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Reproduction of the Blacktip Shark in the Gulf of Mexico

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Abstract
Reproductive and age data were collected for Blacktip Sharks *Carcharhinus limbatus* in the Gulf of Mexico from fishery-dependent and -independent sources from 2006 to 2011 for stock assessment. A total of 757 Blacktip Sharks were sampled for reproductive analysis (399 females, 358 males), of which 741 were aged. Additional length and age data from a previous age and growth study on Blacktip Sharks in the Gulf of Mexico (207 females, 161 males) were incorporated into the size- and age-at-maturity analyses. The results indicated that Blacktip Sharks in the Gulf of Mexico have a synchronous, seasonal reproductive cycle and that females exhibit a biennial ovarian cycle. Male and female mating and parturition peaked from March to May. Length at 50% maturity was estimated to be 105.8 and 119.2 cm FL for males and females, respectively, while age at 50% maturity was calculated as 4.8 and 6.3 years. Near-term pups averaged 38 cm FL, and gestation was approximately 12 months. Litter size was 4.5 pups per female, and fecundity was found to increase with both maternal size and age. Maternal body size—but not age—had a positive influence on offspring fitness. This represents the first comprehensive reproductive study of Blacktip Sharks in the Gulf of Mexico.

Reproductive parameters, such as fecundity, size and age at maturity, and reproductive periodicity and synchrony are used by fisheries scientists to estimate the productivity and rebound potential of a fish stock. Stock assessments are reliant upon current, stock-specific estimates because reproductive output often differs by area (Walker 2005) and can be affected by density dependence due to fishing mortality (Walters et al. 2000; Rose et al. 2001). Unfortunately, timely and regional reproductive information is rare for many elasmobranch species throughout the world. Even more rare are reproductive studies that also estimate the ages of elasmobranchs; most age-at-maturity estimates are back-calculated using the von Bertalanffy (von Bertalanffy 1938) equation for size at maturity (Casey et al. 1985; Carlson et al. 2006; Barreto et al. 2011).

The Blacktip Shark *Carcharhinus limbatus* is a common coastal species that occupies tropical and subtropical waters worldwide (Compagno et al. 2005). In U.S. waters, it ranges from Massachusetts to Florida in the western North Atlantic Ocean and throughout the Gulf of Mexico (McEachran and Fechhelm 1998). Previous studies in the Gulf of Mexico have mostly focused on age and growth and used back-calculation methods to estimate age at maturity (Branstetter 1987; Killam and Parsons 1989; Carlson et al. 2006). These studies have estimated size at maturity ranges for Gulf of Mexico Blacktip Sharks of 103–110 and 117–132 cm FL for males and females, respectively. Back-calculated ages at maturity in these studies were estimated to be 4–5 years for males and 5–8 years for females. Sizes and ages were also estimated by Carlson et al. (2006) for Blacktip Sharks in the western North Atlantic Ocean: median maturity estimates were 117 cm FL (5.0 years) for males and 126 cm FL (6.7 years) for females. While Carlson et al. (2006) provided maturity information, the Blacktip Sharks in that study were only classified as mature or juvenile based on internal examination and no further reproductive information was collected. The most comprehensive reproductive study for Blacktip Sharks in the eastern United States was in the

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western North Atlantic Ocean (Castro 1996). Sizes at maturity were estimated to be 118 and 127 cm FL for males and females, respectively, with females exhibiting a biennial ovarian cycle and an 11-month gestation period. Mating and ovulation were estimated to occur in May and June, agreeing with previous observations of the reproduction of Blacktip Sharks in the same area (Springer 1940; Bigelow and Schroeder 1948; Clark and von Schmidt 1965).

Tagging evidence and genetic information indicate that the western North Atlantic and Gulf of Mexico populations of Blacktip Sharks are separate (Keeney et al. 2003, 2005; Bethea et al. 2012), with little mixing occurring between the two basins. For this reason, the Blacktip Shark is managed as two stocks: the Gulf of Mexico and Atlantic Ocean stocks. The objectives of this study were to provide the first detailed reproductive analysis for the Blacktip Shark in the Gulf of Mexico for stock assessment and to test for differences in size and age at maturity between the current study and Carlson et al. (2006).

