and the INP of Mexico as a means of gaining a better understanding of the early life history of the Kemp's ridley sea turtle (Caillouet et al., 1997).

Using the methods described previously, we prepared stained thin-sections from the humeri. Without prior knowledge of the animal's history, the number of visible LAGs was quantified for each bone and a minimum age estimated. Our age estimates were then compared to the age information available for each animal.

Indirect validation of annual growth marks

Peabody (1961) and Castanet et al. (1993) suggested that the correlation between the width of the last zone formed and the date of death provided an indirect means of validating that deposition of the LAG occurs annually and at the same time of year for an individual population. We applied this method to 76 wild Kemp's ridley sea turtles for which humeri displayed between one and five LAGs. Each of these animals had stranded dead along the Atlantic coast between Maryland and North Carolina. Thin-sections were prepared of the humeri as described above. We quantified the width of the last zone formed by measuring the outside diameter of the whole section (D_O) and the diameter of the last competed LAG (D_L), between the lateral edges of the bone on an axis parallel to the dorsal edge. The amount of bone growth after the last LAG (D_O–D_L) was plotted against the Julian stranding date, with the assumption that stranding date approximated date of death. Least-squares linear regressions were fitted to the data.

Validation of the relationship between LAG diameter and body size

In order to relate GM diameters to somatic growth rates, there must be a constant proportionality between bone growth and somatic growth (Chaloupka and Musick, 1997). To address this proportionality, we took eight morphometric measurements of 240 wild loggerhead and 262 wild Kemp's ridley humeri, using digital calipers or a tape measure when dimensions were beyond the range of the calipers. Measurements of maximum length, longitudinal length, proximal width, distal width, deltopectoral crest width, lateral diameter at sectioning site, ventral to dorsal thickness at sectioning site, and mass were recorded. We compared these measurements with the carapace length, measured as standard straight-line length (SCL) from the nuchal notch to the posterior end of the posterior marginal, using a least-squares linear regression. For mass, the data were natural-log transformed to form a linear regression.

Results

Known-age Kemp's ridley sea turtles

Three Kemp's ridley sea turtles captive for one year and then released were recovered 4.5 to 6.5 years after hatching (Table 1). Sample LK-1 had minimal resorption and four complete GMs, each comprising one zone followed by a LAG. An additional zone was seen at the periphery and the LAG that would complete this last GM was not yet visible at the outer edge of the humerus cross-section. From GM counts and death date, we estimated the age of this animal accurately at five years (Fig. 1). Sample LK-2 retained five completed and one incomplete GM; however, we observed a large area of resorption in the interior region of the cross-section that potentially obscured additional GMs. We aged this animal at a minimum of 5.5 years, the actual age being 6.5 years. Sample LK-3 displayed four completed GMs and one incomplete mark. Without prior knowledge of this animal's age, we estimated the age accurately at 4.5 years based on layer count and time of death.

Ten of the Kemp's ridley sea turtle samples were tagged and released after hatching, and no time was spent in captivity (Table 1). Results from these ten recovered animals allowed us the opportunity to study and interpret the early GM patterns in noncaptive animals. The first year mark for Kemp's ridley sea turtles appeared to be a poorly defined annulus, as evidenced by LK-4 (Fig. 2A). In turtles greater than two years old, similar first year marks also appeared more or less distinctly (Figs. 2B and 3). Additional marks, which can only be interpreted as supplemental lines given the age of the animal, appeared between GM one and the outer edge of the bone in LK-6 (Fig. 2B) and LK-10. Specimens

LK-7 and LK-8 were difficult to interpret and in our initial assessment we underestimated age by one year. In both of these samples, the LAG representing the end of the second GM was very close to the outer edge of the bone cross-section and was difficult to differentiate from the edge. Hence these samples were not counted in the initial assessment. Because both of these animals died in the fall, there would have been a full growing season, and hence a growth zone, following the completion of the second GM. Both of these animals were recovered dead in Cape Cod, Massachusetts, during the fall of 1999 when record numbers of cold-stunned sea turtles stranded in that region.

Humerus cross-sections from LK-9 through LK-13 (Fig. 3) showed poorly defined annuli at the end of the first GM—annuli similar to the poorly defined annulus in LK-4 (Fig.2A). Subsequent GMs in these humerus cross-sections contained well-defined LAGs. Without prior knowledge of these animals' history we accurately aged each of them from GM counts and stranding date. Specimens LK-9 through LK-13 demonstrated clearly that well-defined LAGs were deposited at the end of year two and in subsequent years, providing evidence that any lines between the year-one annulus and the year-two LAGs were supplemental.

