Abstract—Skeletochronological data on growth changes in humerus diameter were used to estimate the age of Hawaiian green seaturtles ranging from 28.7 to 96.0 cm straight carapace length. Two age estimation methods, correction factor and spline integration, were compared, giving age estimates ranging from 4.1 to 34.6 and from 3.3 to 49.4 yr, respectively, for the sample data. Mean growth rates of Hawaiian green seaturtles are 4–5 cm/yr in early juveniles, decline to a relatively constant rate of about 2 cm/yr by age 10 yr, then decline again to less than 1 cm/yr as turtles near age 30 yr. On average, age estimates from the two techniques differed by just a few years for juvenile turtles, but by wider margins for mature turtles. The spline-integration method models the curvilinear relationship between humerus diameter and the width of periosteal growth increments within the humerus, and offers several advantages over the correction-factor approach.

Age and growth of Hawaiian green seaturtles (Chelonia mydas): an analysis based on skeletochronology

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The Hawaiian population of the green seaturtle (Chelonia mydas) provided some of the first published growth data (Balazs, 1979, 1980, 1982) for this species. These early data showed how slowly seaturtles grow and how long a female must survive simply to lay her first clutch of eggs. Twenty or more years to reach sexual maturity seemed biologically unrealistic, yet slow growth and late maturity has been repeatedly confirmed for some seaturtle species, e.g. Bahamian C. mydas (Bolten et al., 1992), West Atlantic Caretta caretta (Parham and Zug, 1998). Slow growth and the resulting delayed maturity greatly affect the demography of a population (Crouse et al., 1987; Chaloupka and Musick, 1996). An understanding of growth and growth-pattern variation in seaturtles is an important prerequisite to the development of population models that are required to guide seaturtle population recovery and conservation.

Green seaturtles within the Hawaiian Islands contribute to the larger Indo-Pacific C. mydas gene pool, yet the nesting females of the Hawaiian population comprise a distinct genetic unit and contain a unique mtDNA haplotype (Bowen et al., 1992). Except during their posthatching pelagic phase, the great majority of Hawaiian green seaturtles reside in coastal waters, primarily around Hawaii, Kauai, Maui, Molokai, Oahu, and other islands in the southeastern part of the Hawaiian chain. Most reproduction takes place at French Frigate Shoals in the Northwestern Hawaiian Islands (Balazs, 1980; Wetherall et al., 1999). The population of Hawaiian Chelonia mydas has benefited from over two decades of intense conservation management (Balazs, 1998). Despite important early research on the Hawaiian population of C. mydas (Balazs, 1982), the patterns of growth within and among the geographic habitat components of this population have remained incompletely documented (Balazs et al., 1994, 2000). The chief reason for this has been a lack of methods to age sea turtles. This deficiency has been overcome recently by the development of skeletochronological techniques that estimate age from the number of growth increments formed on the humerus (Parham and Zug, 1998).
Our goal in this study was twofold. First, we used humerus growth-increment data to estimate ages of a sample of Hawaiian green sea turtles from various locations in the archipelago and developed a growth model for the general Hawaiian population; geographic variation in growth will be addressed in a subsequent paper. Second, we compared two different methods of deriving the age estimates, the so-called “correction-factor” method described by Parham and Zug (1998) and a newer approach, the “spline-integration” method, introduced in the present study.

Materials and methods

Our sample consisted of 104 individuals of C. mydas, collected from the islands of Hawaii, Kauai, Lanai, Maui, Oahu, and the Northwestern Hawaiian Islands; the Oahu sample predominated with 64 individuals. All individuals were measured to the nearest 0.1 cm straight carapace length (SCL). The smallest individual was a 5.3-cm-SCL hatchling. The smallest posthatchling was estimated to 0.1 cm straight carapace length (SCL). All individuals were measured to the nearest 0.1 cm straight carapace length (SCL). The smallest individual was a 5.3-cm-SCL hatchling. The smallest posthatchling was a pelagic juvenile (of assumed Hawaiian origin) recovered from the former squid drift net fishery north of the island chain. All other posthatchling turtles were from coastal Hawaiian waters, found stranded dead and retrieved by the National Marine Fisheries Service’s Hawaiian Islands Sea Turtle Stranding Network. The salvaged turtles ranged from 28.7 to 96.0 cm SCL. The sample was divided into eight 10-cm size classes; representation was roughly equivalent for the middle six classes (Fig. 1). The 30–39 cm sample contained only turtles in the upper quartile of this size class. Each turtle was necropsied and its right humerus removed for skeletochronological examination. The necropsy data included a complete set of carapace measurements, organ condition evaluations, and information on fibropapilloma tumor occurrence and severity; see Work and Balazs (1999a, 1999b) for details on the entire data set. In addition to the skeletochronological data, we used only the SCL measurements and tumor-evaluation observations in the present study.