METHODS

Sampling.—Blacktip Sharks were sampled for aging and reproductive analysis in the Gulf of Mexico from 2006 to 2011. The majority of reproductive samples were obtained by certified fisheries observers aboard commercial longline vessels in the Gulf of Mexico. Additional samples were also collected from a fishery-independent gill-net survey in order to obtain juvenile Blacktip Sharks not captured by commercial vessels (Figure 1). A full description of the gear and fishing methods can be found in Hale and Baremore (2010).

Fisheries observers sampled gonads and vertebrae from Blacktip Sharks in an opportunistic fashion when fishing activity and sea conditions were favorable. All Blacktip Sharks were measured for FL (cm) in a straight line from the tip of the nose to the fork in the tail. For females, the right ovary, both oviducal glands, and both uteri were saved, while for males the claspers, both testes, both epididymides, and the seminal vesicle were saved. As the Blacktip Sharks sampled were commercial

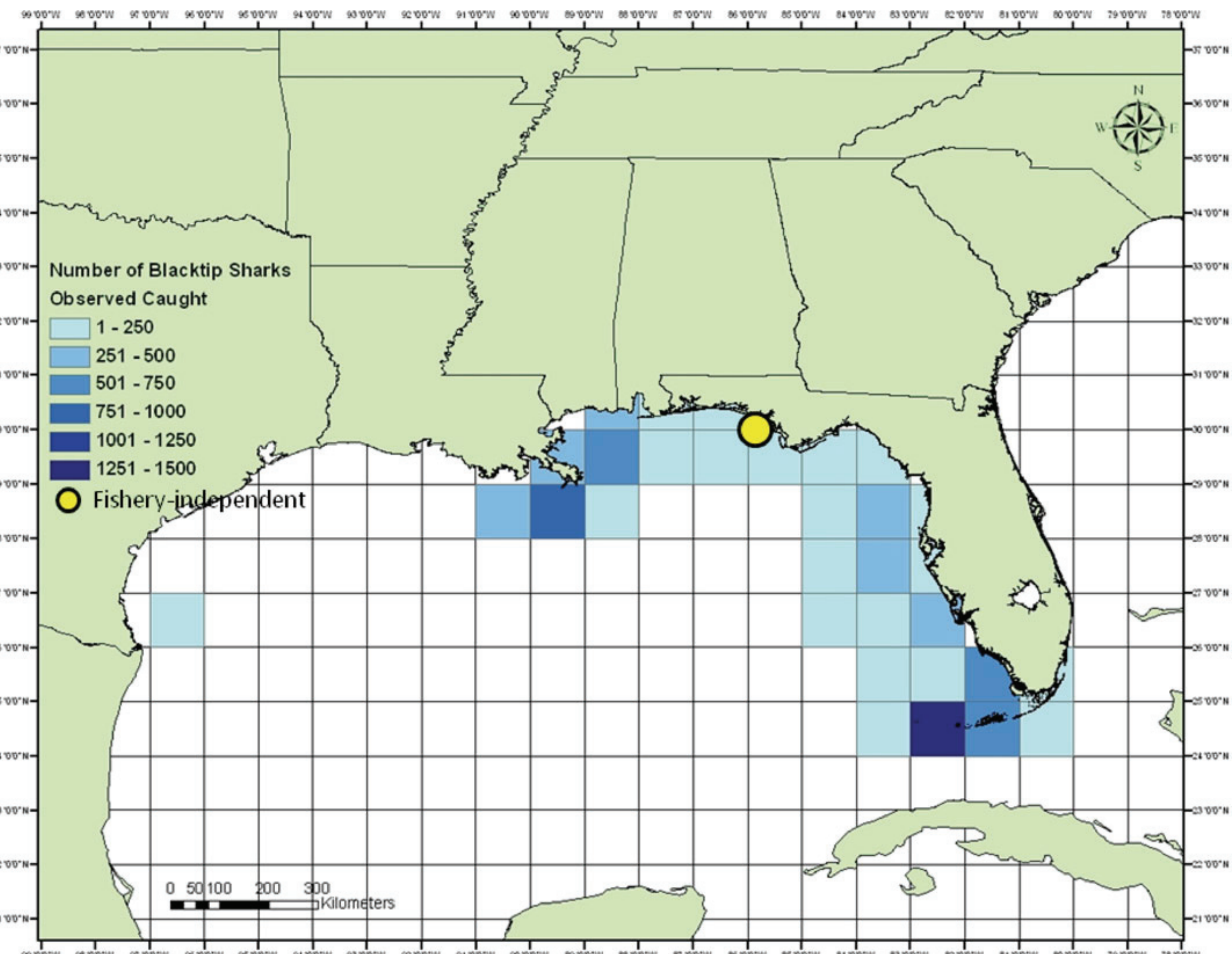

FIGURE 1. Capture locations in the Gulf of Mexico for Blacktip Sharks sampled for reproduction and age analysis from 2006 to 2011. The shaded grids indicate areas sampled by commercial bottom longline gear, the circle the area sampled by a fishery-independent gill-net survey.

products, the vertebrae were removed from the discarded portions of the carcasses in the cervical region of the spinal column. Reproductive and vertebral samples were either frozen or kept on ice and then shipped to the National Marine Fisheries Service's Panama City Laboratory for processing. The reproductive samples were processed immediately, while the vertebrae were catalogued and frozen until they were cleaned and sectioned. For vertebrae preparation and aging methods, see Passerotti and Baremore (2012).

Reproductive analysis—Gonad measurements were taken by fishery biologists. Ovary length, width (mm), and weight (g) were measured for females, along with the width of the oviducal glands and one uterus. Occasionally, one to two follicles were observed to be notably larger than the majority of ovarian follicles, meaning that the traditional method of measuring the maximum follicle diameter could misrepresent the true reproductive condition of the ovary (e.g., a pregnant female with