DETERMINATION OF GROWTH SPURTS

IN HAWAIIAN GREEN SEA TURTLES

USING SKELETOCHRONOLOGY

AND HISTOLOGICAL ANALYSIS OF GONADS

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI`I AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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ABSTRACT

The population of Hawaiian green sea turtles (*Chelonia mydas*) has steadily increased since its protection under the Endangered Species Act of 1978. However, a more complete understanding of the state of recovery of Hawaiian green turtles is stymied by lack of certainty regarding age structure of the population. Based on the observed slow growth rates of juveniles, current assessments place age to maturity in Hawaiian green sea turtles at 35-40 years. However, it is possible that dynamics such as growth spurts associated with the maturation process have been missed. Studies such as skeletochronology and histological analysis of the gonads have provided data on growth rates and maturity of marine turtles, but comparative analysis using both techniques has never been completed. Combining data from both techniques have resulted in showing that growth spurts occur throughout the life span of Hawaiian green turtles indicating that using mean annual growth rates may overestimate age to maturity.

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Introduction

Many Pacific green sea turtle populations are in serious decline (Seminoff 2002), but the Hawaiian green sea turtle population (*Chelonia mydas*) represents one of the few remaining stocks with a viable population size of nesting females (Balazs and Chaloupka 2004a). Although recovery of the Hawaiian green sea turtle is occurring, the population is still federally listed under the Endangered Species Act as a threatened species. The lack of information on turtle growth rates within the diverse geographic habitats hinders decision-making in the management of this protected species. Particularly with the emerging public debate about whether the population of Hawaiian green turtles has recovered enough to be delisted, growth and growth-pattern variations in sea turtles are important demographic parameters to understand in recovering and conserving a population (Zug et al. 2002).

Estimates from growth models predict age- and size-based growth rates using data from capture-mark-recapture field studies. Unfortunately these field studies require a long period of time and effort in order to provide an accurate assessment of growth rates. With the addition of skeletochronology, confirmation can be made to justify age estimates. Skeletochronology provides a means to estimate growth and is used to analyze the number of growth increments formed within the humerus (Zug et al. 2002), long bones (Kumbar and Pancharatna

2001), teeth (Dellabianca et al. in press, Laws 1952, Scheffer 1950), and phalanges (Eggert and Guyetant 1999, Leclair et al. 2000, Lima et al. 2000) and has been successful in estimating age and growth in numerous species of reptiles and amphibians (Castanet 1994, Smirina 1994) and mammals (Dellabianca et al. in press, Scheffer and Myrick 1980). However, this tool needs validation through various methods such as studying known-age turtles, capture-mark-recapture studies, and incorporating fluorescent markers (Snover and Rhodin 2008). The annual nature of the growth increments has been validated in several herpetological species such as lizards (Roitberg and Smirina 2006), tortoises (Castanet and Cheylan 1979, Curtin 2006), and in loggerhead, Kemp's ridley, and green sea turtles (Coles et al. 2001, Goshe et al. 2010, Klinger and Musick 1992, Snover and Hohn 2004, Snover et al. 2011) by using known-age turtles and fluorescent markers.

Morphological changes in the genders occur as the turtle approaches maturity (Spotila 2004). Two indicators of maturation in males include the elongation of the tail and the curving and elongation of the flipper claws. For mature males, the carapace, top shell, is wider than and not as domed as the females.
In addition, reproductive biology and endocrinology of sea turtles have been examined and used to determine developmental stages. Gonadal developmental stages have proven successful in determining growth stages in the male green sea turtles. Histologically analyzing the gonads provide confirmation of the size-

related changes in the reproductive organs. For reptiles, sexual maturity is based primarily on size and not age (DeNardo 1996). By using histological and immunohistochemical analysis in sea turtles, Otsuka et al. (2008) determined that growth can be determined independent of body size measurements indicating that the carapace length may not always be an accurate indicator of age of maturity. Another tool that has recently been used is histological analyses of gonads (Blanvillain et al. 2008, Blanvillain et al. 2010, and Otsuka et al. 2008) which provide definitive data on sexual maturity. Other techniques for assessing maturity include the use of ultrasonography and laparoscopy, and the softness of the plastron (Blanvillain et al. 2008).

Age estimates of Hawaiian green sea turtles indicate that turtles resident to different foraging habitats may reach adult size at different ages (Balazs 1980). Twenty or more years to reach sexual maturity seems improbable, but slow growth and late maturity have been repeatedly confirmed for some sea turtle species (Bowen et al. 1992, Goshe et al. 2010, Parham and Zug 1997, Snover 2002). Expected age at maturity was estimated to be about 35-40 years for the four populations sampled at the southeastern end of the Hawaiian Archipelago (Figure 1), but it might be greater than 50 years for the Midway population due to slower growth rates due to limited food availability and cooler water surface temperatures (Balazs and Chaloupka 2004a).

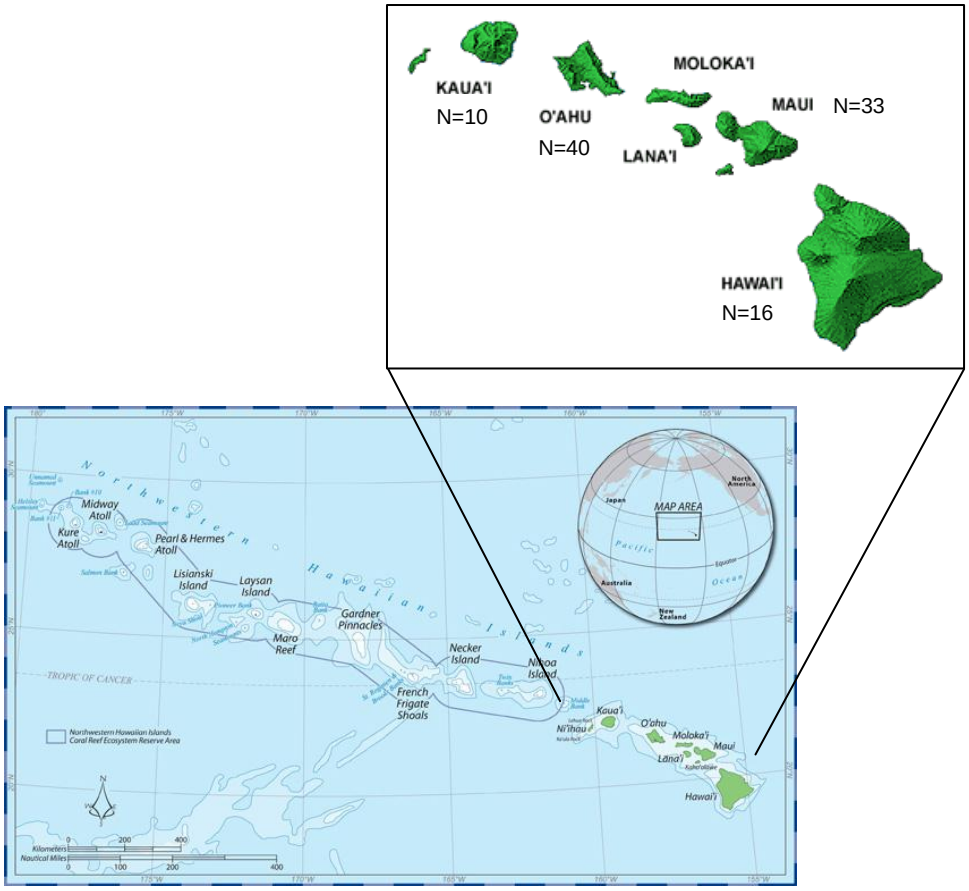

Figure 1. The Hawaiian Archipelago (NOAA) with a break-out map of the main Hawaiian Islands and corresponding number of strandings per island.

However, there are challenges in the use of skeletochronology, particularly in larger size classes where bone resorption results in loss of growth rings thus complicating the interpretation of age. The present study uses both skeletochronology and histological analysis of gonads to estimate growth spurts and sexual maturity in Hawaiian green turtles. I hypothesize that, while average growth rates in large juveniles/sub-adults are very low (<1.5 cm/yr), individuals may experience surges in growth rate during this stage, lasting only 1 or 2 years,

that move them through this stage class faster than predicted from average growth rates, and causing them to reach the size of sexual maturity faster than previously predicted. Also, I hypothesize that since growth spurts may be detected in some of the large juvenile/sub-adults, the maturation process may be initiated at these earlier size classes.

Material and Methods

The Marine Turtle Research Program of the Pacific Islands Fisheries Science Center (PIFSC) has a sea turtle stranding and salvage program that has been in existence since 1982, but formally since 1990 (Chaloupka et al. 2008, Murakawa et al. 2000). Stranded sea turtles are defined as dead, injured, sick, tumored, or abnormally behaving turtles (PIFSC per comm). Calls are reported to PIFSC and/or their collaborators and then, if necessary, the turtle is recovered. Dead turtles are stored in PIFSC's freezer for future necropsies to collect morphometrics and samples and determine the cause of mortality. Live turtles are evaluated by a veterinarian and a biologist to determine treatment options, but if it has a poor prognosis it is humanely euthanized then necropsied (Chaloupka et al. 2008). As of 2011, a total of over 6,000 sea turtle stranding cases from the Hawaiian Archipelago have been reported to PIFSC.

For this study, a total of 99 humeri (Figures 2, 3, and 4) and 70 gonads were collected. 33 testes were histologically analyzed along with the visual examination of 37 ovaries. The straight carapace length (SCL) and humerus were collected from salvaged dead Hawaiian green sea turtles for skeletochronological analysis. The SCL was measured from the nuchal notch to the posterior end of the posterior marginal with an aluminum tree caliper (Haglőf©) and recorded in centimeters (Wyneken 2001). Turtles were then

categorized into size classes based upon the SCL as follows: less than 65cm were classified as juveniles; greater than 65 and equal to 81cm as sub-adults; and greater than 81cm as adults (Balazs 1980). These size classes were devised from Hirth (1971) with the modification of the sub-adult class from 40 to 65cm as it has been observed that sexual dimorphism occurs at 65cm for Hawaiian green sea turtles (Balazs 1980). Hatchling measurements were taken with a digimatic plastic caliper (Mitutoyo©) and recorded in millimeters. It is not possible to determine the gender of a turtle by its external appearance for individuals smaller than 81cm SCL. The gender of larger turtles can be determined by the length of the tail as adult males have tails that extend well beyond the carapace and the cloaca will be closer to the tip, whereas, in adult females the tail does not extend too much further from the edge of the carapace (Wyneken 2001).

