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Use of skeletochronological analysis to estimate the age of leatherback sea turtles *Dermochelys coriacea* in the western North Atlantic

Larisa Avens[1,*], J. Christopher Taylor[2], Lisa R. Goshe[1], T. Todd Jones[3], Mervin Hastings[3,4]

[1]NOAA Fisheries, Southeast Fisheries Science Center, Center for Coastal Fisheries and Habitat Research, 101 Pivers Island Road, Beaufort, North Carolina 28516, USA

[2]National Ocean Service, Center for Coastal Fisheries and Habitat Research, 101 Pivers Island Road, Beaufort, North Carolina 28516, USA

[3]University of British Columbia, Department of Zoology, 6270 University Blvd., Vancouver, British Columbia V6T 1Z4, Canada

[4]Conservation and Fisheries Department, Ministry of Natural Resources and Labour, Government of the British Virgin Islands, Road Town, Tortola, British Virgin Islands

ABSTRACT: Although growth rate and age data are essential for leatherback management, estimates of these demographic parameters remain speculative due to the cryptic life history of this endangered species. Skeletochronological analysis of scleral ossicles obtained from 8 captive, known-age and 33 wild leatherbacks originating from the western North Atlantic was conducted to characterize the ossicles and the growth marks within them. Ages were accurately estimated for the known-age turtles, and their growth mark attributes were used to calibrate growth mark counts for the ossicles from wild specimens. Due to growth mark compaction and resorption, the number of marks visible at ossicle section tips was consistently and significantly greater than the number visible along the lateral edges, demonstrating that growth mark counts should be performed at the tips so that age is not underestimated. A correction factor protocol that incorporated the trajectory of early growth increments was used to estimate the number of missing marks in those ossicles exhibiting resorption, which was then added to the number of observed marks to obtain an age estimate for each turtle. A generalized smoothing spline model, von Bertalanffy growth curve, and size-at-age function were used to obtain estimates of age at maturity for leatherbacks in the western North Atlantic. Results of these analyses suggest that median age at maturation for leatherbacks in this part of the world may range from 24.5 to 29 yr. These age estimates are much greater than those proposed in previous studies and have significant implications for population management and recovery.

KEY WORDS: Sea turtle · Leatherback · *Dermochelys coriacea* · Skeletochronology · Skeletal growth mark · Line of arrested growth · LAG

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INTRODUCTION

Knowledge of juvenile stage durations, age at maturation, and reproductive longevity are essential for understanding the life history of any organism (Stearns 1992). Furthermore, the ability to relate age to reproductive effort is integral to studies of population dynamics, which in turn can inform management strategies for those organisms considered at risk (Sæther et al. 1996, Chaloupka & Musick 1997, Heppell et al. 2003a). Given the Vulnerable and Endangered status of most sea turtle populations worldwide (IUCN 2008), the need for age data to accurately characterize the status of these species is vital for conservation efforts (Heppell et al. 2003a,b).

Historically, studies of sea turtle age and growth have predominantly focused on cheloniid sea turtle species such as green turtles *Chelonia mydas* and log-

*Email: larisa.avens@noaa.gov

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gerheads *Caretta caretta*. As this field of research has developed, our understanding of the time frames over which individual marine turtles exist has undergone a significant transformation. Early on, these marine turtles were thought to possibly transition from hatchlings to mid-sized juveniles within a single year (Carr 1967). Extrapolation of short-term, rapid growth rates observed for captive juvenile sea turtles (Caldwell 1962, Uchida 1967, Hirth 1971) and wild turtles during the summer growing season (Mendonça 1981) suggested ages at maturation ranging from 4 to 15 yr.

Subsequent growth rate data obtained from captive marine turtles reared under semi-wild conditions (Frazer & Schwartz 1984) and wild mark-recapture studies conducted over longer time scales (Limpus 1979, Limpus & Walter 1980, Frazer & Ehrhart 1985, Braun-McNeill et al. 2008) indicated greater time to maturation than previously thought. Similarly, skeletochronology (the analysis of skeletal growth marks) has been applied to obtain age data (Zug et al. 1986, 1995, 2002, Klinger & Musick 1995, Parham & Zug 1997, Zug & Glor 1998, Snover 2002, Snover & Rhodin 2008), often resulting in markedly increased estimates of stage durations and age at maturity. For loggerhead and green turtles, the 'lost year' has been revealed to be the 'lost years'; estimates of oceanic stage duration range from 8 to 14 yr for loggerhead (Snover 2002, Bjorndal et al. 2003) and 3 to 6 yr for green turtles (Zug & Glor 1998). Predictions of age at maturation for these species now range from 30 to 40 yr (Limpus 1979, NMFS SEFSC 2001, Snover 2002, Zug et al. 2002, Heppell et al. 2003b) and are as high as 40 to 50 yr in the case of Hawaiian green turtles (Balazs & Chaloupka 2004).

Despite the advances made with cheloniid marine turtles, estimates of the time required for 6 cm leatherback sea turtle *Dermochelys coriacea* hatchlings (van Buskirk & Crowder 1994) to grow into 145 cm or greater adults (TEWG 2007) range widely. Whereas early analyses extrapolating the rapid growth rates observed for captive turtles yielded 2, 3, or 6 yr to maturity (Deraniyagala 1952, Birkenmeier 1971, Bels et al. 1988), more recent captive growth data indicate that leatherbacks may not mature until 12 to 18 yr of age (Jones 2009). The presence of vascularized cartilage in leatherback limb bones, a characteristic associated with rapid growth and more typical of endotherms, has suggested the potential for early maturation, perhaps between 3 and 6 yr of age (Rhodin 1985, reviewed by Snover & Rhodin 2008). However, genetic analyses describing possible mother–daughter relationships in a Caribbean nesting population estimate 12 to 14 yr to maturation (Dutton et al. 2005).

Skeletochronological analysis of putative growth marks observed at the lateral edge of sectioned scleral ossicles (small bones surrounding the pupil of the eye; see Fig. 1) yielded an average age at maturation of 13 to 14 yr for Pacific leatherbacks (Zug & Parham 1996). Subsequent analyses have supported the hypothesis that marks in the scleral ossicles of loggerhead and Kemp's ridley sea turtles represent annual cycles (Avens & Goshe 2007). However, these analyses also demonstrated that, due to lateral growth mark compression and central resorption, growth mark counts taken at ossicle section tips are far greater than those obtained laterally, suggesting that leatherback age might previously have been underestimated (Avens & Goshe 2007).