large, seemingly vitellogenic follicles). Without histology, it can be difficult to distinguish vitellogenic from atretic follicles, and these definitions should only be used with histological evidence. Therefore, we began to measure the five largest ovarian follicles (mm) for each female. A repeated measures-analysis of variance (rANOVA) was used to test for differences in measured follicle diameters for mature females. An rANOVA can be used to test the equality of means of a sample when the same measurement is repeated over time or within a group. If follicle diameters were significantly different from one another (i.e., one or more follicles was significantly larger than the other measured follicles), the average of the five follicle diameters was calculated and used for analysis. This ensured that the significantly larger follicle(s) did not misrepresent the ovarian condition. If the rANOVA results were not statistically significant, only the maximum follicle diameter (MFD) was used for all analyses. The clasper calcification state was recorded for males, as were testis length (mm), width (mm), and weight (g) and the width of one epididymis. The seminal vesicle was examined to characterize semen (thin, clear versus viscous, white).

All Blacktip Sharks were assigned a stage based on their reproductive characteristics (Walker 2005; Baremore and Hale 2012). stages were based on measurements and qualitative examination of the gonads. Female Blacktip Sharks were assigned stages from 1 to 7 as follows: (1) juvenile, no development of ovary or oviducals and thread-like uteri; (2) juvenile, developing, with some ovarian follicles >5 mm and more prominent uteri; (3) mature, not pregnant; (4) mature, sperm packets visually confirmed in the uterus; (5) mature, ovulating (both yolked, ovarian follicles and fertilized uterine eggs present); (6) mature, pregnant; and (7) mature, postpartum with distended uteri and internal umbilical scars. Males were staged from 1 to 3 as follows: (1) juvenile; (2) mature; and (3) mature, running ripe with distended seminal vesicles containing copious amounts of viscous semen. Females at stage 3 and greater were considered mature. Mature versus immature status in males was determined by clasper calcification alone: mature males had fully calcified claspers, while immature males did not.