Where possible, we selected tumor-free individuals for the present analysis because our goal was to examine the overall growth pattern for normal Hawaiian Chelonia mydas. Because the prevalence of fibropapillomatisis is high in the wild Hawaiian population (Murakawa et al., 2000), we included in our sample individuals with fibropapillomas, but otherwise appearing normal, in order to ensure adequate representation in the larger size classes. Healthy animals were those showing no evidence of weight loss or other indicators of illness and no evidence of disruption or retardation of normal growth. Individuals with tumors represented 27% of the 50–59 cm, 50% of 60–69 cm, 76% of 70–79 cm, 73% of 80–89 cm, and 17% of 90–99 cm SCL size classes (Fig. 1). Our skeletochronological data derived from cross-sections (0.6–0.8 mm thick) from the middle of the humeral shaft just distal to the deltopectoral crest and at the narrowest diameter of the diaphysis (Zug et al., 1986). On each specimen, we counted the number of visible growth layers and measured the widths (long-axis diameters) of the humerus at each successive growth cycle and the width of the resorption core. Bone sections were taken from mid-shaft, the narrowest location of the bone, because the humerus retains the greatest number of periosteal growth layers there, and hence this location permits the most accurate estimation of the number of growth cycles (periosteal layers) and the relative rates of growth (=successive humerus diameters).

We used two procedures for estimating the total number of growth layers, and hence age, of each turtle. In the correction-factor (CF) method, as described in Parham and Zug (1998), the turtle’s age is estimated as the number of growth layers observed in the outer region of the humerus section plus the predicted number of resorbed growth layers represented in the remodeled core of the humerus. The latter, unobservable component is estimated as C (R – R_A), where R is the radius of the absorption core, R_A is the radius of a hatchling’s humerus (before the beginning of increment formation), and C is the so-called correction factor. The correction factor is a constant “aging rate” (yr/mm) assumed to apply to the resorption core, and calculated as the reciprocal of the mean growth layer width in small turtles. The mean growth layer width was estimated from 129 periosteal growth layer widths observed in 34 turtles.

Figure 1

Size (straight carapace length) distribution of the Hawaiian Chelonia mydas skeletochronological sample. The members of each class are segregated into individuals without (shaded bar) and with (black bar) fibropapilloma tumors. Tumors in our sample are present only in larger turtles.
with SCLs <60 cm and resorption core diameters <19.0 mm. Selecting only small turtles with minimum core diameters reduces the frequency of the narrower periosteal layers found in the outer margin of the humerus in larger turtles; hence, it reduces the possibility of overestimating the number of layers in the resorption core. The resulting correction factor was used to estimate the number of resorbed periosteal layers.

A second method, spline integration (SI), is introduced here. The SI method uses a scatterplot smoothing spline (Härdle, 1990; Hastie and Tibshirani, 1990) to model the relationship between the aging rate and humerus diameter. Once the aging function is estimated, a turtle's age is estimated by integrating the spline over the total diameter of the turtle's humerus section. The method of modeling increment width patterns in hard parts and of estimating age by the integration of the resulting aging function was first formalized by Ralston and Miyamoto (1983) for a Hawaiian snapper and first applied to sea turtles by Zug et al. (1995). In those applications, the aging rate was a parametric function of size. In our analysis, we modeled the aging rate nonparametrically by fitting a smoothing spline to pairs of observations of growth-layer width and humerus diameter. The SI approach uses the same source of data as the CF method but without selection. Lines of arrested growth (LAG) delimit each observable growth layer. Increment width is measured as the difference between the humerus diameters at the outer LAG and the inner LAG. Assuming an increment represents one year of growth, each increment width measurement provides a measure of the humerus growth rate (mm/yr) and its reciprocal, a measure of the aging rate (yr/mm) at the observed humerus diameter (the mean diameter of the pair of LAGs). The skeletochronological sample yielded 269 such observations of aging rate and humerus diameter. The aging rates were grouped in 1-mm intervals of humerus diameter and averaged. A cubic smoothing spline was fitted to the mean aging rates by using S-PLUS (MathSoft, Inc., 1999). The age (yr) of each turtle was estimated by integrating the aging spline from its origin to the observed outside diameter of the humerus section.

To assess the effect of the estimation method on age estimates, the data were divided into 10-cm SCL groups. Within each group a Student's t statistic was used to test the hypothesis that the two methods give equal age estimates.