Known-age loggerhead sea turtles

The first known-age loggerhead sea turtle, CC-1, was 29.4 years old. Eleven LAGs were discernible around the circumference of the bone cross-section (Fig. 4A), although the LAGs become too compressed on the lateral edges of the bone to be differentiated; hence counts were made on the ventral and dorsal edges.(Fig. 4). Tracing the LAGs from the lateral to the ventral edge of the bone, we observed that these LAGs at some point became bifurcating and splitting LAGs and we interpreted each branch as a separate LAG. An additional nine LAGs can still be seen within the resorption zone in most areas of the bone (Fig. 4B). On the dorsal side of the cross-section, at least four less-distinct LAGs or annuli could still be observed; these had been

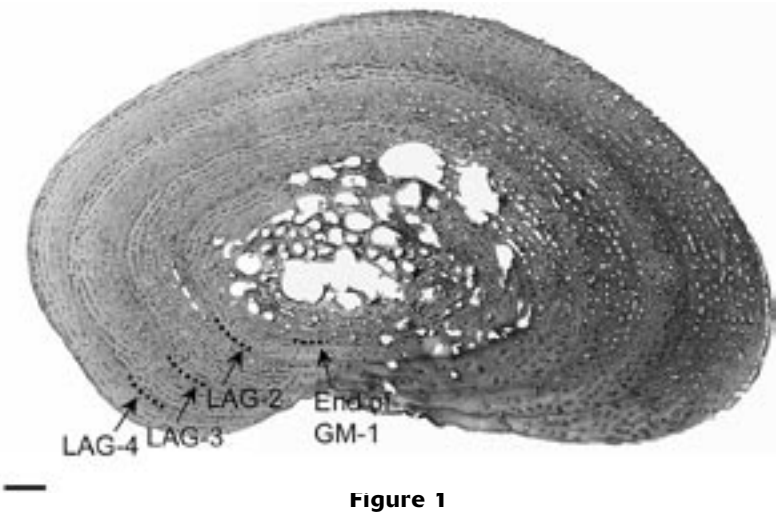

Figure 1

Image of a humerus cross-section from a headstart Kemp's ridley (*Lepidochelys kempii*, LK-1) sea turtle. GM-1 refers to growth mark one; LAG-2, LAG-3, and LAG-4 refer to the lines of arrested growth ending growth marks two, three, and four. Curved dashed lines highlight GM-1 and the LAG. Black bar represents 1 mm in length. This specimen was 5.0 years old.