Here, we present a skeletochronological analysis of scleral ossicles obtained from leatherbacks in the western North Atlantic Ocean, to further characterize these structures and the growth marks within them. We analyzed ossicles from captive-reared leatherbacks of known age to calibrate interpretation of the deposition pattern and frequency of growth marks in the ossicles of wild turtles. We then analyzed ossicles obtained from wild leatherbacks to estimate age at maturation for the species in this region.

MATERIALS AND METHODS

Sample collection. From 2001 to 2008, scleral ossicles were obtained from leatherback sea turtles that stranded dead, were debilitated and later euthanized, or died in the course of permitted research; no turtles were purposefully sacrificed in the course of the present study. Eight juvenile leatherbacks obtained as hatchlings from the British Virgin Islands (Lambert's Bay Beach: 18.4167° N, 64.5833° W) that were part of a captive rearing study (Jones 2009) died in the course of this research between 0.10 and 1.41 yr of age (mean ± SD, $\bar{X}$ = 0.79 ± 0.51 yr). These turtles were reared indoors under a combination of ambient and full-spectrum fluorescent lighting, with water temperature maintained at 24 ± 1°C and salinity at 28 to 33 ppt (Jones 2009). One eye was collected from each of the sea turtles, along with straight line carapace length (SCL) as measured in cm from the nuchal notch of the carapace to the tip of the caudal peduncle (9.71 to 34.7 cm, $\bar{X}$ = 28.1 ± 15.6 cm).

For each wild, stranded sea turtle, participants in the national Sea Turtle Stranding and Salvage Network (STSSN) collected either one or both eyes. Observers typically recorded curved carapace length (CCL) and whenever possible, necropsies were conducted to ascertain sex. For analyses necessitating direct comparison of carapace length for both captive and wild leatherbacks, CCL for wild sea turtles was converted to SCL using the following formula derived from 17 sea turtles for which both CCL and SCL were available:

SCL = 0.9781CCL – 0.7714 (r^2 = 0.9982). Samples were obtained from a total of 33 wild leatherbacks: 6 hatchlings, 2 small juveniles (16.6 and 27.3 cm CCL), and 25 large juveniles and adults ranging from 122 to 173 cm CCL ($\bar{X}$ = 147.5 ± 13.9). Of the larger sea turtles, 10 were female, 9 male, and 6 were of unknown sex. With the exception of 1 leatherback stranded in the Gulf of Mexico, the remainder were distributed along the US Atlantic coast as follows: Massachusetts (7), New Jersey (2), Virginia (2), North Carolina (11), South Carolina (1), and Florida (9, including the 6 hatchlings).

Sample processing. Ossicles were dissected from surrounding tissue, maintaining position within the ossicle ring in cases where the orientation of the eye relative to the head was known. After all ossicles had been removed from a given eye, they were photographed using a digital camera with a macro lens (Canon EOS 10D digital camera with Canon Telephoto EF 100 mm f/2.8 USM Macro Autofocus lens) before further processing, to document the characteristics of individual ossicles and the entire ring (Fig. 1). Ossicles were then immersed alternately in tap water and a 1:1 bleach:water solution and gently scraped with a metal probe to remove any remaining tissue.

Ossicles were fixed in 10% neutral buffered formalin (Fisher Scientific), rinsed in tap water, decalcified by immersion in RDO (Apex Engineering Corporation), and then allowed to soak in water overnight to remove any decalcifier remaining in the tissue. A freezing stage microtome (Leica Microsystems) was used to obtain 25 µm thick sections (plane of sectioning shown in Fig. 1), which were stained using modified Mayer's hematoxylin (Myrick et al. 1983). Stained sections were mounted in 100% glycerin on microscope slides under glass coverslips sealed with Cytoseal 280 (Thermo Fisher Scientific).

Fig. 1. *Dermochelys coriacea*. Leatherback skull, showing location of ossicle ring. Dashed line through ossicle denotes plane of sectioning

Analysis. Hematoxylin-stained ossicle sections were viewed under a trinocular compound microscope (Olympus BX41, OPELCO) at 10× magnification. Sequential, partial digital images of each ossicle were obtained using a Colorcube-12 Color CCD, 1.4 megapixel digital camera (OPELCO) and then stitched together in Adobe Photoshop LE (Adobe Systems) to form a high-magnification composite image to be used for further analysis. Ossicle and growth mark measurements were obtained using Microsuite Basic image analysis software (OPELCO). For comparisons of growth mark counts and measurements, the Mann-Whitney *U*-test was used when sample sizes differed, and the Wilcoxon test was applied to pair-wise comparisons (Zar 1996). Relationships between ossicle and carapace lengths were compared among wild and captive turtles, accounting for multiple ossicle measurements within individuals by using unbalanced repeated measures.

Counts of lines of arrested growth (LAGs), which delimit the outer boundaries of skeletal growth marks (Castanet et al. 1993), were conducted by a single observer (L. Avens). Initial examination of the wide tips of ossicles obtained from wild, stranded leatherbacks revealed a large number of marks whose deposition appeared to follow a distinct pattern: one or more lighter, diffuse marks followed by a darker, distinct mark (Fig. 2). It seemed unlikely that each of these marks represented an annual cycle, as this would have resulted in unexpectedly high age estimates (e.g. 63 marks observed for a 156 cm CCL female, just above the mean size for nesting females).

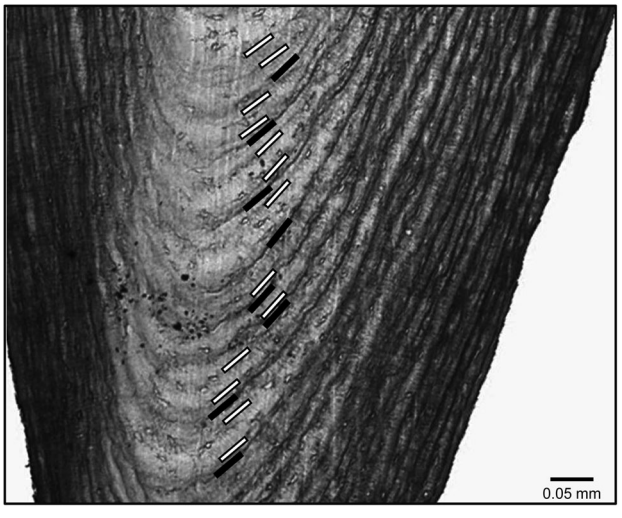

Fig. 2. *Dermochelys coriacea*. Partial, high-magnification (10×) image of wide ossicle tip obtained from a leatherback 156 cm in curved carapace length, showing alternating diffuse light (white bars) and well-defined dark (black bars) lines of arrested growth

