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ANNULAR GROWTH LAYERS IN JUVENILE LOGGERHEAD TURTLES (*CARETTA CARETTA*)

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ABSTRACT

Juvenile loggerhead turtles (*Caretta caretta*) were tagged with tetracycline to establish a chronology for deposition of periosteal bone growth layers. Eight recaptures, encompassing intervals of 1 to 3 years, were used to demonstrate that these growth layers were typically deposited on an annual basis. However, the number of scorable growth layers represents an underestimate of true age because earlier growth layers are obscured by expansion of the medullary cavity during growth. The number of absorbed growth layers was extrapolated from calculations based on juvenile bone growth patterns. Most (83%) of these corrected age estimates for juvenile loggerheads, 40 to 80 cm straight line carapace length, were between 5 and 15 years.

Marine turtles are long-lived, slow-growing animals (Frazer, 1983; 1986; Zug et al., 1986), but published estimates of age at sexual maturity and growth rates vary widely. For captive loggerhead turtles (*Caretta caretta*), estimates of age at sexual maturity range from six to seven years (Caldwell, 1962; Uchida, 1967) to 22 years (Frazer and Schwartz, 1984). Mendonca (1981) estimated 10–15 years to maturity based on a population of immature loggerheads in Mosquito Lagoon, Florida. Frazer and Ehrhart (1985) calculated age at maturity for recaptured loggerheads using both logistic and von Bertalanffy growth models. This study, also conducted in Florida, indicated an age of 12–30 years at sexual maturity. Limpus (1979) estimated 30+ years for age at maturity based on a tagging study of Australian loggerheads.

Juvenile loggerheads exhibit growth layers in their foreflipper bone, as indicated by humeri cross sections (Zug et al., 1986). These growth layers may provide valuable information about growth if the pattern of deposition can be documented. To determine whether growth layers are deposited on an annular basis, we injected a known time label, oxytetracycline, into juvenile loggerheads. Tetracycline has proven effective for this purpose in other vertebrates (Smirna, 1974; Castanet and Naulleau, 1974; Gruber, 1982) because it deposits in growing bone at time of injection and will subsequently fluoresce under ultraviolet light (Harris, 1960). Fluorescence remained visible up to three years in tetracycline-marked captive farm-reared green turtles (Frazier, 1985). In this study the time label was applied to turtles in a wild population. When a marked turtle is recovered, the rate and type of bone deposition during a known time interval can be observed. This approach can validate humerus periosteal growth as the product of seasonal periods of alternate slow and rapid growth.

METHODS

Since 1979 researchers at the Virginia Institute of Marine Science (VIMS) have monitored the sea turtles which seasonally inhabit Chesapeake Bay (Musick et al., 1984; Lutcavage and Musick, 1985; Musick, 1988). Ninety percent of stranded turtles are loggerheads. Over 80% of loggerheads examined are between 40 cm and 80 cm carapace length (Klinger, 1988), well below size at maturity of 90 cm (Baldwin and Loften, 1959). The dominance of this size class in Chesapeake Bay provided an unusual opportunity to study large numbers of juvenile loggerheads.

Tetracycline Mark and Recapture.—During the period 1983–1987, 169 live loggerheads (caught in stationary nets by Chesapeake Bay fishermen) were examined. These animals were measured, tagged with Monel (International Nickel Co.) tags on each foreflipper and injected with 25 mg·kg⁻¹ oxytet-

racycline (LA-200 Liquamyacin, Pfizer). The injection was given intramuscularly on the ventral shoulder of either foreflipper. Turtles were routinely released immediately after tagging.

Subsequently, humeral bone biopsies were taken from recaptured individuals. Biopsied bone plugs (3 mm in diameter) were processed into both decalcified and undecalcified sections. Tetracycline bands were observed under ultraviolet light in undecalcified sections and growth layers were observed under transmitted white light in decalcified sections. Undecalcified bone sections were processed with methyl methacrylate (Chappard et al., 1983) and 50–70 μm ground sections were mounted on microscope slides. Fluorescent and growth layers were observed under a binocular microscope at 10×. Measurements were taken from the fluorescent layer to the periosteum and from the fluorescent layer to the bone medullary cavity (Fig. 1a, c). Subsequent sections were decalcified with an eight percent formic-hydrochloric acid solution and histologically prepared with Harris hematoxylin and eosin-y stain to highlight growth layers. All growth layers were measured from the (external) periosteum to the (interior) medullary cavity providing an accurate comparison of layer position. Total growth was measured from the medullary cavity to periosteum and compared with the undecalcified section to test possible stretching of tissue during histological processing.