Nonparametric growth models were estimated based on the CF- and SI-derived age estimates and associated carapace lengths, by using the same S-PLUS procedure employed for the SI-method aging spline. The validity of the growth models was judged qualitatively by comparing growth predicted by the models with observed growth in a sample of 171 Hawaiian green turtles tagged and recaptured in waters around Molokai (Balazs et al., 1999).

To assess uncertainty in the SI-based growth curve, the 269 pairs of aging rate and mean humerus diameter data were resampled 100 times, and the SI procedure applied to each bootstrap replicate data set. The 100 aging curves derived in this manner generated a bootstrap distribution of estimated age for each turtle. Nonparametric growth curves were then fitted to each derived data set, producing bootstrap distributions of predicted mean length at age. Empirical confidence intervals for the predicted mean length at age were approximated by using percentiles of the latter bootstrap distributions.

A linear regression of SCL on outside humerus diameter was estimated for the 104 sample turtles. The slope of the linear predictor was applied to the 269 humeral increments to estimate a corresponding set of carapace increments, presumed to represent annual growth. These growth rate estimates were summarized in box plots over 10-cm intervals of SCL. Mean growth rate as a function of estimated age was also estimated by computing finite differences of the SI-based growth model.

Results

Patterns in humerus growth and aging

Carapace length has a strong linear association with humerus diameter (Y =0.643 + 2.326X [where Y =SCL cm; X =humerus diameter mm], r²=0.98, P <0.001, n=104 including the hatchling). Humerus growth-increment width, on the other hand, is nonlinearly associated with humerus diameter at the point of growth (Fig. 2). Specifically, growth increments tend to be larger when the turtles are smaller (i.e. at smaller humerus diameters) and decline as the turtles grow. Variation in humerus increment width (growth rate) shows the same pattern. The estimated aging rate, as the reciprocal of growth rate, increases as the turtles grow. The aging rate does not increase uniformly (Fig. 3). Rather, it increases gradually in small turtles, plateaus over a broad range of length for mid-size turtles, increases abruptly as turtles approach maturity, and maintains an increased rate as the mature turtles grow.

Age and growth-rate estimates

In the CF-method analysis, the correction factor, C, was estimated as 1.14 yr/mm. The resulting age estimates range from 4.1 to 34.6 yr (n=70; excluding the hatchling with age zero). The smallest turtle in the sample had the lowest age estimate and the two largest turtles, the highest estimates. Only 68% could be aged by the CF method. Skeletochronology requires a pattern of distinct layering within the bony element examined. Such patterns are most evident in the smaller, presumably younger, individuals, and the frequency of individuals with distinct periosteal layers decreases as body size increases. In selecting specimens for the CF analysis, growth layers were sufficiently distinct to estimate the number of resorbed layers, and hence the age, in decreasingly fewer turtles: 89% of turtles in the 30–69 cm SCL group, 72% in the 70–79 cm group, 38% in the 80–89 cm group, and 29% in the >89 cm group were used in the CF analysis. Of the individuals for which we were unable to obtain an estimate of resorbed layers, a nearly equal number (48%) had fibropapillomas. The prevalence of tumors for the CF-aged subsample (31%) was somewhat less than in the total sample (37%). Importantly, the tumor prevalence in the subsam-
Figure 2
Humerus growth increment width (the increase in humerus diameter) in relation to humerus diameter (mean of the inner and outer diameters). Mean diameter better reflects the size of the humerus during the entire growth interval than does outer diameter.

Figure 3
Estimated "aging rate" (average of the reciprocals of the humerus growth-increment widths) in relation to humerus diameter and a fitted smoothing spline. The expected age at a given humerus diameter is obtained by integrating the spline up to the specified diameter. Not all members of the sample could be aged by the CF method; see explanation in "Materials and methods" section.
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Figure 4
Relationship between observed carapace length and estimated age (squares) for the two methods of age determination: correction factor (A) and spline integration (B). Fitted growth models (curves) are cubic smoothing splines.

Table 1
Mean age estimation for correction-factor and spline-integration methods for 10-cm length groups. Estimates of mean age vary significantly between estimation methods for turtles in the middle length groups. ** indicates a significant difference.