Although the small size of the ossicles sometimes made it difficult to resolve the fine-scale pattern of LAG deposition, concurrent skeletochronological analysis of larger leatherback phalanges (finger bones) processed using the same methods as for sea turtle humeri (Avens & Goshe 2007) revealed remarkable spatial consistency for the LAG groupings (Fig. 3). Such a regular pattern would not be expected if the marks were deposited as a result of sporadic events such as prey scarcity or harsh environmental conditions. Therefore, for the purpose of these analyses, we instead hypothesize that the LAG groupings result from the repeated, multiple migrations undertaken by North Atlantic leatherbacks each year among breeding/nesting areas and various foraging areas around the ocean basin (e.g. Hays et al. 2004, Eckert 2006). Growth mark deposition corresponding with migrations has been found in North Atlantic tuna species (Compeán-Jimenez & Bard 1983). Also, annual cycles are reflected as multiple marks in the hard structures of many vertebrates (Casselman 1983, Klevezal 1996, Hohn 2002, Cailliet et al. 2006). Based on these findings, each LAG group was conservatively counted as 1 annual mark, delimited by the dark, distinct LAG, and these counts were used to obtain our age estimates. Independent counts were done both laterally and at the wide tips of ossicle sections (Fig. 4) at 5 d intervals or longer, until a consensus count was obtained (within 3 or 4 counts). Average percent error was calculated for these counts as described by Beamish & Fournier (1981).

Ossicles from the 8 captive leatherbacks were analyzed without prior knowledge of age. Ages assigned through skeletochronology were then compared with actual ages, and the appearance of the annual marks in the ossicles of the known-age leatherbacks were then used to characterize early marks deposited in the ossicles of the wild turtles.

Although some wild leatherback ossicles exhibited no resorption, and age for these turtles could be calculated directly from LAG counts, in many ossicles resorption had destroyed early LAGs. The correction factor protocol described by Zug & Parham (1996) was used to calculate the number of lost marks. This involved measuring LAGs throughout those wild ossicles in our sample exhibiting little to no resorption, in order to characterize early LAG growth patterns. Total LAG lengths could not be measured because lateral LAG compression made it impossible to follow marks around their entire circumference; therefore, LAG radii were measured instead (Fig. 4). The relationship between LAG number and LAG radius was then modeled to predict the number of missing LAGs in each resorption core, which was added to the observed number of LAGs to yield an age estimate for each turtle.

Median ages at maturation were estimated assuming 3 carapace lengths reported for nesting female leatherbacks in the western North Atlantic (Stewart et al. 2007, TEWG 2007): (1) 125 cm as the minimum size; (2) 145 cm as the average size at first nesting; and (3) 155 cm as the average size of nesting females. Sex-specific analyses were not conducted due to the large number of unknown-

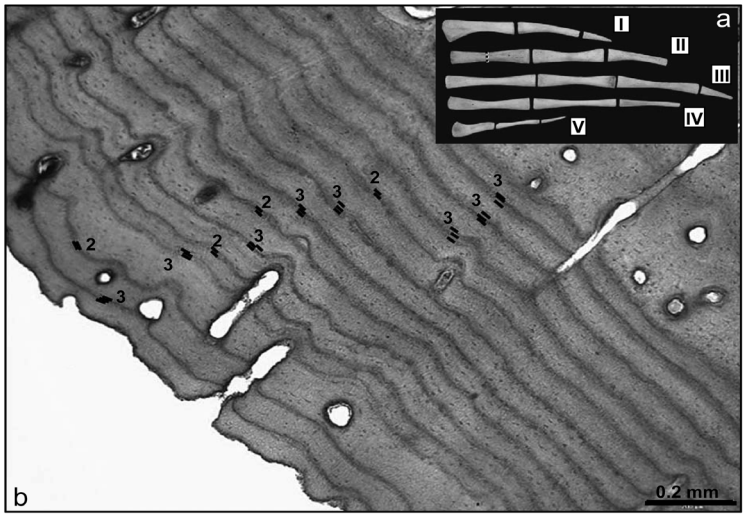

Fig. 3. *Dermochelys coriacea*. (a) Phalanges from right, front flipper of a leatherback 144.5 cm in curved carapace length. Roman numerals signify phalange number; phalanges in 'I' constitute the group of fingerbones positioned closest to the flipper's anterior edge. Dashed line in 'II' denotes plane of sectioning. (b) Partial image of phalange section (4×) demonstrating spatial regularity of line of arrested growth groupings. Black lines and corresponding numbers denote the location and number of marks within growth mark groups

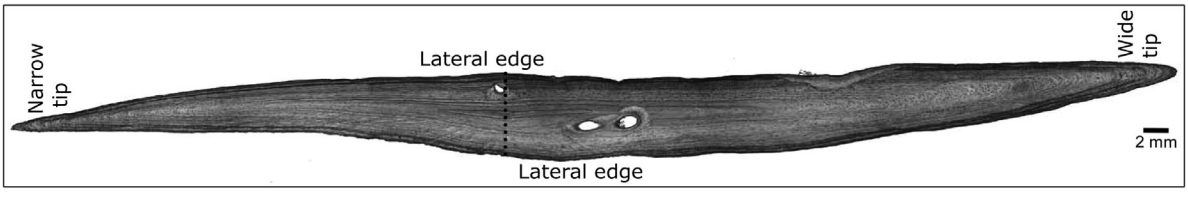

Fig. 4. *Dermochelys coriacea*. Entire ossicle section showing lateral edges, and narrow and wide tips. Growth mark and/or line of arrested growth radius measurements were taken starting from the location of the dotted line, which represents the average location of the core mark center relative to the wide tip (proportionally 0.56 total ossicle length)

sex turtles in the sample. Two methods were used to model the growth of leatherbacks. First, size at estimated age was fit using a non-parametric smoothing spline model and a von Bertalanffy growth curve. Smoothing splines were fit using the generalized cross-validation procedure to optimize the fit, while minimizing the chance of oversmoothing (Wood 2001), and confidence regions were generated using Bayesian posterior probabilities. Second, a parametric model was fit to the data adopting the following form of the von Bertalanffy growth model:

$$\mathrm{CCL} = L_{\infty}\{1 - e^{[-k(\mathrm{Age} - t_0)]}\}$$

where CCL is the curved carapace length, L_{∞} is the maximum length, k is the intrinsic growth rate, and t_0 is the intercept parameter (von Bertalanffy 1938). The model was fit using non-linear least squares; confidence regions were constructed using a delta method for estimating uncertainty using the means and variance–covariance matrix of parameter estimates. As an alternative method to characterize uncertainty, we estimated model parameters and model predictions by randomly resampling from the data with replacement (bootstrap), and refitting the von Bertalanffy model to the sample data 10 000 times. To maintain general consistency in the shape of the fitted model, we assumed that each resampling would contain a hatchling (age = 0, carapace length = 6 cm; van Buskirk & Crowder 1994). Confidence regions were calculated using the central 95% of the predictions of size at estimated age. For both the smoothing spline and von Bertalanffy models, predictions were generated and estimated ages were determined that included the assumed size at maturation (i.e. 125, 145, and 155 cm CCL) within the 95% confidence regions.

Finally, an age-maturation function was estimated using a generalized smoothing spline logistic model incorporating estimated ages and assumed sizes of mature nesting females (where individuals greater than assumed size are considered mature). The generalized smoothing spline was fit and confidence bounds estimated using Bayesian posterior probabilities (Bishop et al. 2006, Zug et al. 2006).

RESULTS

Ossicle rings from leatherbacks for which we received entire eyes contained between 10 and 13 ossicles ($\bar{X}$ = 12.1 ± 1.1, n = 29). The relationship between SCL and ossicle length (OL) was linear and there was no significant difference between the slopes for wild and captive leatherbacks ($p > 0.70$). The equation describing the grouped data was OL = 0.0551 SCL + 1.472 ($r^2 = 0.9216$).

Preliminary observations using stained sections from all ossicles within individual eyes and/or rings revealed a great deal of variation in the amount of resorption;

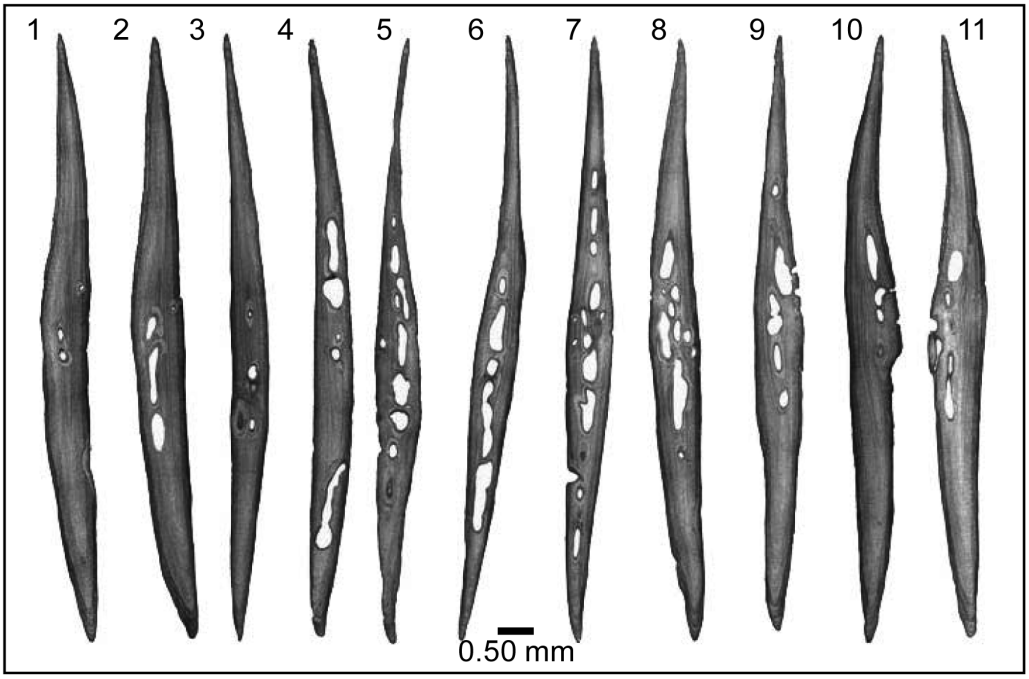

Fig. 5. *Dermochelys coriacea*. Sections of all ossicles from a single eye, displaying resorption levels ranging from minimal (e.g. ossicle 1) to extensive (ossicle 5)

whereas some ossicles exhibited little to no growth mark resorption, others were almost entirely remodeled, with few LAGs remaining (Fig. 5). For those ossicle rings where orientation relative to the head was known (n = 13), no significant relationship was found between ossicle position and the number of retained LAGs ($r^2 = 0.004$, $p > 0.20$, n = 159 ossicles). This lack of predictive power made it necessary to process every ossicle from each sea turtle to ensure that the best ossicle (i.e. that with the least resorption) was found and analyzed.

The ossicles with little to no resorption at the center contained a distinct mark (Fig. 6) similar to the core mark described for Kemp's ridley and loggerhead sea turtles (Avens & Goshe 2007) and which appears to be deposited at hatching for these species. Comparison of leatherback core mark and leatherback hatchling ossicle lengths revealed no significant difference (Mann-Whitney *U*-test, $p > 0.20$, n = 12 hatchling ossicles, n = 11 core marks), indicating that the leatherback core mark is also a hatch mark and should be used as the starting point for estimating age, representing age 0.

Average percent error for grouped growth mark counts was 4%. Comparison of LAG counts made laterally with those from the wide tips of ossicle sections revealed a highly significant difference (Wilcoxon paired sample test, $p < 0.0005$, n = 18). Without exception, a greater number of marks was visible at the wide tip and therefore all counts used for age analyses were obtained from this region.