Stranded Loggerhead Collection.—Straight line carapace length (CLS) measurements were taken on all dead loggerheads stranded in Chesapeake Bay during the 1983–1985 seasons. Humeri were removed, air dried and measured by methods described by Zug et al. (1986). Humeri were initially cut with a Raytech rock saw just below the deltopectoral crest (Zug et al., 1986). The sectional bone was cut into thin (1–3 mm) wedges with a Buehler isomet 11-1180 low speed saw and 11-4244 high concentration diamond wafering blade. Sections were decalcified and stained with the procedure described above. Seven μm sections were cut on an AO 820 microtome. Slides were stained with Harris hematoxylin and eosin-y. The growth layers were counted during three independent trials. Whenever possible, layers were counted from the medullary cavity (core of spongy cancellous bone) to the periosteum both on the short and long axis. Minimum and maximum counts for short and long axes were recorded and means for each set of measurement trials were calculated. In addition, measurements were taken from the focus of the medullary cavity to each line of decelerated growth (winter layer) and periosteum (radii length). Regression between the straight carapace length (CLS) and radial length provided back calculations of length at age using the Fraser-Lee formula (Everhart and Youngs, 1981):

$$L' + C = S'/S(L - C)$$

where, L' = length at first growth layer, C = correction factor related to the expected value (y intercept), S' = length of radius from the focus to the first layer of arrested growth, S = maximum radius length and L = length at capture.

RESULTS

Recapture of Tetracyclined Loggerheads.—Fourteen bone biopsies were taken from thirteen loggerhead turtles tagged with tetracycline and recaptured from 1985 to 1988 (one was recaptured twice). Nine of the biopsy bone sections (from eight individuals) revealed a distinct fluorescent band. The unmarked cases may reflect an insufficient amount of tetracycline at the time of injection, incorrect position of biopsy plug (transverse removal produces a blurred or unreadable band) or degradation of the outer edge of the bone during histological preparation. Notably, all the bone plugs which lacked a tetracycline band were from 1-year recaptures, and at least in one case fragmentary fluorescence was noted at the exterior edge.

Table 1 lists recaptured data for biopsied loggerheads. The relative positions of growth layers and tetracycline bands are summarized in Table 2. In 1985 our first biopsy was performed on a turtle (120-84) recaptured 393 days (1.08 years) after injection. In the undecalcified section, the tetracycline band was visible on the short axis 41 μm from the periosteum. On the decalcified section, the first and second growth layers were 22 μm and 52 μm from the periosteum, respectively. Thus, the tetracycline band was observed between the outer two growth layers. Another recapture (65-84) was biopsied 467 days (1.28 years) after injection. Again, the tetracycline band, 50 μm from the periosteum, was visible between the two outer growth layers, 22 μm and 64 μm from the periosteum, respectively.