Because resting mature females (stage 3) can be difficult to distinguish from developing juveniles (stage 2), measurements of the MFD (mm), ovary weight (OWT; g), uterus width (UW; mm), and oviducal width (OW; mm) were used in conjunction with qualitative characteristics to determine maturity status. Differences among MFD, OWT, UW, and OW values were tested by stage to determine which measurements provided the best indication of maturity. An ANOVA was used to test for differences among stages, with post hoc pairwise comparisons using Tukey's least-square difference (LSD) test (Tukey 1949) unless observations failed the assumption of homogenous variance among stages (Bartlett's test, $P < 0.05$). When it was determined that variances were not homogenous, a Kruskal–Wallis test was used to test for differences among stages. Pairwise comparisons among stages were performed with a Wilcoxon rank-sum test with Holm–Sidak correction (Holm 1979). The Holm–Sidak correction method is considered to be more powerful than the Bonferroni correction for *P*-values while still controlling type I error (Aickin and Gensler 1996).

Only clasper calcification was used to distinguish juvenile from mature males in this study. However, in some cases researchers may find it difficult to obtain claspers from all males (i.e., if receiving samples from an independent source). Measurements of testis width, testis weight, and epididymis width may be used to help determine the maturity status of male Blacktip Sharks based on internal examination alone. Differences among testis width, testis weight, and epididymis widths by stage were tested using the same methods described previously for females.

To assess the reproductive seasonality and synchrony of Blacktip Sharks in the Gulf of Mexico, plots of gonad measurements were constructed. Average female OWT, MFD, and OW and average male testis weight and epididymis width were plotted by month. For females, only those for stage 3 (mature, resting) females were plotted because only nongravid females underwent vitellogenesis during spring months. Stage 2 and 3 males (mature and running ripe) were plotted to examine the reproductive cycle of males. If heteroscedasticity was detected, the differences in all measurements by month were tested with a Kruskal–Wallis test and a post hoc Wilcoxon rank sum test. Otherwise, an ANOVA was used to test for differences, with post hoc pairwise comparisons performed with Tukey's LSD test. The percentage of mature females and males in each reproductive stage was examined graphically by month to further assess the seasonality of reproduction.

The fecundity and gestational characteristics of Blacktip Sharks in the Gulf of Mexico were examined. Fecundity was calculated as the average number of embryos per female for all stage 6 females that were sampled with intact uteri. The sex ratio of embryos in utero was tested for significant difference from 1:1 using a χ^2 analysis, as was the number of embryos in each uterus. Regressions of the number of embryos by maternal FL and age were used to determine whether fecundity increased with the size and age of females. The stretch total length (STL; cm) of embryos were plotted by month to determine size at birth and length of gestation. Because the caudal fins of embryos <5 cm were not forked, STL was used as a standard measurement.

Reproductive output was investigated using the size and weight of near-term embryos (herein called pups) in relation to maternal size and age. Near-term pups were defined as those in utero between February and May, based on observational data. The relationships between maternal FL, age, and weight (kg) and pup STL, weight (kg), and condition factor, and the total weight of the litter (kg) were examined using linear and nonlinear regression analyses. As adult sharks were not weighed, maternal weight was obtained using a published length–weight equation (SEDAR 2006). Condition factor (K) for embryos was defined as

$$K = \frac{\text{weight(g)} \cdot 100}{\text{STL}^3}.$$

When $K > 1$, a fish is considered to be in "good" (well-fed) condition in relation to its size (Froese 2006). The "best" regressions were determined by examining the P-values, R^2, and residual square errors (RSE) of the models. Only complete litters (i.e., uteri intact, no evidence of aborted pups) were examined.

Length and age at maturity.—Size and age at maturity were calculated for males and females using the logistic regression

$$y = \frac{1}{1 + \exp[-(a + bx)]},$$

where x = FL or age, with binomial maturity data (0 = juvenile, 1 = mature). The size (L50) and age (A50) at which 50% of individuals were mature was calculated as ($y = -\frac{a}{b}$), and standard errors (SEs) for the parameters were calculated. Sex was added as a factor to the logistic model to determine whether there were differences in parameter estimates between the sexes using a χ^2 test of likelihood ratios. A maternity ogive was also calculated to determine the size and age at female maternity using binomial data (0 = juvenile or mature but not in maternal condition, 1 = mature and in maternal condition). Maternal condition was defined as females classified as stage 6 that were concurrently pregnant during 1 year's cycle (Walker 2005) and took the reproductive periodicity into account by excluding females that are not actively reproducing.