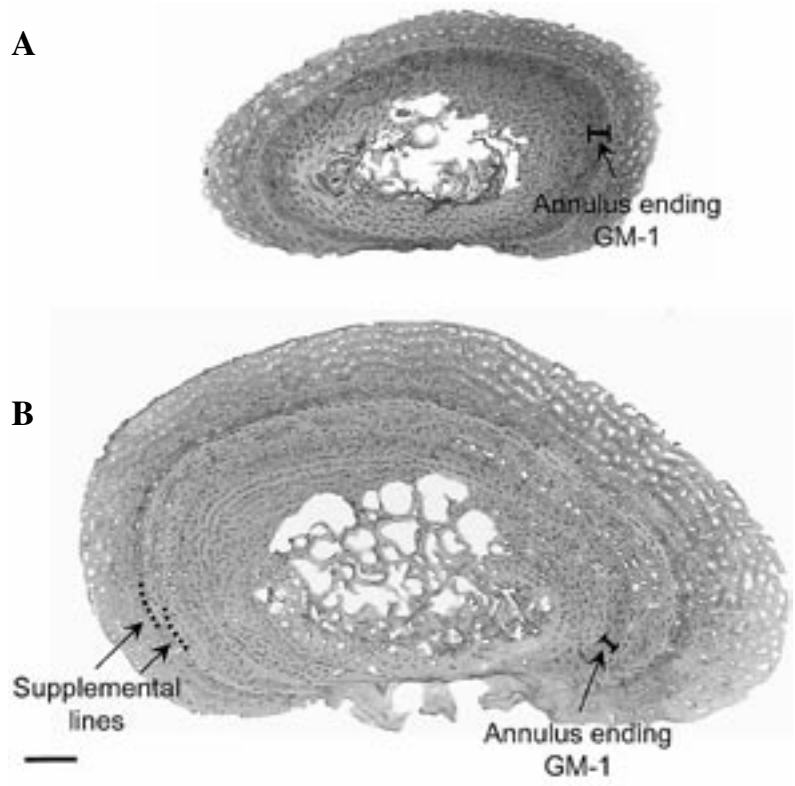

Figure 2

Images of humeri cross-sections of two coded-wire–tagged Kemp's ridley sea turtles (*L. kempii*). GM-1 refers to growth mark one. Black bar represents 1 mm for both images. (**A**) Specimen LK-4 was 1.27 years old. (**B**) Specimen LK-6 was 1.72 years old.

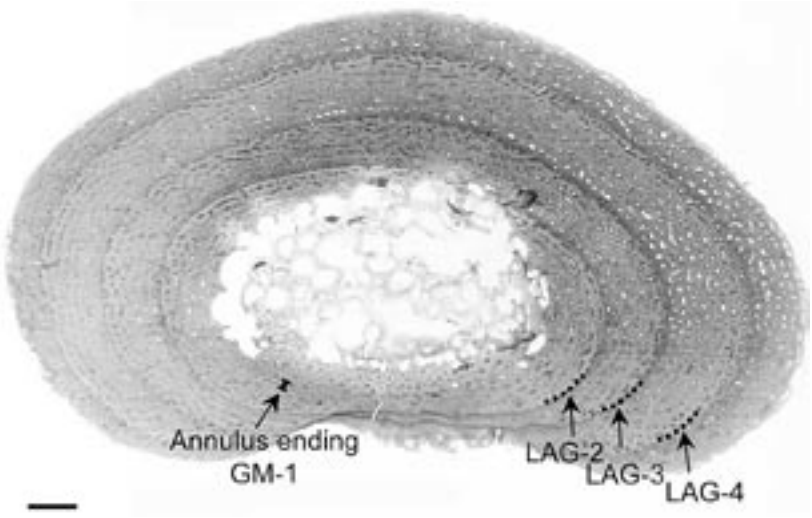

Figure 3

Image of humerus cross-section from a coded-wire–tagged Kemp's ridleys (*L. kempii*). Black bar represent 1 mm in length. GM-1 refers to growth mark one; LAG-2, LAG-3, and LAG-4 refer to the lines of arrested growth ending growth marks two, three, and four. Curved black lines highlight LAGs or annuli. This specimen, LK-13, was 4.75 years old.

resorbed in all other parts of the bone (Fig. 4C). There had been a great deal of remodeling within the bone and much of the inner portion of the bone had been resorbed. Summing all of these GMs, we gave a minimum age estimate of 24 years without prior knowledge of the history of the animal. The outermost 20 GMs contained well-defined LAGs that were spaced close together, whereas the four interior-most visible GMs contained LAGs or annuli that were spaced farther apart (Fig. 4). The number of layers completely resorbed was five.

A second known-age loggerhead sea turtle, CC-2, was eight years old. We assigned a minimum age estimate of five years. Just outside of the resorption area was a series of three LAGs that were very close together (Fig. 5). In our initial analysis, we assumed that three LAGs so close together could not each be deposited annually and we interpreted the triple LAGs as a single LAG with an anomalous appearance. We re-evaluated this assumption after learning its history. The animal was in captivity for two years and then released at 42.7 cm SCL in October 1994. Counting back from the outside of the bone, the outermost of the triplet LAGs would represent spring 1996. Given this evidence, our best interpretation of this bone section was that the innermost of the triplets of LAGs indicated release and was therefore not an annual mark. The next LAG was likely deposited the following spring (1995) and was likely an annual mark. The third of the closely spaced LAGs likely represented spring 1996, indicating that the animal did not grow significantly in its first year in the wild (Fig. 5). Following the three closely spaced LAGs, there were four additional indistinct LAGs or annuli that represented the remaining years at large. The outermost of these was very close to the edge of the bone, indicating that the animal did not grow much, if at all, during the last summer of its life.

Indirect validation of annual growth marks

For Kemp's ridley sea turtles, there was a significant increase in the amount of bone deposited after the last LAG from 20 June to 30 November (Fig. 6). The LAGs near the outer edges of the bones were fully visible in strandings that occurred after 20 June. Earlier detection of the outer LAGs was unlikely because a certain amount of bone formation must occur following the LAG before it can be discerned from the edge. There was not a significant relationship between bone growth and date from 1 December to 19 June. The slope of this regression was very close to zero (b=−0.003), indicating no trend, either increasing or decreasing, in the amount of bone deposited during this time (Fig. 6).

