Impact of exceptional growth rates on estimations of life-stage duration in Hawaiian green sea turtles

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ABSTRACT: The Hawaiian green sea turtle *Chelonia mydas* population has steadily increased since its protection under the US Endangered Species Act of 1978. However, an understanding of their recovery status is stymied by lack of certainty regarding the population age structure. Based on the observed slow growth rates of juveniles, current assessments place age at first reproduction in Hawaiian green sea turtles at 35–40 yr, although a recent study suggests 23 yr for this population. It is possible that somatic growth dynamics such as growth spurts have been missed by traditional mark–recapture studies. Skeletochronology provides annual longitudinal data on growth rates of marine turtles, allowing for the detection of rare but potentially important growth spurts. The present study uses skeletochronology to estimate growth rates and detect the frequency of growth spurts in 30–90 cm straight carapace length (SCL) Hawaiian green sea turtles. We found that growth spurts occurred throughout the life span, but peaked for males from 50 to 59.9 cm SCL and for females from 70 to 79.9 cm SCL. The growth rates were binned into 10 cm SCL size class bins, and 4 methods were used to estimate the mean growth rate for each bin. We found that mean growth rates overestimated life-stage durations for each of the methods, although the expectation of the lognormal distribution gave the least biased results. Our study suggests that infrequent growth spurts are not represented in mean growth rate statistics but that these spurts likely result in faster lifetime growth rates and lower age at first reproduction than has been estimated using traditional methods.

KEY WORDS: *Chelonia mydas* · Age at first reproduction · Growth spurts · Humeri · Skeletochronology

INTRODUCTION

Green sea turtles *Chelonia mydas* are found globally throughout tropical, subtropical, and temperate oceans. Although taxonomically considered one species, genetic and morphological differences allow for a distinction of 11 distinct population segments (DPSs; Seminoff et al. 2015, Federal Register 2016). Under the US Endangered Species Act, 3 DPSs (Central South Pacific, Central West Pacific, and Mediterranean) are listed as endangered and 8 DPSs (Central North Pacific, East Indian-West Pacific, East Pacific, North Atlantic, South Atlantic, Southwest Indian, North Indian and Southwest Pacific) are listed as threatened. Hawaiian green sea turtles are part of the Central North Pacific DPS and remain federally listed under the Endangered Species Act as a threatened population (Federal Register 2016).

Within the Pacific, major nesting grounds are found in Japan (Ogasawara Island), Australia (Heron and Raine Islands), American Samoa (Rose Island), Mexico (Michoacan), Ecuador (Galapagos Islands), and the United States (French Frigate Shoals) (Seminoff et al. 2015). Although many Pacific green sea turtle populations are in serious decline (Seminoff et al. 2002), nesting trends for the Hawaiian green sea turtle population have been steadily increasing since the 1970s (Balazs & Chaloupka 2004a,b, 2006, Cha-

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loupka et al. 2008). While the Hawaiian green sea turtle nesting population is increasing, questions remain whether the population has recovered due to the lack of historical baseline information on population sizes.

Although the Hawaiian green sea turtle is one of the most extensively monitored nesting populations (Balazs & Chaloupka 2006), the diverse geographic habitats make a comprehensive understanding of growth rates and age at first reproduction difficult. Therefore, managing this protected DPS is challenging because there is uncertainty in the data on life-stage durations and age at first reproduction, which are critical for modeling sea turtle populations (Heppell et al. 2003). Growth rate (and its variation) in sea turtles is an important demographic parameter, and is crucial to understanding age at first reproduction and the variability in that age, ultimately leading to a better understanding of population dynamics for recovery and conservation (Heppell et al. 2003, Avens & Snover 2013).

Discrepancies have been noted regarding estimated age at first reproduction in Hawaiian green sea turtles based on juvenile growth rates (Zug et al. 2002, Balazs & Chaloupka 2004a) and through an analysis of recapture records for nesting adult females (Van Houtan et al. 2014). A possible explanation for this discrepancy may be the rare occurrence of brief periods of exceptionally high growth rates, or growth spurts. These growth spurts may occur when an animal that has grown at a reduced rate, due to suboptimal conditions, is exposed to improved conditions (i.e. compensatory growth) (Bjorndal et al. 2003). Growth spurts may also occur in response to hormonal changes such as those associated with puberty (Soliman et al. 2014), although little is known about this mechanism in reptiles (Ball & Wade 2013).

The occurrence of growth spurts contributes to the right-skewed distributions that typify annual growth rates (Vigliola et al. 2000, Avens & Snover 2013), whereby these distributions are bounded by zero on the lower end (for species that do not display negative growth rates), and growth spurts, occurring at relatively low levels, create a long, right-hand tail on the upper end. The importance of the growth rates, which comprise these tails, is likely critical to our understanding of stage duration and age at first reproduction (Avens & Snover 2013). However, these rare growth spurts may not be detected in traditional mark–recapture studies, especially those with shorter durations and limited geographic scope, biasing estimates of age at first reproduction derived from these data.

Skeletochronology is the technique of using growth marks in skeletal structures to obtain information on age and growth rates (Snover et al. 2007, Avens & Snover 2013). It has been used successfully to estimate age and growth in numerous species of reptiles and amphibians (Castanet 1994, Smirina 1994), including sea turtles (see Avens & Snover 2013 for review). The annual deposition of growth marks, as well as the timing of growth mark deposition, has been validated for Hawaiian green sea turtles (Snover et al. 2013, Goshe et al. 2016). In contrast to mark–recapture studies, skeletochronology is able to obtain multiple sequential growth rates from individuals, making it more likely that growth spurts will be detected; however, the statistical methods used to evaluate these growth rates can be critical to their interpretation.

The present study used skeletochronology to (1) estimate annual growth rates, (2) detect the frequency of growth spurts in the 30−90 cm straight carapace length (SCL) size class bins of Hawaiian green sea turtles, and (3) estimate the actual time, in years, to grow from the interior-most measurable growth mark to the time of death, based on the assumption of 1 growth mark per year (Snover et al. 2011). We then used 4 methods, common to both skeletochronology and mark–recapture studies, to estimate average annual growth rates within 10 cm SCL size class bins. Using these size-specific growth rates for each of the 4 methods, we estimated the length of time to grow from the size at the interior-most measurable growth mark to the size at death, and compared those results to the actual time determined through skeletochronology. We discuss the significance of our findings to the current understanding of somatic growth rates, estimates of age at first reproduction, and population growth.

