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Validated Age and Growth of the Bonnethead (*Sphyrna tiburo*) in the Western North Atlantic Ocean

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Abstract

The age and growth of the bonnethead shark, *Sphyrna tiburo*, inhabiting the estuarine and coastal waters of the western North Atlantic Ocean from Onslow Bay, North Carolina, south to West Palm Beach, Florida was examined. Vertebrae were collected and successfully aged from 329 females and 216 males. Sex specific von Bertalanffy growth curves were fitted to length at age data. Female von Bertalanffy parameters were L_∞= 1032 mm FL, k= 0.18, t_o = -1.75, and L_o= 291mm FL. Males reached a smaller theoretical asymptotic length, and had a slower growth coefficient, with von Bertalanffy parameters being L_∞= 778 mm FL, k= 0.30, t_o = -1.50, and L_o= 281 mm FL. Maximum observed age was 17.9 years for females, and 12.0 years for males. Annual deposition of growth increments was verified by marginal increment analysis and validated through recapture of 13 OTC injected wild captured specimens. Annual band deposition was validated for age classes 2.5+ to 10.5+ with times at liberty ranging from 1 to 4 years. Age at 50% maturity was 6.8 years for females, and 4.1 years for males. Von Bertalanffy growth parameters were compared to growth parameters from bonnethead sharks collected in the eastern Gulf of Mexico (GOM) to test for differences. Both female and male bonnetheads in the SAB had a significantly higher theoretical asymptotic length, lower coefficient of growth, and lower estimated mean size at birth. Maximum observed age and age at 50% maturity were also higher for both sexes in the SAB.

Introduction

The bonnethead is a relatively small shark, reaching a maximum size of 150 cm total length. This species inhabits the estuaries and shallow coastal waters of the western Atlantic Ocean from North Carolina to southern Brazil including the Gulf of Mexico (GOM) (Compagno 1984). The 2007 SEDAR listed the bonnethead shark as not overfished, with no overfishing occurring; however, a separate regional assessment for bonnetheads was not conducted due to the paucity of life history information for the South Atlantic Bight region (SAB, defined as the coastal waters of the western North Atlantic Ocean off of the southeastern United States). As a result, the GOM life history parameter estimates were utilized for both regions, which could have led to incorrect conclusions regarding the status of the SAB population. While the stock was not considered to be undergoing overfishing in 2007, overfishing had occurred in previous years (SEDAR 2007). Consequently, the age and growth of the bonnethead in the SAB was listed as a research priority.

Objectives

The objectives of this study are to characterize the age, growth, and size and age at maturity of the bonnethead shark in the SAB and to validate periodicity of vertebral band pair formation. Parsons (1993a) validated band periodicity with OTC in age classes 1-6 years. The majority of these were animals kept in captivity and no fish above age six were validated. Multiple recaptures of greater than seven years were recorded by the South Carolina Department of Natural Resources (SCDNR) Cooperative Atlantic States Shark Pupping and Nursery Habitat Survey (COASTSPAN), indicating age classes greater than six years old would likely be encountered. In order to attempt to validate all age classes, this project attempted to expand upon the OTC validation work completed by Parsons (1993a).

Studies completed by Parsons (1993a), Parsons (1993b), Carlson and Parsons (1997), and Lombardi-Carlson et al. (2003) fully characterized the age, growth and reproduction of the bonnethead in the eastern GOM. Data from SEDAR 13 (2007), prepared by Lombardi-Carlson were utilized to compare the growth parameters determined by this study to the GOM. Spatially, this work covered the range of the bonnethead in the SAB region.

Methods

Collection of specimens

Multiple methods and surveys were used to collect bonnetheads from the coastal waters of the SAB. From April 2007 to November 2012 bonnetheads were collected from the coastal waters of South Carolina as part of the SCDNR COASTSPAN survey. Gears used for collection included gillnets and longlines. The primary gears used for collection were 230 m and 40 m gillnets. Both types of gillnets were 3 m deep and constructed of #177 monofilament with a stretch mesh of 10.3 cm. Initially, bonnetheads were randomly sacrificed for ageing. Once a large sample (100+ individuals per sex) was obtained, individuals were then sacrificed as encountered if they filled a gap in the existing dataset. Specimen collection continued until two male and two female bonnetheads were sacrificed per 1 cm FL increment for all observed lengths. Not all length bins were filled due to growth that took place when participating surveys were not sampling, or bonnetheads were unavailable to surveys due to migration.

Specimens were also provided by the Southeast Area Monitoring & Assessment Program (SEAMAP). The survey's spatial coverage is from Cape Hatteras, North Carolina to Cape Canaveral, Florida. Collections occurred using paired 22.9 m mongoose-type Falcon trawls with tickler chains which were towed for 20 minutes bottom time from the R/V *Lady Lisa*, a 22.9 m St. Augustine shrimp trawler. Nets do not contain TED's or BRD's. Samples collected by SEAMAP were stored on ice and processed by the SCDNR protocol below.

Additional specimens were provided from Florida estuarine waters by a co-investigator. The specimens were collected through sampling with the Florida Fish and Wildlife Research Institute's (FWC-FWRI) Fisheries-Independent Monitoring Program operating in estuarine systems and adjacent coastal waters of Florida's east coast or from recreational and commercial fisheries in the nearshore and offshore waters of this region. The study area on the east coast of Florida ranged from waters near the Georgia-Florida border (latitude ~30° 42' N) south to the Jupiter Island area (latitude ~27° 04' N). Monofilament gillnets (stretch mesh sizes ranging from 50 mm to 150 mm), 183-m center-bag haul seines (37.5 mm stretch mesh), and hook and line methods were used. Sub-samples of vertebrae from specimens collected from 2000 to 2012 were sent to SCDNR for processing.

Morphometrics and reproductive condition

The pre-caudal (PCL), fork (FL) and stretch-total (STL) lengths of all bonnetheads were measured in a straight line along the axis of the body to the nearest mm. Mass was measured to the nearest 0.25 kg using a spring balance. Gross reproductive status was noted and females were considered mature if they had developing pups, vitellogenic follicles (>10mm) and/or developed uteri and oviducal glands (Parsons 1983). Males were considered mature if they had fully calcified claspers, functional siphon sacs, a functional rhipidion and freely rotating claspers (Clarke and von Schmidt 1965). Umbilical scars in neonates were characterized as “umbilical remains”, “fresh”, “partially healed” , ”mostly healed”, and “well healed” according to Pratt et al. (1998).

Morphometric conversions were generated using linear regression for FL to PCL, FL to STL, and vertebral width to FL. Fork length to weight and vertebral diameter to FL conversions were calculated using two parameter power equations. Analysis of covariance (ANCOVA) was used to test for significant differences in morphometric measurements between sexes. If no significant differences were encountered, combined conversions were generated.

Ageing

Vertebral samples were removed from all sacrificed specimens and stored frozen. Each sample comprised a section of up to 12 vertebrae taken from the cervical region of the vertebral column. To prepare vertebrae for analysis, sections were thawed and excess muscle tissue and cartilage was removed from the vertebral column by scalpel. The column was separated into individual vertebra by cutting the connective tissue. Vertebrae were then soaked in 5% sodium hypochlorite for 3 to 15 minutes to remove any excess tissue, rinsed under running tap water for 5 minutes, and stored in 95% ethanol prior to analysis. The vertebra was dried and mounted to a glass slide using Crystalbond 509™ and a 0.4 mm sagittal section containing the focus was removed using a Buehler IsoMet low speed saw. As vertebral sections reached an optimum viewing state during drying , each section was monitored while drying to ensure a preferred viewing state before being mounted and preserved on a glass slide using Cytoseal™-XYL. If allowed to fully dry before mounting and preserving, band pairs may disappear, leading to underestimation of age. Slides were examined using a Nikon SMT-2T dissecting microscope at 20X magnification with a transmitted light source. A Scion™ Model CFW-1310C Color Digital Camera with Image-Pro™ Plus 6.0 digital imaging software was used to record images and count and measure increments.

Slides were selected at random and the number of translucent bands on the corpus calcareum was counted independently by two readers, each without knowledge of the other's reading or of the sex, size or date of capture of the shark from which the section was removed. Opaque bands representing summer growth and translucent bands representing winter growth (Figure 2) were identified following the description and terminology of Cailliet and Goldman (2004). The birthmark, or change in angle of the corpus calcareum was identified and counted as the first band. If there were discrepancies between readings, the section was re-read simultaneously by both readers to resolve the difference. If no agreement was reached, the sample was discarded.

Based on evidence showing synchronized development of embryos, and no pregnant females encountered after the third week of September, a birth date of September 30 was assigned to all individuals (SCDNR unpublished data). In the GOM, Parsons (1993a) found that the first translucent band, which represents winter growth, completes its formation in February at an age of five months. The first opaque band (summer growth) is completed nine months later in November at an age of 13 months. Subsequent winter/summer band pairs were then laid down annually in the same months. In many species of carcharhinid sharks, a change in angle of the corpus calcareum representing differential growth forms shortly after birth (e.g. Loefer and Sedberry 2003). Due to variability in occurrence of a translucent birthmark, the change in the angle of the corpus calcareum was counted as a birthmark/band for individuals with or without a discernible band. The second band representing winter growth was assumed to form five months later (Parsons 1993a). Therefore, for all band counts of one and over:

$$\text{Assigned age} = (\text{Band pair count} - 1.5)$$

In addition to assigned ages, fractional ages were used to generate growth curves. Fractional ages were determined by setting the birth month as month zero, and dividing the capture month by 12. A newborn specimen sacrificed in October (1 band) is considered 0.08 years old (1/12) using fractional age versus 0 using assigned age. A specimen sacrificed in August (2 bands) would be considered 0.92 years old (11/12) versus 0.5 years old with assigned ages. Mean size at birth was determined for males and females through measurements of free swimming neonates with an umbilical stage of open, partly healed or mostly healed.