Age was accurately predicted for the 8 known-age, captive-reared leatherbacks and this allowed characterization of the first annual LAG (LAG 1), which was the first faint, but complete, mark that could be followed continuously starting at the lateral edges down through the wide tip (Fig. 7a). Similar marks outside the core marks were observed in those wild leatherback ossicles that exhibited little resorption (Fig. 7b,c). LAG 1 radius measurements for captive and wild sea turtles were not significantly different (Mann-Whitney *U*-test, $0.10 > p > 0.05$, n = 3 known-age, n = 11 wild). However, captive LAG 1 radius measurements occurred at the larger end of the size distribution, perhaps reflecting the captive sea turtles' probable larger size relative to wild juveniles of the same age. Early LAGs following LAG 1 in the wild ossicles

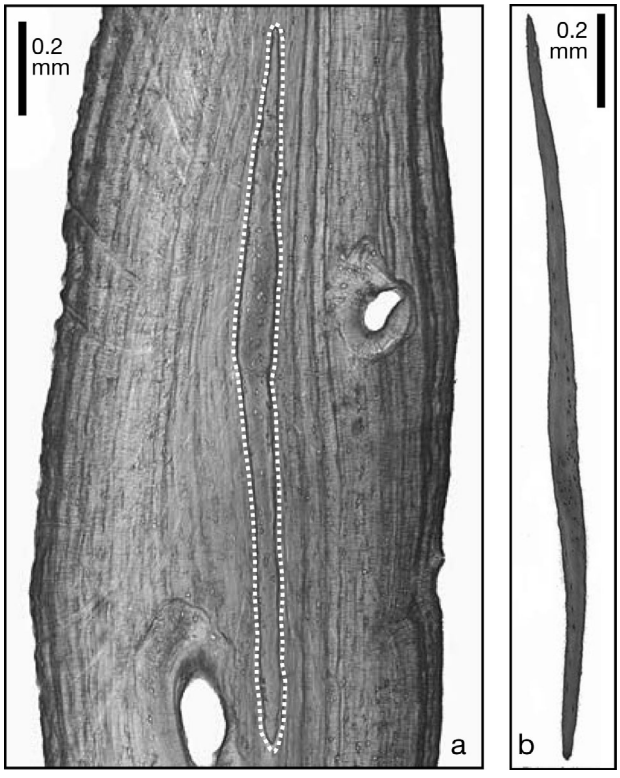

Fig. 6. *Dermochelys coriacea*. (a) Core mark (dotted line) observed at the center of those ossicles exhibiting little to no resorption. (b) Hatchling ossicle section

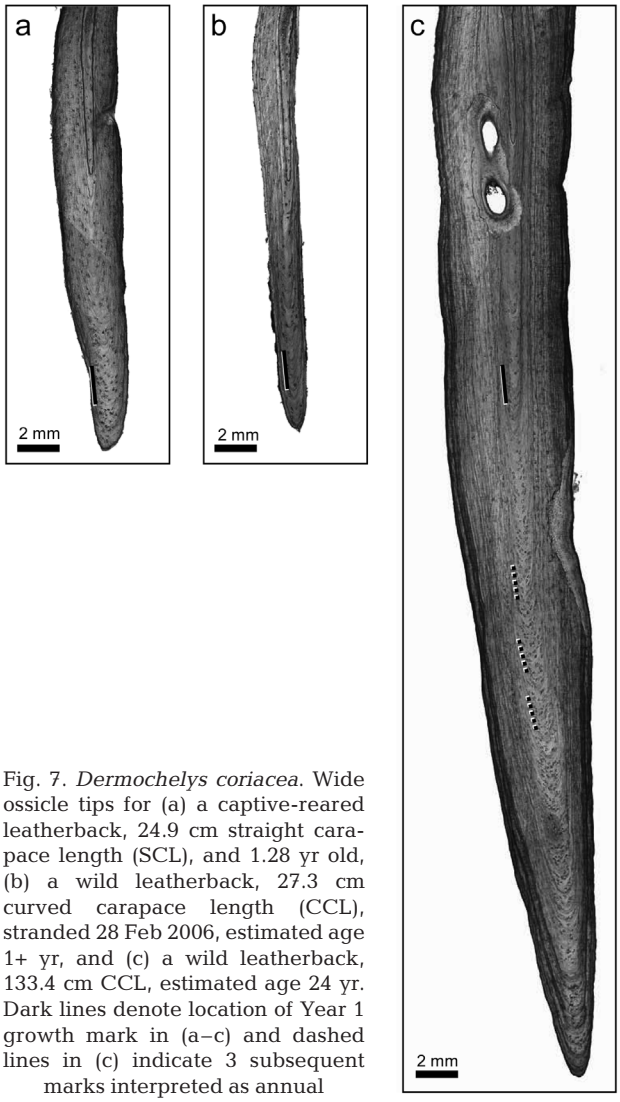

Fig. 7. *Dermochelys coriacea*. Wide ossicle tips for (a) a captive-reared leatherback, 24.9 cm straight carapace length (SCL), and 1.28 yr old, (b) a wild leatherback, 27.3 cm curved carapace length (CCL), stranded 28 Feb 2006, estimated age 1+ yr, and (c) a wild leatherback, 133.4 cm CCL, estimated age 24 yr. Dark lines denote location of Year 1 growth mark in (a–c) and dashed lines in (c) indicate 3 subsequent marks interpreted as annual

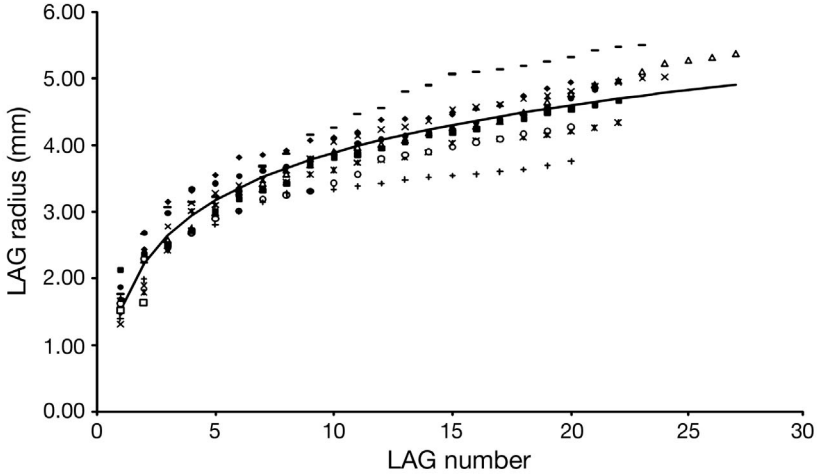

Fig. 8. *Dermochelys coriacea*. Plot of line of arrested growth (LAG) number versus LAG radius (n = 202) for 11 leatherbacks whose ossicles exhibited little to no resorption and in which the earliest growth marks (beginning with LAG 1) were visible. Different symbols represent individual ossicle growth trajectories for different individuals. The relationship between LAG number and LAG radius for the grouped data was best described by the logarithmic equation $y = 1.028\ln(x) + 1.5167$ ($R^2 = 0.862$)

were similar in appearance, lending confidence to their interpretation as annual marks (Fig. 7c).