Historical data.—Additional length, age, and maturity (juvenile versus mature) data from Carlson et al. (2006) were obtained (207 females, 161 males), and maturity ogives were calculated using the methods described above. Both studies had one reader in common for age assignment, so the possibility of aging bias among studies was considered low. Sex and study were added as factors in the logistic model to test for the effects of these parameters on the outcome using a χ^2 test of likelihood ratios. As the differences among the model parameter estimates from the current and previous studies were minimal and not significant ($\text{L50}_{\text{STUDY}}$ $P = 0.38$, $\text{A50}_{\text{STUDY}}$ $P = 0.09$, L50_{SEX} $P = 0.47$, A50_{SEX} $P = 0.42$), age and maturity data were combined with the data for this study. Results are presented for the data from the current study, the historical data, and the combined data.

RESULTS

The catch locations for the Blacktip Sharks sampled mostly ranged from the Florida Keys to the Louisiana coast (Figure 1). A total of 757 (399 females, 358 males) Blacktip Sharks were sampled for reproductive analysis, of which 741 (391 females, 350 males) were aged (Figure 2). Overall, 169 of the females (42%) and 183 of the males (51%) examined were classified as mature. Most of the juveniles were collected by a fishery-independent gill-net survey and most of the adults by a commercial longline fishery, leading to a bimodal length distribution (Figure 2).

The rANOVA showed that follicle diameters were significantly different among the largest five ovarian follicles measured for mature females ($P < 0.001$, $n = 74$), indicating that overall the five largest follicles of mature females were of different sizes. However, when the analysis was restricted to mature females not in reproductive condition (stage 3), the rANOVA results were not significantly different ($P = 0.18$, $n = 27$). Because only stage 3 females were used to assess the seasonality of reproduction, maximum follicle diameter (MFD) is reported for all analyses.

The values for maximum follicle diameter, ovary weight (OWT), uterus width (UW), and oviducal width (OW) showed distinct differences among stages for females (Table 1), while those for testis weight, testis width, and epididymis width were

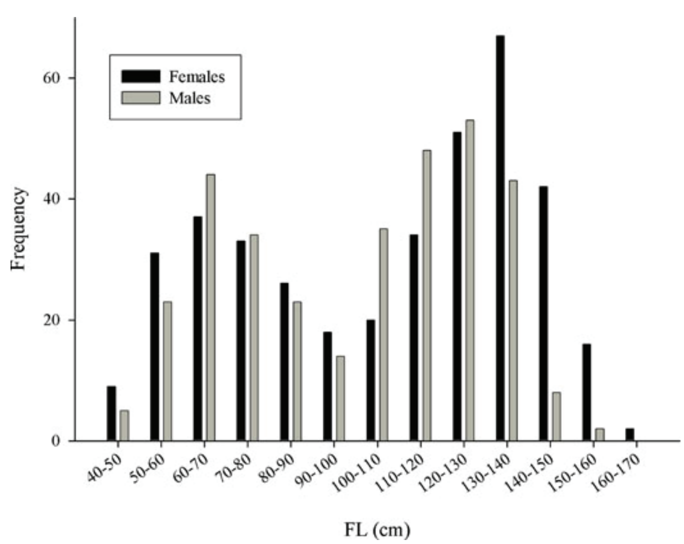

FIGURE 2. Length-frequency distribution of Blacktip Sharks sampled for age, growth, and reproductive analysis in the Gulf of Mexico from 2006 to 2011.

TABLE 1. Average values and SDs for the maximum follicle diameter (MFD), ovary weight (OWT), uterus width (UW), and oviducal width (OW) for each reproductive stage for female Blacktip Sharks in the Gulf of Mexico. Stages are as follows: 1 = juvenile, no development, thread-like uteri; 2 = juvenile, developing; 3 = mature, not pregnant; 4 = mature, sperm in uterus; 5 = mature, ovulating; 6 = mature, pregnant; and 7 = mature, postpartum.