Validation of the relationship between LAG diameter and body size

The regressions of the eight morphometric measurements of loggerhead and Kemp's ridley sea turtle humeri against SCL revealed high correlations between bone dimension and body size (Table 2). Most importantly for purposes of back-calculation, the lateral diameter at the sectioning site of the humerus (distal to the insertion scar of the deltopectoral muscle) and the body length of the animal was highly correlated.

Discussion

Validation of the annual nature of growth marks

Our results supported annual deposition of GMs in loggerhead and Kemp's ridley sea turtles. The headstarted and older CWT Kemp's ridley sea turtles in particular highlighted the likelihood of annual marks. These animals displayed sharp and regularly spaced LAGs that were consistent with the actual ages of the animals. The results from the CWT Kemp's ridley sea turtles also emphasized the difficulties in interpreting early GMs. From these animals we concluded that in general Kemp's ridley sea turtles deposit a poorly defined annulus in their first year and well-defined LAGs starting with the end of the second year and in following years.

For loggerhead sea turtles, only CC-2 spent any time in the wild. The number of GMs deposited after the animal was released (determined from the appearance of the anomalous triplet of LAGs) was consistent with the number of years for which the animal was at large, considering that the first mark was deposited at release. This indicated that not less than one GM was deposited per year, and that additional or supplemental LAGs or annuli indistinguishable from annual lines may be deposited under extreme conditions, such as at the time of release into the wild. Fortunately, in this case, these extreme conditions were not frequent enough to have a serious impact on age estimates. For the life-time captive animal, CC-1, our estimated minimum age was five years shorter than the actual age of 29.4 years and clearly demonstrated that not more than one GM was deposited each year. Because of the relatively large size of the sea turtle humerus, in comparison to phalanges of amphibians, rapprochement did not appear to be a problem in our attempts to discern LAGs. This bone was similar in appearance to adult wild loggerhead and Kemp's ridleys sea turtles with rapprochement of the peripheral LAGs and resorption of most of the interior GMs. Although accurate age estimates cannot be made of these bones through skeletochronology, if rapprochement correlates to the timing of sexual maturity, counts of the compressed GMs can provide valuable information on postreproductive longevity and adult survival. This information can be combined with average age at reproductive maturation for piecing together the life history of sea turtles. Although our sample size for loggerhead sea turtles was very small (two), the size complements a tetracycline-injection study that previously validated annual GMs for juvenile loggerhead sea turtles from Chesapeake Bay (Klinger and Musick, 1992). In addition, an adult loggerhead sea turtle from that same study stranded dead 8.25 years after injection and provided evidence of annual deposition of growth marks in adults (Coles et al., 2001).

The indirect validation results for Kemp's ridley sea turtles highlighted the cyclic nature of bone growth; bone deposition increases from late spring through early summer to fall and no bone deposition occurs from December to spring. From this information we inferred that LAGs form annually in the spring for Kemp's ridley sea turtles that strand along the mid- to southeast U.S. Atlantic coast and that these LAGs are visible at the edges of the bones by late spring to early summer.