The relationship between LAG number and LAG radius within wild leatherback ossicles was described by a logarithmic curve (Fig. 8). Resorption core radius was substituted for LAG radius within the equation to yield an estimate of the number of resorbed LAGs, which was then added to the observed number of LAGs to yield an age estimate for each sea turtle.

The general shapes of the smoothing spline and von Bertalanffy growth functions were nearly identical (Fig. 9). Parameter estimates for the von Bertalanffy growth models were similar between the delta method and bootstrap uncertainty analysis (estimate 95% CI): L_∞ = 169.2 (158.65 to 185.6), k = 0.07 (0.05 to 0.09), t_0 = –0.46 (–0.70 to –0.30). Although it is not common, females as small as 125 cm CCL are occasionally observed on nesting beaches. The 95% confidence regions for the smoothing spline model predicted that turtles of this size are 18 to 22 yr of age (Fig. 9a), while the von Bertalanffy curve estimates ranged from 16 to 20.5 yr (Fig. 9b). Age estimates for sea turtles 145 cm in CCL, a more typical size for first-time nesters, ranged from 24.5 to 29 yr of age for both models (Fig. 9a,b). Finally, the smoothing spline and von Bertalanffy curve predicted that average-sized nesting sea turtles 155 cm in CCL may be >29 or 31 to 43 yr old, respectively (Fig. 9a,b). The age-specific maturation function performed poorly for 125 and 155 cm CCL where sample sizes were low. For 145 cm CCL, the model predicted that median age at maturation would be 27 yr (95% CI = 20 to 30 yr; Fig. 10), which was within the range of estimates predicted by the smoothing spline model and the von Bertalanffy curve.

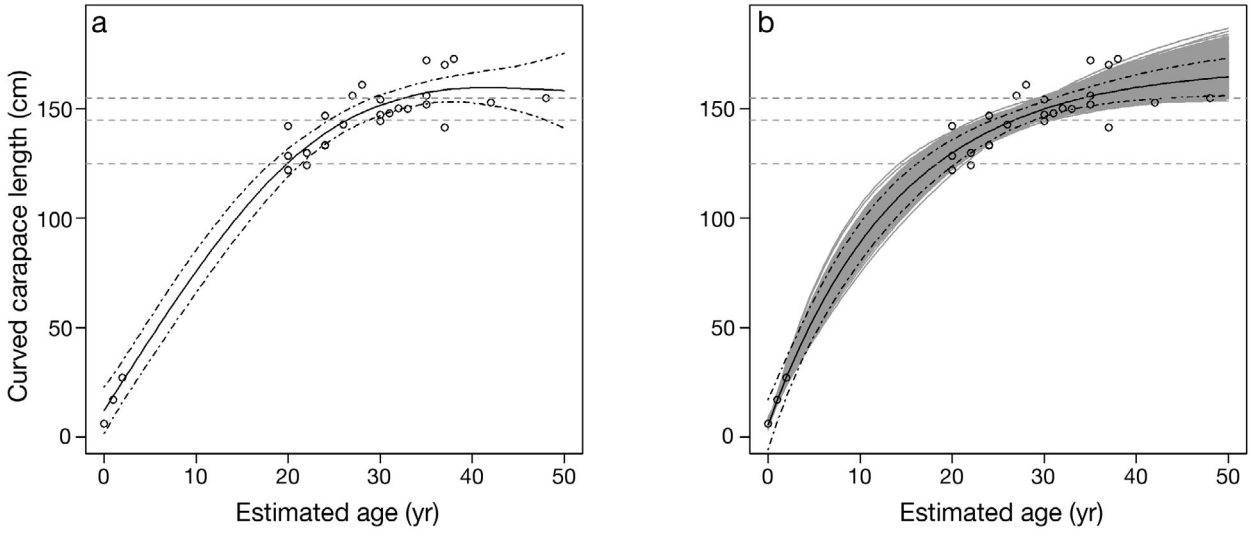

Fig. 9. *Dermochelys coriacea*. (a) Smoothing spline and (b) von Bertalanffy growth curve fit to age estimate and curved carapace length (CCL) data. Dashed lines following the curves denote 95% credible intervals. Horizontal dashed lines correspond to 125, 145, and 155 cm CCL, respectively. Size at estimated age data are shown as open circles. Gray shading in (b) shows results from 10 000 bootstrap permutations of the size at estimated age data

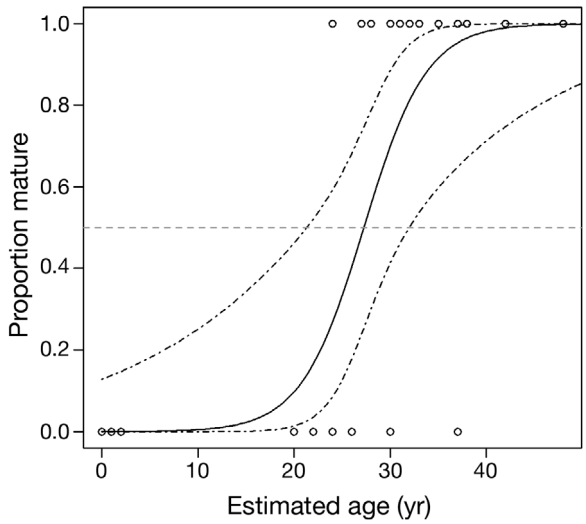

Fig. 10. *Dermochelys coriacea*. Plot of size-at-age function fitted assuming size at first nesting of 145 cm. The model was fit to the data using a generalized smoothing spline (solid line). Size at estimated age data are shown as open circles. Dashed lines following the curve denote 95% credible intervals. Horizontal dashed line corresponds to 0.50 proportion mature

DISCUSSION

The analyses presented here indicate that median age at maturation for leatherbacks in the western North Atlantic Ocean ranges from 24.5 to 29 yr (Figs. 9 & 10). These ages are far greater than the early estimates of 2 to 6 yr (Deraniyagala 1952, Birkenmeier 1971, Rhodin 1985, Bels et al. 1988). However, the lower end of our predicted 16 to 22 yr age range for 125 cm CCL sea turtles does approach the span of 12 to 18 yr that encompasses more recent results from skeletochronology, genetic analyses, and captive rearing (Zug & Parham 1996, Dutton et al. 2005, Jones 2009). Sex-specific differences in growth and maturation have been demonstrated for other marine turtle species, with males tending toward faster growth rates and earlier maturation (Chaloupka & Limpus 1997, Limpus & Chaloupka 1997, Snover 2002). Given that our sample of large juveniles and adults included both males and females, if a similar dichotomy in growth patterns exists for leatherbacks, this would negatively bias our age estimates for nesting females.