MATERIALS AND METHODS

Sample collection

For this study, a total of 267 humeri were collected from stranded Hawaiian green sea turtles that were either recovered dead through the Marine Turtle Biology and Assessment Program (MTBAP) of NOAA's Pacific Islands Fisheries Science Center or euthanized after examination by a veterinarian (Table 1). For 99 of the turtles, sex was determined through examination of the gonads during necropsy and the gonads retained for a different study (Murakawa

Table 1. Sample sizes and description of each sample set of stranded Hawaiian green sea turtles used in this study that were either recovered dead through the Marine Turtle Biology and Assessment Program (MTBAP) of NOAA's Pacific Islands Fisheries Science Center or euthanized after examination by a veterinarian

Purpose of sample set	Sample size
Skeletochronological assessment for growth rates and growth spurt detections	99
Mean hatchling carapace lengths and humerus diameter for Eq. (1)	10
Juvenile/adult paired carapace lengths and humerus diameters used to fit Eq. (1)	158
Total sample size	267

2012). For all of the samples, SCL was measured from the nuchal notch to the most posterior tip of the carapace with an aluminum tree caliper (Haglöf™) (Wyneken 2001) to the nearest 0.1 cm.

In order to back-calculate SCL from skeletochronology growth marks, the relationship between humerus diameter and SCL must be established (Francis 1990, Snover et al. 2007). To do this, we used 168 humeri represented by hatchlings (N = 10) and juveniles and adults (N = 158) that span the full ontogenetic range for Hawaiian green sea turtles (Table 1). Hatchling measurements, SCL (cm) and humerus diameter (mm), were taken with a digimatic plastic caliper (Mitutoyo™). Humerus diameters were measured at the sectioning site and compared to recorded SCLs in order to confirm a predictable and proportional relationship between the humerus and carapace (Snover et al. 2007).

Skeletochronology

Most humeri were collected from the right flipper for standardization; however, in 2 cases the left humerus was collected as the right side was missing. Procedures detailed in Zug et al. (1986) and Snover & Hohn (2004) were used in processing the 99 humeri (Table 1). The humeri were frozen, thawed, flensed, boiled, and dried. They were then weighed to the nearest 0.1 g with an Ohaus™ Navigator digital scale and measured to the nearest 0.1 mm with digital calipers. The humeri were sectioned proximal to the narrowest part of the diaphysis within the deltopectoral muscle insertion scar as this site has the thickest cortical mass for determining the lines of arrested growth (LAGs) or growth marks (Snover & Hohn 2004, Avens & Snover 2013).

Sections 1–3 mm thick were cut with a Buehler Isomet™ low-speed saw. These sections were decalcified for approximately 7 d with Fisher Cal-Ex II™ decalcifier solution, flushed generously with water, and soaked overnight in water to remove any remaining decalcifying solution on the section. Each bone section was then cut into 25 µm sections using a Leica™ microtome with a Physitemp Instruments™ freezing stage. Thin sections were placed back into the decalcifier for up to 12 h to complete the decalcification process. Sections were then soaked in water overnight and stained with Ehrlich's hematoxylin solution (Klevezal 1996). The stained sections were then mounted onto a glass slide in 100% glycerin, covered with a cover slip, and sealed with Permount™ for viewing and archiving.

Sections were photographed at 4× magnification using an Olympus™ BX41 standard laboratory microscope along with an Olympus™ 20MPX digital microscope camera. The iSolutions Lite™ program was used to photograph and save the digital images, which were made into composites using either Adobe™ Photoshop CS3 or Elements software.

LAG measurements, back-calculation and growth rate

The LAGs were identified by microscopically examining the humerus section. LAG diameters were measured across the lateral plane of digital photographs using iSolutions Lite™ software. A broad zone followed by a LAG signified a skeletal growth mark representing 1 yr (Castanet et al. 1977, Snover & Hohn 2004), a phenomenon validated for Hawaiian green sea turtles (Snover et al. 2011).

We used the back-calculation methods presented by Snover et al. (2007) to estimate SCL at interior growth marks (see Goshe et al. 2010). We fit the following equation to the 158 paired measurements of the humerus diameter, at the sectioning site used for skeletochronology, and calculated SCL using least-squares non-linear regression:

$$L = L_{op} + b(D - D_{op})^c \qquad (1)$$

where L is SCL (cm), L_{op} is the mean hatchling SCL (cm), b is the slope of the relationship SCL/humerus diameter (in cm/mm), D is the humerus diameter (mm), D_{op} is the mean hatchling humerus diameter (mm), and c is the proportionality coefficient. We then used the fitted parameter estimates for b and c from Eq. (1) to estimate SCL at each interior LAG using the following relationship (Snover et al. 2007):

$$L_i = [L_{op} + b(D_i - D_{op})^c][L_{final}][L_{op} + b(D_{final} - D_{op})^c]^{-1} \quad (2)$$

where L_i is the predicted SCL at LAG$_i$ (cm), D_i is the diameter of LAG$_i$ (mm), L_{final} is the observed SCL measurement (cm) for each turtle at death, and D_{final} is the observed humerus diameter at the sectioning site (mm). The first term of the equation $[L_{op} + b(D_i - D_{op})^c]$ is the predicted SCL given the LAG diameter from Eq. (1). The second and third terms of the equation, $[L_{final}]$ and $[L_{op} + b(D_{final} - D_{op})^c]^{-1}$, determine the correction factor (ratio) for each turtle using the observed measurement (L_{final}) with the predicted SCL.

All back-calculated SCLs were used to calculate growth rates. Growth rates were computed by subtracting consecutive back-calculated SCLs, such that one annual growth rate could be computed from each pair of LAG diameters. Growth rates were then binned into 10 cm SCL size classes based upon the initial SCL at each LAG.

Average growth rates

We used 3 methods to estimate the average growth rate for each 10 cm SCL size class bin: (1) arithmetic mean and variance of observed growth rates, (2) geometric mean and variance of observed growth rates, and (3) the expectation ($E(x)$) and variance (Var(x)) of the lognormal distribution using the following equations:

$$E(x) = e^{(\mu + \sigma^2/2)}$$
$$\text{Var}(x) = e^{2(\mu + \sigma^2)} - e^{2\mu + \sigma^2} \quad (3)$$

where μ and σ are the mean and standard deviation of the log-transformed data. For each method, standard error (SE) was calculated as (variance)$^{0.5}$/sample size.