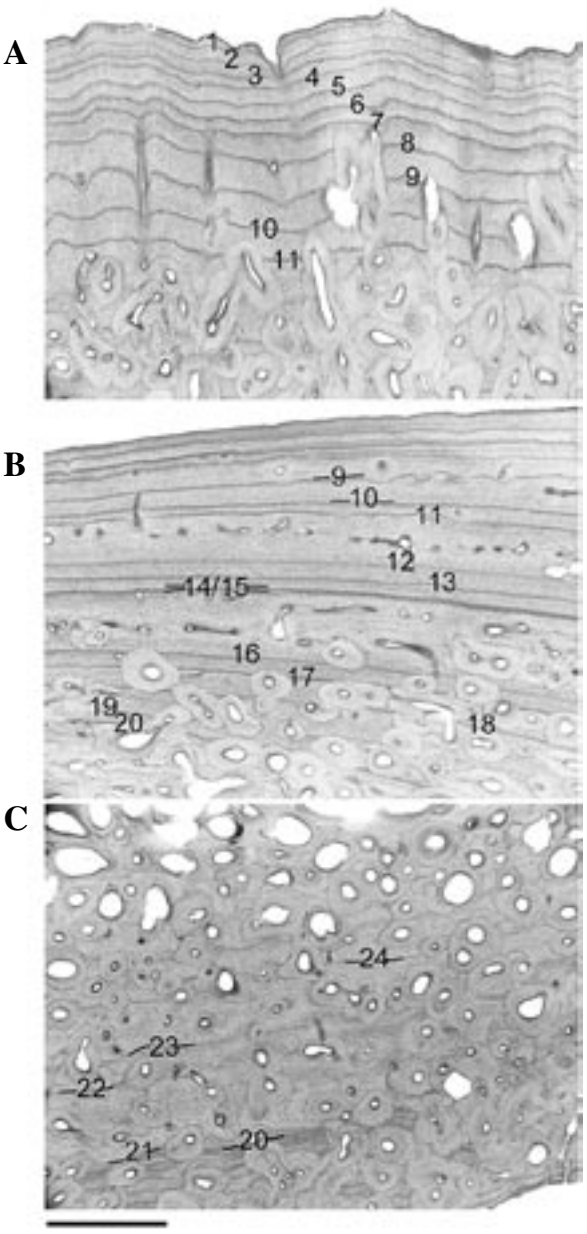

Figure 4

Images of different portions of the humerus cross-sections of CC-1 (*Caretta caretta*). Black bar represents 1 mm in length for all views. (**A** and **B**) The outer edge of the bone is at the top of the photo. (**C**) The outer edge of the bone is towards the bottom of the photo. For all views, lines of arrested growth (LAGs) are labeled with numbers; low numbers represent the most recently deposited LAGs (near the outer edge of the bone) and higher numbers represent the earlier LAGs.

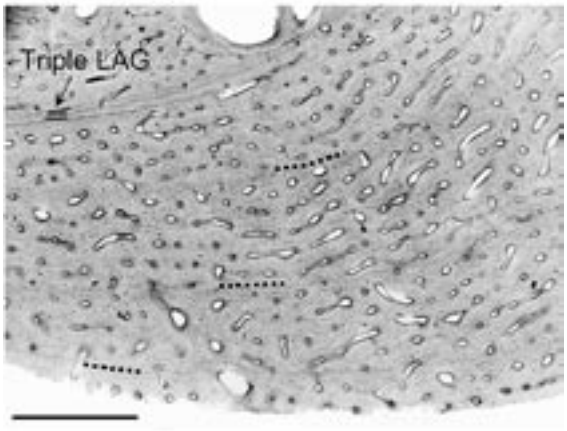

Figure 5

Image of a section of the humerus cross-section of CC-2 (*C. caretta*). Outer edge of bone is towards the bottom of the photo. Solid lines (upper left) highlight a series of triple lines of arrested growth (LAGs); curved dashed lines highlight the three diffuse LAGs. Black bar represents 1 mm in length.

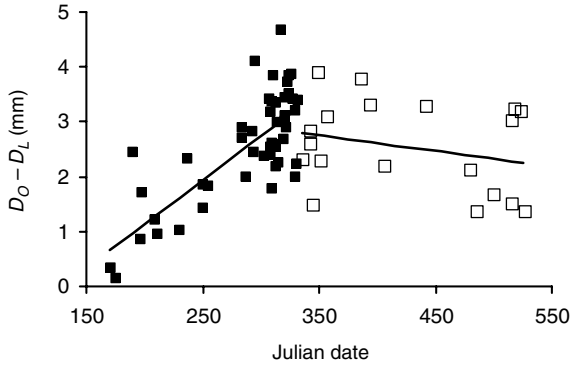

Figure 6

Julian date of stranding plotted against the amount of bone deposited peripherally to the last LAG in Kemp's ridley sea turtles (*L. kempii*; n=76). D_O represents the outside diameter of the humerus, D_L represents the diameter of the last LAG. Julian dates on x-axis equate to 20 June through 19 June; therefore numbers that are greater than 365 represent the Julian date plus 365. Solid lines represent linear regressions that were run separately for 6 months, 20 June to 31 November (filled squares) and 1 December to 19 June (open squares). The regression for the first six months was significant (P<0.006) and the regression for the second six months was not significant (P=0.27).