Growth mark interpretation

As with all skeletochronology studies, the estimates of age at maturation presented herein are dependent upon interpretation of the growth marks within the scleral ossicles. Accurate reads are of course essential, as errors in interpretation can confound age estimates and resulting interpretation of individual and population vital rates (Ardizzone et al. 2006).

Prior to conducting age analyses using hard structures, it is necessary to determine the most appropriate axis along which to conduct growth-mark counts (Lagardère et al. 1995). Zug & Parham (1996) described and analyzed marks visible at the lateral edges of leatherback ossicle sections. However, as predicted from a recent analysis of cheloniid turtle ossicles (Avens & Goshe 2007), mark clarity and spacing was better at the wide tips of leatherback ossicle sections due to lateral mark compression and relatively greater resorption at ossicle centers (Fig. 2). As a result, this was the axis along which we obtained our LAG counts and we recommend that future analyses also focus on this region.

Despite the confidence in the location of the growth mark counts, some uncertainty regarding the frequency of mark deposition in the ossicles remains. Growth-mark deposition has been most strongly linked to variations in ectotherm growth rates that are caused by seasonal temperature fluctuations (e.g. Hutton 1986). However, leatherbacks are atypical for reptiles in that they are able to maintain their body temperature well above ambient (James et al. 2006). In theory, the elevated body temperature and high vascularization of leatherback skeletal elements might allow more or less continuous growth, as is the case for most birds (Turvey et al. 2005), with mark deposition resulting from other factors influencing growth rates. However, hard structures in mammals (reviewed by Klevezal 1996) and tropical reptiles and amphibians (Zug & Rand 1987, Chinsamy et al. 1995, Kusrini & Alford 2006) experiencing less annual thermal variability than the leatherback have been found to contain annual growth marks. Furthermore, controlled laboratory experiments have not revealed a positive relationship between degree of vascularization and growth rates in bird bones (Starck & Chinsamy 2002). Growth mark deposition appears to be predominantly driven by endogenous cycles that function even in the absence of environmental influence (Schauble 1972, Simmons 1992). As a result, it would be expected that the hard structures of leatherbacks, like most other non-avian vertebrates studied to date (Castanet et al. 1993, Secor et al. 1995, Klevezal 1996), would also exhibit annual cycles of deposition. This supposition is supported by the observation that annual growth marks were observed in the captive leatherbacks >1 yr old, although they had been reared under fairly constant environmental conditions (Jones 2009).

Another issue is the interpretation of the light and dark marks within the ossicles. Aside from the influence of endogenous factors on growth mark deposi-

tion, this process can also be affected by physiological and environmental stressors; aestivation, disease, food shortage, and adverse climatic conditions may all alter somatic growth and yield mark formation (Klevezal 1996, Sinsch et al. 2007). Furthermore, long and energetically expensive migrations, such as those exhibited by Atlantic tuna species, have been linked to mark deposition (Compeán-Jimenez & Bard 1983). In many other cases, although the factors underlying deposition are not necessarily well understood, annual cycles are also represented by more than one mark (e.g. salamanders, Castanet et al. 1993; cetaceans [growth layer groups], reviewed by Hohn 2002; terrestrial mammals, Klevezal 1996; chondrichthyans [band pairs], Cailliet et al. 2006; fish otoliths, Casselman 1983).

In the North Atlantic, leatherbacks undertake seasonal migrations that span the ocean basin (Hays et al. 2004, Eckert 2006) and their behavior suggests that foraging may occur to some extent during these movements (Hays et al. 2006). However, if the energetic requirements of continuous, high-speed swimming and diving exceed the level of prey intake during travel, this could result in cessation of somatic growth and mark deposition within the bones during migration. The leatherback's spring/early summer migration to northern foraging areas (periodically after directing considerable resources toward reproduction) might correspond with deposition of the annual, dark LAG, while the lighter marks within the groups could relate to additional migrations undertaken by these sea turtles each year. In light of these precedents, we counted each group of LAGs in leatherback scleral ossicles as a single, annual growth mark, presuming that the darker, more distinct mark demarcated the annual cycle and was deposited in winter/spring (Klevezal 1996), as found for cheloniid sea turtles (Snover & Hohn 2004).

Recent isotopic analyses have begun to investigate potential differences in the microchemistry of the light and dark LAGs, which could provide information about the timing of mark deposition (R. Day unpubl. data). However, additional work is needed to address the frequency of deposition, possibly through bone-marking studies (reviews in Frazier 1985, Klevezal 1996) or bomb radiocarbon analysis (e.g. Campana 1997, Ardizzone et al. 2006).

Delayed maturation and life-history considerations

Although our proposed age at maturation estimate of 24.5 to 29 yr for leatherbacks contrasts sharply with earlier estimates of 2 to 6 yr (Deraniyagala 1952, Birkenmeier 1971, Rhodin 1985, Bels et al. 1988), our results do approach the 30 to 40 yr range established for the larger, cheloniid sea turtle species (Limpus 1979, NMFS SEFSC 2001, Snover 2002, Zug et al. 2002, Heppell et al. 2003b, Balazs & Chaloupka 2004). It may seem difficult at first to draw direct parallels between leatherback life history and that of cheloniid sea turtles because the lineages diverged at least 100 million years ago (Bowen & Karl 2007). Furthermore, leatherbacks display some unusual characteristics, such as their enormous size as adults, and their lack of a true carapace (Eckert & Luginbuhl 1988, Stewart et al. 2007). Also unlike cheloniid sea turtles, leatherbacks are able to maintain their body temperatures well above that of their surroundings, allowing them to forage at high latitudes (Spotila et al. 1997, James et al. 2006). In the course of their movements between these feeding areas and tropical breeding sites, leatherbacks transit over exceptionally long distances (Eckert 2006, Hays et al. 2006). Finally, for all their large size and presumed metabolic requirements, leatherbacks subsist almost solely on an 'energetically improbable' diet of gelatinous prey (Houghton et al. 2006).

