Editorial Comment. – This section presents research reports based on support provided by Chelonian Research Foundation through the Linnaeus Fund, its annual turtle research awards program. Named after CAROLUS LINNAEUS [1707–1778], the Swedish creator of binomial nomenclature, the fund honors the first turtle taxonomist and father of all modern systematics. Linnaeus Fund awards are granted annually to individuals for specific turtle research projects, with either partial or full support as funding allows. Priority is generally given to projects concerning freshwater turtles, but tortoise and marine turtle research proposals are also funded. Priority is given to the following general research areas: taxonomy and systematic relationships, distribution and zoogeography, ecology, natural history, and morphology, but other topics are also considered. Priority is also given to projects that demonstrate potential relevance to the scientific basis and understanding of chelonian diversity and conservation biology. The generally preliminary and summary reports in this section are not formally peer-reviewed, but are nonetheless subjected to editorial review.

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Age Estimation of *Eretmochelys imbricata* by Schlerochronology of Carapacial Scutes. Linnaeus Fund Research Report

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Hawksbill turtles (*Eretmochelys imbricata*) have long been harvested intensively for the carapacial scutes known as tortoiseshell or bekko (Meylan and Donnelly, 1999). Bekko contains alternating layers of α and β keratin (Baden and Maderson, 1970; Webster, 1972) that compose an individual chronology of growth marks (GMs) within the keratin laminae (Zangerl, 1969).

Age estimation and growth rates are standard parameters required for demographic studies of wild populations. Fisheries managers use GMs in otoliths to estimate age in fish stocks and growth rings in scutes are often suited to estimate age in studies of freshwater or terrestrial turtles (Zug, 1991). Studies of age estimation in marine turtles, however, have tended to focus on GMs deposited in long bones rather than scute chronologies (Hohn and Frazier, 1979; Zug and Parham, 1996; Germano and Bury, 1998). Nevertheless, there is clear evidence of annual deposition of keratin in scutes of hawksbills (Tucker and Limpus, 1995), which fulfills a necessary condition for schlerochronology to be a reliable technique for age estimation (Beamish and McFarlane, 1983).

We strongly believe that a detection technique for counting GMs should be consistent with the rigorous technical procedures developed in the fisheries literature. These procedures rely on independent verification by microscopic viewing of specimens to attain accuracy and precision in counts. A practical obstacle to overcome is that hawksbill turtles commonly abrade the carapace on coral reefs, thereby eroding the dorsal layers of keratin at an unknown rate. Thus, any attempt to develop an age estimate from the GMs in scutes requires a systematic investigation of both keratin growth and abrasion.

The present study applied standard schlerochronology techniques to hawksbill scutes to discern patterns of keratin growth and abrasion. We document the occurrence of internal GM patterns of keratin lamina by microscopic examination and compare the results to earlier studies (Carrillo et al., 1998; Kobayashi and Diez, 1998) that counted pigment patterns visible on the keratin surface (Fig. 1).

We adopted the preferred terms for histological structures in hard tissue as used in the fisheries literature that has long-established protocols for skeletochronology of otoliths. Similar terminology is used in chronological studies of reptiles to designate a growth mark (GM) as a couplet of a wide growth zone and a narrow line of arrested growth (Castanet, 1994). The internal GMs within hawksbill scutes can probably be termed annuli because the validation criterion (that one growth ring is deposited once a year) holds at least for hawksbills of the Pacific Ocean (Tucker and Limpus, 1995). We acknowledge that not all populations need have an annual growth cycle.

A preliminary study (Tucker and Dalgleish, unpubl.) found that GMs were evident in hawksbill scutes but noted that counts are affected by scute location and the degree of abrasion. To address these concerns, the present

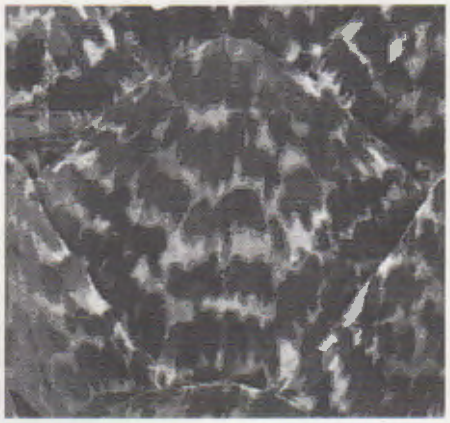

Figure 1. Juvenile hawksbill turtle (*Eretmochelys imbricata*) illustrating a pigment pattern on the scutes. Counts of these pigment patterns have been used by some studies to estimate age (Carrillo et al., 1998; Kobayashi and Diez, 1998).

study set several aims: (1) to characterize the formation and retention of microscopic GMs within and between scutes by comparing counts of annuli from multiple transverse sections, (2) to examine the utility of these microscopic GMs versus macroscopic dorsal pigment bands in scutes for age estimation in turtles of different sizes, and (3) to assess the utility of microscopic GMs as a technique to estimate age structure in wild or harvested populations.