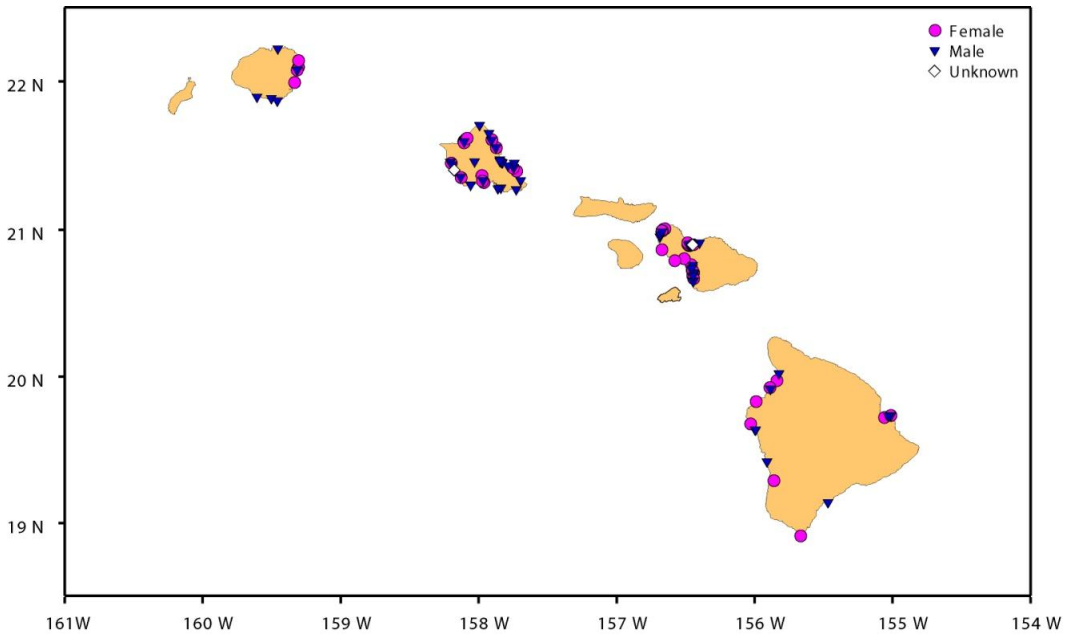

Figure 2. Location of the 99 Hawaiian green sea turtle strandings with corresponding size in SCL. Pink circles represent the females (N=47), blue triangles represent the males (N=50), and the two white diamonds represent the turtles that gender could not be identified.

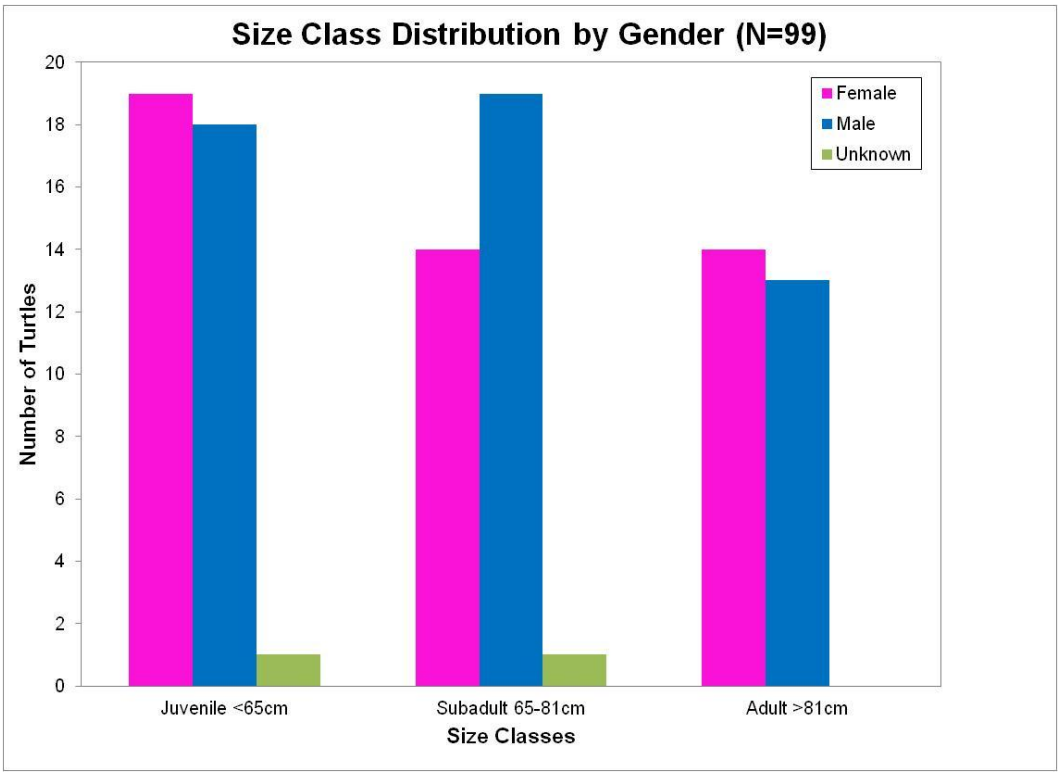

Figure 3. Size classes and genders of the 99 stranded Hawaiian green sea turtles (Juvenile=38, Sub-adult=34, and Adults=27).

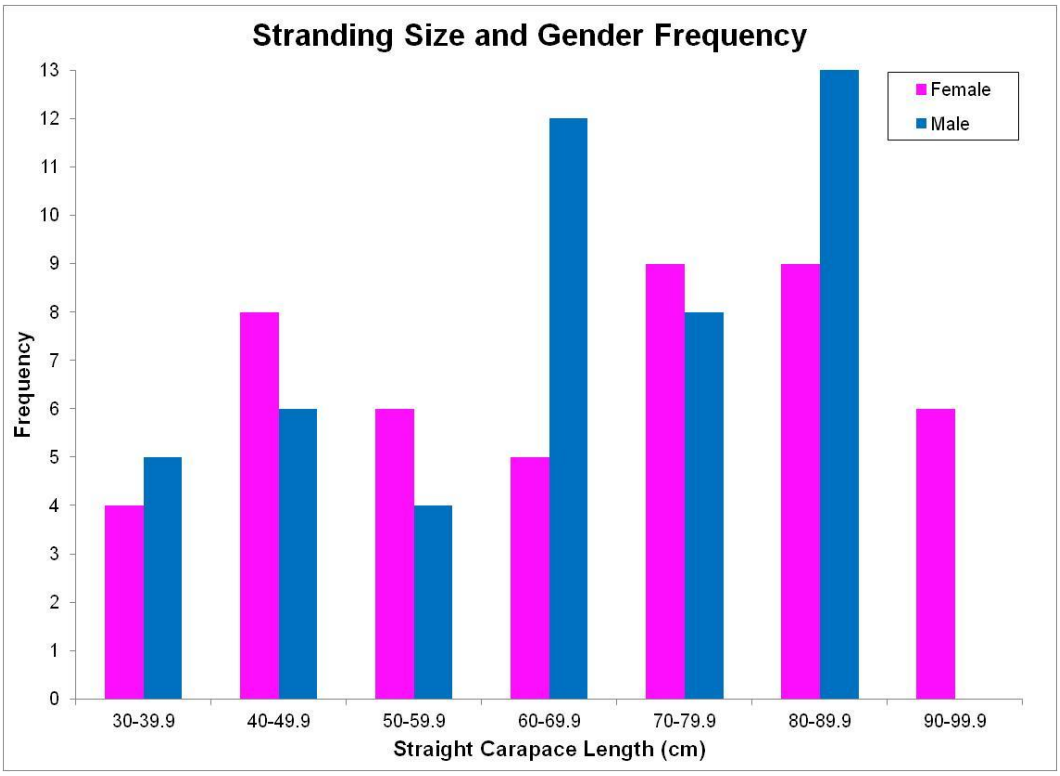

Figure 4. Stranding size and gender distribution of the 99 Hawaiian green sea turtles.

Skeletochronology

The majority of humeri collected were the right for standardization, but in two cases the left humerus was collected as the right side was not available. Procedures detailed in Zug et al. (1986) and Snover and Hohn (2004) were used in processing the humeri. After collection from dead stranded turtles (Chaloupka et al. 2008, Murakawa et al. 2000), the humeri were stored frozen until it could be processed which involved thawing and then flensing, boiling, and drying. Prior to cutting the humeri, measurements (mm) and weights (g) were taken. The weight of the humerus was taken with an Ohaus® Navigator™ digital scale. The humeri sections were sectioned proximal to the narrowest part of the diaphysis within the deltopectoral muscle insertion scar (Figure 5). This site was used because it has the thickest cortical mass for determining LAGs (Snover and Hohn 2004).

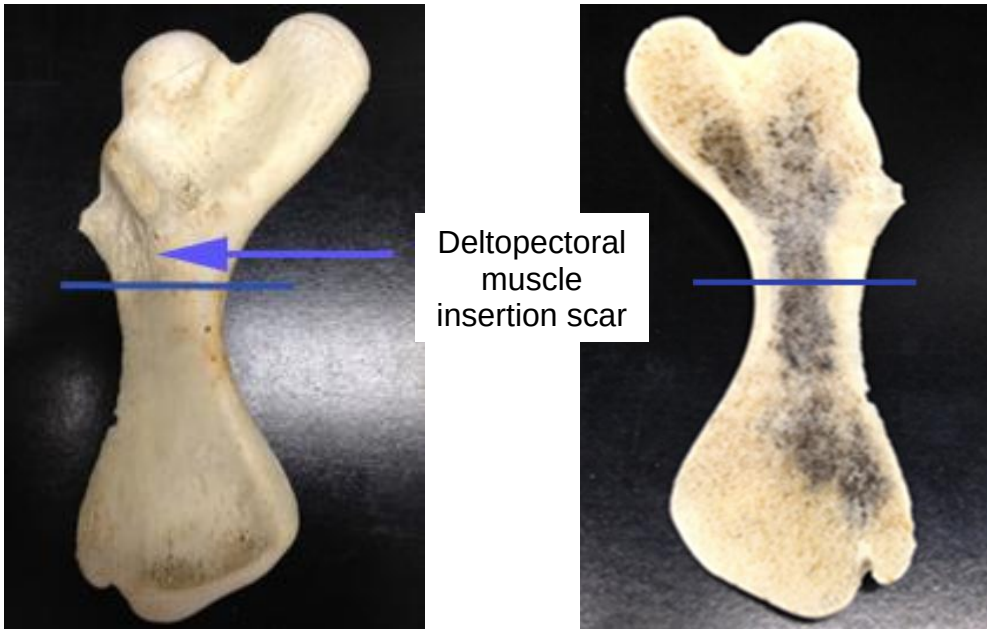

Figure 5. Right humerus of a Hawaiian green sea turtle. Arrow points to insertion scar and line indicates the where the humerus was cut.