Reader precision and bias

Multiple methods were used to examine reader bias and precision. Overall percent agreement (PA= [number agreed/number read] X 100) and percent agreement ± 1 year were calculated to evaluate precision. Percent agreement was also examined in 10 cm FL groups as recommended by Goldman (2004). Age agreement tests were generated and tested for symmetry using Bowker's test of symmetry (Hoenig et al. 1995). Age bias plots (Campana et al. 1995) were used to evaluate reader bias. A subset of 100 randomly selected specimens was also re-read by reader 1 to examine within reader age bias. The index of average per cent error (IAPE; Beamish and Fournier 1981) was calculated to assess between-reader error.

$$\mathrm{IAPE} = \frac{1}{n}\sum_{j=1}^{n}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{\left|x_{ij}-\bar{x}_j\right|}{x_j}\right]$$

where:

n = number of sharks aged;
R = number of times each fish is aged
X_{ij} = *i*th age estimation of *j*th shark at *i*th reading;
$\bar{X}_j$ = mean age calculated for the *j*th shark.

While IAPE assumes standard deviation of age estimates are proportional to the mean of the age estimates, Chang (1982) suggested that the coefficient of variation (CV) should be used to measure precision.

$$\mathrm{CV} = \frac{1}{n}\sum_{j=1}^{n} 100 * \frac{\sqrt{\sum_{i=1}^{R}\frac{\left|x_{ij}-\bar{x}_j\right|^2}{R-1}}}{\bar{x}_j}$$

Age verification

To verify the periodicity of band pair formation, marginal increment analysis was utilized. The marginal increment on each sample was measured from the outer edge of the previous growth band to the outermost edge of the corpus calcareum. Verification of the annual period of band formation was performed using the relative marginal increment ratio (Conrath et al. 2002) as recommended by Cailliet et al. (2006).

$$\text{MIR} = \frac{MW}{PBW}$$

Where:

MIR = marginal increment ratio
MW = margin width
PBW = previous band width

For specimens greater than one year old, the margin width was divided by the penultimate band width (Figure 2)**.** For specimens with less than one year old, the margin width was divided by the distance to the birthmark. One way Analysis of Variance (ANOVA), and Tukey's test for Honestly Significant Differences (HSD) were used to test for significant differences in MIR between months.

Age Validation

In addition to marginal increment analysis for age verification, the periodicity of vertebral band pair formation was validated using recaptured specimens that had been previously injected with OTC. During routine and targeted sampling in the North Edisto river, bonnetheads were captured using gillnets and, if healthy, weighed, tagged and intra-muscularly injected with OTC at a dosage of 25 mg/ kg body weight (Gelsleichter et al. 1998). Recaptured individuals were identified by unique tag numbers, and any recaptures were sacrificed if they had been at liberty for more than 9 months. Specimens were processed following the protocols described above with the exception that vertebrae with assumed OTC reference marks were stored in the dark to prevent degradation of photosensitive OTC reference marks. The vertebral sections were examined under ultraviolet light to determine the presence of an OTC mark. Detected marks positions were compared to the observed band pairs to validate the hypothesis of deposition of one band pair per year.

Growth Models

Observed FL and both fractional and assigned age estimates (years) were utilized to generate von Bertalanffy (von Bertalanffy 1938) growth models. Confidence intervals for all model parameters were generated by bootstrapping (1000 samples).

The von Bertalanffy growth model as adapted by Beverton (1954) and Beverton and Holt (1957) is:

$$L_t = L_\infty(1 - e^{-K(t-t_0)})$$

Where:

L_t = length at age t,

L_∞ = theoretical maximum length,

k = coefficient of growth,

t_o = theoretical age at which length equals zero.

The original growth von Bertalanffy growth model was also fit to data as recommended by Calliet et al. (2006).

$$L_t = L_\infty - (L_\infty - L_o)\, e^{-kt}$$

Where:

L_0= mean length at birth.

Maximum likelihood ratio tests (Kimura 1980) were used to detect differences between different growth models. To examine potential differences in growth parameters between males and females, sex-specific growth curves were estimated. Von Bertalanffy parameters estimated from the GOM and SAB were compared using data from Lombardi (2007), rather than Lombardi et al. (2003). Original FL at age data were used to generate sex specific GOM von Bertalanffy parameters for comparison. New curves were generated as TL at band count were originally used to generate parameters for the GOM, versus FL at age for the SAB. If FL was missing from an aged GOM individual, a GOM specific TL to FL regression from Lombardi (2007) was used to convert measurements. A combined model with all GOM and SAB specimens (sex specific) was also generated using original FL at age data. Model fit was assessed by examination of residuals, Akaike Information Criterion (AIC) and residual sums of squares. Theoretical maximum age was estimated to be the age at which 95% of the theoretical maximum length is reached, using the formula $(5(\ln 2))/k$ (Fabens 1965).

To determine size and age at 50% maturity, a logistic model $Y=1/1(e^{-(a+bx)})$ was fitted to binomial maturity data. Median fork length and age at maturity were determined by $-a/b$ (Mollet et al. 2002). Confidence intervals were generated by bootstrapping (1,000 samples). All statistical analysis and model generation was completed using R statistical software (R Development Core Team 2012).

RESULTS

The majority of specimens were collected from April through October (n=543), with limited samples (n=11) collected in March, November and December. Females were found primarily in estuarine waters (76.5%), with the majority of males collected in nearshore waters (62.9%). A total of 554 specimens were collected with a size range of 245-825 mm FL for males (n=218) and 262 to 1043 mm FL for females (n=336). Samples were collected in coastal and estuarine waters throughout the SAB (Figure 1) with most samples coming from South Carolina (59.3%) and Florida (37.8%). Results of ANCOVA indicate that sex specific linear regressions were unnecessary for morphometric conversions (FL→STL: F=0.61, P=0.55; FL→PCL: F=0.93, P=0.34). Sex specific models were necessary for weight on FL conversions (F=12.74, P<0.001). Data were log transformed and two parameter power equations were generated (Table 1).

Of the 554 specimens aged, nine were discarded because a consensus age could not be reached. Overall percent agreement was 59.5% and percent agreement ± 1 band was 90.8%. Bowker's test of symmetry ($\chi^2 = 35.57$, df = 40, $P = 0.669$) did not indicate bias between Reader 1 and Reader 2 (Table 2). Beamish's APE (3.30%) and Chang's CV (4.66%) suggest that assigned ages are acceptably precise based on resulting CVs all being less than 5%, as proposed by Campana (2001). Age bias plots for Reader 1 vs. Reader 2 revealed no systematic differences between readers (Figure 2), and a subset of 100 samples read twice by Reader 1 showed no bias between readings.

Marginal increment analysis verified annual band formation for ages 0 to 4. Age 0 to 1 year old samples were analyzed separately from age 1-4 year old samples due to differences in MIR ratios. Age 0-1 MIRs (n=55) reflected a shorter growth window for the penultimate band (birthmark to first winter band) than MIRs in older specimens (n=85).Observed age 0-1 MIRs approached, and were even larger than one (Figure 3). A one way ANOVA found significant differences between months (*F*=8.44, *P* <0.001), and Tukey's HSD test found significant differences between April and June-August, and significant differences between May and August (Table 3). The largest observed differences between mean MIR were between April and August**.**

Increments were analyzed beyond age 4, but could not be utilized as margin widths became too small to elucidate seasonal differences. Ratios for ages 1-4 year followed a similar pattern (Figure 3) and a one way ANOVA found significant differences among months (*F*=5.15, *P* <0.001). Tukey's HSD test found significant differences between April and September-November, between May and October/November, and between June and September/October (Table 3). The largest observed differences between mean MIR were between May and October.

A total of 60 bonnetheads were captured, injected with OTC, held at Bear's Bluff National Fish and Wildlife Hatchery and released. Of those, 13 were recaptured, with liberty ranging from 10.5 months to 4.1 years (Table 4)**.** Ages of specimens ranged from 2.8 to 7.0 years at initial tagging (calculated from band counts and time at liberty), and 3.7 to 10.5 years when sacrificed (based on band counts). Six were noted as immature at initial tagging (based on length), two mature, and five were unknown. Five were immature, and eight were mature when sacrificed. All recaptured specimens had an OTC reference mark when viewed under UV light. Twelve of the 13 specimens validated annual band deposition (Figure 4). The specimen that did not show annual band deposition was at liberty for 707 days, yet only grew 16 mm FL (860-866 mm FL). The fluorescent reference mark is visible in close proximity to the edge of the corpus calcareum, and no bands were counted after the reference mark (Figure 5).

Likelihood ratios (χ^2=159.0, d.f.=3, *P* <0.001) indicated that separate growth curves were necessary for male and female bonnetheads. Residual sums of squares and AIC values indicated that fractional age data (Figure 6) produced better fits of the von

Bertalanffy curves than assigned ages (Figure 7). The original von Bertalanffy model, and Beverton and Holt model (1975) produced nearly identical L_∞ and k parameters . All L_∞ and L_0 parameters, and are reported in mm FL (Table 5).

Fractional age L_0 was more realistic than assigned age L_0, when compared to observed mean size at birth. Female observed mean size at birth was 278.3mm FL (range=265-302mm, n=15) and male observed mean size at birth was 274.3 mm FL (range=245-300mm, n=22). The oldest aged female was 17.9 years old, and the oldest male was 12.0 years old. Theoretical maximum age was estimated to be 18.7 for females, and 11.9 for males. The length at 50% maturity for females was 815.9 ±32.2 (mm FL) at an age of 6.7 ±0.3 years. Male's length at 50% maturity was 617.7 ±12.1 (mm FL) at an age of 3.9 ±0.3 years.

Assigned age von Bertalanffy models were used for comparisons of SAB and GOM data due to a poorer fit of GOM data with a fractional age von Bertalanffy model. Both females ($\chi^2 = 34.8$, d.f=3, $P<0.001$) (Figure 8), and males ($\chi^2 = 45.1$, d.f=3, $P<0.001$) (Figure 9) had significantly different von Bertalanffy models. In both sexes, regional differences were driven by significant differences between L_∞ (females χ^2=12.91, $P<0.000$, males χ^2= 8.65, P=0.003) and K (females χ^2=9.26, P=0.002, males χ^2=7.67 P=0.006) (Table 6). Significant differences were not detected for t_0 (females χ^2=0.18, P=0.67, males χ^2=0.16, P=0.69).