<table>
<thead>
<tr>
<th>Length group (SCL, cm)</th>
<th>Sample size</th>
<th>Mean age estimate (yr)</th>
<th>Correction-factor method (CF)</th>
<th>Spline-integration method (SI)</th>
<th>t [df], P</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-40</td>
<td>14</td>
<td>7.3</td>
<td>6.9</td>
<td>1.09 [13], 0.296</td>
<td></td>
</tr>
<tr>
<td>40-50</td>
<td>10</td>
<td>12.9</td>
<td>11.0</td>
<td>4.36 [9], 0.002**</td>
<td></td>
</tr>
<tr>
<td>50-60</td>
<td>13</td>
<td>19.1</td>
<td>15.8</td>
<td>4.66 [12], 0.001**</td>
<td></td>
</tr>
<tr>
<td>60-70</td>
<td>14</td>
<td>22.1</td>
<td>22.1</td>
<td>-0.06 [13], 0.952</td>
<td></td>
</tr>
<tr>
<td>70-80</td>
<td>13</td>
<td>25.3</td>
<td>25.6</td>
<td>-0.67 [12], 0.514</td>
<td></td>
</tr>
</tbody>
</table>

nological data (Fig. 7). In plotting the growth vectors for marked (tagged) turtles, we fixed the origin of each vector by assuming that the carapace length at time of first capture was given exactly by the CF- or SI-based growth curve. The observed length at recapture and time at liberty then determined the endpoint coordinates of the growth vector. Despite considerable variation in the observed growth of the marked turtles, the growth vectors were generally concordant with the growth model predictions (Fig. 7).
Age- and length-specific growth rates

The box plots of the SI-estimated carapace growth rates (Fig. 8) indicated relatively fast growth for smaller turtles, a reduced growth rate remaining fairly constant over intermediate length classes, and declining growth rates in larger, mature turtles. Mean carapace growth rate declined from 4.4 cm/yr for turtles in the 20–30 cm SCL group to less than 1 cm/yr for mature turtles in the 90–100 cm group, and remained around 2.0–2.5 cm/yr for immature juveniles in the intermediate length groups (Table 2, Fig. 8). Differences in mean growth rate among the intermediate length groups were not significant. The average growth rates predicted by first differences of the SI-based growth model (Fig. 9) indicated a similar pattern, as expected, showing a decrease in growth rate during the first decade of life (when Hawaiian green turtles are still foraging in the open ocean or in the early years of their residence in inshore habitats), relatively constant growth during the next 15–20 year interval, and a further decline in growth rate as the turtles approach 30 years of age. The growth rate appears to remain low in older turtles.

Discussion

Age and growth-rate estimates

Age estimates by both methods indicate an age range of 4–49 years for the Hawaiian green seaturtles in their coastal habitats. The pelagic juvenile (28.7 cm SCL) in our sample was about four years old (4.1 and 3.3 yr by CF and SI methods, respectively). The smallest juveniles (35–37 cm SCL) in coastal waters were 6 to 9 years old by the CF
method and 4 to 10 years old by the SI method. These age estimates for Hawaiian greens in the last years of their pelagic developmental stage are similar to those reported for C. mydas populations in the southern Great Barrier Reef (SGBR) (5–6 yr; Chaloupka et al., in press) and for the Atlantic coast of central Florida (3–6 yr; Zug and Glor, 1999). The smallest C. mydas turtle in the Florida sample was 28 cm SCL (several others were less than 35 cm), whereas the smallest SGBR turtle was 38.5 cm CCL (Limpus and Chaloupka, 1997) and the smallest Hawaiian specimen was 34.8 cm SCL, indicating an earlier shift from pelagic to benthic life in Florida greens.

The growth of the juvenile turtles predicted by both CF and SI models is consistent with the growth observed in the Molokai mark-recapture sample, but predictions of the CF model depart from the tag-recapture results in older turtles (Fig. 6). Mean growth rates for smaller (30–60 cm) turtles estimated from our transformed humerus increment data (Table 2) were about half as high as growth rates reported for turtles of the same size in most Atlantic and Caribbean locales (based on tagging and skeletochronology; Tables 2 and 3 in Zug and Glor, 1999). Our estimates were similar to the observed growth rates of tagged turtles in Kiholo Bay, Hawai’i (Balazs et al., 2000) and nearly twice as high as rates observed in some other Pacific samples (Galápagos, Heron Island; Tables 2 and 3 in Zug and Glor, 1999). Subsequent studies of Australian populations (Limpus and Chaloupka, 1997; Chaloupka et al., in press) have shown a mid-juvenile growth rate more similar to our estimates; however, growth rate is associated with a growth surge in the Australian turtles over a narrow mid-juvenile length range (50–60 cm SCL). Such a spurt in growth was not evident in our SI-based growth curve, and growth rates were fairly constant in the 40–80 cm size classes (Fig. 8).