Sections were cut between 1-3mm using a Buehler Isomet™ low speed saw then placed into histocassettes into the Fisher Cal-Ex II© decalcifier solution. Once the humeri section was decalcified, for about 7 days, it was flushed generously with water then soaked overnight in water to remove any remaining decalcifying solution on the section. The humeri section was then cut into 25µm sections using the Leica© freezing stage microtome then decalcified overnight to prepare the section to receive the staining solution. After decalcification the 25µm section was soaked overnight in water. The sections were then stained using the Ehrlich's Hematoxylin solution (Klevezal 1996) then soaked in water for 20 minutes. The stained sections were then examined using a Nikon© stereozoom microscope to ensure that the lines of arrested growth (LAGs) were visible.

Sections were then transferred into glycerin first by a 50% solution of glycerin and water then finally 100% glycerin at 15 minute intervals. The stained sections were then mounted onto a glass slide in 100% glycerin, covered with a cover slip, and then sealed with Permount® for viewing and archiving. Once the humeri sections were completely processed, the LAGs were counted. The humeri sections were then photographed at 40x 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. The photographs were then composited using either Adobe® Photoshop CS3 or Elements software. Once the composites were made, analysis for LAGs was performed. While viewing the section with the microscope, marks were made on the identified LAGs using Adobe® Photoshop CS3. The marked LAGs were measured using the iSolutions Lite© software and saved in a Microsoft® Excel spreadsheet for manipulating the data.

Calculating Growth Rates

The lines of arrested growth (LAGs) were determined by microscopically examining the humerus section to identify the thin lines that appear darker than the surrounding tissue. A broad zone followed by a LAG represents a skeletal growth mark that is expected to represent one year (Castanet et al. 1977, Snover and Hohn 2004). For Hawaiian green sea turtles, it has been validated by Snover et al. (2011) that growth increments were annular. A predictable and proportional relationship needs to be demonstrated between the bone and the carapace (Snover et al. 2007) and in this study the straight carapace length (cm) and humerus minimum width (mm) from 167 Hawaiian green sea turtles were plotted (Figure 6). Ten hatchlings were used in this study and the minimum hatchling straight carapace length (SCL) was 5.1cm and humerus sectioning site (MW) was 2.6mm.

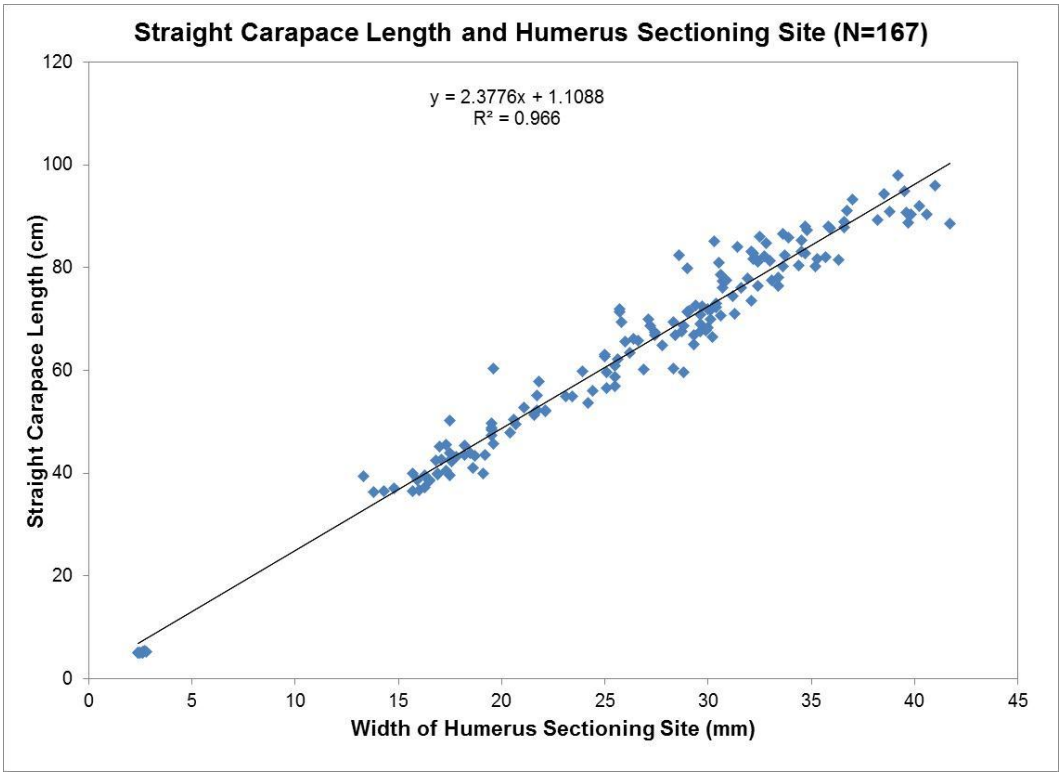

Figure 6. Straight carapace length and humerus diameter of 167 Hawaiian green sea turtles (blue diamonds). Solid line represents the fit of the linear regression.

In addition, the relationship between straight carapace length (SCL) growth and humerus medial width growth from hatching must be confirmed. This relationship is assumed by the proportionality between the growth rates of the humerus and carapace (Vigliola et al. 2000). Snover et al. (2007) adapted the proportionality equation from Vigliola et al. (2000) by changing the length of the fish (L_{op}) to the carapace length of a hatchling sea turtle as follows:

$L = L_{op} + b(D - D_{op})^c$ (Equation 1, Figure 7)

where L is the estimated SCL (cm) of the turtle, L_{op} is the minimum hatchling SCL (cm), b is the slope of the relationship (cm/mm), D is the humerus diameter (mm), D_{op} is the average hatchling humerus diameter (mm), and c is the proportionality coefficient. The residuals were also plotted with the biological intercepts of minimum hatchling SCL (cm) and minimum hatchling humerus diameter (mm, Figure 8).

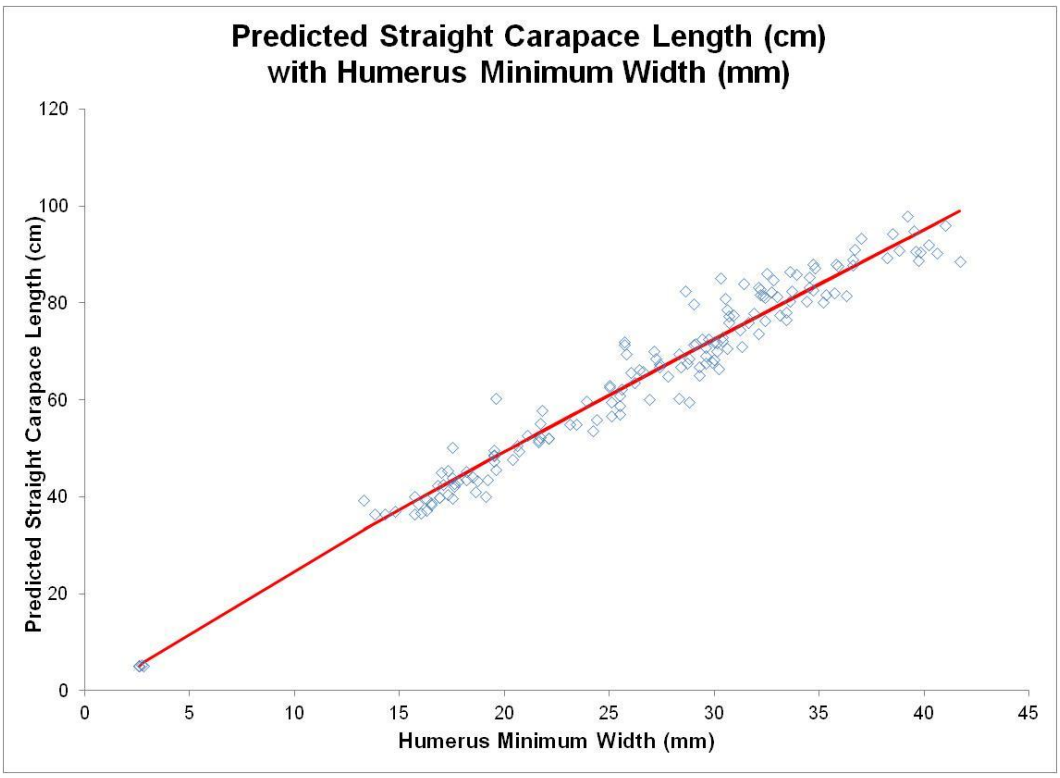

Figure 7. Predicted straight carapace length (cm) with humerus minimum width (mm) of 167 Hawaiian green sea turtles (open blue diamonds) using Equation 1. Solid red line represents the fit of the allometric relationship.

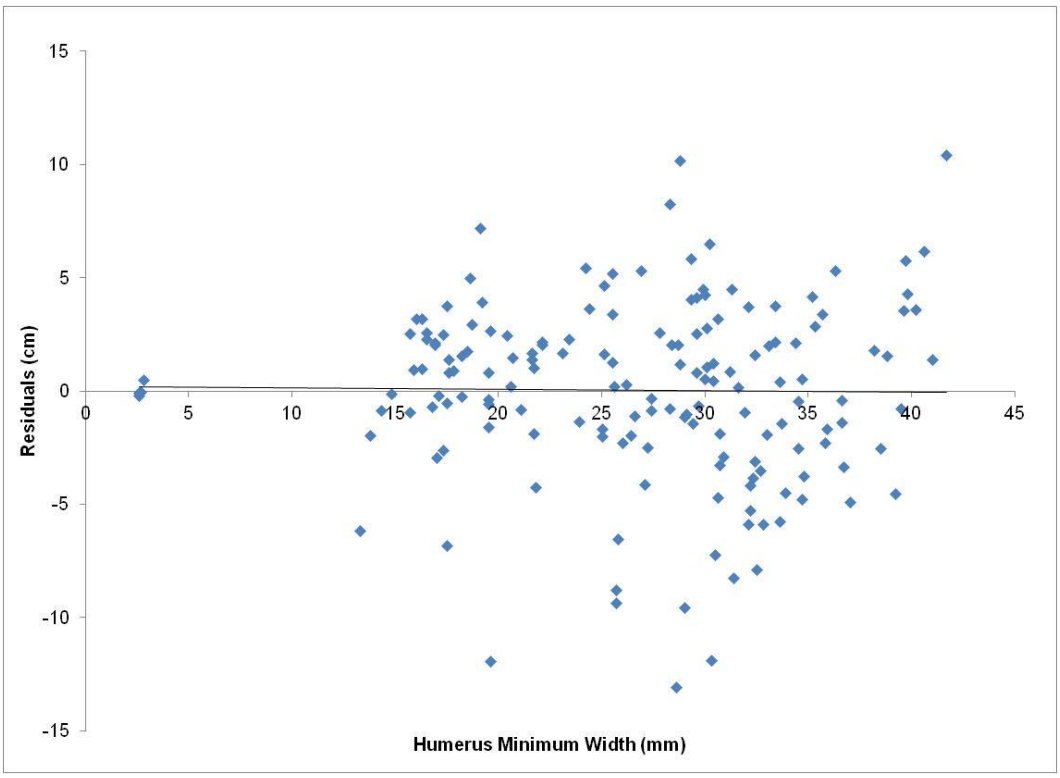

Figure 8. Residual plots of the allometric Equation 1.