In 1987, biopsy plugs were recovered from seven tetracycline marked loggerheads—four 1-year recaptures, one 2-year recapture and two 3-year recaptures.
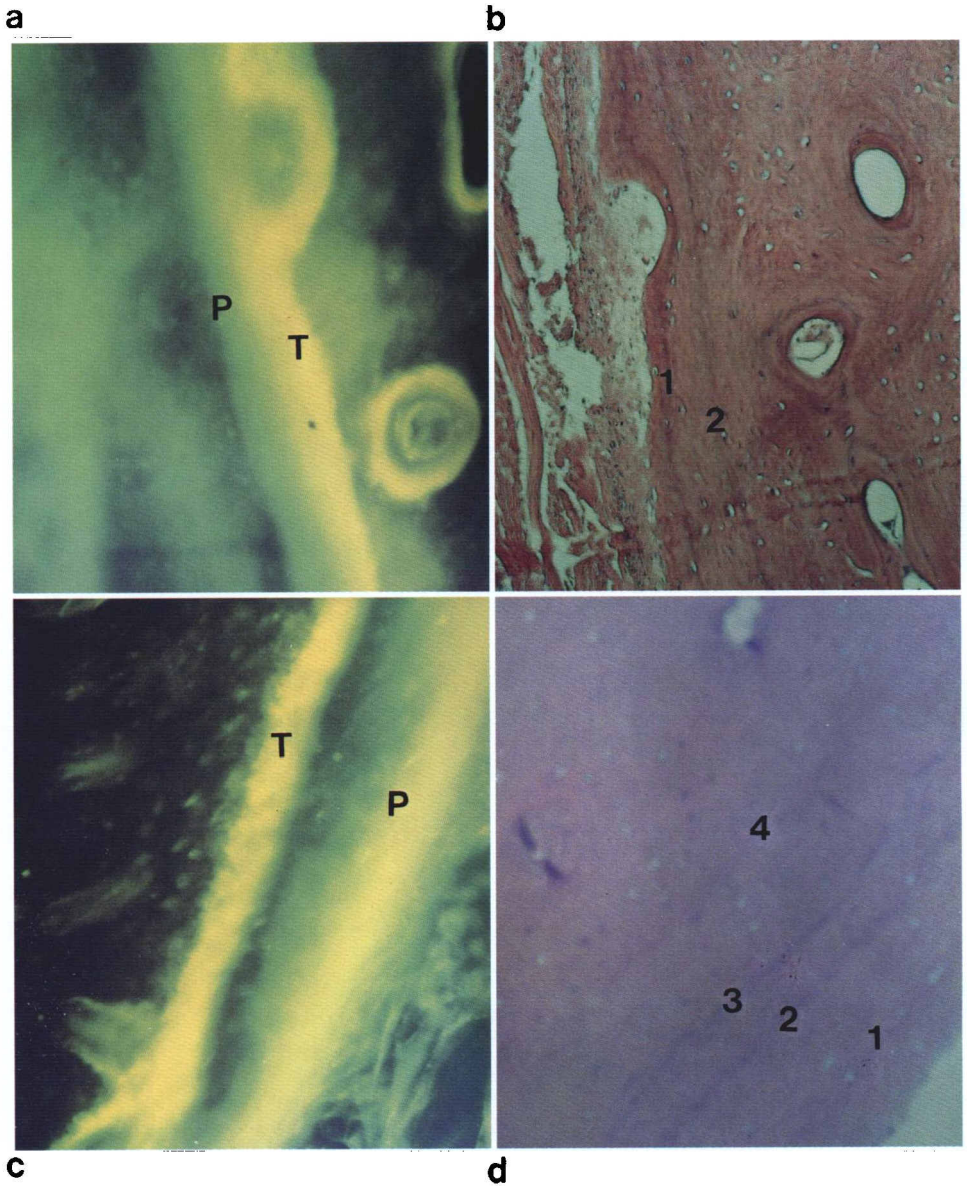
Figure 1a–d: a, tetracycline band (T) observed in the bone biopsy of a 1-year recapture (30-86). Periosteum (P) is also indicated. b, bone growth layers in decalcified section of a 1-year recapture (30-86). Layer numbers are indicated. c, tetracycline band (T) of a 3-year recapture (122-84). d, bone growth layers in decalcified section of a 3-year recapture (122-84).

The loggerheads recaptured after 1 year (30-86, 36-86, 45-86 and 53-86) revealed one growth layer outside the tetracycline mark (Fig. 1a, b). Specimen 42-85, recaptured after 2 years, had two growth layers which measured 12 μm and 19 μm from the periosteum. The tetracycline mark was observed at 22 μm from the periosteum. A 3-year recapture, 122-84, had a total of eight growth layers in the decalcified section. The four outer growth layers were 4 μm, 10 μm, 15 μm and 67 μm from the periosteum. The tetracycline band, 18 μm from the periosteum, was between the third and fourth growth layers. Loggerhead 120-84 was also

Table 1. Recapture data of tetracycline tagged loggerheads including straight line carapace length (CLS) and the number of growth layers observed in resultant bone biopsies