Our age and growth estimates pertain to the Hawaiian population as a whole, because the sampled turtles originated from locations throughout the archipelago. Some variation in age and growth between island foraging groups is likely, given the extensive latitudinal range of the habitats and the associated variation in physical and biological parameters affecting growth (Balazs, 1982). A geographic analysis of age and growth will be the subject of a future study. Future study will also investigate the
effects of fibropapillomatosis and gender on growth rates among the Hawaiian population.

**Age-estimation methods**

A key assumption of both the CF and SI methods is that each estimated humerus growth layer represents 1 year of growth. This assumption has been validated only recently (Hohn and Snover\(^1\)). Hawaiian turtles tagged and injected with tetracycline have been recaptured, and these turtles show the appropriate number of LAGs for the years since their receipt of tetracycline. Furthermore, strong support is provided by the consistency of the growth model predictions with observed growth in tagged Molokai turtles. Additional justifications have been advanced in other studies of seaturtle humerus LAG formation (e.g. Zug and Glor, 1998; Coles et al., 2001).

\(^1\) Hohn, A., and M. Snover. 2001. Personal commun. Beaufort Laboratory, Southeast Fisheries Science Center, Beaufort, NC.

Both the CF and SI methods require histological preparation and analysis of humerus sections. But they use the same skeletochronological data in independent and different ways to estimate the total number of humerus growth layers. The CF method assumes that a constant humerus growth rate (the "correction factor") applies to the resorption core regardless of the diameter of the core and despite the fact that periosteal increment width decreases with length of the turtle (Fig. 2). The correction factor, C, is estimated from a subset of the skeletochronological data taken from juvenile turtles only, i.e. excluding larger turtles likely to have narrower increments in the outer region of the humerus. Even so, the CF estimates of age for juvenile turtles appear to be biased upward (Fig. 5), suggesting that the constant correction factor also failed to reflect the effect of wider increments deposited in the early years of life. In age estimation, the CF method can be applied only to turtles displaying a complete set of periosteal layers, i.e. distinct LAGs, from the resorption core to the outer margin of the humerus.
The SI method models increment-width variation over the entire distance from humerus center to outer margin by using a nonlinear, nonparametric smoother. The model is estimated from all available sample data with two or more LAGs and associated diameter measurements without regard to size of the turtle. The SI model can be applied to age all turtles for which the outside diameter of the humerus section has been measured.

In the skeletochronological sample we studied, the CF method gave age estimates significantly higher than the SI method for turtles of intermediate length (Table 1), but expected differences for such turtles were no greater than about 2 years. On the other hand, based on current data the CF-based model gives much lower age estimates than the SI-based model for turtles longer than about 86 cm SCL (Fig. 5). Thus although either method may suffice for aging juvenile Hawaiian green seaturtles, only the SI method appears to provide support for inferences about growth in turtles larger than about 80 cm. The CF method is computationally simpler, because it involves only linear regression rather than fitting and integrating a nonparametric smoother, and this consideration may recommend it to some users.

Based on our experience with Hawaiian green seaturtle data, the main issue in judging the two techniques appears to be the assumption with the CF method that humerus growth is linear. In reality, it is curvilinear, and the SI method explicitly models this curvilinearity. Moreover, the SI method makes fuller use of available humerus increment data than the CF method. Further comparisons of the methods with additional skeletochronological data sets are recommended.

**Ecological précis**

1. *Chelonia mydas* within the Hawaiian Islands is a component of the larger Indo-Pacific *C. mydas* gene pool, yet the nesting females of the Hawaiian population comprise a distinct genetic unit and contain a unique mtDNA haplotype (Bowen et al., 1992).

2. Except for the posthatching pelagic phase, the green seaturtles of the Hawaiian coastal waters are year-round residents, and all known individuals reproduce within the Hawaiian island chain, predominantly on the beaches of the Northwestern Islands at French Frigate Shoals (Balazs, 1998; Wetherall et al., 1999).

3. Skeletochronological age estimates indicate that Hawaiian juveniles exit the pelagic phase between the ages of 4 to 10 years.

4. In coastal waters, juveniles 10 years and older possess a relatively constant growth rate until about 28 to 30 years (approximately 80 cm SCL), then growth begins to slow as individuals attain sexual maturity.

5. The mean SCL of nesting females is 92 cm (range 81-106 cm; Balazs, 1980), suggesting ages of 30 or more years at first nesting for some individuals.
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Figure 9
Growth rates of Hawaiian Chelonia mydas in relation to age and carapace length, estimated by taking first differences of the SI-based growth model.
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