The body proportionality hypothesis (BPH, Francis 1990) was applied to sea turtles by Snover et al. (2007) in order to back-calculate SCLs. This hypothesis accounts for either the isometric or allometric relationship using a proportional method. Since the relationship between the SCL and the MW shows a correlation, a back-calculation model using Equation 1 can be used to estimate carapace lengths (Snover et al. 2007):

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

where L_i is the predicted straight carapace length at LAG_i (SCL, cm), L_{op} is the average hatchling SCL (cm), b is the relationship of the slope (cm/mm), D_i is the diameter of LAG_i (mm), D_{op} is the hatchling humerus minimum width (mm), c is the proportionality coefficient, L_{final} is the observed SCL measurement (cm), and D_{final} is the observed humerus minimum (mm) width. The first term of the equation, $[L_{op} + b(D_i - D_{op})^c]$ determines the proportional relationship between the humerus growth (minimum width) and the increase in SCL. The second and third terms of the equation, $[L_{final}][L_{op} + b(D_{final} - D_{op})^c]^{-1}$, determine the correction factor (ratio) for each turtle using the observed measurement (L_{final}) with the predicted SCL.

For each composited section, the diameters of all LAGs found were measured, allowing for the back-calculation of SCLs using Equation 2. All back-calculated

SCLs were used to calculate growth rates. Growth rates were computed by first subtracting the initial SCL from the next back-caculated SCL. Then with each subsequent back-calculated SCL, the SCL was subtracted from the previous SCL so that growth rates could be computed from each LAG diameter. Growth rates were then binned into 10-cm size categories based upon the initial SCL.

Gonad Histology

Gonads were collected from 70 salvaged dead Hawaiian green sea turtles for histological and visual examination. As previously described, the gonads were categorized into three size classes as determined by the SCL. The gonads were first visually examined (Wyneken 2001) to identify the gender (Figures 9 and 10) then collected and fixed in Fisher Formalde-Fresh® solution. After at least 24 hours, the gonads were checked to ensure that the tissues were not degrading and, if necessary, additional fixative was added. After the gonads were fixed for at least 1 month, the gonads were then transferred into 70% Ethanol. Within the 37 ovaries, the five largest follicle diameters were measured (mm) and photographed if smaller than 3mm. Following methods from Perez et al. (2010), follicle diameter sizes were classified as follows: <1mm juvenile, 1-3mm sub-adult, and >3mm adult. Female turtles were classified as sexually mature based on having egg follicles >3mm diameter (Perez et al. 2010). Testes were measured (mm) and weighed (g). A total of 33 testes were sectioned and sent to the Imaging and Core Facilities of the University of Hawaii for paraffin mounting and hematoxylin-eosin staining. Four of the testes were determined to be too degraded for use. Of the 29 viable testes, 4 were from the juvenile size class, 17 from the sub-adult size class, and 12 from the adult size class. Once the sections were mounted each slide was photographed then composited with graphics software. The testes were histologically analyzed for presence of

maturing spermatozoa within the seminiferous tubules which in indicative of the male being sexually mature. The seminiferous tubules expand and spermatozoa migrate toward the center.

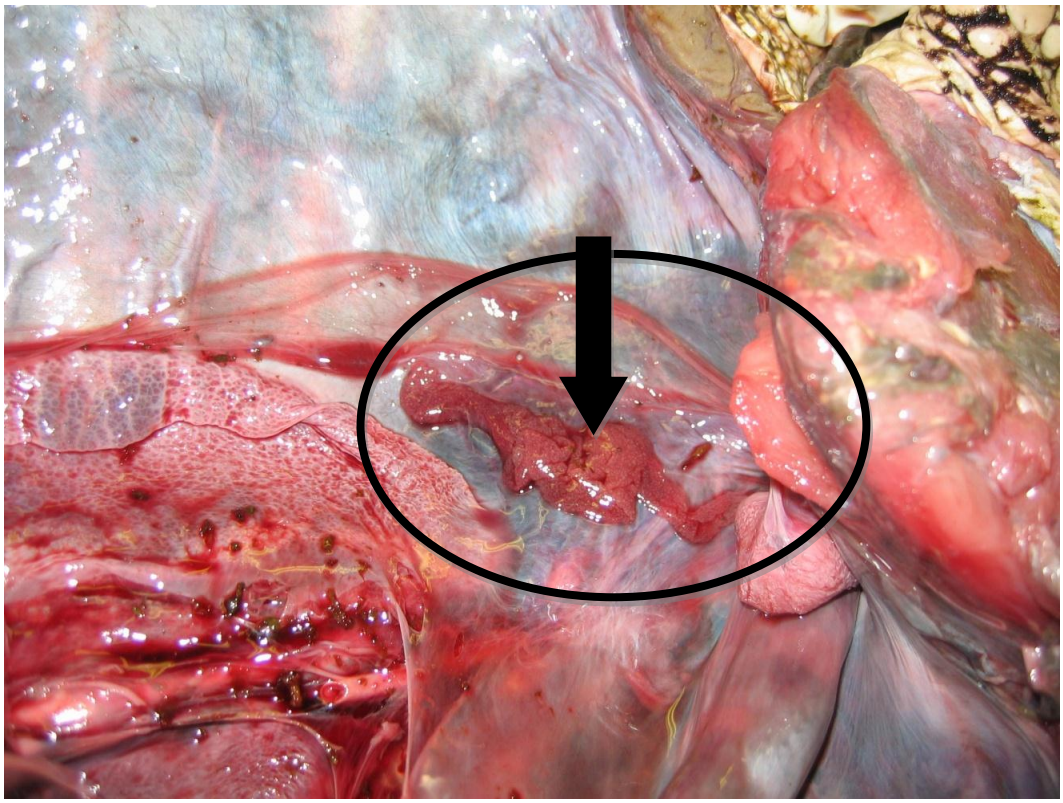

Figure 9. Gonad of a female Hawaiian green sea turtle. Note the tortuous border and rugose appearance.

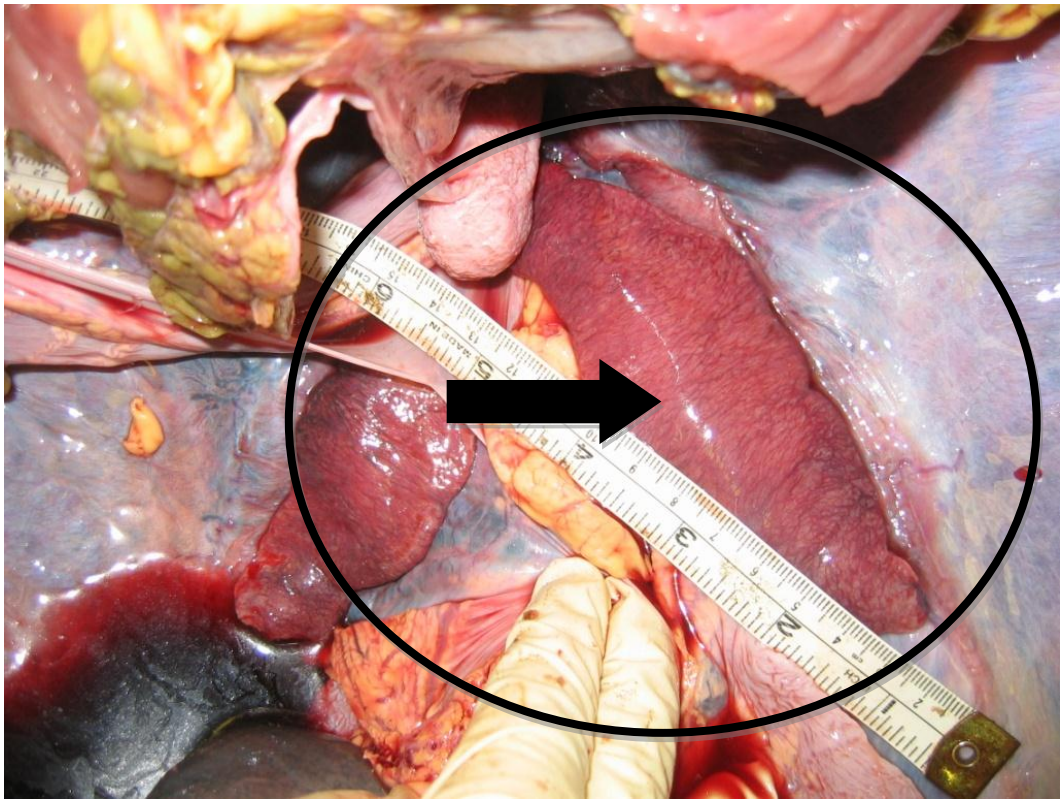

Figure 10. Gonad of a male Hawaiian green sea turtle. Note the regular smooth surface and relatively straight borders.

Results

Skeletochronology

The size range of turtles collected was 36.4cm to 97.9cm SCL. All humeri composites were analyzed by defining the lines of arrested growth (LAGs, Figure 11). Two turtles were not used in the growth rate analysis because one turtle, a 77.8cm SCL female (Figure 12), had no LAGs and another turtle, a 86.5cm SCL male, only had one LAG, and one turtle had an unknown gender. The remaining 96 turtles revealed at least two LAGs within the humeri composites (Figure 13) and were used for growth rate analysis.

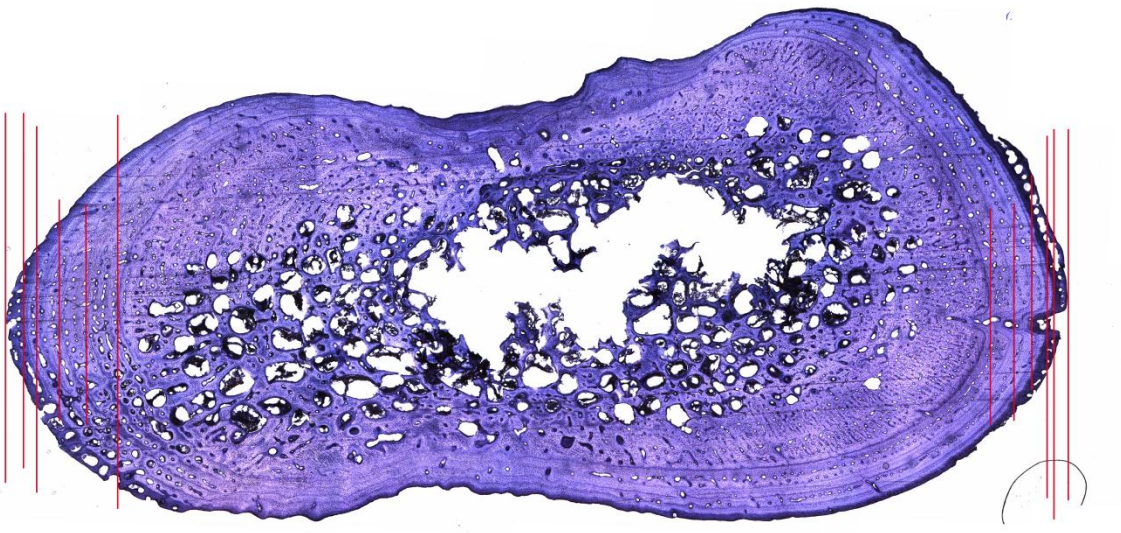

Figure 11. Lines of arrested growth (LAGs), noted by the red lines, found in the humerus section of a 43.9cm straight carapace length turtle.