Most studied species of reptiles and amphibians deposit GMs within their bones (Castanet et al., 1993; Smirina, 1994). For some of these species, the annual nature of the GM has been validated (e.g., Tucker, 1997; de Buffrénil and Castanet, 2000; Trenham et al., 2000). For others, it is consistent with their ecology that the marks must represent annual events (Castanet et al., 1993). Growth marks observed in loggerhead (Zug et al., 1986; Zug et al., 1995; Coles et al., 2001), Kemp's ridley (Zug et al., 1997), and green (Zug and Glor, 1998; Zug et al., 2002) sea turtles are similar in structure to those observed in other species of reptiles and amphibians. Drawing on previous studies of reptiles and amphibians, validation studies on sea turtles, and the evidence presented in this article, we assert that GM in bones of sea turtles are likely deposited primarily with an annual periodicity.

Given these results, on the surface it seems contradictory that in two validation studies annual GMs could not be confirmed. For serpentine species, Collins and Rodda (1994) injected brown snakes with a fluorescent marker and kept them in captivity for one year under two different feeding regimes. Five or six GMs varying in distinctness were identified beyond the fluorescent marks in bone cross-sections. Statistical analyses showed that these marks may relate to shedding events. It is unclear if the GM pattern prior to captivity was similar to what was seen after the fluorescent mark. The forced feeding component of that study may have induced higher growth rates than would be found in nature, causing the shedding events to appear as histological marks in the bone.

In a sea turtle study, Bjorndal et al. (1998) did not find GMs in the humeri of green sea turtle bones. They suggested that the tropical marine habitat of the study population (approximately 21°07′N) allowed for continual activity and growth and inhibited GM formation. However, GMs have been clearly demonstrated in green sea turtles from the coastal waters of Florida (approximately 29°N) (Zug and Glor, 1998) and Hawaii (approximately 22°N) (Zug et al., 2002). Other studies of reptiles and amphibians in tropical and warm temporate climates have reported distinct GMs in species that remain active year-round (i.e., do not hibernate or estivate) (Patnaik and Behera, 1981; Estaban et al., 1996; Guarino et al., 1998).

Interpretation of anomalous LAGs

Although our sample sizes were small, especially for loggerhead sea turtles, several characteristics were noted in the analyses of the samples that would affect how anomalous LAGs are interpreted. Three interpretations of double and bifurcating LAGs are provided. The first interpretation is that if double LAGs appear frequently in individual bones and throughout the sample, they likely indicate an ecology that has two growth cycles per year (Castanet et al., 1993). In this case the two LAGs are distinct from each other over the entire bone cross-section. This pattern was observed in the newt *Triturus marmoratus* living at a high altitude where the animals had both winter and summer dormancy periods (Castanet and Smirina, 1990; Caetano et al., 1985; Caetano and Castanet, 1993). The second interpretation of double LAGs is that they result from a brief

Table 2

Regressions equations and statistics from correlations between dimensions of the humerus and notch-to-tip straight carapace length (SCL, cm) in loggerhead and Kemp's ridley sea turtles. All F statistics are significant at $P<0.005$.

Humeral measurement	Model equation	SE slope	F	r^2
Loggerhead sea turtles (n=243)				
Maximal length (ML, mm)	$SCL = 0.44 \times ML + 5.97$	0.0064	4814	0.95
Longitudinal length (LL, mm)	$SCL = 0.47 \times LL + 4.85$	0.0064	5381	0.96
Proximal width (PW, mm)	$SCL = 1.06 \times PW + 7.31$	0.015	4857	0.95
Deltopectoral crest width (DCW, mm)	$SCL = 1.69 \times DCW + 6.04$	0.026	4069	0.94
Site of sectioning width (SW, mm)	$SCL = 2.38 \times SW + 5.48$	0.037	4110	0.94
Site of sectioning thickness (ST, mm)	$SCL = 4.13 \times ST + 11.62$	0.080	2682	0.92
Distal width (DW, mm)	$SCL = 1.28 \times DW + 5.43$	0.021	3684	0.94
Mass (M, g)	$\ln(SCL) = 0.30 \times \ln(M) + 2.94$	0.0022	18905	0.99
Kemp's ridley sea turtles (n=262)				
Maximal length (ML, mm)	$SCL = 0.43 \times ML + 4.69$	0.0040	10970	0.98
Longitudinal length (LL, mm)	$SCL = 0.47 \times LL + 3.11$	0.0039	14772	0.98
Proximal width (PW, mm)	$SCL = 1.12 \times PW + 4.39$	0.010	12390	0.98
Deltopectoral crest width (DCW, mm)	$SCL = 1.69 \times DCW + 3.35$	0.017	10200	0.98
Site of sectioning width (SW, mm)	$SCL = 2.48 \times SW + 2.74$	0.033	5715	0.96
Site of sectioning thickness (ST, mm)	$SCL = 4.16 \times ST + 4.79$	0.072	3306	0.93
Distal width (DW, mm)	$SCL = 1.36 \times DW + 0.227$	0.013	11435	0.98
Mass (M, g)	$LN(SCL) = 0.30 \times LN(M) + 2.89$	0.0023	16305	0.98