Materials and Methods. — Scute specimens were obtained from the Queensland Museum, specimens confiscated by the Australian Quarantine and Inspection Service, and indigenous turtle harvests in the Solomon Islands and Fiji. Where possible, turtles were measured (curved carapace length to the nearest 0.1 cm) and sex determined. For Fijian specimens, only the raw scute was available and approximate CCLs were derived from regressions given by Limpus and Miller (1990). We typically used a hacksaw or bandsaw to excise small sections (ca. 1.5 cm wide) of scute suitable for sectioning. However, we also trialed a commercial "hot-knife" and a high-speed Dremel motor tool equipped with a cut-off disc to remove scute sections. We also experimented with removal of scute sections from a frozen carcass (Queensland Museum holdings) to judge the feasibility of the methods for use on live turtles. Careful test removals of scute material resulted in minimal abrasion of underlying carapacial tissue, but no more than has been observed in nature.

Samples were processed on a Leitz 1600 rotary diamond saw and mounted (ImPruv UV-cured Resin 363) on glass slides for examination by standard light microscopy. Serial sections of 80–300 μ were taken initially to establish the optimum thickness for clear identification of GMs. The degree of pigmentation influences the ease with which GMs can be identified but a 120 μ section gave adequate resolution for most specimens. Scute sections typically occupied several fields of view and to facilitate counting of GMs and reduce counting errors, we used 35 mm photography combined with digital scanning to obtain an enlarged paper print of an image. Several other options are available including video-capture or digital photography and, if required, the counting process can easily be automated (e.g., BonyParts 4.0; Brittnacher, 2001). Graphical models of keratin lamination were constructed in a graphics package.

During the initial phases while we sought to standardize the thickness of a specimen, we evaluated interpretations and between-reader reliability by counting GMs on each slide three times to derive a covariance, precision, and average percent error on successive viewings (Beamish and Fournier, 1981; Chang, 1982). Plots of GM counts against carapace length were evaluated to determine when erosion became a limiting factor on the accuracy of an age estimate (as based on maximum counts).

Results. — In all of the transverse sections we viewed, unambiguous evidence of periodic GMs as alternating narrow opaque lines and wide translucent zones could be identified in the stratified keratin structure (Fig. 2). New scute growth formed from a ventral basal layer of active keratin deposition. The active layer displaces the previous layers of beta keratin upward and away to the rear. In juvenile and subadult turtles, the surface area of the active growth layer also increases, enlarging with skeletal carapace growth. With growth, the vertical thickness of each successive keratin lamina was similar, but the horizontal shifting of layers became diminished. Thus, in large hawksbills, the process of scute growth was largely from vertical stacking rather than a stacking and horizontal displacement of keratin as seen in smaller turtles.

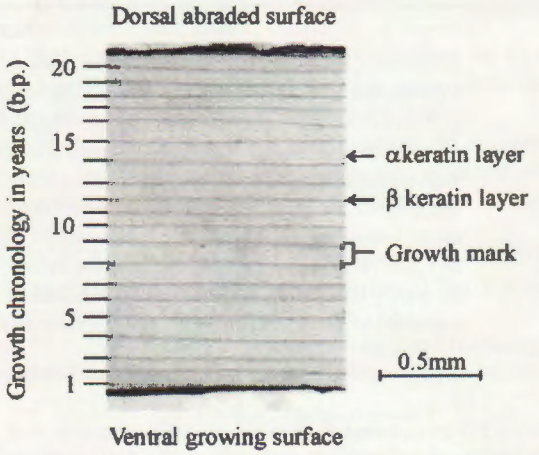

Figure 2. Periodic growth marks (GMs) appear as couplets of alternating wide translucent zones (α keratin) and narrow opaque lines (β keratin) in the stratified structure of a hawksbill carapacial scute.

A low coefficient of variation in visual counts of any given specimen confirmed the reliability for GM counts by independent observers or upon sequential viewing. Since counts derived by direct viewing will reliably identify a GM, a greater concern was whether counts were conducted on a scute from a carapace position that was prone to wear and tear.