We used each of these average annual growth rates per SCL size class bin to estimate the expected time to grow from the size at the interior-most measured LAG to the size at death for each turtle. For example, for a turtle that was estimated to be 32 cm SCL at the interior-most measured LAG and 76 cm SCL at death, we estimated time to grow from 32 to 76 cm SCL as:

$$\text{Expected time} = 8/GR_{30} + 10/GR_{40} + \\ 10/GR_{50} + 10/GR_{60} + 4/GR_{70} \quad (4)$$

where GR$_{30}$ is the average annual growth rate (cm yr^{-1}) for turtles with initial SCLs between 30 and 39.9 cm and similarly for the remaining GR terms. The unit for the numerator in each term is cm.

For a fourth method of estimating total growth time from the interior-most measured LAG to death, we used the spline integration method and curve from

Zug et al. (2002). We found the area under the curve in Zug et al. (2002) by using the diameter of the interior-most measured LAG and the full humerus diameter.

Error in estimating time to grow

Each LAG was assigned a calendar year based on time of death assuming a rate of deposition of 1 LAG per year (Snover et al. 2011). Observed time to grow was estimated by assuming LAGs are deposited in late winter/early spring for Hawaiian green sea turtles (Goshe et al. 2016). To standardize this timing, we assumed a date of 1 February, with the year corresponding to the year assigned to the LAG. Observed time, from the interior-most measured LAG to the exterior of the bone, was calculated as the difference between the date assigned to the interior-most measured LAG and the stranding date, assumed to be representative of time of death (Snover et al. 2007, Snover et al. 2013).

We compared the expected time to grow from Eq. (4), for each of the 3 methods of estimated mean annual growth rates per SCL size class bin, to the observed time to grow as described above. We also compared the expected time to grow using the cubic smoothing spline from Zug et al. (2002) to the observed time to grow.

Growth spurts

For this analysis, we defined a growth spurt as an annual growth rate in the 90th percentile of the distribution of growth rates (see 'Results'), or an annual growth rate greater than 3.725 cm yr^{-1}. The frequency of the growth spurts was then determined for each 10 cm SCL size class bin for both sexes. We then compared the error between actual and estimated time to grow (see 'Error in estimating time to grow', above) for turtles with and without one or more growth spurts.

RESULTS

Collected samples and skeletochronology

For the skeletolochronology assessment of growth rates, humeri were collected from 48 females, 50 males, and 1 turtle of unknown sex (Table 1, Fig. 1). The size range of turtles collected was 36.4 to 97.9 cm SCL (Fig. 1). The analyses in this study did not differentiate
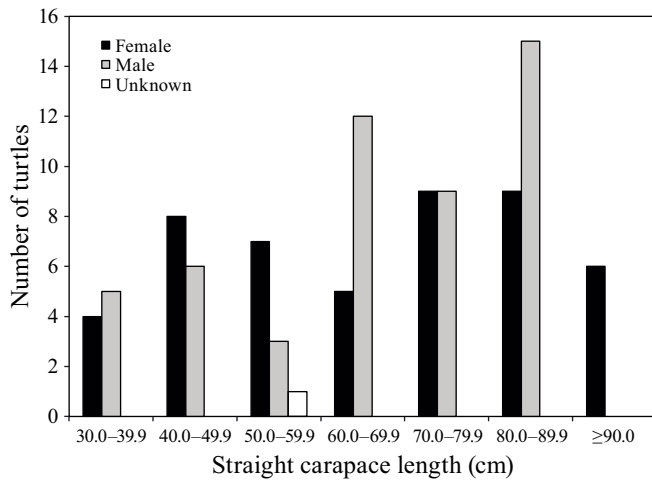
Fig. 1. Summary of the size and sex of the Hawaiian green sea turtles *Chelonia mydas* used in this study. This graph shows roughly equal numbers of females and males across the full size range of turtles

between fibropapillomatosis (FP) tumor severity; however, we note that 50.5% of turtles presented with FP (17.6% for SCLs less than 60 cm and 67.7% for SCLs greater than 60 cm). The causes of stranding (from highest to lowest prevalence) were FP, unknown, fishing gear involvement, shark attack, boat impact, suspect drowning, emaciation, and human take. Paired humerus diameters and SCL were obtained from an additional 168 stranded turtles ranging in size from 5.1 to 97.9 cm SCL to show a proportional relationship between the SCL and humeri growth for all SCL size class bins (Table 1, Fig. 2A). The mean hatchling SCL was 5.1 cm, and mean hatchling humerus diameter was 2.6 mm (range 5.1–5.4 cm SCL and 2.4–2.8 mm humerus diameter). All humeri image composites were analyzed by defining the LAGs (Fig. 3). Three turtles were not used in the growth rate analysis be-
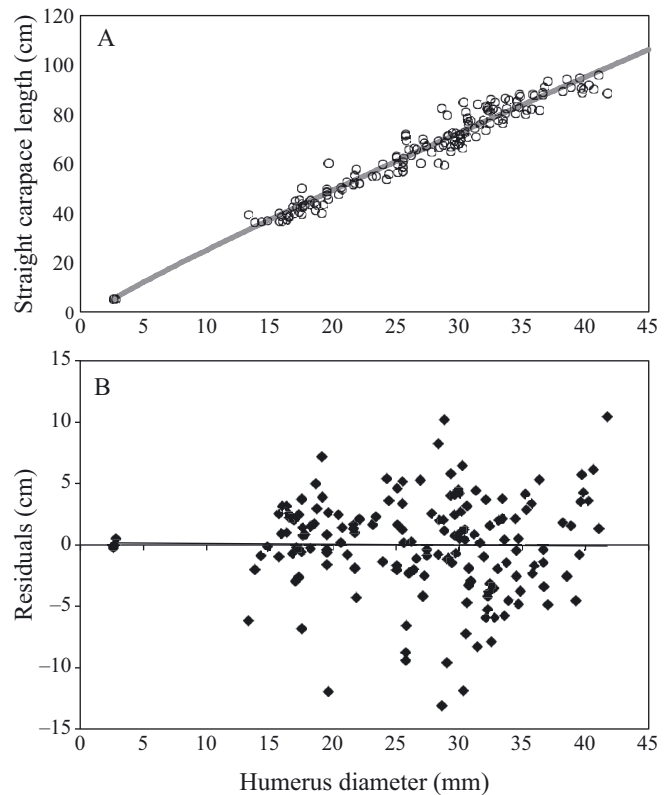