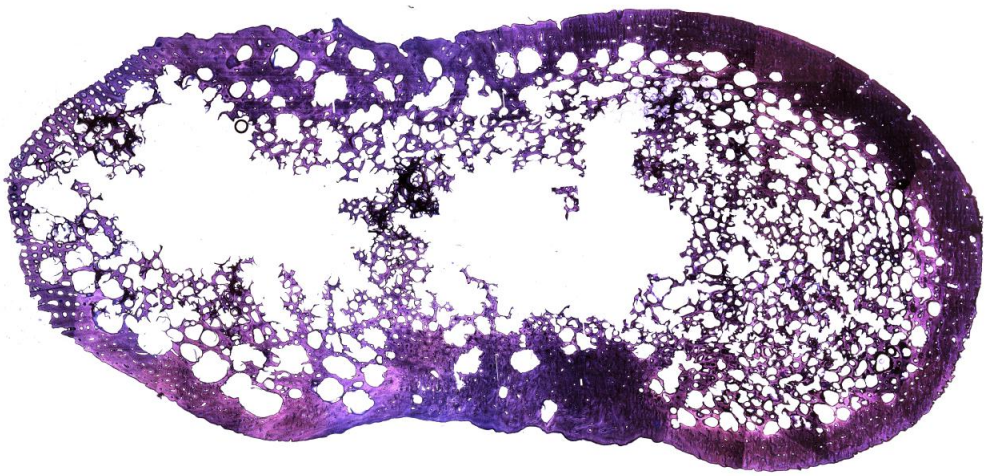

Figure 12. No lines of arrested growth (LAGs) were found in the very cancellous humerus section of a 77.8cm straight carapace length turtle.

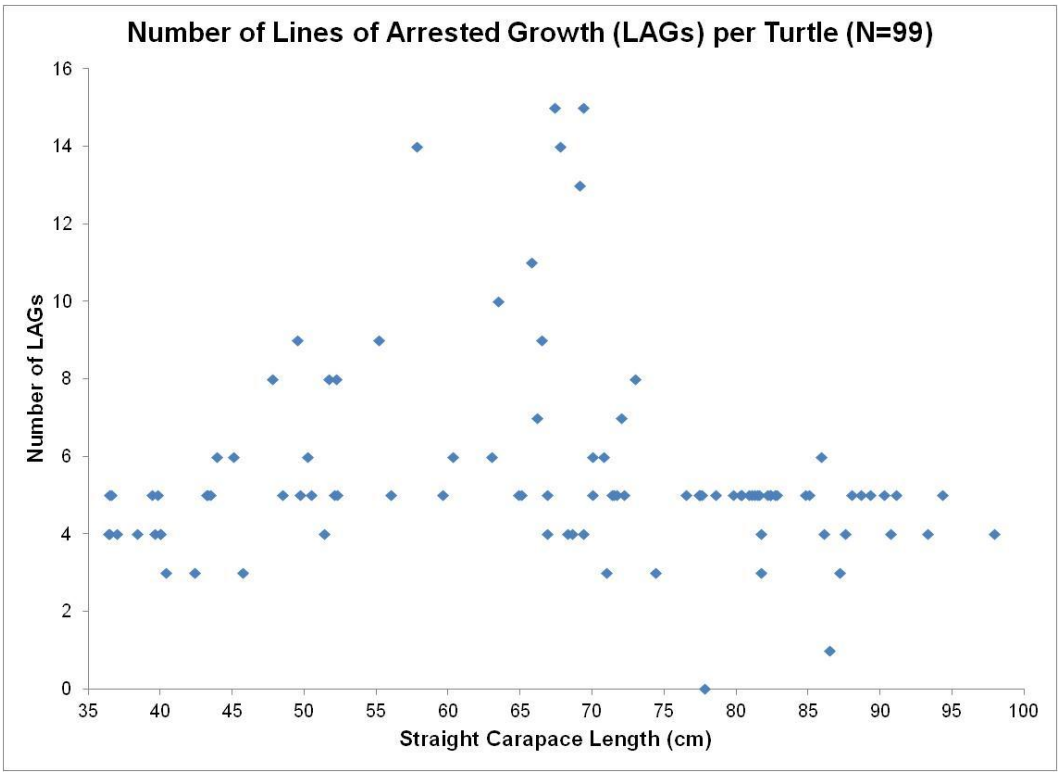

Figure 13. Amount of lines of arrested growth (LAGs) revealed in each Hawaiian green sea turtle humerus section.

Determination of Growth Spurts

A total of 297 growth rates were calculated and examined for growth spurts (Figure 14). Growth rates were binned into 10-cm size classes based upon the initial straight carapace length (cm SCL, Table 1). Growth spurts were determined by two methods. The first method was comparing the 75% quartile of the mean of all growth rates to the individual growth rates. If the individual growth rate was larger than the 75% quartile it was then defined as a growth spurt. There were a total of 74 (24.9%) growth spurts found. Of the 74, 39 were females (52.7%) and 35 were males (47.3%).

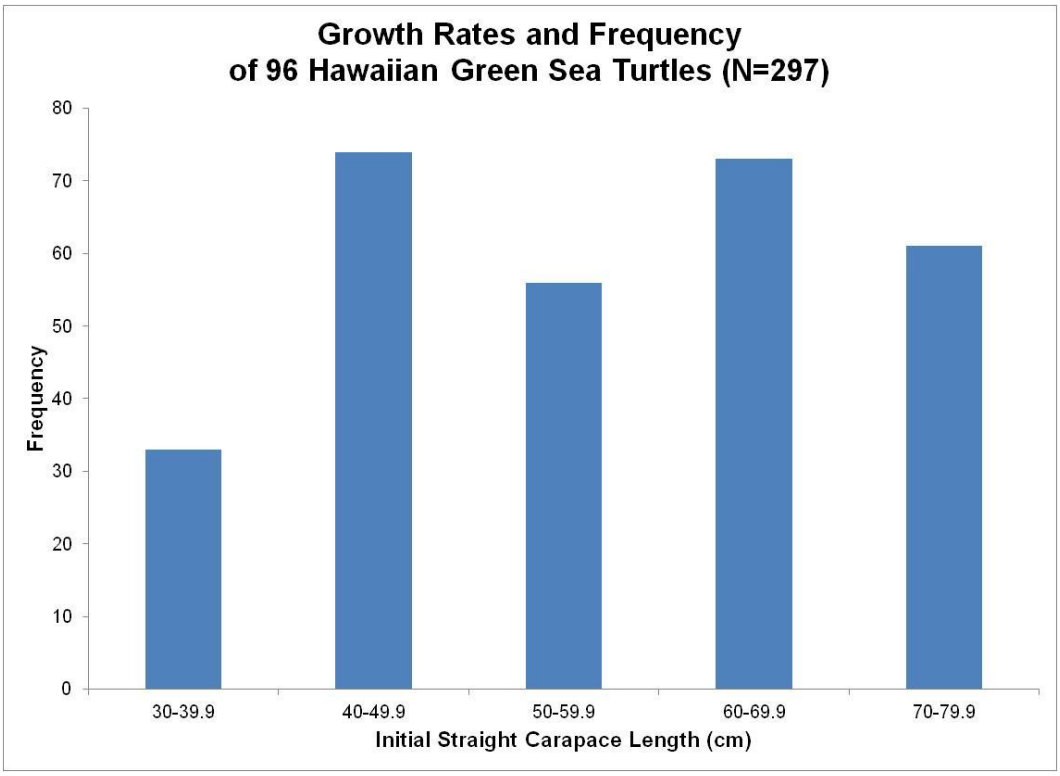

Figure 14. Growth rates calculated from 96 Hawaiian green sea turtles (N=297).

Table 1. Mean ± SD growth rates (cm yr^{-1}) of Hawaiian green sea turtles binned by 10-cm initial size classes.

Initial Size Class (cm)	Mean ± SD	N
30-39.9	1.278032 ± 2.562303	33
40-49.9	1.26745 ± 2.411132	74
50-59.9	1.45503 ± 2.318816	56
60-69.9	1.16329 ± 2.240526	73
70-79.9	1.149014 ± 2.798034	61

The second method was to calculate the difference between the observed times to grow with the expected time to grow for the 96 Hawaiian green sea turtles. The observed time to grow was calculated using the first LAG year, assumed to be February of the first visible LAG, and subtracting it to the final date which was the stranding date. To calculate the expected time to grow, for each humerus section, all size classes were identified and the mean annual growth rate recorded. For each size class, the initial size was subtracted from the largest SCL and the difference remaining was multiplied by the mean annual growth rate to calculate the expected time to grow for the size class bin. Once all size class bins were calculated, the times were added to compute the total expected time to

grow (yr, Figure 15). Within the calculations for the 96 Hawaiian green sea turtles, 78 (81.3%) had a negative and 18 (18.7%) had a positive observed-to-expected difference. For the 78 turtles, 40 (51.3%) were females (Figure 16) and 38 (48.7%) were males (Figure 17). The longer the time period the greater the negative difference between the observed and the expected time to grow (Figure 18).