Surface abrasion was evident in all larger animals and the exposure to abrasion varied both within and between scutes. The imbricated arrangement of scutes resulted in more abrasion at the posterior edge of a scute. The anterior regions of a carapace tended to display greater effects of abrasion. Thus, the greatest loss of GMs due to abrasion occurred on the posterior section of scutes on the anterior portion of the carapace. The highest counts of GMs were recorded in the middle of a sagittal strip of vertebral scutes 3–5. Despite these variations in exposure to surface abrasion, visual clarity of subsurface GMs was not affected.

Pigment band-patterns evident on the dorsal aspect of scutes (Fig. 1) often have a distinctive pattern that has been used by other investigators for age estimation (Carrillo et al., 1998; Kobayashi and Diez, 1998). These pigment bands were obscured or abraded on scutes of larger animals. Furthermore, the microscopic sections indicated that pigment bands were not actually a component of stratified

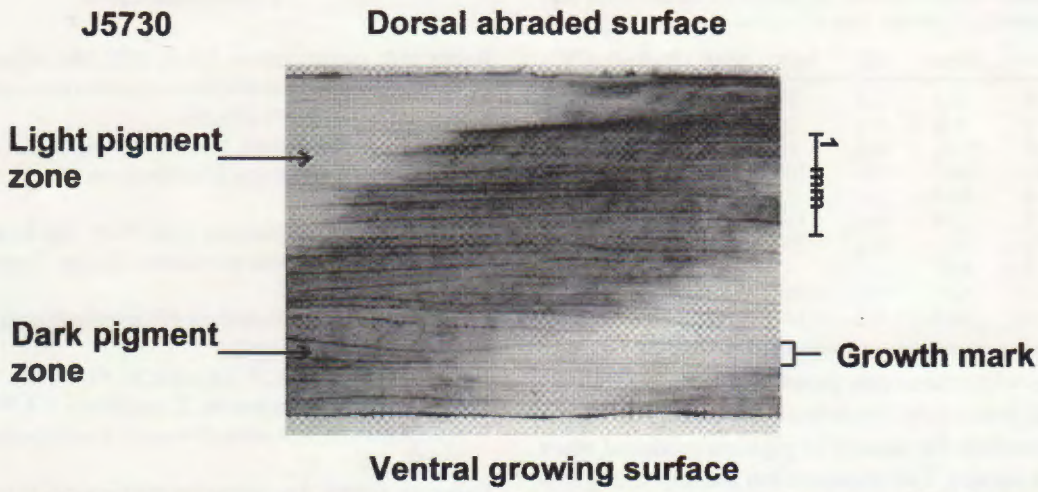

Figure 3. Microscopic view (sagittal section) of growth marks formed from stratified keratin can be seen to pass through pigmented regions within the scute of a hawksbill turtle. Hence, pigment bands are questionable as indicators of annual growth, particularly with older turtles, even if they are evidence of periodic growth.

keratin because the GMs clearly bisected the pigmented areas (Fig. 3) and therefore, pigment bands did not actually represent chronological age.

GM counts were correlated with increasing body size (Pearson r = 0.61), yet 63% of the variance was unexplained (r^2 = 0.37) because of vast differences in GM counts at a given size (Fig. 4). The highest count was 60 GMs in a 65.2 cm turtle, which was well below the minimum breeding size for the species. The increased variance with size suggested that retention of GMs was inconsistent among turtles and probably due to individual differences in erosion. A rough approximation of potential losses from surface abrasion came from the range (maximum-minimum count) of GM counts for turtles of a given size (Table 1, Fig. 4). For example, ranges progressed from a mean difference of 6 GMs at 30 cm, to 15 GMs at 40 cm, 13 GMs at 50 cm, 16 GMs at 60 cm, 40 GMs at 70 cm, and 30 GMs at 80 cm. The ranges represented several decades of a growth chronology that might be missing in subadult or adult size classes.

Discussion.—A major finding is that GMs were readily identified in hawksbill scutes by standard schlerochronology methods. Moreover, the technique revealed a more extensive growth chronology than previously identified by the pigment banding method. A maximum of 60 GMs was recorded by microscopic examination whereas a maximum of 20 was given by the pigment band method (Carrillo et al., 1998). Was there a 3-fold difference in GM detection because one technique gave increased resolution or because of different growth dynamics among Atlantic and Pacific hawksbills? The first assertion is definitely true, but the latter hypothesis requires more detailed comparisons of data from growth studies. Several such growth studies are in progress (Chaloupka and Limpus, 1997).