Turtle No.	Tetracycline date	CLS (cm)	Biopsy date	CLS (cm)	Total growth layers
65-84	7/12/84	81.4	9/25/85	83.2	7
30-86	6/27/86	70.0	7/22/87	70.3	9
53-86	10/15/86	63.1	7/8/87	64.8	10
36-86	7/3/86	68.2	7/20/87	68.7	10
45-86	8/6/86	55.0	9/1/87	58.1	5
42-85	6/6/85	83.4	7/22/87	86.5	14
122-84	8/13/84	65.1	7/22/87	68.2	8
120-84	8/13/84	82.1	9/10/85	82.5	nd*
			7/22/87	82.9	10

* nd = not determined.

recaptured 3 years after tetracycline injection. The four outer growth layers were 5 μm, 14 μm, 20 μm and 56 μm from the periosteum. The fluorescent band, 42 μm from the periosteum was deposited between the third and fourth growth layers (Fig. 1c, d). Total growth measurements were very similar for calcified and undecalcified tissue preparations in all recaptures, such that tissue stretching was considered negligible.

Growth Layers and Age Estimation of Stranded Loggerheads.—In the course of Chesapeake Bay turtle surveys, humeri were obtained from 90 dead (stranded) individuals. Based on the results of the tetracycline data, growth layer counts from humeri cross sections were used to estimate age for these immature loggerheads. Short axis readings were used for further analysis since growth layers on the long humeral axis were often unreadable. Seventy eight percent of examined turtles had between six and ten growth layers. However, the distance between growth layers was highly variable among analyzed individuals (Table 2).

Despite the demonstration of annual growth layer deposition in juvenile loggerheads, one confounding factor prevents a direct assignment of age from the number of observed growth layers; the expansion of the interior medullary cavity during growth. In larger turtles, this spongy core can be wider than the entire humeral cross section of smaller turtles, such that earlier growth layers are most certainly lost or resorbed during growth. Therefore, to correlate actual layer count with age, back calculations were used to estimate length at time of first growth layer. Log linear regression of bone radius length against carapace length was

Table 2. Distance (μm) between relevant growth layers in relation to tetracycline band, as measured from the periosteum. All measurements were taken from the short axis at 10× magnification

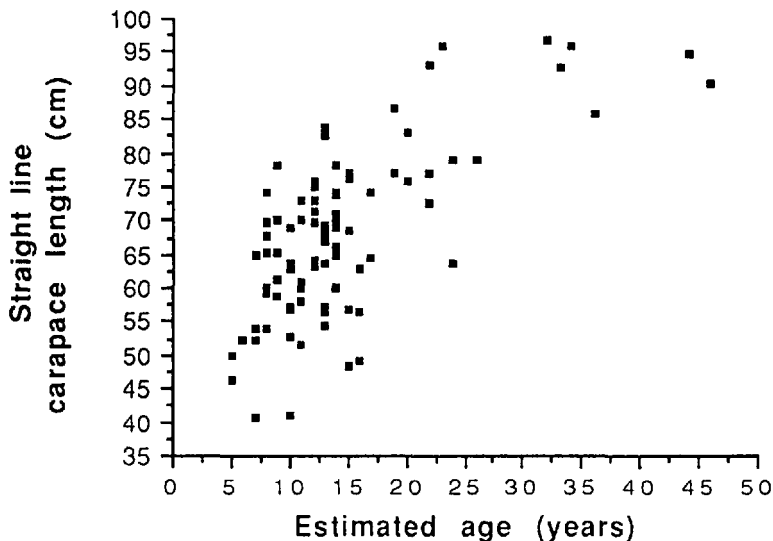| Turtle No.	Duration (years)	Distance (μm) of periosteum to					Tetracycline band
		layer 1	layer 2	layer 3	layer 4	core	
65–84	1.28	22	64	103	137	270	50
30–86	1.07	7	18	20	80	468	11
53–86	0.81	1	10	32	74	490	3
36–86	1.05	1	7	27	84	530	3
45–86	1.07	24	40	76	98	118	35
42–85	2.13	12	19	29	69	190	22
122–84	2.94	4	10	15	67	231	18
120–84	1.08	22	52	nd*	nd	nd	41
	2.90	5	14	20	56	230	42

*nd = not determined.