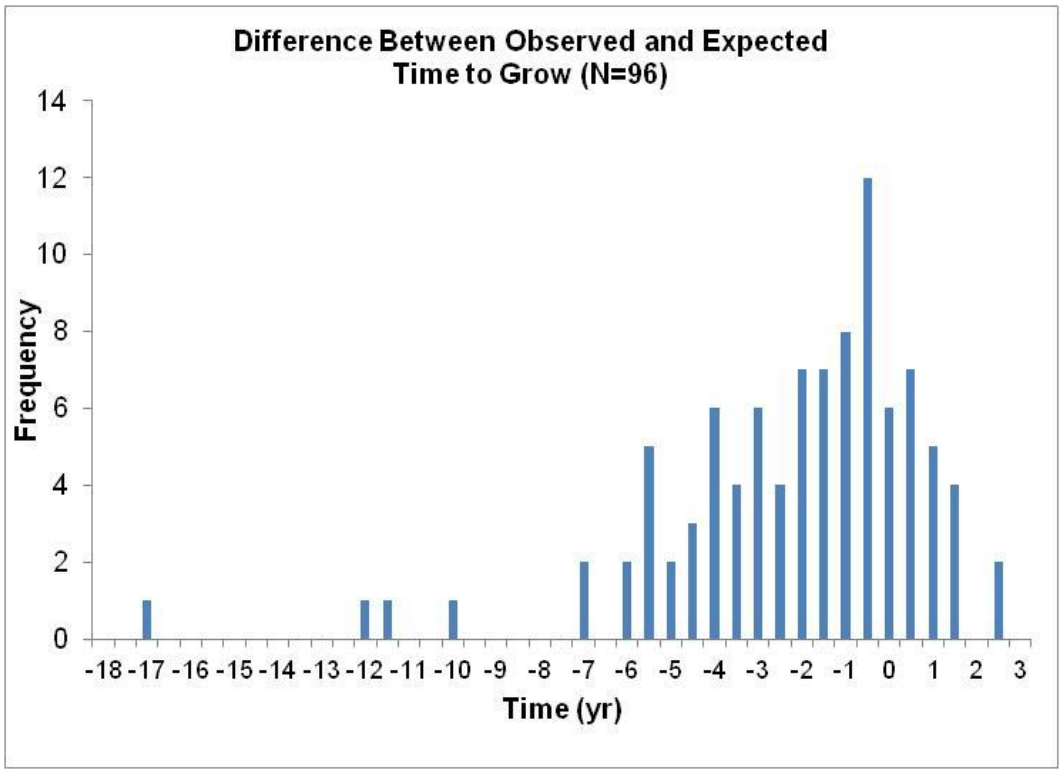

Figure 15. Difference between the observed and the expected time to grow (N=96).

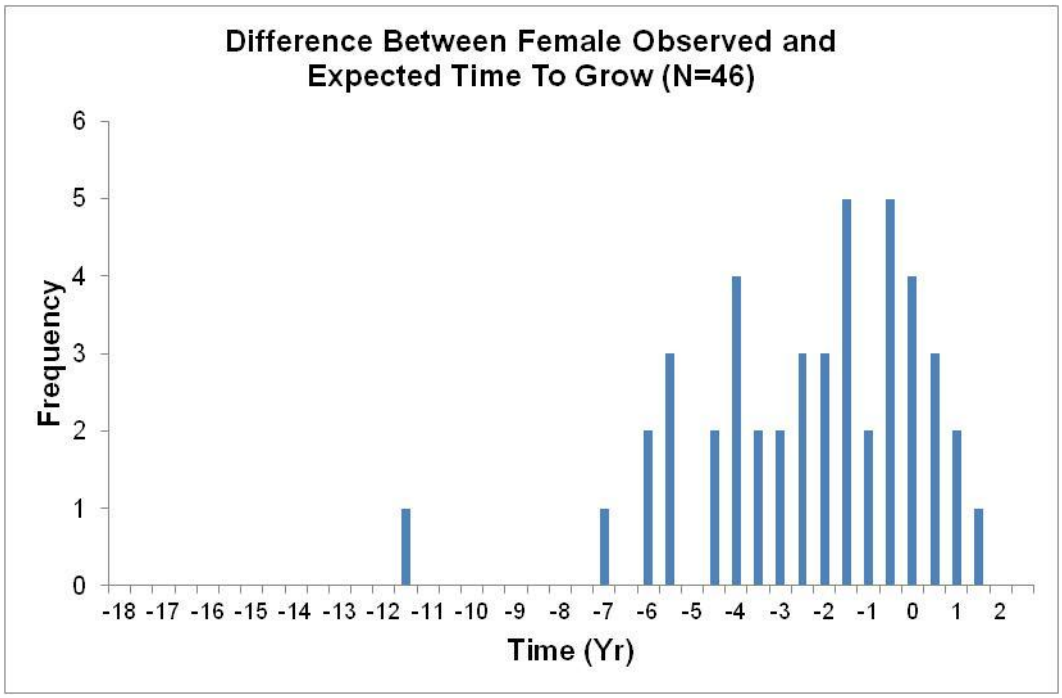

Figure 16. Difference between the female observed and the expected time to grow (N=46).

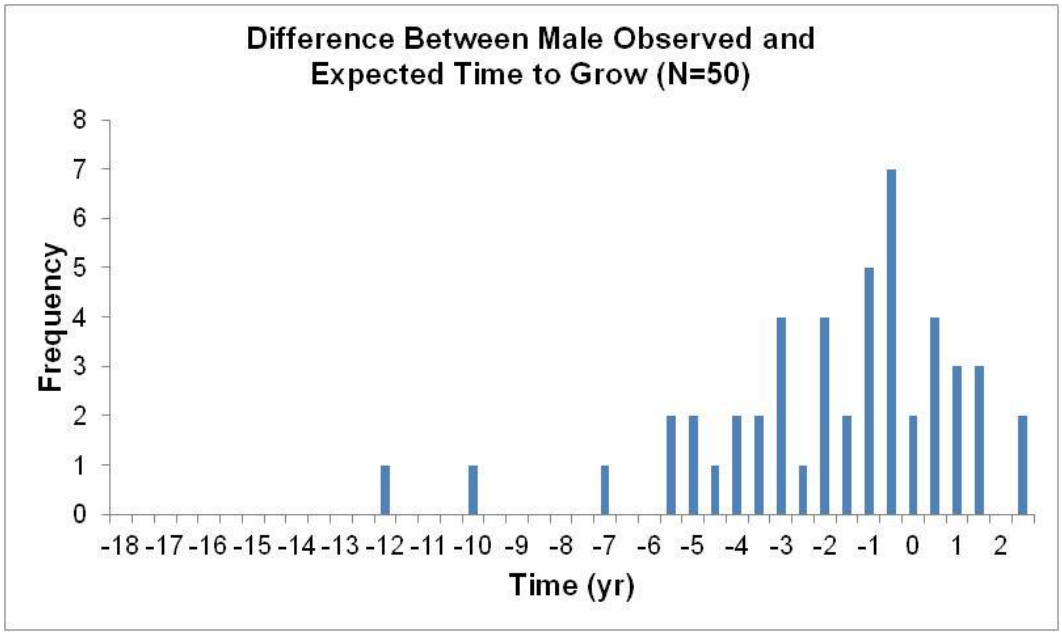

Figure 17. Difference between the male observed and the expected time to grow (N=50).

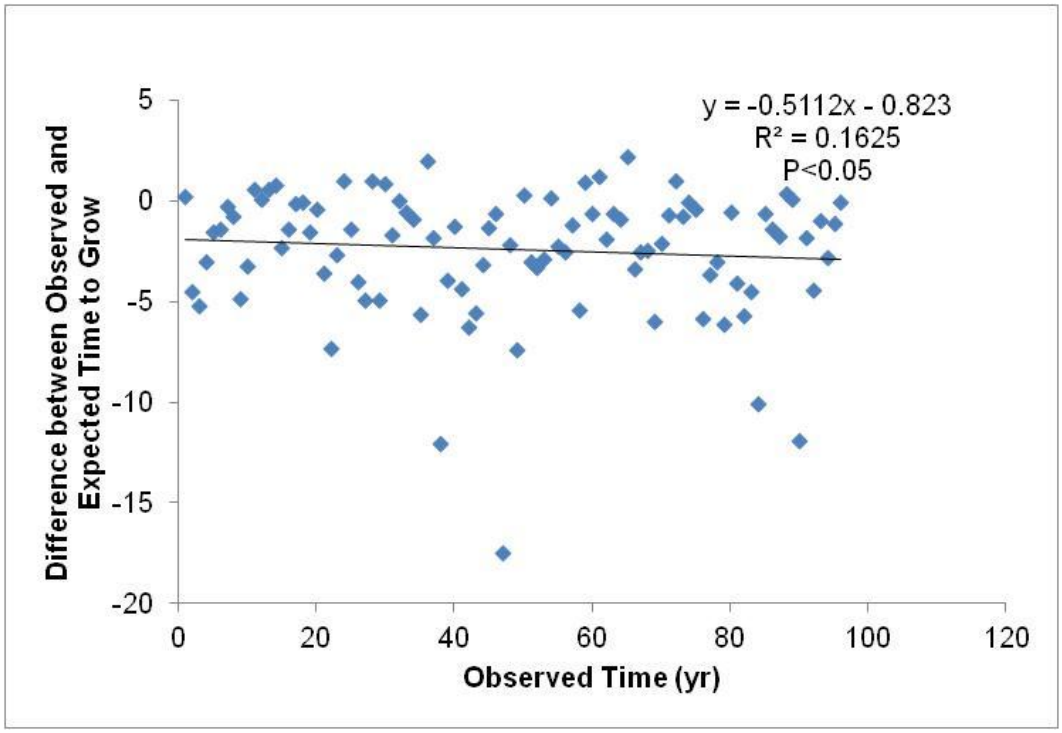

Figure 18. Observed time of growth for 96 Hawaiian green sea turtles (blue diamonds) with the difference between the observed and expected time to grow. Solid line represents the fit of the linear regression.

Gonad Histology

The follicle diameter size increased as SCL increased (Figure 19). Of the thirty-seven ovaries examined for follicle diameter size, 17 were juveniles (range 0.31–1.12mm), 9 were sub-adults (range 0.80–2.46mm), and 11 were adults (range 5.20–24.10mm, Figure 20). Eight female turtles, from the adult size class, had follicle diameters larger than 3mm classifying them as sexually mature. Seventeen juveniles, 10 sub-adults, and 2 adults were classified as sexually immature.