Significant differences were also detected between the combined GOM/SAB models (Table 5) and the SAB models. Both female ($\chi^2 = 19.6$, d.f=3, $P<0.001$) (Figure 10) and male ($\chi^2 = 25.9$, d.f=3, $P<0.001$) (Figure 11) models were determined to be significantly different. In females, differences were driven by t_0 (χ^2=5.64, P=0.018). Significant differences were not detected for L_∞ ($\chi^2 = 0.15$, P=0.699) and k ($\chi^2 = 0.04$, P=0.841). For males differences were detected between L_∞ ($\chi^2 = 4.00$, P=0.046) and k ($\chi^2 = 5.60$, P=0.018), differences were not detected between t_0 (χ^2=0.00, P=1.00).

In addition to OTC recaptures, eight previously tagged sharks were recaptured and sacrificed during the course of the study (Table 4). Time at liberty ranged from 351 to 3263 days at large. Recapture B2916 was already at asymptotic length when tagged 9 years prior to recapture, average growth was 3.1 mm/year, and age was estimated at 15.7 years (Figure 12). Both sacrificed and OTC recaptures were plotted against the generated female von Bertalanffy curve for comparison of observed versus modeled growth (Figure 13).

DISCUSSION

Estimates of precision and bias, marginal increment analysis, as well as OTC tagging studies support the use of vertebrae for ageing bonnethead sharks. Precision was relatively high, with a minimum of >84% agreement ± 1 year for all 10 cm size classes, and an overall agreement ± 1 year of 90.8%.

Our use of OTC tagging expands on the results presented by Parsons (1993a). While predominantly captive reared bonnetheads were injected with OTC for validation of the periodicity of growth band formation in Parsons (1993a), this study used all wild captured, and wild recaptured specimens. In addition to the validation of age 1.5+ to 4.5+ year old sharks completed by Parsons, we further validated annual band deposition for females for ages 2.7 to 10.5 years old. Band deposition was validated across as many as four years. Validation however, was not achieved for any male bonnetheads, as recaptures of male bonnetheads were rare.

Band formation was found to be aberrant in specimen 531833. Two bands would be expected to be evident after the OTC reference mark; however, these bands were not present. This specimen was likely reaching asymptotic growth as growth had slowed to 8mm/year. Under-ageing also likely occurred in the ageing of long-term recapture B2916. This individual was already near maximum-recorded length when it was initially tagged. Over 9 years of liberty its average growth was 3.1mm/year. Ageing produced an estimate of 17 bands (15.7 years old at recapture, 6.7 years old at initial tagging). If the length at initial tagging were applied to the von Bertalanffy equation with the parameters generated by this study, this specimen could have been as old as 14.5+ at initial tagging (23.5+ at sacrifice). Analysis of fish aged at 6.5+ years old reveals a range of 723-890 mm FL ($\bar{x}$=827.5 mm), suggesting B2916 was likely underaged as it was 90 mm larger than the largest aged 6.5+ year old bonnethead.

These two anomalies cast doubt over the ability to properly age individuals that have reached asymptotic length. As the growth of an individual slows, so too does vertebral growth, making it difficult to count bands that may be present on the edge. Therefore, while annual band deposition was validated for female age classes 1.0-10.5, under-ageing may still occur for individuals that have reached asymptotic length. Several other recaptured specimens had slow to no growth, including one individual that was measured at -1 mm/year (likely due to measuring error). However, maximum observed age estimates for this study were similar or equal to the theoretical maximum ages, indicating that the observed under ageing is likely uncommon.

Marginal increment ratios were only available from March to November, as no specimens from age 0 to 4 years old were collected during the winter months. Age 0-1 MIRs approached and were even over 1.0 for the month of August. While a normal MIR should be <1.0, this result can be explained by parturition date, formation of the first winter band, as well as environmental effects on early growth. Bonnetheads are born in late September and the birthmark forms shortly after in October. The first winter band is assumed to form five months later in February. This early fall parturition date is unique among viviparous sharks found in the coastal waters of the Western Atlantic. Evidence suggests that parturition could occur prior to or during fall adult migration. For example, numerous post-partum females were documented in SC waters, and free swimming neonates with open umbilical scars were captured in both SC and Georgia. The combination of slow initial growth after parturition, migration to primary nursery grounds shortly after birth, as well as slower growth (documented in other elasmobranches) with colder water temperatures (Calliet et al. 1986 and Branstetter 1987), likely results in slower initial growth over the first five to six months of life for bonnethead neonates. Marginal increment growth after the first winter band takes place in warmer spring and summer months where conditions could be better for rapid growth. Therefore, measured growth between the birthmark and first winter band may be smaller than the margin width, and a MIR over 1.0 would not be unexpected. The MIR analysis verifies that annual bands are formed in age 0-4 year old classes. Unfortunately, the month of winter band completion could not be confirmed, however, our data does not refute Parson's (1993a) hypothesis of winter band completion occurring in February. Given the verification of MIRs and validation achieved by OTC tagging, we determined that vertebrae were an appropriate structure to use in the ageing of bonnetheads.

This study modeled growth using both fractional and assigned ages. The use of fractional ages improved model fit and produced more realistic estimates of L_o for von Bertalanffy growth functions. Fit was likely better because fractional ages helped to capture seasonal growth. While this impact is likely negligible in older year classes, in younger year classes, before asymptotic length is reached, this seasonal growth could be important to model fitting.

Measured growth from OTC and non-OTC recaptures was used to verify modeled growth. Plots of OTC and non-OTC recaptured individuals over the female von Bertalanffy curve (Figure 11) show slight variation in observed growth, however, the recaptures lend additional verification to the generated curve. The plot of recapture B2916 appears to confirm the assertion that it was likely under-aged.

While we do not feel that under-ageing was a major problem in this study, it is important to note that there is evidence that it occurred. Age under-estimation of up to 50% has been documented in porbeagle sharks , *Lamna nasus*, (Francis et al. 2007) and Australian school sharks, *Galeorhinus galeus*, (Kalish and Johnston 2001) using bomb radiocarbon. These species have far greater longevity than bonnethead, and if under ageing occurred in this study, it likely only affects older age classes and occurs at a much lower level than observed in these studies.

Great care was taken to preserve vertebrae at an optimum viewing state for ageing. We found when vertebrae were first sectioned, little to no bands were visible; however, after a period of several minutes, an optimum viewing state was reached. If the section was not fixed to maintain that state and allowed to continue drying, it was observed that some bands would fade or completely disappear. While this was not the reason for the under aging that we documented, it bears noting, as previous studies have allowed vertebrae to fully dry before polishing or staining.

The life history parameters estimated in this study were significantly different than those reported by Lombardi (2007) for the GOM. Maximum observed age and age at 50% maturity were double the estimates reported for the GOM. Length at 50% maturity was larger for females from the SAB (815.9 mm FL) than GOM females (662.6 mm FL). Male length at 50% maturity was larger for the SAB (617.7mm FL) compared to the GOM (572.4 mm FL). Observed maximum size was larger for both sexes for the SAB.

Differences in von Bertalanffy models were driven by significant differences in L_∞ and k. Bonnetheads in the SAB grew at a slower rate and reached a larger asymptotic length. Theoretical ages at length zero (t_o) were not significantly different, but were larger in the GOM than the SAB.

Mean length at birth (L_o) was not originally calculated by Lombardi et al. (2003), but for comparative purposes, we calculated L_o using the original von Bertalanffy equation with length at age data from the GOM (Table 5). Estimated mean lengths at birth (L_o) were larger for the GOM than SAB. Observed mean lengths at birth for the SAB were within the confidence intervals for L_o parameter estimates.

Once bonnetheads recruit to an estuary in SC they exhibit a high degree of site fidelity, returning to the same estuary each year after migrating out of the system (W Driggers *pers comm.*). Given the high level of site fidelity seen in larger juveniles and adults, we would expect that there would be genetic evidence of stock structure, however preliminary work has found no significant differences in haplotype frequencies between the GOM and SAB (DM Chapman *pers comm.*). Gene-flow was observed to be high along the coastline from South Carolina to Texas.

Significant regional differences in age and growth have been documented by this study. Bonnetheads in the SAB were found to grow more slowly to a larger size, take longer to mature, and reach an older age than populations from the GOM. The annual periodicity of growth band deposition was validated for a large portion of female age classes, and several long-term recaptures further verified age estimates and generated growth curves. Given the significant differences in important life history characteristics between regions, and lack of stock mixing shown by tagging studies, we recommend, if possible, that stocks be managed and assessed separately.

LITERATURE CITED

Beamish, RJ, and DA Fournier. 1981. A method for comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Sciences 38:982-983.

Beverton, RJH. 1954. Notes on the use of theoretical models in the study of the dynamics of exploited fish populations. United States Fishery Laboratory, Beaufort, North Carolina, Miscellaneous Contribution (2), 159 pp.

Beverton, RJH and SJ Holt. 1957. On the dynamics of exploited fish populations. United Kingdom Ministry of Agriculture and Fisheries, Fisheries Investigations (Series 2) 19, 533 pp.

Branstetter, S. 1987. Age and growth of newborn shark held in laboratory aquaria, with comments on the life history of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Copeia 2: 291-300.

Cailliet, GM, RL Radtke, and BA Weldon. 1986. Elasmobranch age determination and verification: a review. Iin T. Uyeno, R. Arai, T. Taniuchi and K. Matsura (eds). Indo-Pacific Fish Biology: proceedings of the second international conference on Indo-Pacific Fishes, pp. 345-360. Ichthyology Society. Japan, Tokyo.

Cailliet, GM & KJ Goldman. 2004. Age determination and validation in chondrichthyan fishes. In Carrier, J., J.A. Musick, & M.R. Heithaus. Biology of sharks and their relatives. CRC Press LLC, Boca Raton, FL, pp. 399-447.

Cailliet, GM, WD Smith, HF Mollet, and KJ Goldman. 2006. Age and growth of chondrichthyan fishes: the need for consistency in terminology, verification, validation, and growth function fitting. Environmental Biology of Fishes 77: 211-228.