Fig. 2. Relationship between straight carapace length (cm) and humerus diameter (mm) for 167 Hawaiian green sea turtles *Chelonia mydas*. (A) Open circles represent the data and the solid line represents the fit of the allometric relationship given by Eq. (1). (B) The residuals from the fit of allometric Eq. (1); the solid line represents a linear regression showing no trend in the residuals

cause a 77.8 cm SCL female had no discernible LAGs, an 86.5 cm SCL male was excluded because it only had 1 visible LAG (and 2 or more LAGs are necessary to calculate a growth rate), and the third turtle with a 51.4 cm SCL was of unknown sex. The 77.8 and
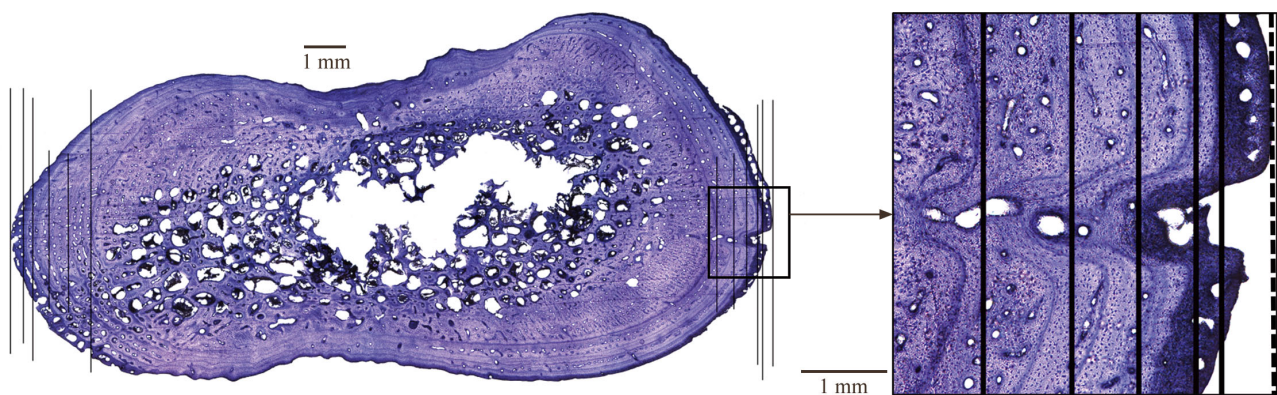

Fig. 3. Lines of arrested growth (LAGs), appearing as darker purple lines, found in the humerus section of a 43.9 cm straight carapace length (SCL) female turtle. Black vertical lines highlight the location of the LAGs and their spacing, with the dashed line indicating the outer edge of the humerus. In the inset, 5 LAGs are visible

Table 2. Summary of the lines of arrested growth (LAGs) identified in each Hawaiian green sea turtle *Chelonia mydas* used in this study. SCL: straight carapace length

SCL (cm)	Sex	0	1	3	4	5	6	7	8	9	10	11	13	14	15	Grand total
30.0–39.9	Female				3	1										4
	Male				2	3										5
40.0–49.9	Female			2	1	3	2		1							9
	Male			1	1	2				1						5
50.0–59.9	Female					3	1		1					1		6
	Male					2			1	1						4
	Unknown				1											1
60.0–69.9	Female				1	2					1				1	5
	Male				3	1	2	1		1		1	1	1	1	12
70.0–79.9	Female	1		1		4	1	1	1							9
	Male			1		7	1									9
80.0–89.9	Female			1		7	1									9
	Male		1	1	3	10										15
>90.0	Female				3	3										6
Grand total		1	1	7	18	48	8	2	4	3	1	1	1	2	2	99

86.5 cm SCL turtles had very large resorption cores due to the remodeling of the innermost part of the bone (Snover & Hohn 2004). The remaining 96 turtles revealed at least 2 LAGs (range 2–15) within the humeri and were used for growth rate analyses (Table 2).

Back-calculation and growth rates

Results of the non-linear least-squares regression fit of Eq. (1) to the data indicated that there was an allometric relationship between SCL and humerus diameter (Fig. 2A). The parameter estimates *b* and *c* were 3.13 (0.21 SE) and 0.93 (0.02 SE), respectively.
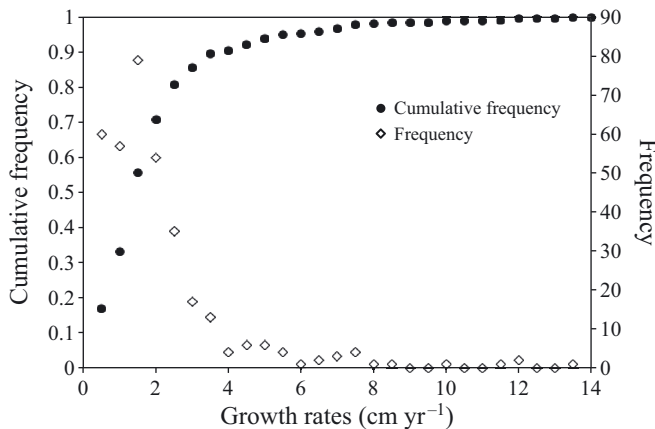

Fig. 4. Distribution of growth rates for Hawaiian green sea turtles *Chelonia mydas* used in this study. Growth rates are binned by 0.5 cm; values on the *x*-axis represent the upper value of the bin (e.g. 4 cm yr⁻¹ represents growth rates between 3.5 and 4 cm yr⁻¹). Solid circles represent the cumulative frequency; open diamonds represent the frequency

A *t*-test showed that the slope of the linear regression of the residuals against SCL was not significantly different from zero (slope = −0.0066, df = 165, p > 0.05; Fig. 2B). Using Eq. (2), we were able to back-calculate SCLs for 447 LAGs, resulting in 350 annual growth rate estimates for SCLs ranging from 17.9 to 97.5 cm. No subset of turtles disproportionately skewed the growth rates as average growth rates per size class bin were used.

The distribution of growth rates showed an extreme right-hand tail (Fig. 4). The highest frequency of growth rates was between 1 and 1.5 cm yr⁻¹. Approximately 10% of the growth rates were greater than 3.725 cm yr⁻¹, and the maximum observed growth rate was 13.0 cm yr⁻¹ (Fig. 4).