interruption of hibernation (Hemelaar and van Gelder, 1980). In this instance little bone deposition would occur and the layers would not be distinct from each other over the entire bone, thus giving the appearance of a bifurcating LAG (Hemelaar and van Gelder, 1980). The third interpretation of double or bifurcating LAGs is that they result from extreme decreased growth over the active period, which places annual LAGs very close to each other and in some cases they appear to merge (de Buffrénil and Castanet, 2000).

With the first two interpretations, a double or bifurcating LAG would be counted as one for the purposes of age estimation, whereas the third interpretation would necessitate counting each LAG or bifurcating branch separately. Coles et al. (2001) interpreted a bifurcating LAG as one LAG in an adult loggerhead sea turtle that was recovered 8.25 years after it had been injected with oxytetracycline. In cross-sections of the humerus, Coles et al. (2001) reported seven LAGs following the tetracycline mark, six plus the bifurcating LAG. The animal was marked on 20 June 1989 and recovered dead on 22 September 1997. It is reasonable to assume that, as with Kemp's ridley sea turtles from the same region, the LAGs form in the spring, and Coles et al. (2001) showed that the oxytetracycline mark overlaid one of the LAGs—likely the LAG deposited in spring of 1989. Therefore, there should have been eight LAGs deposited after the tetracycline mark, not seven, each representing the spring of years 1990 through 1997. In this case, then, the bifurcating mark in this bone should be counted as two LAGs.

Similarly, for splitting LAGs, where numerous thinner LAGs branch out from what appears to be one thick LAG, Francillon-Vieillot et al. (1990) examined different bones from the same animal and determined whether each thin LAG comprising splitting LAGs should be counted as one LAG. In our analysis of the adult loggerhead sea turtle, CC-1, we observed several bifurcating and splitting LAGs, each of which eventually split into two or more thinner LAGs. We counted each of the thin LAGs as one. Because the LAG count was close to the actual age of the animal, this interpretation appears to have been appropriate for compressed LAGs in adult humeri.

The question remains as to whether this is the appropriate interpretation for double or bifurcating LAGs in juveniles. Wild loggerhead growth rates have been monitored in an ongoing mark-recapture study in Pamlico and Core Sounds in North Carolina (Epperly et al., 1995). Epperly et al. (1995) currently have 65 growth rates for 49 juvenile loggerhead sea turtles between 45.1 and 81.0 cm SCL at initial capture that were at-large for one year (±0.1 year). The mean annual growth rate for all of the animals is 2.09 cm/yr. However, of the 65 growth records, 11 of them displayed an annual increase of 0.3 cm or less in SCL (Braun-McNeill[1]). Hence it is not uncommon for juvenile loggerhead sea turtles to

[1] Braun-McNeill, J. 2004. Personal commun. Center for Coastal Fisheries and Habitat Research, National Marine Fisheries Service, NOAA, 101 Pivers Island Rd., Beaufort, NC 28516

grow little or not at all over the course of a year. Using the equation for width at sectioning site from Table 2, we found that the increase in bone diameter for these 11 animals was ≈0.13 mm or less, which places the LAGs very close together. Because it not uncommon for sea turtle to exhibit little or no growth over a year, LAGs spaced closely together very likely represent distinct years as also determined by de Buffrénil and Castanet (2000). Although the sample sizes are still small for a definitive answer, our results indicate that counting the LAGs individually is the correct interpretation of double or bifurcating LAGs in juvenile as well as adult loggerhead sea turtles.