Campana, SE 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59: 197-242.

Carlson, JK and GR Parsons. 1997. Age and growth of the bonnethead, *Sphyrna tiburo*, from northwest Florida, with comments on clinal variation. Environmental Biology of Fishes 50: 331-341.

Campana, SE, MC Annand, and JI McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American Fisheries Society 124:131-138.

Chang, WYB (1982). A statistical method for evaluating the reproducibility of age determination. Canadian Journal of Fisheries Aquatic Science 39: 1208-1210.

Clarke, E, and K Von Schmidt. 1965. Sharks of the central gulf coast of Florida. Bulletin of Marine Science 15:13-83.

Compagno, LGV 1984. FAO species catalogue, Vol. 4. Sharks of the world, Part 2: Carcharhiniformes. FAO Fish Synopsis 4 (125), Part 2: 251-655.

Conrath, CL, J Gelsleichter, and JA Musick. 2002. Age and growth of the smooth dogfish (*Mustelus canis*) in the northwest Atlantic Ocean. Fishery Bulletin 100:674-682.

Fabens, AJ. 1965. Properties and fitting of the von Bertalanffy growth curve. Growth 36: 883-902.

Francis, MP, SE Campana, CM Jones. 2007. Age under-estimation in New Zealand porbeagle sharks (Lamna nasus): is there an upper limit to ages that can be determined from shark vertebrae? Marine and Freshwater Research 58: 10-23.

Gelsleichter, J, E Cortes, CA Manire, RE Hueter, and JA Musick. 1998. Evaluation of toxicity of oxytetracycline on growth of captive nurse sharks, *Ginglymostoma cirratum*. Fisheries Bulletin 96: 624-627.

Goldman, KJ 2004. Age and growth of elasmobranchs fishes. In: Musick, J.A., Bonfil R. (eds) Elasmobranch fisheries management techniques. Asia Pacific Economic Cooperation, Singapore, 370 pp., pp. 97-132.

Hoenig, JM, MJ Morgan, CA Brown 1995. Analyzing differences between two age determination methods by tests of symmetry. Canadian Journal of Fisheries Aquatic Science 52: 364-368.

Kalish J, and Johnston J. (2001). Determination of school shark age based on analysis of radiocarbon in vertebral collagen. In "Use of the Bomb Radiocarbon Chronometer to Validate Fish Age". (Ed. JM Kalish.) pp 116-129. Final Report FRDC Project 93/109. Fisheries Research and Development Corporation, Canberra.

Kimura, DK. 1980. Likelihood methods for the von Bertalanffy growth curve. Fishery Bulletin 77: 765-776.

Loefer, JK & GR Sedberry. 2003. Life history of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) (Richardson, 1836) off the Southeastern United States. Fisheries Bulletin 101:75-88.

Lombardi-Carlson LA, E Cortes, GR Parsons, CA Manire (2003). Latitudinal variation in life-history traits of bonnethead sharks, Sphyrna tiburo, (Carcharhiniformes, Sphyrnidae), from the eastern Gulf of Mexico. Marine and Freshwater Research 54: 875-883.

Lombardi-Carlson LA. 2007. Life history traits of bonnethead sharks, *Sphyrna tiburo*, from the eastern Gulf of Mexico. SEDAR13-DW-24, SEDAR, North Charleston, SC.

Mollet, HF, JM Ezcurra, and JB O'Sullivan. 2002. Captive biology of the pelagic stingray, *Dasyatis violacea* (Bonaparte, 1832). Marine and Freshwater Research 53: 531–541.

Parsons, GR. 1983. The reproductive biology of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Richardson). Fisheries Bulletin. 81 (1): 61-73.

Parsons, GR 1993a. Age determination and growth of the bonnethead shark, *Sphyrna tiburo*: a comparison of two populations. Marine Biology 117: 23-31.

Parsons, GR 1993b. Geographic variation in reproduction between two populations of the bonnethead shark, *Sphyrna tiburo*. Environmental Biology of Fishes 38: 25-35.

Pratt, HL, C McCandless, N Kohler, C Jensen, G Ulrich, and M Gilligan. 1998. Report of the 1998 Apex Predators Program Cooperative Shark Pupping and Nursery (COASTSPAN) Survey. Apex Predators Program, USDOC, NOAA, NMFS Narragansett Lab, Narragansett, Rhode Island.

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

SEDAR. 2007. SEDAR 13 – HMS Small Coastal Sharks. SEDAR, North Charleston SC. Available online at: http://www.sefsc.noaa.gov/sedar/Sedar_Workshops.jsp?WorkshopNum=13

Von Bertalanffy, L. 1938. A quantitative theory of organic growth (inquiries on growth laws. II). Human Biology 10: 181–213.

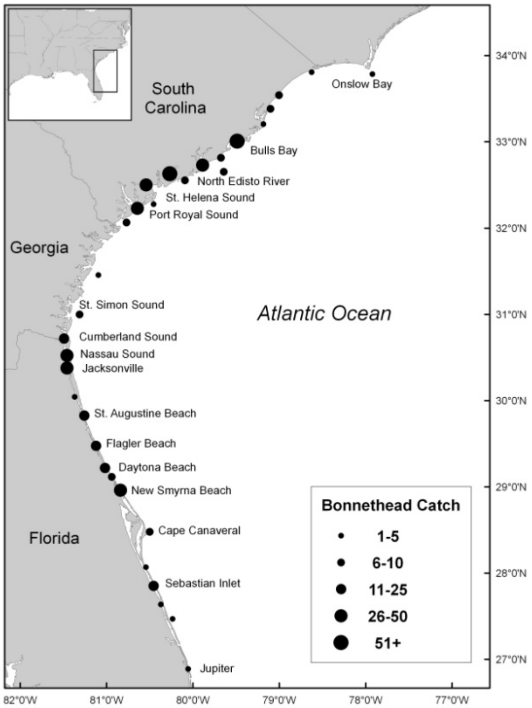

Figure 1. Map of South Atlantic Bight with sampling locations and abundance indicated by black circles.

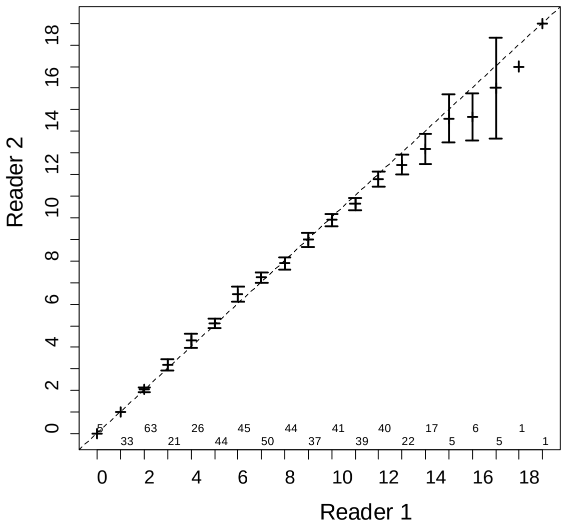

Figure 2. Intra-reader age bias plot for Reader 1 versus Reader 2 band counts with 95% confidence intervals. Numbers represent numbers of bonnetheads aged per year class, the dash is the mean of age estimates, and error bars represent 95% confidence intervals. The dotted line indicates a one to one relationship.

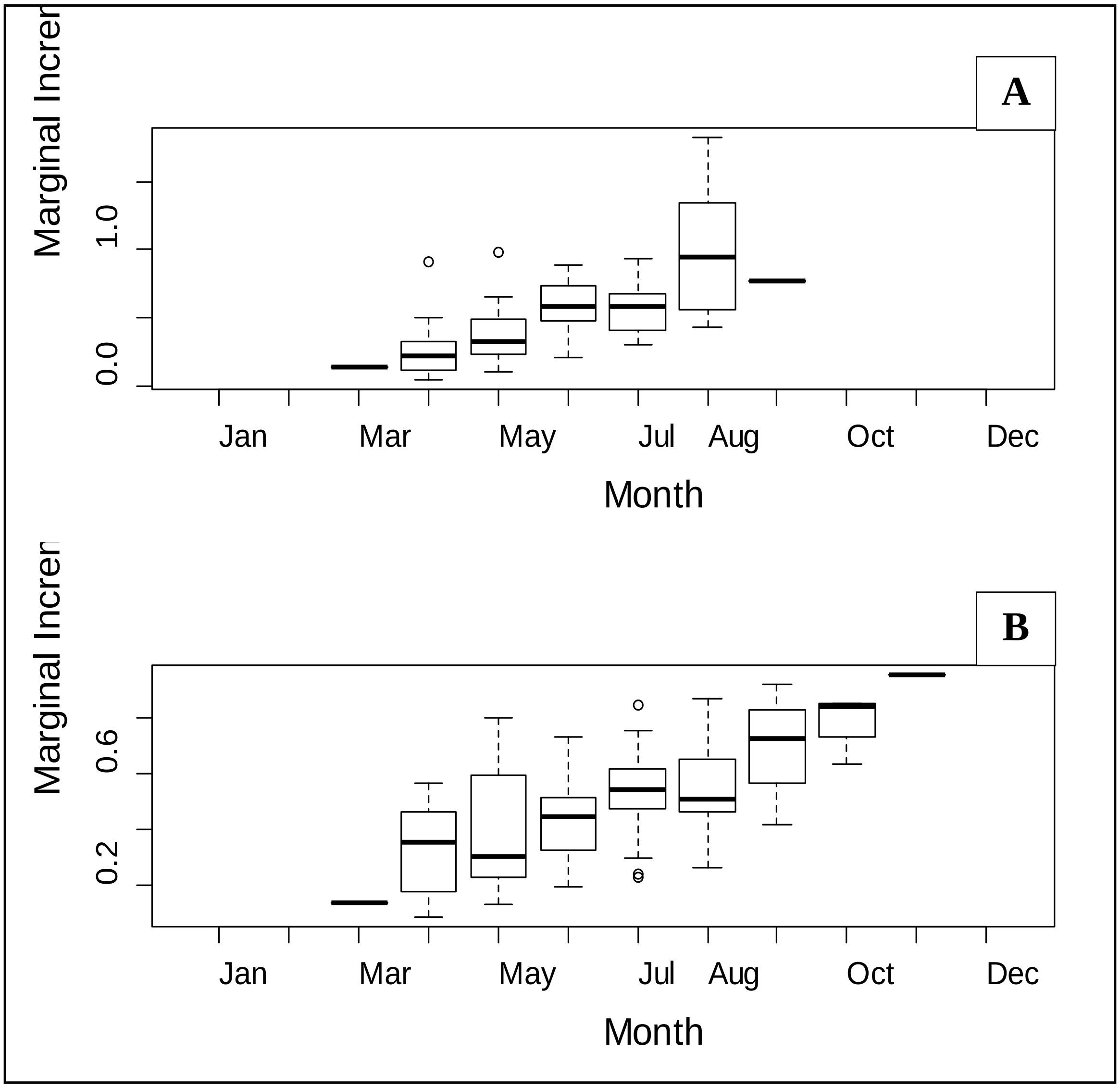

Figure 3. Box plot of monthly average marginal increment ratio (MIR) for age 0 to 1 year old (A), and 1-4 year old bonnetheads (B), (sexes combined). Solid black lines equate to median monthly MIR, the top and bottom of the box equates to 75% and 25% quartiles. Whiskers encapsulate the remaining upper and lower 25% MIR values. Individual points are considered outliers.