Average growth rates

We found a range of average annual growth rates per 10 cm SCL size class bin, depending on the method used to calculate the mean (Table 3). The expectation of the lognormal distribution consistently resulted in higher mean growth rates compared to the other methods. Using all methods to estimate the time it would take to grow from 30 to 90 cm, we found results ranging from approximately 33 to 49 yr. To estimate the same duration using the spline integration method from Zug et al. (2002), we used the relationship from Eq. (1) to estimate average humerus diameters at 30 and 90 cm SCL, which were 12.0 and 37.8 mm, respectively. Using the relationship in Fig. 3 of Zug et al. (2002), the time to grow from 30 to 90 cm SCL is 31.3 yr.

Table 3. Average annual growth rates (cm yr^{-1}) per size class bin. Sizes are straight carapace length (SCL, cm). Average annual growth rates were calculated using 3 methods: the arithmetic mean of the raw data (Arithmetic); the geometric mean of the raw data (Geometric); and the expectation of the lognormal distribution as given in Eq. (3) (Expectation). Standard errors are given in parentheses and N is the growth rate sample size from the 96 turtles in the present study. Average growth rates using the spline integration method (SI) were reported in Zug et al. (2002) for both sexes combined. Size-specific annual growth rates were used to calculate the time to grow from 30 to 90 cm SCL; 95% confidence intervals are given in brackets

	Initial size	Arithmetic	Geometric	Expectation	N	SI
All data combined	30–39.9	2.39 (0.46)	1.40 (0.52)	2.49 (0.65)	32	3.5 (0.44)
	40–49.9	1.86 (0.18)	1.35 (0.29)	2.05 (0.27)	75	2.1 (0.15)
	50–59.9	2.03 (0.29)	1.42 (0.32)	2.05 (0.29)	54	2.3 (0.14)
	60–69.9	1.58 (0.11)	1.22 (0.25)	1.68 (0.29)	78	2.2 (0.11)
	70–79.9	1.56 (0.19)	1.04 (0.35)	1.66 (0.27)	57	2.1 (0.22)
	80–89.9	1.56 (0.31)	1.04 (0.35)	1.47 (0.23)	43	1.3 (0.14)
	90–99.9	0.52 (0.14)	0.42 (0.69)	0.53 (0.14)	8	0.6 (0.11)
Time to grow from 30 to 90 cm SCL (yr)		33.6 [27.0–45.3]	49.0 [32.3–108.8]	32.5 [29.2–41.1]		30.0 [24.7–35.1]
Females	30–39.9	2.45 (0.68)	1.55 (0.79)	2.66 (1.03)	13	–
	40–49.9	1.69 (0.18)	1.27 (0.38)	1.86 (0.31)	40	–
	50–59.9	1.79 (0.33)	1.27 (0.50)	1.90 (0.43)	24	–
	60–69.9	1.78 (0.20)	1.41 (0.42)	1.91 (0.60)	27	–
	70–79.9	1.91 (0.36)	1.29 (0.49)	1.97 (0.45)	26	–
	80–89.9	1.71 (0.48)	1.09 (0.50)	1.63 (0.38)	24	–
	90–99.9	0.52 (0.14)	0.42 (0.69)	0.53 (0.14)	8	–
Time to grow from 30 to 90 cm SCL (yr)		32.9 [24.3–50.8]	47.3 [27.2–219.8]	31.1 [26.7–46.3]		
Males	30–39.9	2.14 (0.61)	1.31 (0.68)	2.45 (0.88)	18	–
	40–49.9	1.78 (0.19)	1.37 (0.42)	2.05 (0.39)	34	–
	50–59.9	2.22 (0.45)	1.54 (0.42)	2.19 (0.40)	30	–
	60–69.9	1.47 (0.13)	1.13 (0.31)	1.57 (0.32)	51	–
	70–79.9	1.27 (0.18)	0.88 (0.47)	1.40 (0.32)	31	–
	80–89.9	1.37 (0.34)	0.98 (0.49)	1.31 (0.27)	19	–
	90–99.9	–	–	–	0	–
Time to grow from 30 to 90 cm SCL (yr)		36.2 [28.0–56.1]	50.7 [29.5–∞[a]]	34.3 [26.0–55.8]		

[a]Lower 95% confidence interval of growth rates for geometric mean was less than 0 cm yr^{-1}, making it impossible to calculate an upper 95% CI value for time to grow

Error in estimating time to grow

All estimation methods resulted in a negative slope between error, the observed minus the expected time to grow, and the amount of growth, indicating that expected time to grow is increasingly overestimated as the amount of growth increases (Fig. 5). The steepest slope, indicating the highest error, was found using the geometric mean to estimate average annual growth rates (Fig. 5B). The shallowest slope and lowest error were found using the expectation of the lognormal distribution to estimate mean annual growth rates (Fig. 5C). For the expectation of the lognormal distribution, the error was 0.1702 yr cm^{-1} (slope of the regression) (Fig. 5C). Therefore, on average, the overestimation of time to grow using the expectation of the lognormal distribution is 0.17 yr for every 1 cm of carapace length. Intercepts for all estimation methods were positive, indicating that expected time to grow typically underestimated actual time to grow for increases in carapace length of less than about 10 cm.