Similarly, our results indicate the same interpretation for double or bifurcating LAGs in juvenile Kemp's ridley sea turtles. The CWT Kemp's ridley sea turtles, samples LK-7 and LK-8, displayed LAGs near the outer edge of the bone and a small amount of bone was deposited after the LAGs. These animals were each 2.25 years old and had one-year marks visible in the humeri but no LAGs or annuli other than those at the periphery. Other CWT samples clearly indicated that LAGs are deposited at the end of the second GM. The indirect validation results demonstrated that LAGs were visible in bone tissue by late spring or early summer. It seemed that the LAGs at the outer edge of the LK-7 and LK-8 bones were the LAGs ending the second GM and that very little growth occurred over the subsequent growing season. Both of these animals were recovered as dead strandings resulting from a major cold stun event in Cape Cod, Massachusetts, in 1999; hence their growth rates may have been anomalous in their last year of life. Had these animals survived the cold stun event, they would have deposited a year-three LAG very close to year two, giving the appearance of a double or bifurcating LAG.

Another anomaly in skeletochronology, supplemental lines, may form as a result of temporary stressful environmental events such as droughts. In support of this, Rogers and Harvey (1994) noted a supplemental line in 11 of 43 specimens of the toad *Bufo cognatus,* and in 10 of these animals the supplemental line was within a growth zone that corresponded to a drought year. Most skeletochronology studies that have noted the presence of supplemental lines have indicated that supplemental lines are easily identified as such because they are less distinct and do not appear around the entire circumference of the bone. In general, the same has been observed in sea turtles. Supplemental lines do appear but are generally easily differentiated from LAGs by appearance. An exception to this was the presence of supplemental marks in one- to two-year-old Kemp's ridley sea turtles. These marks were similar in appearance to the first year annuli. We were able to identify these marks as supplemental only by the observation of known-age animals. In addition, there appeared to be a supplemental line in CC-2 that represented when the animal was released; hence, highly stressful events may cause the deposition of nonannual lines, but these events are likely to be relatively rare in wild turtles and not likely to interfere significantly with age estimations.

Resorption of early growth marks

The loss of the early GMs due to endosteal resorption and remodeling of the interior region of the bone is a limiting factor in the application of skeletochronology to sea turtles. From our findings, it was possible to accurately age juvenile Kemp's ridley sea turtles up to at least 5 years from GM counts and this may be true for other sea turtle species (e.g., Bjorndal et al., 2003), with the possible exception of the leatherback sea turtle (Zug and Parham, 1996). Because sea turtles have distinct life-cycle stages, we suggest that in order to age a population of sea turtles, one must acquire an ontogenetic series of samples spanning all sizes and stages. Average duration can be determined for each ontogenetic stage and the approximate age of older animals with extreme resorption can be estimated. Because GM patterns appear to mimic somatic growth rates, once growth through each life-cycle stage is understood, backcalculation techniques can be used to estimate the number of layers resorbed.

Conclusions

For many species, skeletochronology is not a perfect method for age estimation. As GMs are histological expressions of variation in rates of osteogenesis (Castanet et al., 1993), external factors and individual variation will affect the appearance of the marks (Castanet et al., 1993, Esteban et al., 1996, Waye and Gregory, 1998). Endosteal resorption also serves to confound this technique and is the primary difficulty in the application of the technique to sea turtles. However, the evidence presented in the present study gives strong support to the concept that GMs are deposited on an annual basis in sea turtles and that the spatial pattern of the GMs correspond to the growth rates of the animal. The GMs therefore provide invaluable information on age and growth that cannot otherwise be easily obtained, and age determination by skeletocronology is valid and appropriate for the study of sea turtles.

Acknowledgments

We thank L. Crowder, S. Heppell, A. Read, and D. Rittschof for their valuable comments on earlier versions of this manuscript. A. Gorgone, B. Brown and J. Weaver provided assistance with the preparation of the humeri. Most of the humeri were received through the Sea Turtle Stranding and Salvage Network, a cooperative endeavor between the National Marine Fisheries Service, other federal and state agencies, many academic and private entities, and innumerable volunteers. We especially thank R. Boettcher and W. Teas. In addition, humeri were received from F. Swartz at the University of North Carolina–Chapel Hill Institute of Marine

Science, B. Higgins at the National Marine Fisheries Service–Galveston Lab, the Virginia Marine Science Museum Stranding Program, the Maryland Department of Natural Resources, and the Massachusetts Audubon Society in Wellfleet. Funding was provided by the National Marine Fisheries Service. All work was done under and complied with the provisions of the Sea Turtle Research Permit TE-676379-2 issued by the U.S. Fish and Wildlife Service.

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