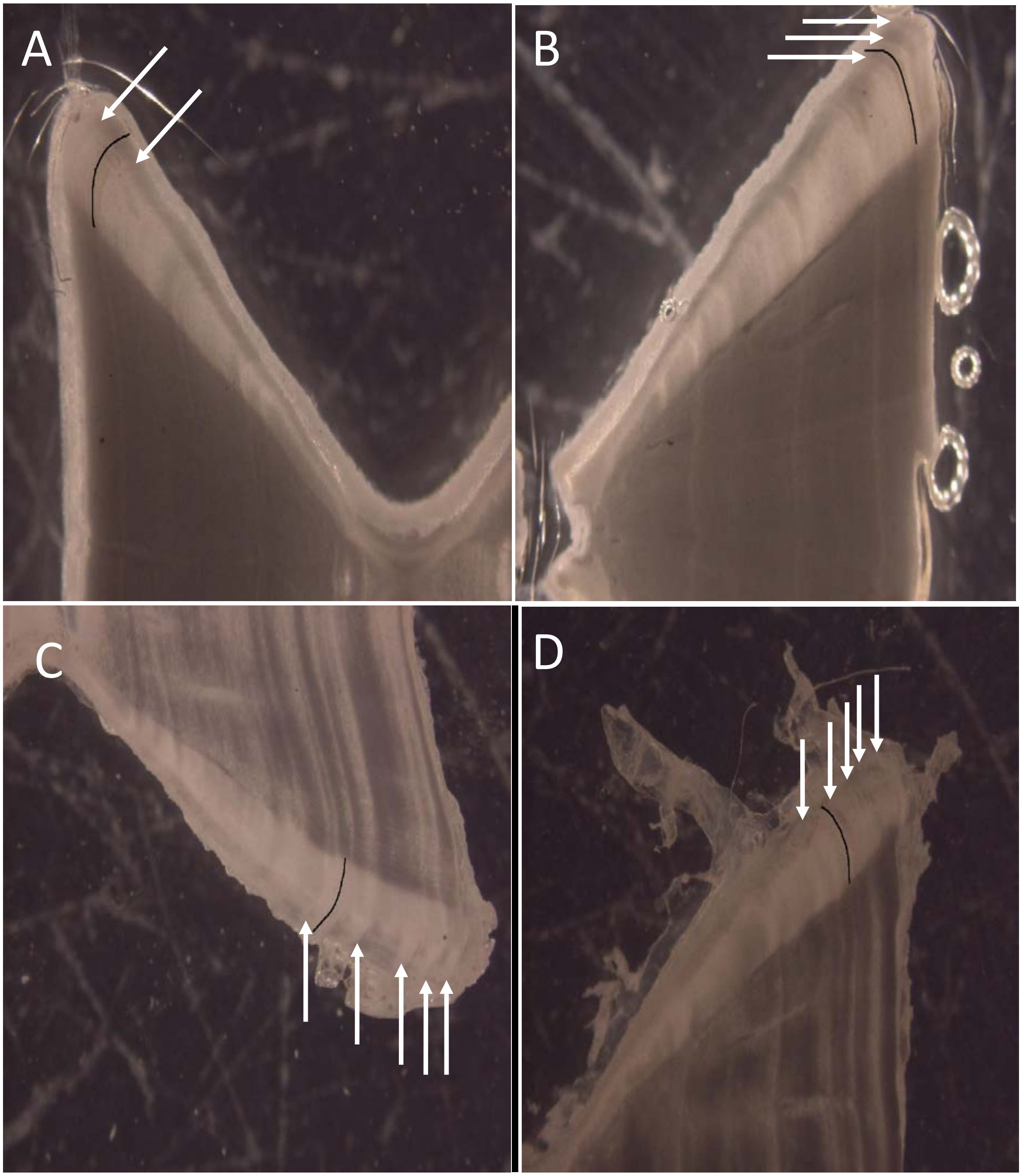

Figure 4. Sectioned vertebrae showing oxytetracycline (OTC) reference marks (black line) and winter bands around reference mark (white arrows). A). Recapture 532720, at liberty 322 days, length at injection was 650 mm FL, length at sacrifice was 713 mm FL, 1 band after reference mark. B). Recapture 532714, at liberty 706 days, length at injection was 802 mm FL, length at sacrifice was 835 mm FL, 2 bands after reference mark. C). Recapture 531799, at liberty 1415 days, length at injection was 756 mm FL, length at sacrifice was 916 mm FL, 4 bands after reference mark. D). Recapture 531845, at liberty 1512 days, length at injection was 830 mm FL, length at sacrifice was 924 mm FL, 4 bands after reference mark.

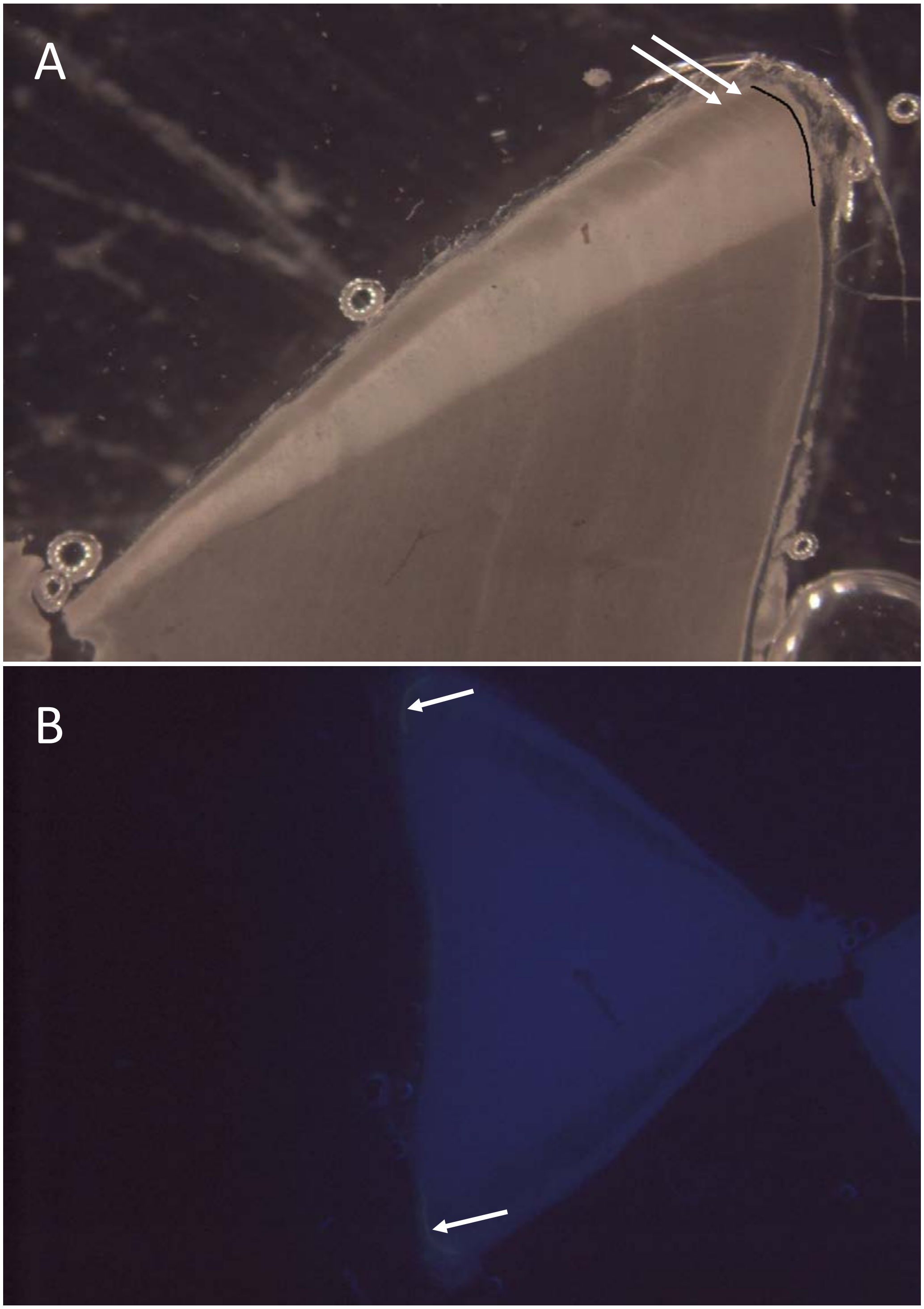

Figure 5. Sectioned vertebral images from recapture 531833 showing oxytetracycline (OTC) reference marks and no visible bands after reference mark. A). Image showing OTC reference mark (black line) and counted winter bands prior to reference mark (white arrows). B). Image illuminated by ultraviolet light showing actual OTC reference marks (noted by white arrows). Liberty was 707 days, length at injection was 860 mm FL, length at sacrifice was 876 mm FL.