Despite these singular qualities, many fundamental similarities exist between leatherbacks and their closest extant relatives. Resting metabolic rates of leatherbacks do not differ from those of green sea turtles (or other reptiles); their elevated body temperature results instead from anatomical adaptations that very effectively retain behaviorally generated heat (Wallace & Jones 2008). In addition, leatherbacks display an iteroparous reproductive strategy similar to that of cheloniid sea turtles, in which females lay 2 to 10 nests each reproductively active year at intervals of 2 to 5 yr (van Buskirk & Crowder 1994). Nesting occurs on oceanic beaches and, as for all other marine turtle species, stochastic environmental conditions combined with predation can yield low nest and/or hatchling survival (Frazer 1987, Leslie et al. 1996). Once in the water, hatchling leatherback and cheloniid turtles alike are vulnerable to predators, and survival rates can be low (Gyuris 1994, Spotila et al. 1996, TEWG 2007, but see Stewart & Wyneken 2004). By contrast, large juvenile and adult survival probabilities are estimated to be relatively high (Frazer 1986, 1987, Troëng et al. 2007, TEWG 2007).

Given this extensive concurrence, it is possible that the selective pressures leading to the life-history characteristics described above, including delayed maturation, may have acted similarly for all marine turtle species. Growing to and maintaining a large size is energetically expensive and only worthwhile if this increased size also yields a corresponding increase in reproductive success (Gadgil & Bossert 1970, Stearns 1992, Charnov et al. 2001). Total egg output does increase with body size for green (Broderick et al. 2003) and leatherback (Price et al. 2006) sea turtles,

indicating that such a benefit may exist. Large adult size can confer a size refuge from predation (Takada & Caswell 1997), yielding increased adult survival rates, which in turn have been found to correlate positively with age at maturation (e.g. for squamate reptiles, Shine & Charnov 1992). Low juvenile survival can result in selection against reproductive effort at smaller sizes and/or younger ages (Law 1979, Michod 1979, Reznick et al. 2002) so that all available energy is devoted to growth during this time, to move beyond the vulnerable stage(s) (Takada & Caswell 1997). The effects of stochastic environmental conditions on reproductive success are thought to select for bet-hedging, where organisms are long-lived to distribute reproductive effort over space and time, maximizing the probability of offspring survival over the course of the reproducer's life span (Stearns 1992, Sæther et al. 1996). Finally, the trait of delayed age to maturity in sea turtles might simply be phylogenetically constrained (Stearns 1984, 1992, Dunham & Miles 1985), as is thought to be the case for their metabolic rates (Wallace & Jones 2008).

It should be noted, however, that despite the convergence of delayed age at maturation estimates among the larger sea turtle species, the growth rates of leatherbacks remain accelerated relative to those of cheloniid turtles (Snover & Rhodin 2008). Although Atlantic leatherback hatchlings are only slightly larger than those of loggerhead or green sea turtles, the mean carapace length of nesting female leatherbacks exceeds that of the other 2 species by approximately 50% (Buskirk & Crowder 1994, Stewart et al. 2007). As a result, it appears that leatherbacks are still conferred some advantage by reaching large size more quickly, perhaps related to the thermoregulatory capacity that allows them to exploit a foraging niche not available to other sea turtle species.

Implications for conservation

Accurate information relating age to reproductive effort is essential to understanding population dynamics and assessing the effectiveness of potential management strategies (Sæther et al. 1996, Chaloupka & Musick 1997, Heppell et al. 2003a). When age at maturation is underestimated, as has occurred on a number of occasions for various fish species, this can introduce inaccurate assumptions regarding population resilience to negative impacts, which in turn can have disastrous management implications (see Summerfelt & Hall 1987, Secor et al. 1995, Cailliet et al. 2006 for reviews).

The age at maturation estimates presented here for leatherbacks may seem unexpectedly high relative to earlier published values (Deraniyagala 1952, Birkenmeier 1971, Rhodin 1985, Bels et al. 1988, Zug & Parham 1996, Dutton et al. 2005). However, the tendency toward increasing estimates of age at maturation as the techniques used to study age and growth are refined over time is consistent with the pattern seen for hard-shelled marine turtle age estimates over the past several decades (Caldwell 1962, Uchida 1967, Hirth 1971, Limpus 1979, Mendonça 1981, NMFS SEFSC 2001, Snover 2002, Zug et al. 2002, Heppell et al. 2003b, Balazs & Chaloupka 2004). Discrepancies among results from the various leatherback age studies are likely due to a number of factors. Early studies of captive leatherback hatchlings reared in constant conditions and fed unusual diets yielded rapid growth rates not necessarily representative of wild individuals, and analyses of these data did not always take into account growth rate decay through ontogenetic stages (Deraniyagala 1952, Birkenmeier 1971, Bels et al. 1988). The results of our skeletochronological analysis of leatherback ossicles indicate that growth mark compaction and resorption may have resulted in a lower estimate of age at maturation for an earlier Pacific leatherback skeletochronology study (Zug & Parham 1996), although population-specific differences in age and growth are possible. Finally, although DNA fingerprinting data for a Caribbean nesting population provide compelling evidence for a minimum reproductive age of 12 to 14 yr, the mixture of possible mother–daughter and sister–sister relationships can confound interpretation of lineages (Dutton et al. 2005).

Leatherbacks are listed as Critically Endangered worldwide (IUCN 2006), with nesting populations in the Pacific having undergone catastrophic collapse in the past few decades (Chan & Liew 1996, Spotila et al. 1996, 2000, Hitipeuw et al. 2007, Sarti Martínez et al. 2007). By contrast, some western North Atlantic nesting populations are increasing dramatically, while others remain stable (TEWG 2007), or are perhaps even decreasing (Troëng et al. 2007). The increasing trends are encouraging and in some cases have been attributed to recently implemented protective measures taking effect over the presumed short generation time of the leatherback (Dutton et al. 2005). However, some conservation efforts that have been in place for 15 to 20 yr in the Pacific have yet to yield an increase in nesting populations (Huppert & Mittleman 1993, Hitipeuw et al. 2007, Sarti Martínez et al. 2007). Although this lack of recovery might be due to continued low survival rates, it is also possible that sufficient time has not passed for the hatchlings and juveniles impacted by these recovery efforts to have matured and returned to nest.

Despite some uncertainty associated with the estimates of age at maturation proposed here, they might

serve as a caution for researchers and managers, as they carry with them potentially significant implications for the future of this species. Leatherbacks face many threats to their survival, including habitat degradation and destruction, directed take, and fisheries bycatch (TEWG 2007). Therefore, if these turtles do require decades to reach maturity, then the ability of populations to recover from such negative impacts is going to be diminished relative to current expectations.

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