Growth spurts

Using the 90th percentile of the distribution of growth rates from Fig. 4, a total of 24 (8.1% of all growth rates) growth spurts were found, with 58.3% occurring in females and 41.7% in males (Fig. 6); 6 turtles had 2 or more growth spurts. No spurts were found for females in the 60–69.9 cm SCL size class bin. We compared the error between observed and expected time to grow for turtles with and without growth spurts and found the error to be significantly lower for turtles without growth spurts (Student's t-test, p < 0.005; Fig. 7). For all methods, the mean error was less than 1 yr for turtles without growth spurts, and overestimated by more than 2 yr for turtles with growth spurts (Fig. 7).
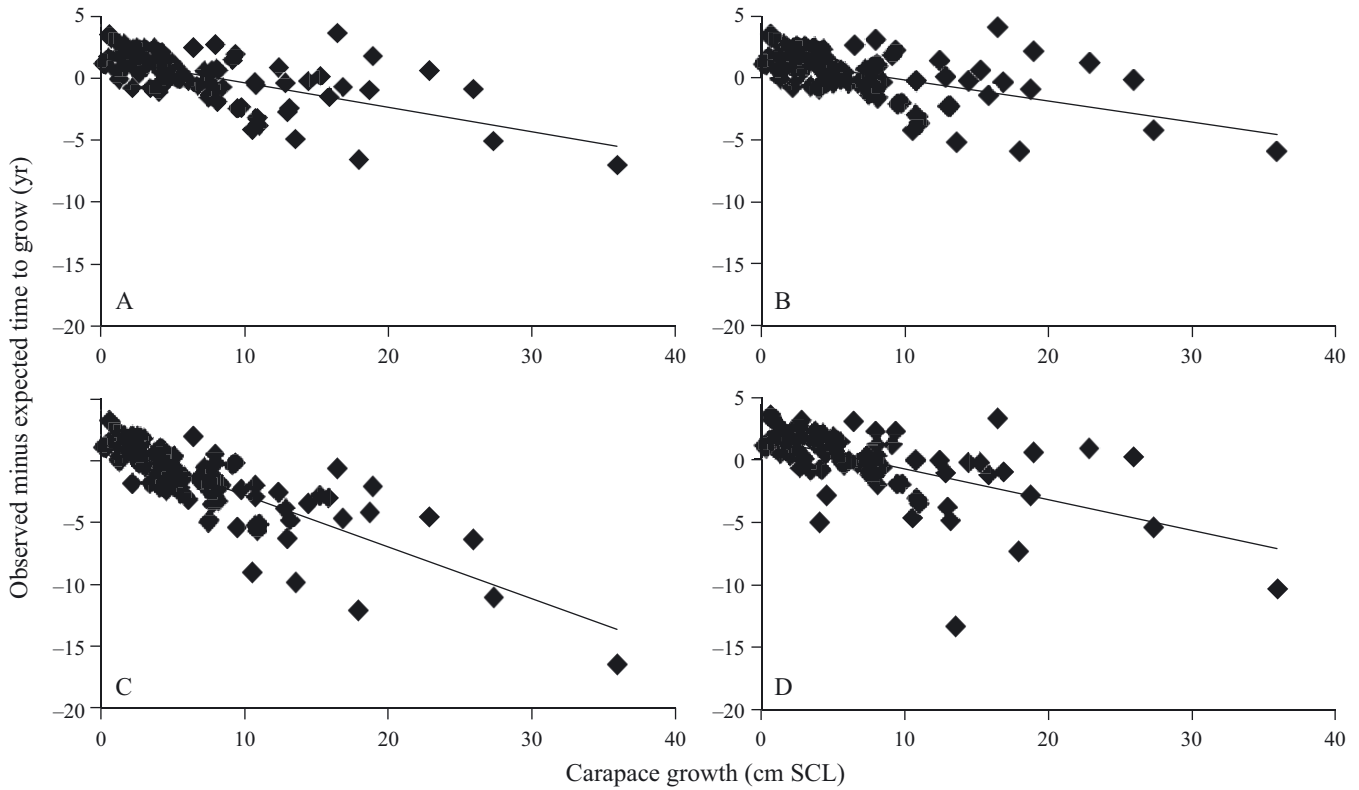
Fig. 5. Error in estimation methods for expected time to grow for Hawaiian green sea turtles *Chelonia mydas* used in this study. For each graph, solid lines represent a linear regression through the data and all regressions were significant (p < 0.001). (A) Average annual growth rates estimated by an arithmetic mean of raw (untransformed) data (regression slope = −0.20). (B) Average annual growth rates estimated by a geometric mean of raw (untransformed) data, which is equivalent to log-transforming the data (regression slope = −0.42). (C) Average annual growth rates estimated from the expectation of a lognormal distribution with parameters μ (the mean of the log-transformed growth rates) and σ^2 (the variance of the log-transformed growth rates; regression slope = −0.17). (D) Total time to grow from the diameter of the interior-most measured LAG to the humerus diameter, estimated using the spline integration method of Zug et al. (2002; regression slope = −0.25)
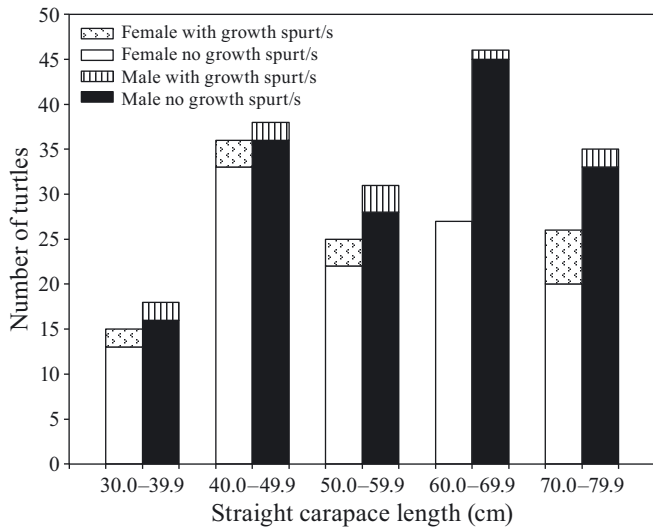

Fig. 6. Summary of the size and sex of the Hawaiian green sea turtles *Chelonia mydas* exhibiting growth spurts, defined as annual growth rates greater than 3.725 cm yr^{-1}

DISCUSSION

Our results highlight how both the method used to estimate mean growth rates and the prevalence of growth spurts can influence our ability to extrapolate life-stage durations from partial growth chronologies. The estimated time to grow from 30 to 90 cm SCL varied by 16.5 yr depending on the method used to determine the mean growth rate. Eight percent of the turtles in our sample experienced at least one annual growth spurt greater than 3.725 cm yr^{-1}; the mean error rate in estimating time to grow from mean growth rates for these turtles was as large as 5.8 yr, compared to less than 1 yr for turtles without growth spurts. These results emphasize the importance of incorporating the prevalence of growth spurts in skeletochronological and mark–recapture analyses, as well as evaluating the impact that summarizing growth rate means has on estimating time to grow and back-calculating the number of growth
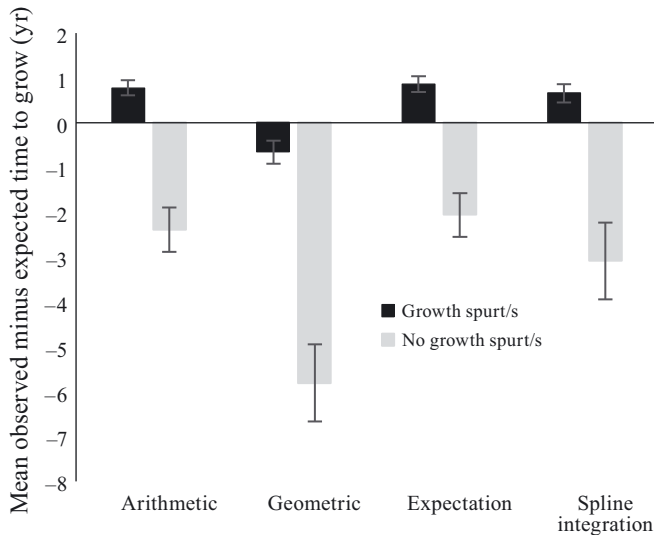
Fig. 7. Error rates in estimation methods for expected time to grow for Hawaiian green sea turtles *Chelonia mydas* with and without growth spurts. Average annual growth rates were estimated by: an arithmetic mean of raw (untransformed) data; a geometric mean of raw (untransformed) data, which is equivalent to log-transforming the data; the expectation of the lognormal distribution with parameters μ (the mean of the log-transformed growth rates) and σ^2 (the variance of the log-transformed growth rates); and the spline integration method of Zug et al. (2002). For each method, the mean difference between observed and expected time to grow was significantly different for turtles with and without growth spurts (Student's *t*-test, p < 0.005)