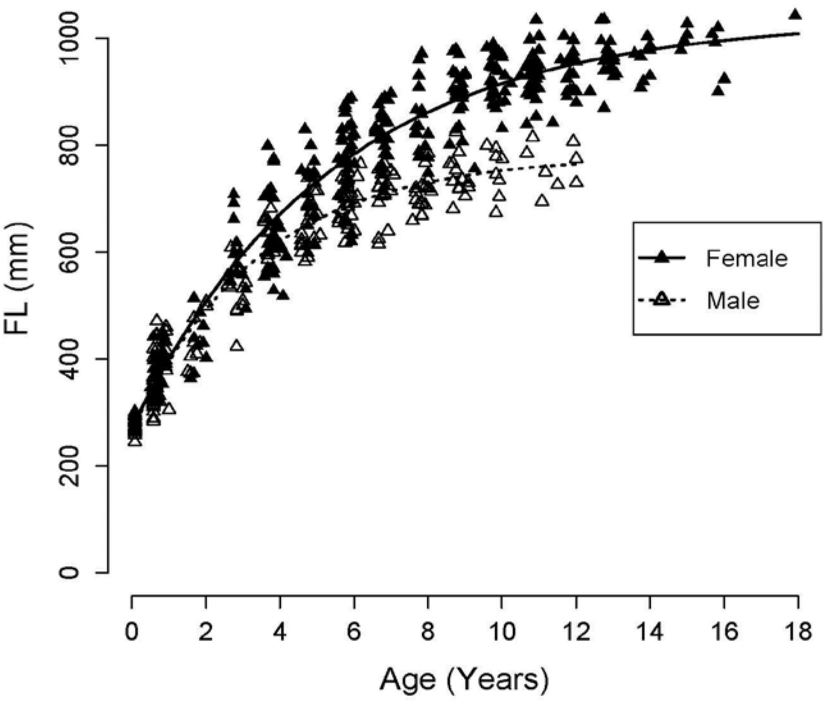

Figure 6. Von Bertalanffy growth model for bonnetheads inhabiting the South Atlantic Bight fitted to fork length at fractional age data for female and male bonnetheads. Females: L_∞=1035.8, k=0.18, L_o=271.5, t_o=-1.64, n=329, Males: L_∞=780.5, k=0.29, L_o=265.6, t_o=-1.42, n=216

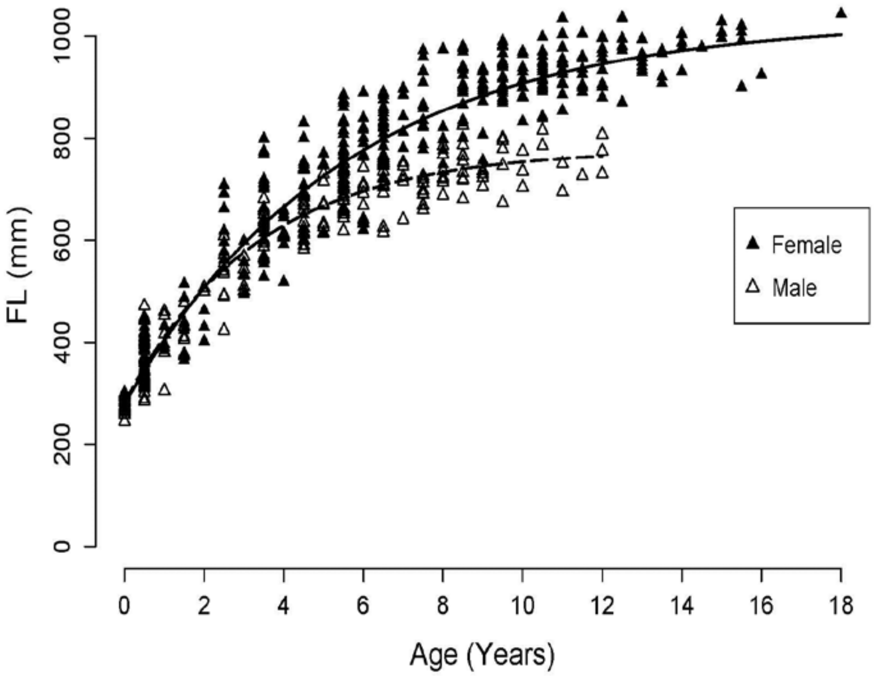

Figure 7. Von Bertalanffy growth model for bonnetheads inhabiting the South Atlantic Bight fitted to fork length at assigned age data for female and male bonnetheads. Females: L_∞=1032.4, k=0.19, L_o=290.8, t_o=-1.76, n=329, Males: L_∞=778.4, k=0.30, L_o=280.9, t_o=-1.50, n=216

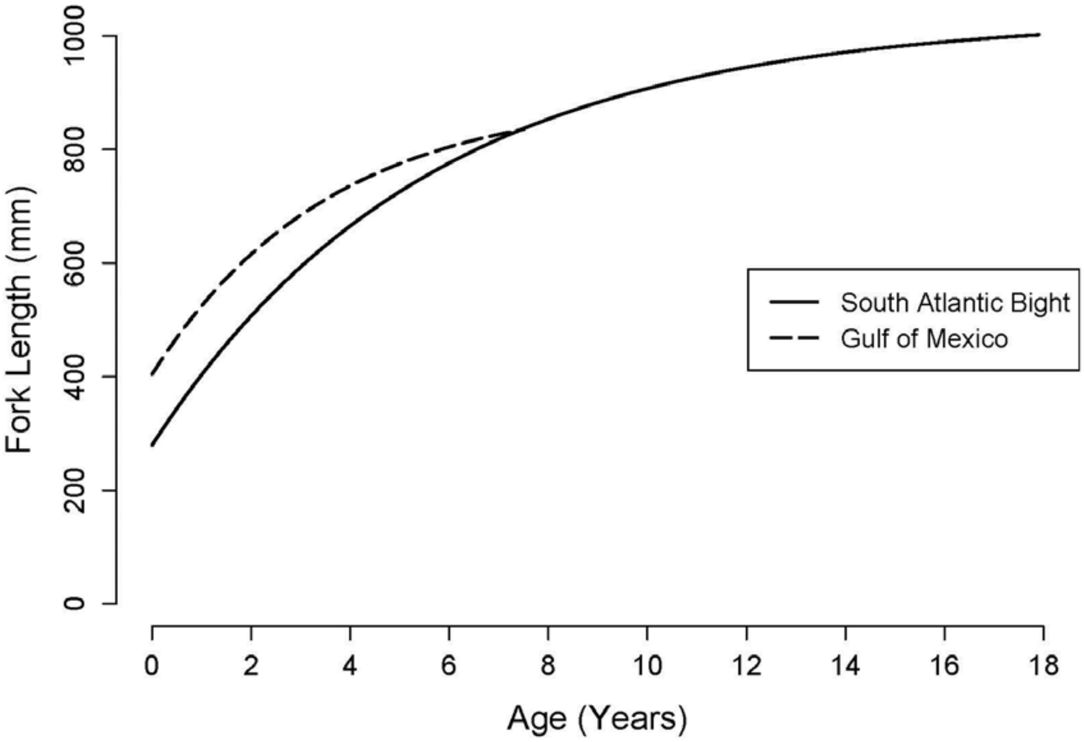

Figure 8. Female von Bertalanffy curves for the South Atlantic Bight and the Gulf of Mexico.

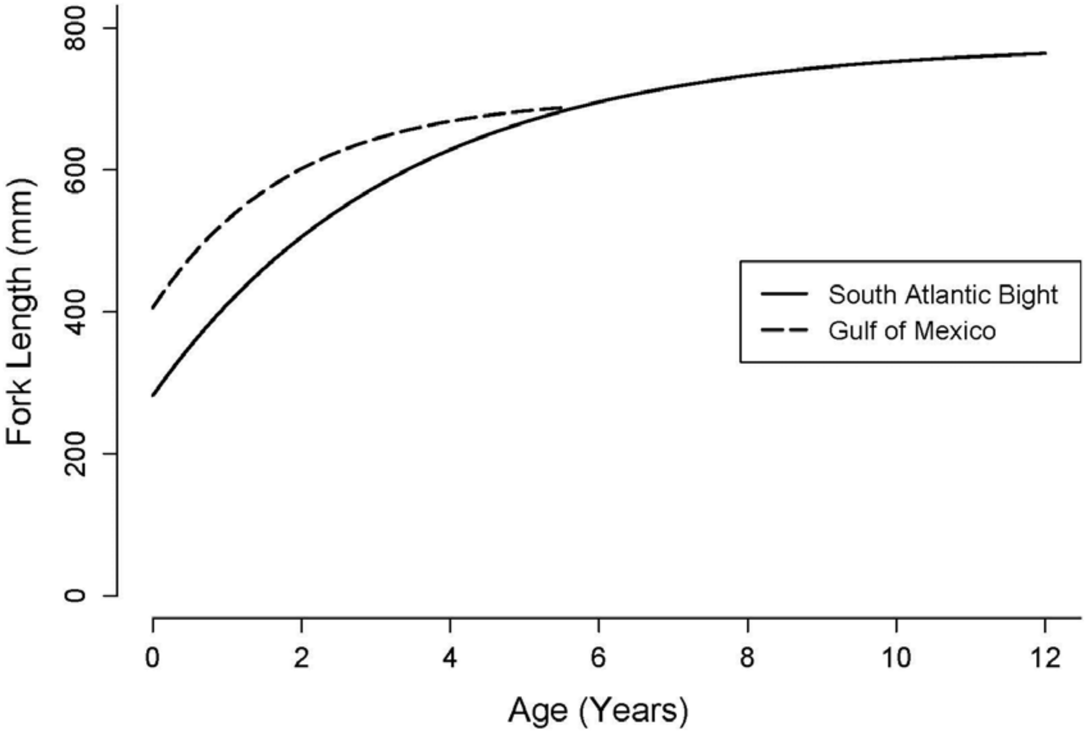

Figure 9. Male von Bertalanffy curves for the South Atlantic Bight and the Gulf of Mexico.