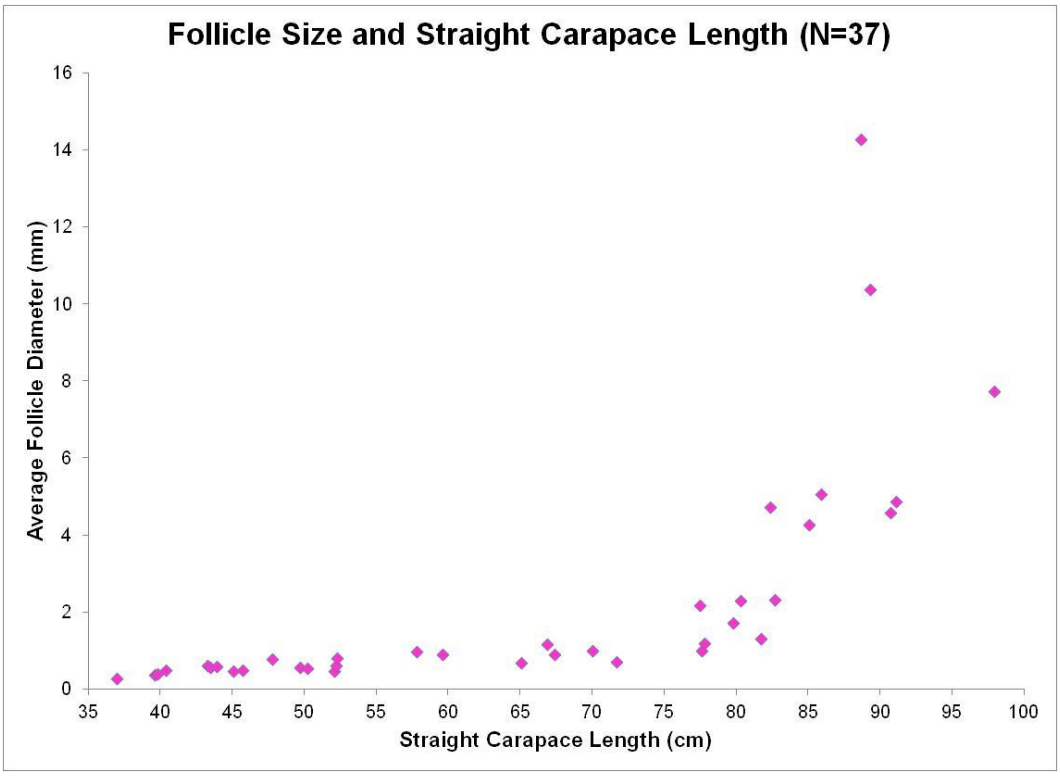

Figure 19. Follicle size and straight carapace length for 37 female Hawaiian green sea turtles.

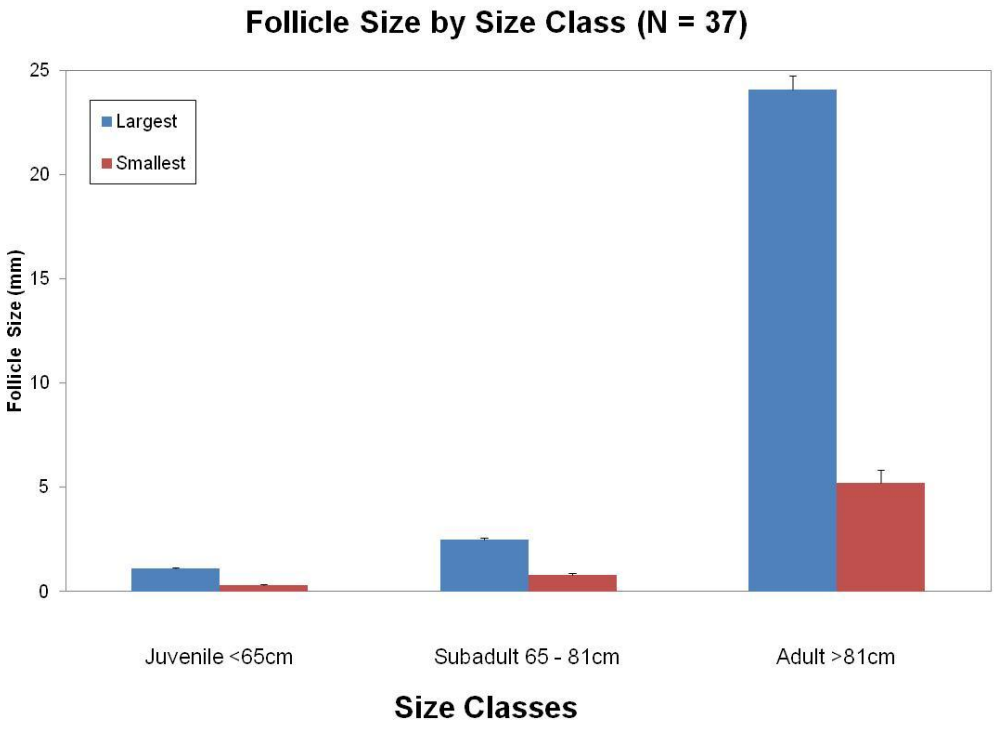

Figure 20. Follicle size by size class of 37 female Hawaiian green sea turtles.

The testes weight increased as SCL increased (Figure 21). Of the 33 testes, the presence of maturing spermatozoa was detected in 3 sub-adults and 8 adults and 4 testes were too degraded to determine spermatozoa maturity (Figure 22).

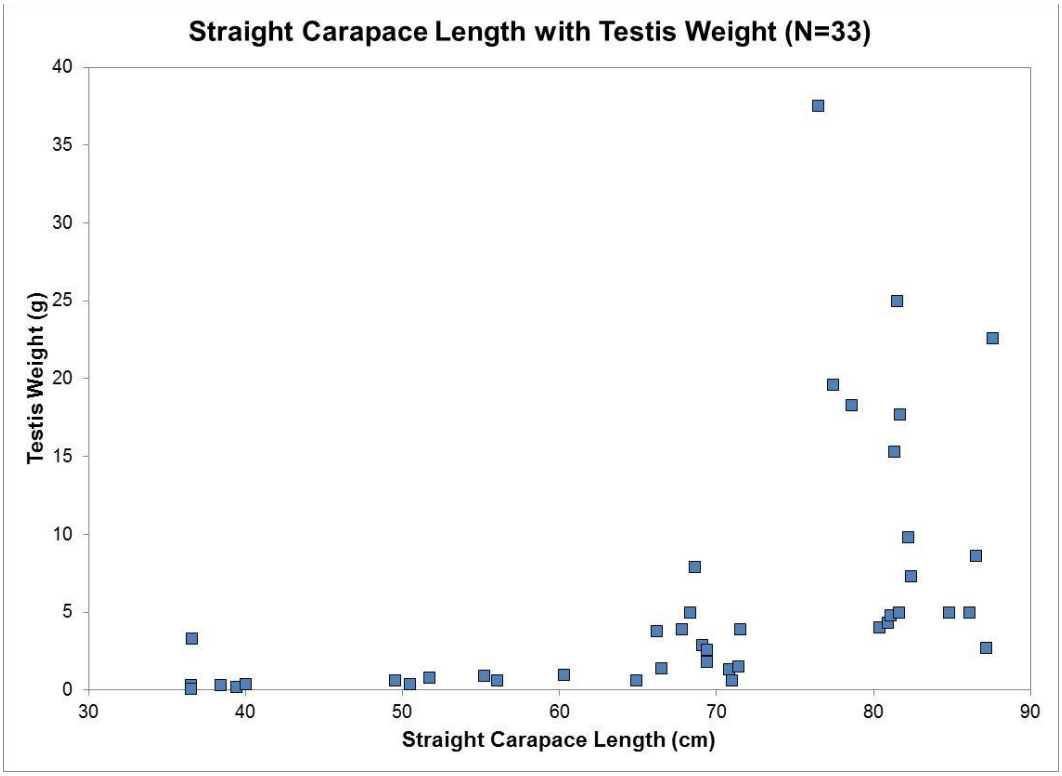

Figure 21. Testis weight and straight carapace length of 33 male Hawaiian green sea turtles.

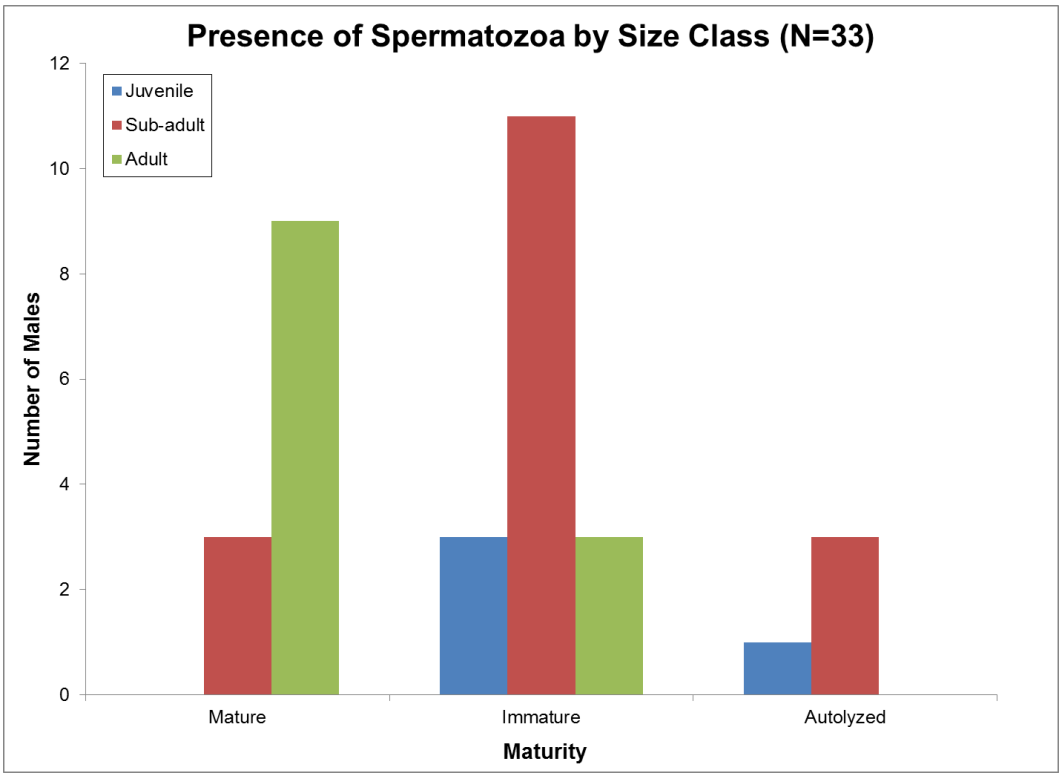

Figure 22. Spermatozoa presence in 33 male Hawaiian green sea turtles.

Comparative Analysis of Growth Rates and Sexual Maturity

Using growth rates and gonad size, both follicle diameter and testes mass indicate that there are 3 phases in growth: 1) flat line indicating no growth in small juveniles; 2) slight incline where growth is starting to increase in larger juveniles; and 3) an increase in larger sizes typical of adults. Plotting the percent of growth rates that are spurts (based on the 75% quartile), the highest percentage of spurts precedes the onset of the large sub-adults for females (Figure 23) and the large juvenile in the males (Figure 24).