marks lost in the resorption core. In contrast to our results, Avens et al. (2012), using a similar approach, found a minimal difference between stage durations estimated from LAG counts compared to summations from size-class-specific growth rates for juvenile green sea turtles collected in northwestern Florida. In the present study, we included all individuals, regardless of health status and foraging location, and differences in growth rates and the quality of foraging habitats have been demonstrated for Hawaiian green sea turtles (Balazs & Chaloupka 2004a, Wabnitz et al. 2010). Hence, it is possible that the prevalence of growth spurts is influenced by the location and environmental conditions. The northwestern Florida study comprised cold-stunned juveniles that were otherwise healthy and associated with abundant foraging areas (Avens et al. 2012).

Due to the presence of growth spurts within our data, we achieved very different stage durations depending on the method used to calculate mean growth rates. Using the geometric mean, we estimated that it should take approximately 49 yr (95% CI: 32.3–108.8) for an individual to grow from 30 to 90 cm SCL. In contrast, using the expectation of the

lognormal distribution, we estimated 32.5 yr (95% CI: 29.2–41.1) for the same growth interval, a mean difference of 16.5 yr. In comparing the differences between actual and estimated time to grow for individual turtles, we found that the geometric mean resulted in an error rate of 0.42 yr cm^{-1}, compared to 0.17 yr cm^{-1} for the expectation of the mean for the lognormal distribution. Hence, for a 60 cm increase in length, the geometric mean could overestimate time to grow by 25 yr, while the expectation of the mean would overestimate this value by 10 yr. While both methods result in an overestimation of time to grow, the expectation of the mean produces less of a bias.

The presence of growth spurts within an individual significantly impacted the error rate in estimating time to grow for individual turtles. We detected at least one annual growth spurt, greater than 3.725 cm yr^{-1}, in 8.1% of the turtles in our samples, although given the restricted number of growth marks visible for many of the turtles, the actual prevalence of growth spurts within the population may be much higher. For the turtles with no detectable growth spurt, the mean error between observed and estimated time to grow using the expectation of the mean was 0.86 yr, compared to 2.05 yr for turtles with growth spurts. Hence, while using the expectation of the mean improves our ability to estimate stage durations from mean growth rates, methods that specifically incorporate frequency of growth spurts would further improve these estimates.

Our results have implications for estimating growth rates from mark–recapture studies. Mark–recapture uses cross-sectional data, often with only 1 or 2 yr of growth data (i.e. Balazs & Chaloupka 2004a). Applying growth rates based on only 1–2 yr of growth may lead to an underestimation of mean growth rates. Since recapture rates of turtles are highly variable (Piovano et al. 2011), using just 1–2 yr of growth provides an incomplete life history and is likely to miss rare growth spurts. Therefore, long-term datasets are more accurate in estimating life-stage durations as they are more likely to capture the variability in and full distribution of growth rates, but such studies are quite laborious and time-consuming, requiring multiple recaptures of animals (Bjorndal et al. 2000, Balazs & Chaloupka 2004b, Kubis et al. 2009, Eguchi et al. 2012, Avens & Snover 2013, Sampson et al. 2015, Goshe et al. 2016).

In contrast, skeletochronology provides longitudinal growth rate data for each individual turtle with a more complete temporal assessment of growth rates because the LAGs provide the ability to back-calculate sizes at multiple earlier times. Back-calculating sizes

require an understanding of the relationship between humerus diameters and carapace length across the population's ontogeny, which necessitates an adequate sample size of paired humeri and carapace lengths from hatchling to adult. Another key drawback of skeletochronology is the loss of early growth marks due to bone resorption (Zug et al. 1986). Statistical models have been developed to estimate the number of growth marks lost in resorption cores in order to assign age to individual turtles (Parham & Zug 1998, Zug et al. 2002). These methods rely on size-specific mean growth rates to relate the diameter of the resorption core to the likely number of resorbed growth marks. We recommend that back-calculation methods be developed that incorporate the expectation of the lognormal distribution to estimate mean growth rates. This method produced the least amount of error between observed and estimated times. We also recommend that similar analyses be conducted for the distribution of growth rates (or increase in humerus diameter between LAGs) to detect the frequency of growth spurts, and that back-calculation methods be developed that incorporate these frequencies.

While estimating the age at first reproduction was not a goal of this study, we can make inferences based on our results and assess the impact of growth spurts on this metric. Mean size at first reproduction in female Hawaiian green sea turtles is estimated at 89.7 cm SCL (Balazs et al. 2015). The mean age of a 30 cm SCL juvenile Hawaiian green sea turtle was estimated to be 5 yr by Zug et al. (2002), and turtles in this size range often retain their full complement of LAGs without resorption, removing the potential for bias due to estimating numbers of lost LAGs (Goshe et al. 2010). Using the mean growth rates calculated with the expectation of the mean, we would estimate age at first reproduction to be 37.5 yr (95% CI: 34.2– 46.1 yr). As our results showed that mean growth rates generally overestimated actual time to grow, we consider this estimate to be high. We found an error rate of -0.17 yr cm^{-1} for mean growth rates calculated using the expectation of the mean. In other words, age is overestimated by 0.17 yr for every 1 cm of growth. Applying this error rate to our estimates of time to grow from 30 to 90 cm SCL would suggest an overestimation of 10.2 yr (0.17 yr cm^{-1} × 60 cm) and result in mean age at first reproduction for turtles that experience growth spurts to be 27.3 yr (95% CI: 24.0–35.9 yr).