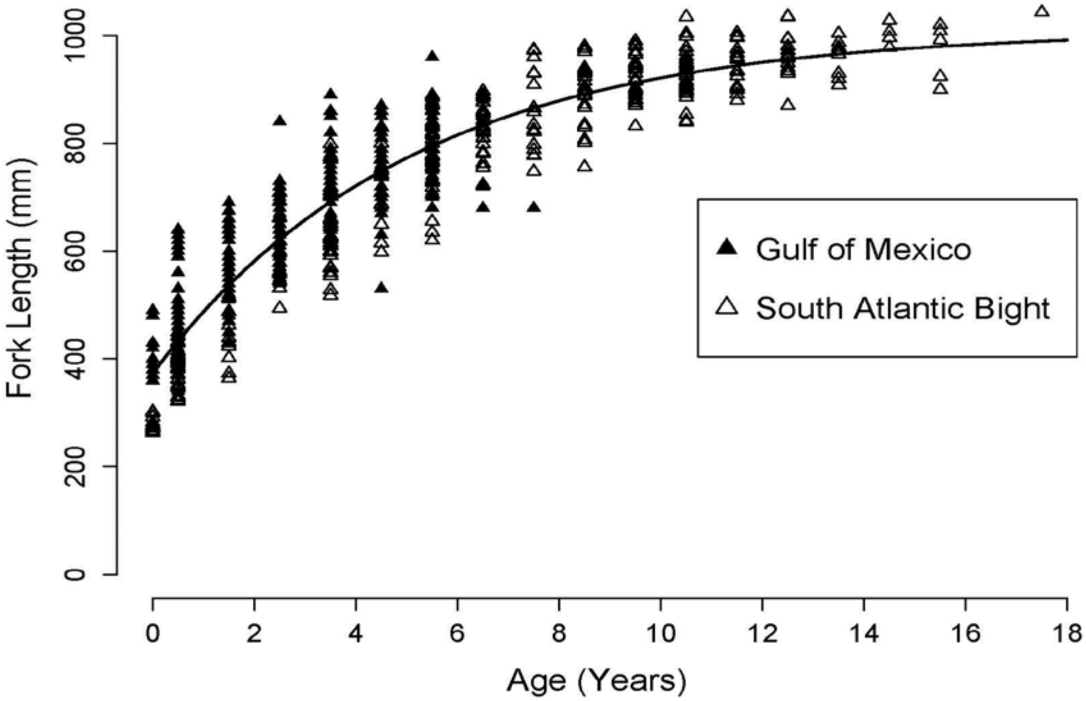

Figure 10. Combined von Bertalanffy model for female bonnetheads with fork length at assigned age data for the Gulf of Mexico and South Atlantic Bight.

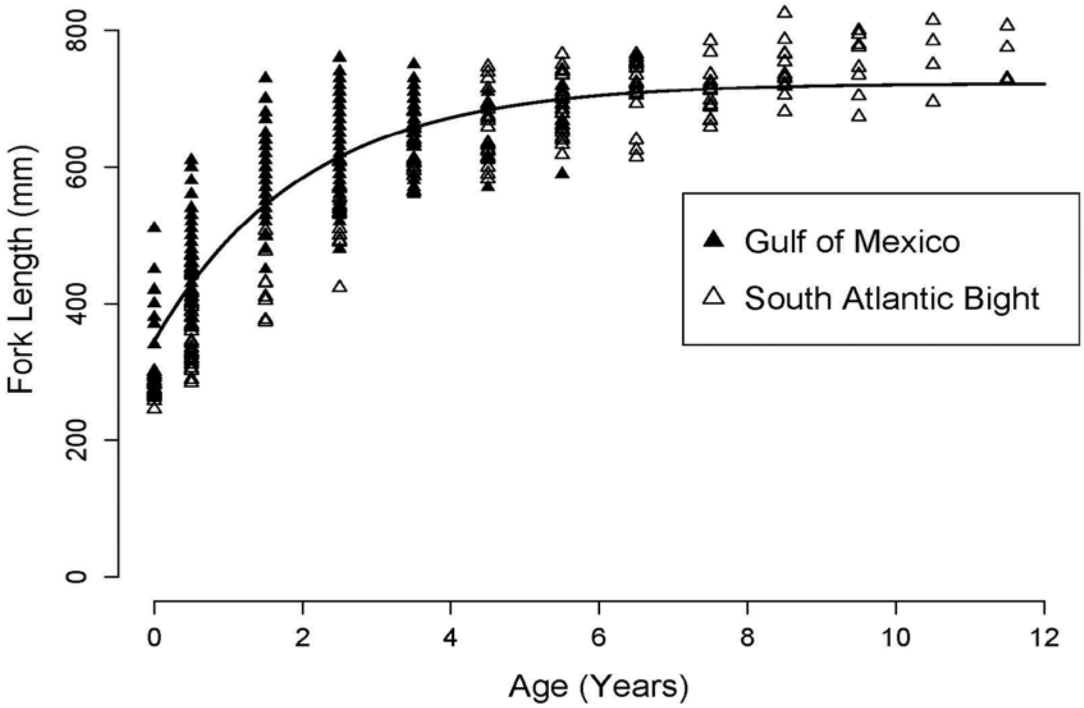

Figure 11. Combined von Bertalanffy model for male bonnetheads with fork length at assigned age data for the Gulf of Mexico and South Atlantic Bight.

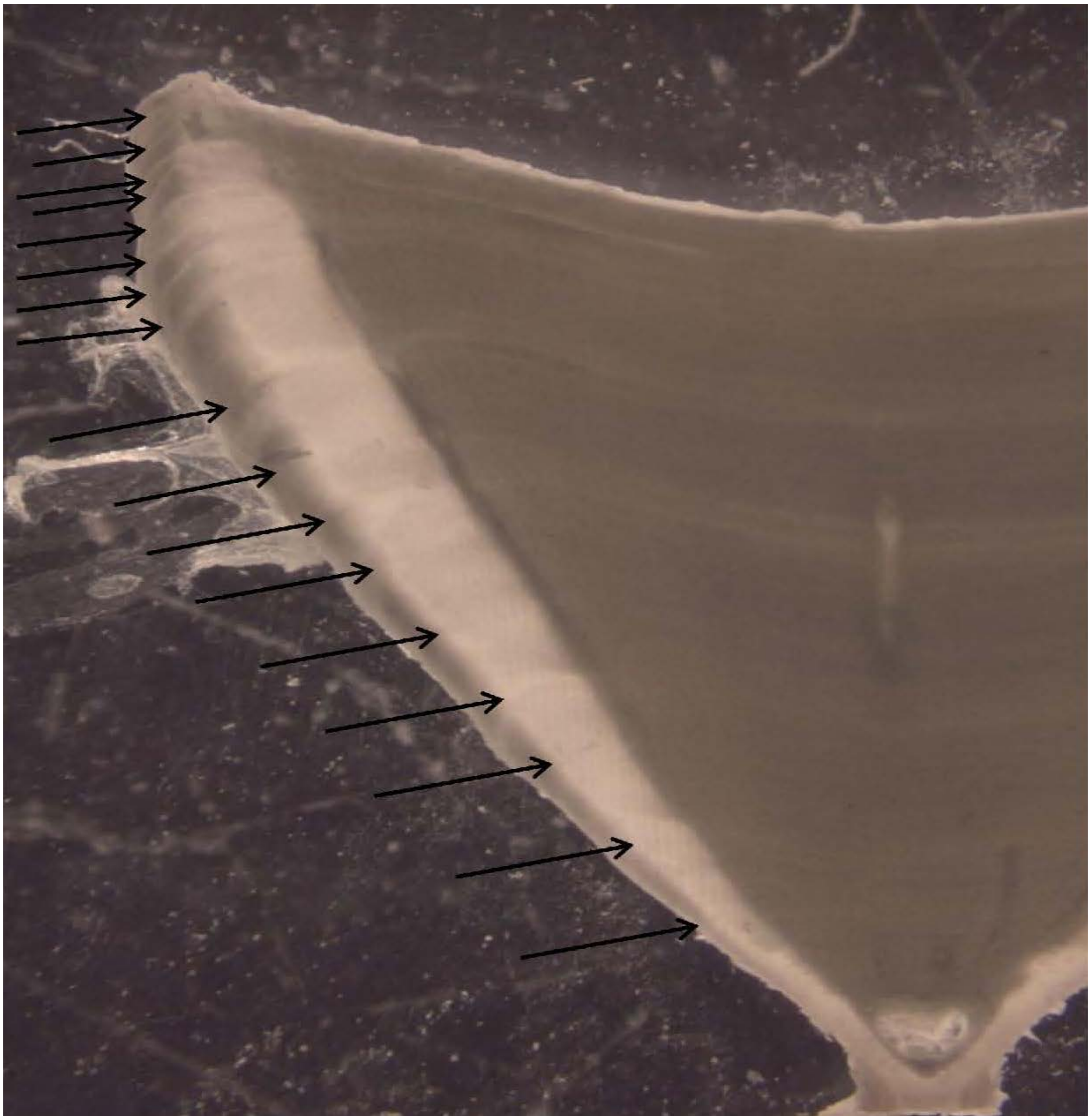

Figure 12. Sectioned vertebrae from long term recapture B2916 showing band count (denoted by black arrows).