	MFD (mm)		OWT (g)		UW (mm)		OW (mm)	
Stage	Average	SD	Average	SD	Average	SD	Average	SD
1	6.9	3.3	15.7	8.4	6.8	5.1	14.8	9.7
2	10.5	3.8	23.6	10.6	20.2	8.8	22.5	5.8
3	17.0	8.7	70.5	41.1	36.9	11.4	30.3	5.0
4	25.9	10.7	79.3	30.1	45.5	15.6	32.8	7.3
5	36.6	7.7	114.2	38.0	68.1	15.0	42.7	8.6
6	13.5	7.8	61.8	35.5	122.7	28.5	29.7	4.4
7	13.6	7.8	49.3	39.0	42.4	26.5	26.0	9.2

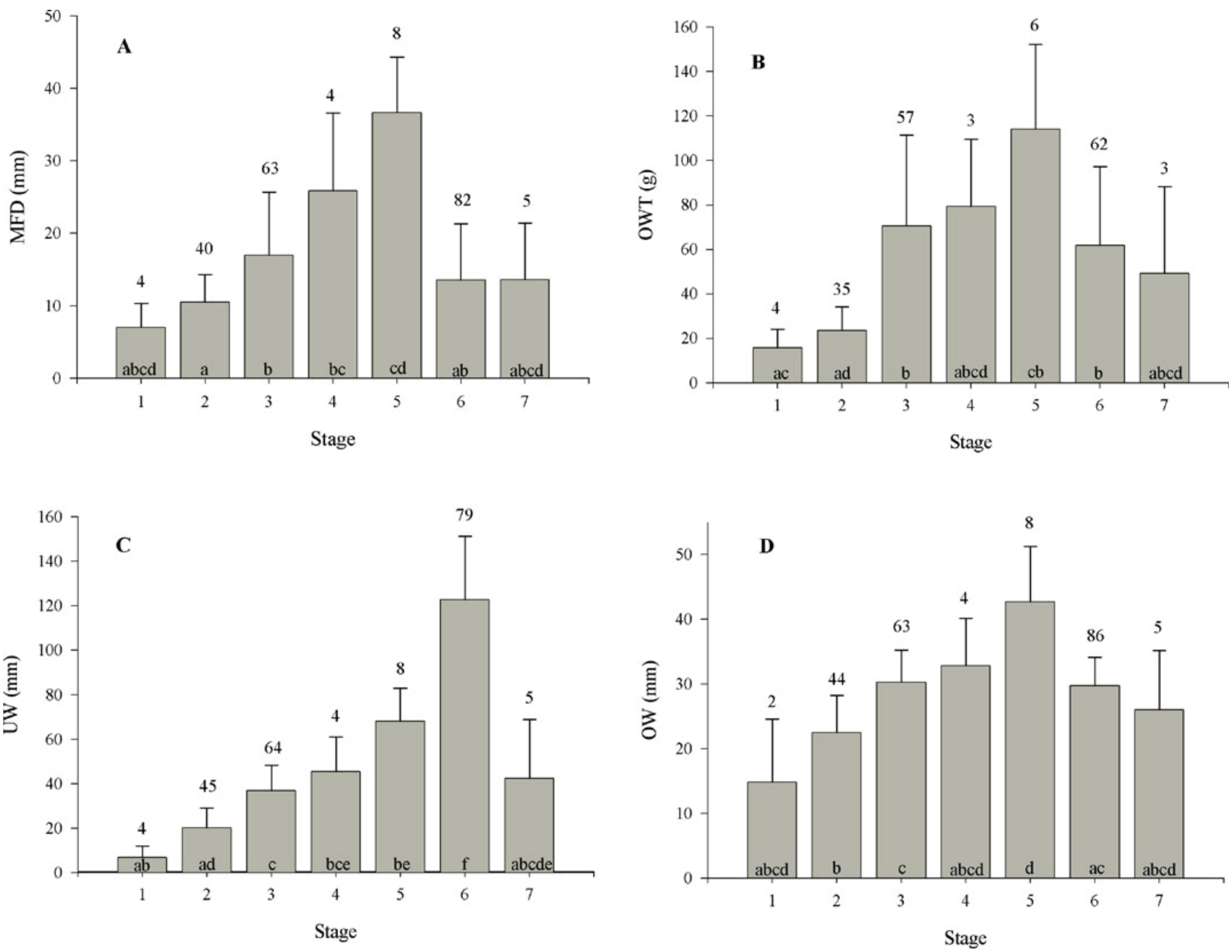

FIGURE 3. Average **(A)** maximum follicle diameter (MFD), **(B)** ovary weight (OWT), **(C)** uterus width (UW), and **(D)** oviducal width (OW) for female Blacktip Sharks sampled for reproductive analysis, by stage. Stages are as follows: 1 = juvenile, no development, thread-like uteri; 2 = juvenile, developing; 3 = mature, not pregnant; 4 = mature, sperm detected in uterus; 5 = mature, ovulating; 6 = mature, pregnant; and 7 = mature, postpartum. Sample sizes are listed above the standard deviation error bars. Values with the same lowercase letters are not significantly different (Wilcoxon rank-sum test: $P > 0.05$).

TABLE 2. Average values and SDs for testis width and weight and epididymis width for male Blacktip Sharks in the Gulf of Mexico, by stage (1 = juvenile, 2 = mature, and 3 = mature, running ripe).

	Testis width (mm)		Testis weight (g)		Epididymis width (mm)	
Stage	Average	SD	Average	SD	Average	SD
1	22.6	8.9	33.9	26.9	17.1	4.3
2	32.2	12.7	76.2	59.9	23.1	6.0
3	40.0	8.8	115.1	55.2	30.5	5.5

well-defined for the three male stages (Table 2). Significant differences in MFD, OWT, UW, and OW values were found among stages (Kruskal–Wallis test: $P < 0.001$; Figure 3). The average MFD for stage 2 and stage 3 females was 10.5 and 17.0 mm, respectively, while the OW averaged 22.5 (stage 2) and 30.3 mm (stage 3). All measurements were significantly different between stage 2 and stage 3 females (Wilcoxon rank-sum test: $P < 0.05$). Significant differences were also found among stages for male testis weight, testis width, and epididymis width (Kruskal–Wallis test: $P < 0.001$; Wilcoxon rank-sum test: $P < 0.05$; Figure 4). Though there was some overlap in the values among stages, especially among mature animals, these measurements can be used to accurately assign reproductive stage when taken together and with qualitative observations.