Van Houtan et al. (2014) found a similar bias with a positive correlation between amount of growth and time to grow using the skeletochronology size–age relationship based on the spline integration method of Zug et al. (2002). The dataset consisted of adult females that were tagged and measured as juveniles and subsequently 'recaptured' as nesting adults. They used Zug et al.'s (2002) size–age relationship to estimate age at first capture for these turtles, and then added the known time at large between first capture and nesting to estimate overall age at first reproduction. Van Houtan et al. (2014) found that age at first reproduction increased with increasing size at first capture. Using these data, we can estimate an error rate of 0.24 yr cm^{-1} ([40.7−31.4]/40 cm) for the size– age relationship generated using the spline integration method to estimate the number of LAGs lost in the resorption core, a value that is very similar to the 0.25 yr cm^{-1} error rate we found using the spline integration method. Van Houtan et al. (2014) assumed that this error rate would be consistent across all size classes, resulting in a mean estimate of 23 yr of age at first reproduction. However, as mentioned previously, smaller turtles retain most or all LAGs, with minimal resorption cores, which will minimize the error rate between actual and estimated age. For example, Goshe et al. (2010) found that green sea turtles up to 44.8 cm SCL retained all LAGs, allowing age to be estimated from direct counts. Hence, it is possible that age at first reproduction was underestimated by Van Houtan et al. (2014).

We assert that, once the bias is understood and taken into account, skeletochronology benefits our understanding of age and growth by providing long-term time series of growth rates for individual turtles that span multiple habitats, including information on growth rate variability. Furthermore, using skeletochronology provides growth rates that are consistent 1-yr intervals, in contrast to the growth records used by Balazs & Chaloupka (2004a), which represented varying amounts of time, 12 mo or more, between captures. The mean growth rates observed in Hawaiian green sea turtles in the present study, for the 40– 80 cm SCL size class bins, were 1.66–2.05 cm yr^{-1} for both sexes combined, higher than the range of 0.8– 1.8 cm yr^{-1} found by Balazs & Chaloupka (2004a) in a mark–recapture study, but comparable to the mean growth rates of 2.1–2.3 cm yr^{-1} from a skeletochronology study of the same population (Zug et al. 2002).

Our sample size of growth spurts was small, and we were unable to detect a significant difference between sexes in the frequency of spurts within size classes. However, our results do suggest a higher frequency of growth spurts in the 70 to 80 cm SCL size classes for females, while growth spurt frequencies were relatively uniform across all SCL size classes for males. We also detected slightly lower growth rates in males compared to females. Using the expectation

of the mean, we estimated the time to grow from 30 to 90 cm SCL for males at 34.3 yr (95% CI: 26.0–55.8 yr) compared to 31.1 yr (95% CI: 26.7–46.3 yr) for females.

We detected growth spurts in all juvenile size classes between 30 and 90 cm SCL. The mechanism(s) that cause growth spurts are uncertain; however, compensatory growth has been detected in green (Bjorndal et al. 2000) and loggerhead *Caretta caretta* sea turtles (Bjorndal et al. 2003). Strictly speaking, compensatory growth is defined as a period of accelerated growth following an episode of reduced growth rates, potentially due to lack of adequate forage (Wilson & Osbourn 1960, Jobling 1994). Balazs & Chaloupka (2004a) found growth spurts in immature Hawaiian green sea turtles, and Casale et al. (2011) also observed periods of fast growth following slow growth in loggerheads. Undernutrition has been observed in Hawaiian green sea turtles at Kaloko-Honokohau on Hawaii Island (Wabnitz et al. 2010), suggesting these turtles may compensate via compensatory growth to reach normal weight and length (Mitchell 2007).

In addition to compensatory growth, it is possible that growth spurts are regulated through hormonal mechanisms such as the pubertal growth spurt (Soliman et al. 2014), although very little is known about this mechanism in reptiles (Ball & Wade 2013). While no studies have been conducted regarding this phenomenon in sea turtles, Lance et al. (2015) found a rise in testosterone levels associated with a growth spurt in male alligators *Alligator mississippiensis*. In support of this mechanism for Hawaiian green sea turtles, we detected the majority of the growth spurts for females in the 70 to 80 cm SCL size category, and Murakawa (2012) found increasing follicle sizes for juvenile females greater than 65 cm SCL, suggesting a relationship between growth spurts and the onset of sexual maturity. While Murakawa (2012) also found increasing weights in testes for juvenile males greater that 65 cm SCL, we did not detect a pulse in growth spurts for these size classes, potentially due to the small sample size. The relationship between growth rates and sexual development in sea turtles requires further study, as this could have important implications for estimating age at first reproduction for both sexes.

While compensatory growth and pubertal growth spurts can increase growth rates, disease has been known to negatively affect growth rates (Zug et al. 2002). Severely afflicted turtles with FP, a tumor-forming disease, have been found to have decreased growth rates (Chaloupka & Balazs 2005). In the present study, all turtles were used regardless of tumor severity and, as expected, FP was more prevalent in the larger sizes (60–89.9 cm SCL) as previously noted by Chaloupka & Balazs (2005). We did not differentiate between turtles with and without FP in our analysis. The prevalence of the disease within our sample size may in part explain the differences between the growth rates of the present study and those of other studies on the same population. However, the purpose of our study was to examine the impact of growth spurts on our ability to estimate stage durations from mean annual growth rates. Therefore, considering the severity of FP for individuals is outside of the scope of this study, but may be addressed in another study.

Conclusions and conservation implications

In this study, we demonstrate that while some methods are more accurate than others, all of the methods used overestimated the time to grow when more than 10 cm of carapace length growth is being estimated. The results provide evidence that exceptional growth rates, while relatively rare, allow turtles to grow through size class bins faster than expected based on knowledge regarding mean growth rates. Our work provides new information on the potential impact of growth spurts on estimates of age at first reproduction that should be considered in any status assessment of this population of green sea turtles. The results emphasize the importance of incorporating the prevalence of growth spurts in skeletochronological and mark–recapture analyses, as well as evaluating the impact of summarizing growth rate on estimating time to grow and back-calculating the number of growth marks lost in the resorption core. Conducting a robust population assessment of the Hawaiian green sea turtle is critical, as the population, which uses annual nesting females as a proxy for the total population, has increased by nearly 5.4% a year since the 1970s (Balazs et al. 2015) when protections were enacted. Fully understanding the demographics and status of this threatened species would require the incorporation of uncertainty around growth rate and age at maturity.

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