Figure 2. Estimated age (years) of juvenile loggerheads. N = 90.

significant ($r^2 = 0.51$, F = 31.74, $P < 0.001$). Length at the first growth layer calculated with the Fraser-Lee equation was linearly regressed with length at capture. Since the correlation was significant ($r^2 = 0.72$), that is, larger lengths at capture had larger estimated length at growth layer one, layer loss into the interior medullary cavity is probably occurring on a continual basis. Estimates of growth layer loss were determined with a log linear regression ($y = 1.6905x^{0.8823}$) of radius length against observed layer number (Ketchen, 1975). The smallest loggerhead reported during collection (40.5 cm CLS), with a medullary cavity diameter of 1.02 mm, was used as a reference or point of no layer loss. The difference in core diameter from larger turtles was used in the regression equation to calculate the number of resorbed layers. For each humeral cross section, the calculated number of resorbed layers was added to the number of observed layers, yielding an estimate of true age for loggerheads in Chesapeake Bay. After correcting for resorbed layers, 83% of juvenile loggerheads (those in the 40–80 cm CLS range) had estimated ages of 5 to 15 years (Fig. 2). A few larger turtles (>80 cm CLS), possibly representing occasional subadult visitors to Chesapeake Bay, had estimated ages of 13 to 46 years (Fig. 2). In a companion report (Klinger and Musick, in prep.), we analyze this data with conventional growth curves to generate estimates of age at sexual maturity.

DISCUSSION

The temporal pattern of growth layer deposition must be established in order to use skeletal hard parts for age determination (Beamish and McFarlane, 1983; Brothers, 1987). In the case of juvenile loggerheads sampled in Chesapeake Bay, the demonstration of annular deposition in bone layers allows a greater understanding of age and growth patterns.

In all cases where a fluorescent tag was visible, growth layer number and pattern was consistent with annular deposition. We conclude that growth layers are deposited on an annual basis in juvenile (40–80 cm CLS) loggerheads, at least in the temperate Chesapeake Bay area. However, it is possible that annular growth layers are not deposited in other life history stages. If hatchlings inhabit a tropical

or warm temperate pelagic habitat, as is widely believed (Carr, 1986), growth at that stage may be of a continuous (noncyclic) type, or may reflect patchiness in food availability. Alternately, growth layers in mature females may result from calcium depletion during egg formation. In these animals bone growth layers may indicate calcium alteration during nesting occurrences, rather than yearly growth layers (Colin Limpus, pers. comm.). Nonetheless, these data indicate that annular growth layers are deposited during the juvenile life history stage. Linear regression of back calculated length against length at capture strongly suggests that the first few growth layers in juveniles (whether annular or not) are obscured or absorbed by subsequent expansion of the medullary core. This resorption process probably continues throughout all growth stages.

It was not possible to determine if smaller loggerheads (<40 cm CLS) also absorb growth layers since no specimens available in this study were less than 40.5 cm. Back calculations were based on the smallest turtle, which (for the purposes of this analysis) was assumed to be the point of initial layer loss. Capture of smaller turtles might demonstrate that layer loss occurs at earlier stages. Therefore, analysis of turtles in the 10 to 40 cm size range is needed to completely document loggerhead growth rate.

Age estimates for marine turtles are difficult to generate due to suspected long lifespans and slow growth rates (Balazs, 1982; Limpus, 1979). Growth rates among individuals in Chesapeake Bay were highly variable, consistent with conclusions drawn from past studies on juvenile loggerheads (Bjorndal and Bolten, 1988; Mendonca, 1981). This variation in growth rate may be attributed to differences in feeding success between individuals, especially in the early life history stages.

Annular growth layers, as demonstrated by tetracycline tagging, can increase our understanding of age and growth in these animals. However, several mediating factors, including growth layer resorption and habitat switching (in successional life stages) must be considered in any analysis of age and growth based on bone layering and deposition. Our study establishes for the first time the annual deposition of growth layers in a wild population of juvenile loggerhead sea turtles. Analysis of these annual growth layers confirms that loggerheads grow and mature slowly relative to other vertebrates. These data provide an additional perspective on conservation issues; rookery protection strategies might require decades to yield tangible results, and depleted populations of this threatened species will take many years to recover.

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