Schlerochronology may have limitations but its constraints are due to GMs lost via epidermal abrasion, rather than a lack of GM detection. Certainly, further efforts to better quantify GM loss from abrasion are warranted. Methods to back-count the missing GMs are potentially applicable to scute chronologies (Zug and Parnham, 1996). In addition, further work is required to determine the annual periodicity of these GMs, whether they are indeed always deposited annually or whether they may also represent non-annual periodic growth zones.

A second key finding of our study was a lack of correlation between pigment bands and GMs. For all size classes of turtles, there were laminae of keratin that transected the visible pigment bands. Even if pigmentation bands were periodic features in young turtles (and conjecture remains on this point), discrete pigment bands became indistinct in larger turtles once keratin lamination was more a vertical stacking process rather than a horizontal displacement process. Hence, the use of pigment bands to estimate age of hawksbills must be viewed with skepticism for large animals. Since even small hawksbills had GMs that transected the pigment bands, it must also be questioned whether these pigment bands accurately reflect age in small individuals. Thus, studies will need to document the orientation of pigment bands with respect to keratin laminae and how this changes with ontogeny. The relative position of pigment

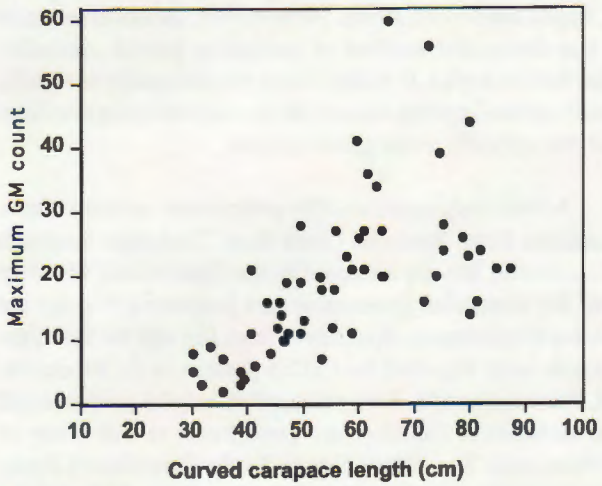

Figure 4. Maximum counts of growth marks (GM) plotted against carapace length in hawksbill turtles.

Table 1. Growth marks counted in hawksbill turtles from size classes, grouped by 5 cm size bins of curved carapace length.

Size class	*n*	Mean	SD	Min	Max	Range	CV
30-35	4	6.3	2.8	3	9	6	44.1
35-40	5	4.2	1.9	2	7	5	45.8
40-45	5	12.4	6.1	6	21	15	37.3
45-50	11	14.5	5.6	10	28	18	31.7
50-55	4	14.5	5.8	7	20	13	33.7
55-60	8	22.4	9.6	11	41	30	91.4
60-65	8	26.5	6.1	20	36	16	37.4
65-70	1	60.0	-	-	-	-	-
70-75	4	32.8	18.4	16	56	40	56
75+	9	24.4	8.6	14	44	30	35.2

bands moves with time as new growth displaces older layers upwards and posteriorly. Epidermal cells must also have a capacity to mediate the amount of pigment produced when laying down keratin. This phenomenon was especially evident in larger turtles whose carapace had a mottled appearance from multi-layered patches of pigment with different coloration. The combination of spatial variation in pigment deposition and subsequent scute growth produces substantial variation in patterns of carapace coloration.

The visible pigment-bands on Cuban hawksbill turtles suggested that a maximum age of hawksbills was about 17 yrs (Carrillo et al., 1998). On the contrary, insights from our study suggest that additional GMs may simply have gone undetected, as we observed up to 60 GMs in turtles that were not yet adult size. Further work is clearly needed to resolve the differences between these two technologies. Any demographic study that was based upon age estimates from GM detection could obviously come to highly divergent conclusions, depending upon the respective technique (and its associated assumptions) that was chosen (see Heppell, 1996).

We conclude that schlerochronology offers a significant improvement over pigment banding for counting the GMs in hawksbill scutes. Schlerochronology is a basic and proven method for evaluating scutes from hawksbill turtles of all size classes. Extensions of this pilot study may prove useful to recover additional data from stockpiles of scutes concerning the growth chronology of harvested hawksbills (Limpus and Miller, 1990). Furthermore, the results suggest a non-destructive method of estimating growth chronologies for live turtles. Both directions would contribute significantly to the ongoing conservation and management efforts for this critically endangered species.

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