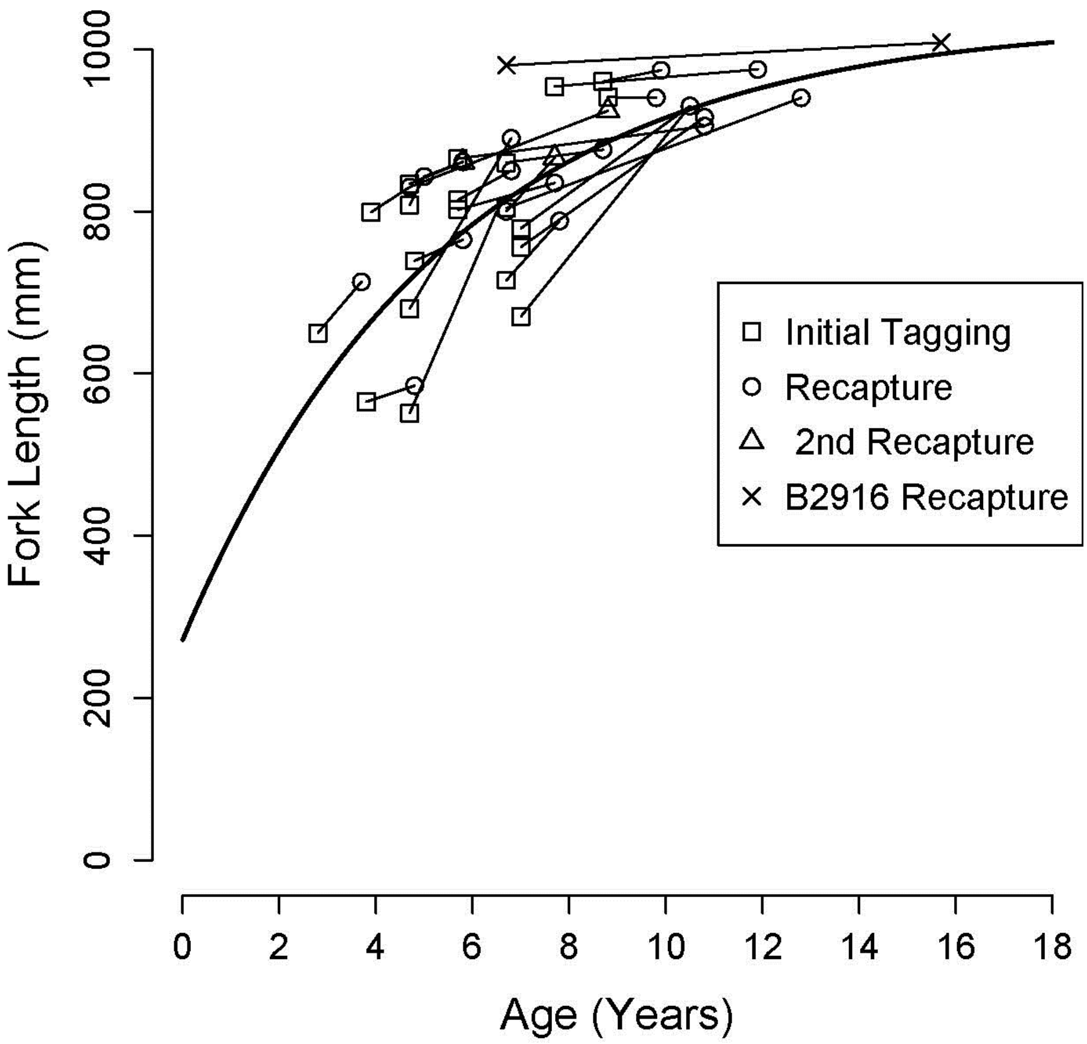

Figure 13. Von Bertalanffy growth curve for female bonnetheads inhabiting the South Atlantic Bight with all aged recaptures (Table 5) plotted.

Table 1. Morphometric conversions for length and weight of bonnetheads in the South Atlantic Bight. STL=stretch total length (cm), FL= fork length (cm), PCL= precaudal length (cm), and WT= weight (kg).

Conversion	Sex	Equation	R^2	n
FL→STL	Combined	STL= 1.198(FL) +3.91	0.994	2747
FL→PCL	Combined	PCL= 0.925(FL) -0.90	0.999	507
FL→WT	Female	$WT=3.462\times 10^{-6} * FL^{3.208}$	0.984	200
FL→WT	Male	$WT=4.482\times 10^{-6} * FL^{3.126}$	0.968	155

Table 2. Results for tests of precision and bias of bonnethead ageing including: Percent agreement, Percent agreement plus or minus (±) one year 1, Bowker's Test (χ^2, degrees of freedom and p-value), Beamish's Average Percent Error and Chang's Coefficient of Variation.

Reader Comparison	Percent Agreement	Percent Agreement ±1	Bowker's Test χ^2	Bowker's Test degrees of freedom	Bowker's Test p-value	Beamish's Average Percent Error	Chang's CV
Reader 1 vs. Reader 2	59.45	90.83	35.57	40	0.669	3.30	4.66
Reader 1 vs. Final	80.00	97.98	27.49	25	0.332	1.33	1.88
Reader 2 vs. Final	73.94	93.57	28.11	35	0.789	2.19	3.09
Reader 1 vs. Reader 1	63.00	99.00	8.87	15	0.884	2.71	3.83

Table 3. P-values for Tukey's test of Honestly Significant Differences on marginal increment ratios by age (years old) and month. Asterisks (*) denote significantly different p-values.

Age	Month	July	August	September	October	November
0-1	April	<0.001*	<0.001*	0.311		
0-1	May	0.352	0.005*	0.808		
1-4	April	0.159	0.334	0.002*	0.003*	0.049*
1-4	May	0.364	0.665	0.003*	0.006*	0.098
1-4	June	0.877	0.934	0.043*	0.034*	0.166

Table 4. Recaptures of oxytetracycline OTC injected and non-OTC tagged bonnetheads from South Carolina's Cooperative Atlantic States Shark Pupping and Nursery Habitat Survey. Initial lengths at tagging (mm), lengths at recapture (sacrifice), growth (mm), liberty (days), maturity status, back calculated age (years) at initial tagging, age at recapture, and bands after OTC reference mark for OTC injected samples.*indicates multiple recaptures, **indicates recaptured prior to OTC injection, # indicates derivation from expected band count after OTC reference mark.

Tag #	Initial FL (mm)	Recapture FL (mm)	Growth (mm)	Days at Large	Maturity at Recapture	Age at Initial Tagging	Recapture Age	Bands after OTC mark
				OTC Tagged Recaptures				
532720	650	713	63	322	imm	2.8	3.7	1
531774	739	765	26	332	imm	4.8	5.8	1
531801	814	850	36	360	imm	5.7	6.8	1
531809	715	788	73	372	imm	6.7	7.8	1
531829*	808	843/861	35/53	121/372	mat	5.7	6.8	1
531839	834	861	27	372	mat	4.7	5.8	1
532714	802	835	33	706	mat	5.5	7.7	2
531833	860	876	16	707	mat	6.7	6.7	0#
532709	680	890	210	776	imm	4.7	6.8	2
531783	670	930	260	1329	mat	7.0	10.5	4
531793	779	930	151	1329	mat	7.0	10.5	4
531799	756	916	160	1415	mat	7.0	10.8	4
531845**	830	924	94	1512	mat	4.7	8.8	4
				Non-OTC Tagged Recaptures				
B13223	565	685	80	351	imm	3.8	4.8	
B2603	941	940	-1	356	mat	8.8	9.8	
B2620	960	974	14	417	mat	8.7	9.9	
B11584*	551	800	249	680		4.7	6.7	
B11584*	551	866	315	1062	mat	4.7	7.7	
B13929	954	975	21	1526	mat	7.7	11.9	
531845**	799	924	125	1817	mat	3.9	8.8	
B11084	865	905	40	1853	mat	5.7	10.8	
B1933	804	940	136	2138	mat	6.9	12.8	
B2916	980	1008	28	3263	mat	6.7	15.7	

Table 5. Results from von Bertalanffy growth models generated to fork length (FL) in mm at assigned and fractional age (years) for bonnetheads inhabiting the South Atlantic Bight and FL at assigned age for the Gulf of Mexico and combined model. Sex (M=male, F=Female), growth parameters and size and age at 50% maturity plus or minus (±) 95% confidence intervals, observed maximum age (years) and size (mm FL) as well as theoretical maximum age are reported. *Gulf of Mexico parameters estimated using original fork length at age data. Results reported in Lombardi (2007) used total length and band count.

Parameter	Sex	South Atlantic Bight Assigned Age	South Atlantic Bight Fractional Age	Gulf of Mexico (Lombardi 2007)	Combined Gulf of Mexico and South Atlantic Bight
Sample Size	M	216	216	245	461
	F	329	329	254	583
L_∞ (mm)	M	778.4±22.0	780.5±21.8	703.3±51.3*	723.1±16.7*
	F	1032.4±26.4	1035.8±27.5	894.9±94.6*	1009.4±26.9*
k	M	0.30±0.04	0.29±0.04	0.54±0.20*	0.50±0.07*
	F	0.19±0.02	0.18±0.02	0.28±0.10*	0.20±0.02*
t_o	M	-1.50±0.21	-1.42±0.20	-1.60±0.31*	-1.29±0.22*
	F	-1.76±0.23	-1.64±0.24	-2.13±0.63*	-2.33±0.25*
L_o (mm)	M	280.9±13.8	265.6±14.8	406.3±24.6*	344.7±17.2*
	F	290.8±19.2	271.5±19.5	404.1±23.8*	373.3±15.1*
Age at 50% Maturity	M	3.9±0.3 yrs	4.1±0.3 yrs	1.7±0.2 yrs*	2.3±0.2 yrs*
	F	6.7±0.3 yrs	6.8±0.4 yrs	2.9±0.2 yrs*	4.6±0.4 yrs*
Length at 50% maturity (mm FL)	M	617.8±12.1	617.8±12.1	572.4±10.5	583.1±9.4
	F	815.9±32.2	815.9±32.2	662.6±10.3	729.0±16.2
Observed maximum size (mm FL)	M	825	825	808	825
	F	1043	1043	952	1043
Observed maximum age	M	12.0 yrs	12.0 yrs	5.5+ yrs	12.0 yrs
	F	18.0 yrs	17.9 yrs	7.5+ yrs	17.9 yrs
Theoretical maximum age	M	11.6 yrs	11.9 yrs	6.4 yrs*	6.9 yrs*
	F	18.4 yrs	18.7 yrs	12.3 yrs*	17.5 yrs*