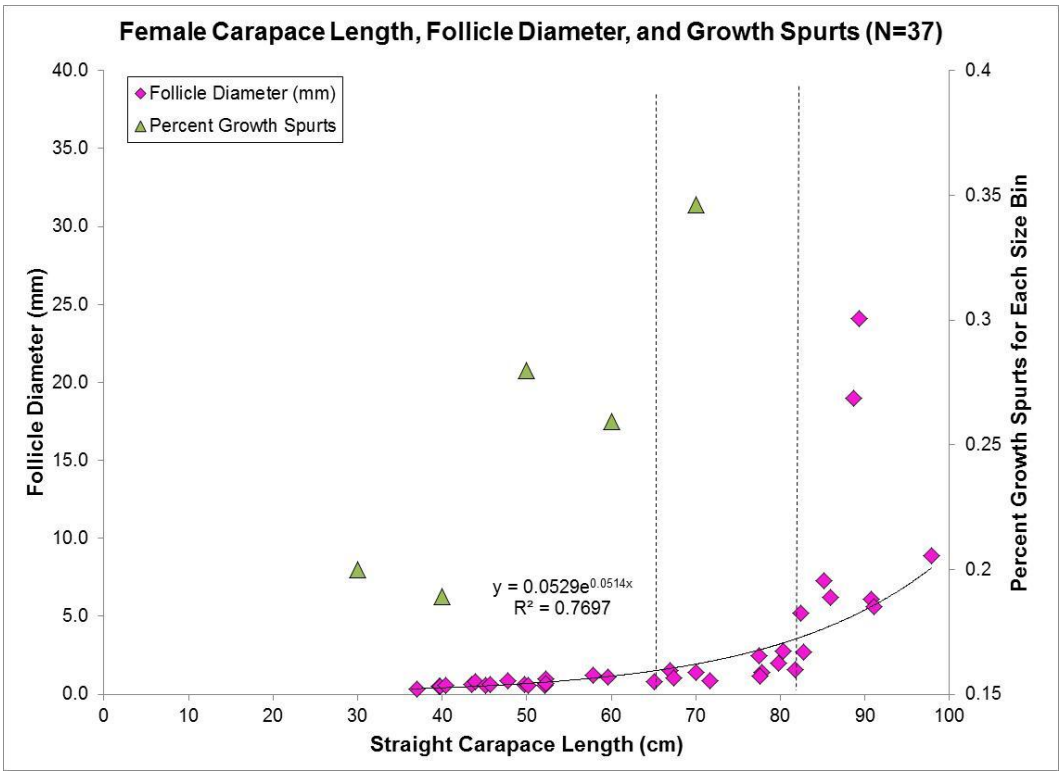

Figure 23. Female straight carapace length (cm), follicle diameter (mm), and percent of growth spurts within each size bin category. Dotted lines separate the three size classes of juvenile (<65cm), sub-adult (65-81cm), and adult (>81cm).

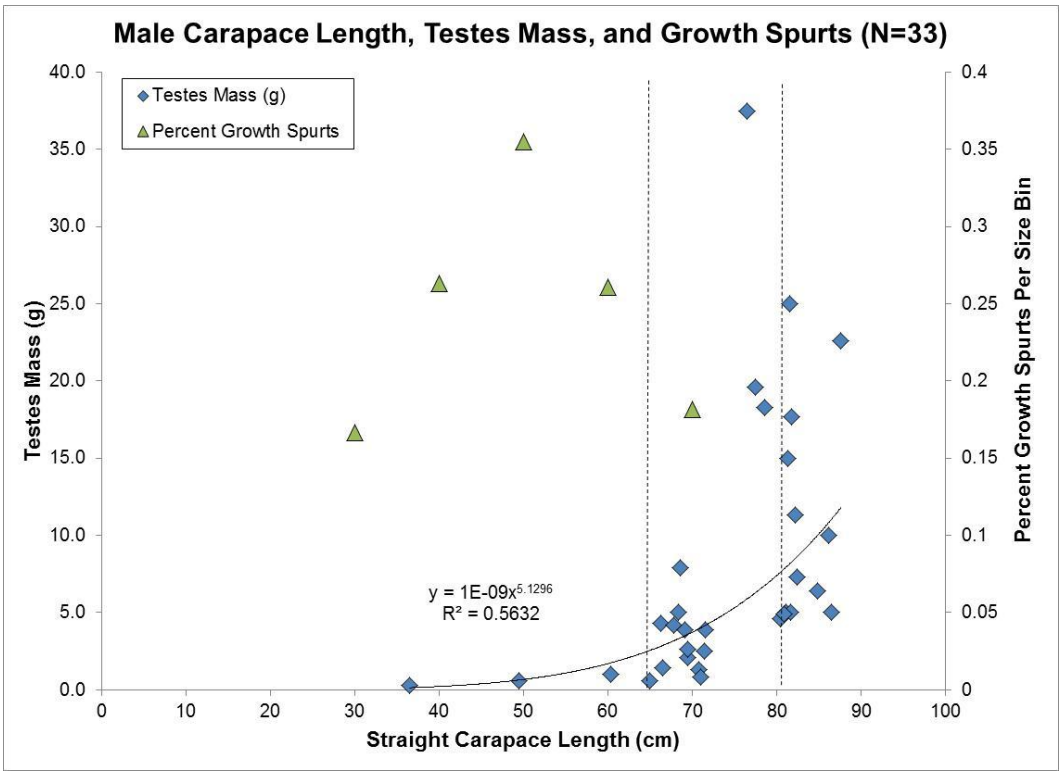

Figure 24. Male straight carapace length (cm), testes mass (g), and percent of growth spurts within each size bin category. Dotted lines separate the three size classes of juvenile (<65cm), sub-adult (65-81cm), and adult (>81cm).

Discussion

Snover et al. (2007) demonstrated that carapace length could be estimated or back-calculated from measurements of the lines of arrested growth (LAGs). Hence, somatic growth can be determined from successive LAGs. The mean growth rates observed in Hawaiian green sea turtles in this study were similar to the range of 0-2.5cm/yr as found by Balazs and Chaloupka (2004a). But the means are lower than an earlier study by Zug et al. (2002) which predicted mean growth rates ranging from 4-5cm/yr in the early juvenile size class. Although it was expected to see the difference between the expected and observed time to grow, to be centered around zero, the differences were skewed towards the negative numbers indicating that using mean annual growth rates can overestimate age sometimes even as high as ten years. This overestimation may be due to compensatory growth which allows an animal/organism to have increased growth or growth spurts after experiencing reduced growth rates (Bjorndal et al. 2003). It is essential to note that using data based on only 1-2 years of growth may also misconstrue the age estimation as applying those growth rates over many size classes will overestimate the time to grow. Also, sometimes the LAGs are not clearly defined and the resorption core may be quite large reducing the number of LAGs that are visible (Zug et al. 1986, Parham and Zug 1997). This could possibly increase the mean annual growth rate further indicating that age estimates could be overestimated.

Gonadal development for this study was defined by morphometrics rather than hormonally-driven changes. The relationships between straight carapace length and follicle diameter or testis weight show that an increase in carapace length coincided with the increase in gonad size indicating that as the carapace size increases the onset of sexual maturation of the gonad occurs. The correspondence of size classes to the skeletochronology and maturity generally worked with a couple exceptions. All but 2 adult females were considered sexually mature as the egg follicle diameters were >3.0mm. The two adult females were <83.0cm straight carapace length (SCL) suggesting that they had just entered the adult size class. No sub-adult females were sexually mature. A 76.5cm SCL sub-adult male was found with maturing spermatozoa, whereas an 84.8cm SCL adult had none. All but 3 of the adult males plus 3 sub-adults had maturing spermatozoa in the seminiferous tubules. The 3 sub-adults were >75.0cm SCL nearing the adult size class suggesting a possibility that adulthood could be reached as the turtle nears 80.0cm SCL. More interestingly a juvenile of 60.0cm SCL was found with maturing spermatozoa (Figure 23, T. Work pers. comm.).

Skeletochronology has been used to estimate age and growth rates in many sea turtle species and gonads have been used to determine growth-related changes. The stage of maturity was determined by evaluating the gonads relative to the

state of maturity seen in the histology. There appears to be growth spurts occurring throughout the life span of the turtle as predicted time to grow showed an overestimation of time. There is also a potential difference in growth between the sexes in that female growth rates seem to peak in the 70-80 cm range while males peak in the 50-60 cm range. Similar to what Chaloupka and Limpus (1997) and Limpus and Chaloupka (1997) found in the Great Barrier Reef, there appears to be a sex-specific difference in growth rates and that the female growth rates peak higher than males. Using both methods to assess the stage of maturity is valuable as it provides greater resolution to the size classes that are currently being used in the assessment of the population and also looks at sex differences. As mentioned in Balazs and Chaloupka (2004b), it has taken 30 years to increase the nearly depleted Hawaiian green sea turtle population. Up to this point, it has not been known whether or not gender and state of maturity influence growth rates. Gender specific growth rates can assist stock assessment and conservation of the Hawaiian green sea turtle. However, more research needs to be performed within the late juvenile and early sub-adult size classes of the males as maturing spermatozoa has been found in a size class where it's not expected. The late sub-adult size class is obviously a very critical time for both genders in transition to sexual maturity.

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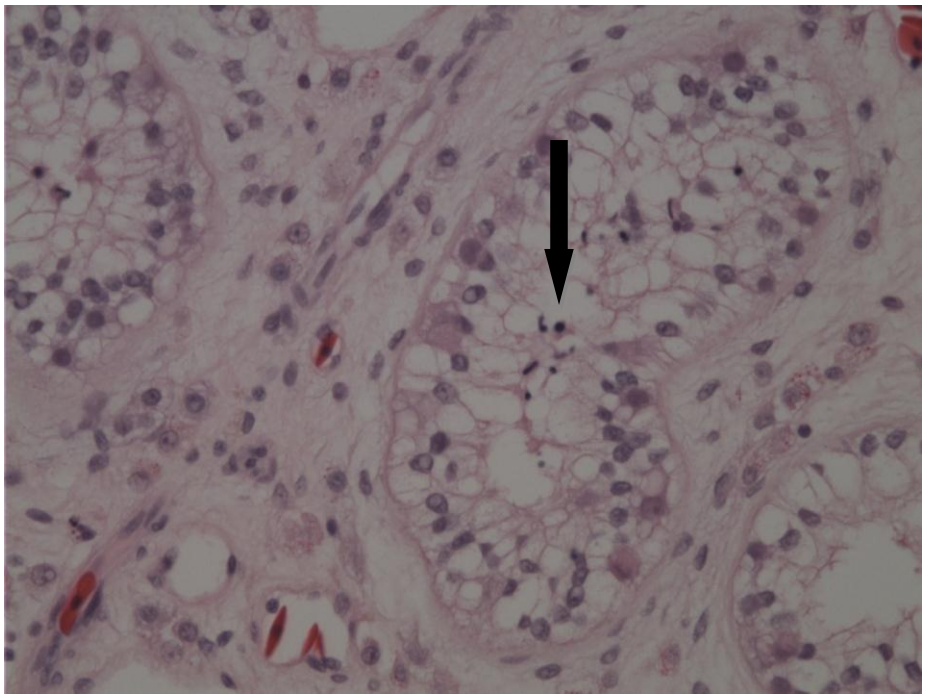

Figure 23. Testes section from a 60.4cm straight carapace length male Hawaiian green sea turtle with maturing spermatozoa (arrow, T. Work pers comm.).

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