Reproduction was seasonal and synchronous, with the peak in mating and ovulation occurring from March through May. Reproductive measurements were highest from February through May for stage 3 females, with a drastic decline in June for most measurements (Figure 5). All measurements were heteroscedastic for females; Kruskal–Wallis tests showed significant differences in OWT, OW, and MFD by month; however, post hoc tests were not conclusive, likely due to the large range of values and small sample sizes by month. Therefore, pairwise comparisons were not reported for females by month. Stage 2 and 3 males had the highest testis weight from February through May, while epididymis width peaked in May–June (Figure 6). For males, only epididymis width was not heteroscedastic; pairwise comparisons showed a significant decline in testis weight after May, while epididymis width decreased significantly between July and August.

A graph of female stages by month showed that stage 4 (sperm present) and stage 5 (ovulating) females occurred as early as March, though ovulation most frequently occurred during June (Figure 7A). No near-term females were observed in June, indicating that pupping occurred in May and most newly pregnant females (stages 5, 6) were gravid by July. Stage 3 (running ripe) males first occurred in February, and no stage 3 males were observed after July (Figure 7B).

Of the 169 females that were classified as mature, 86 (51%) were pregnant. Because approximately half of the mature females were pregnant, and because pregnant females showed no signs of vitellogenesis during gestation, the ovarian cycle is most likely biennial. Gestation was approximately 12 months

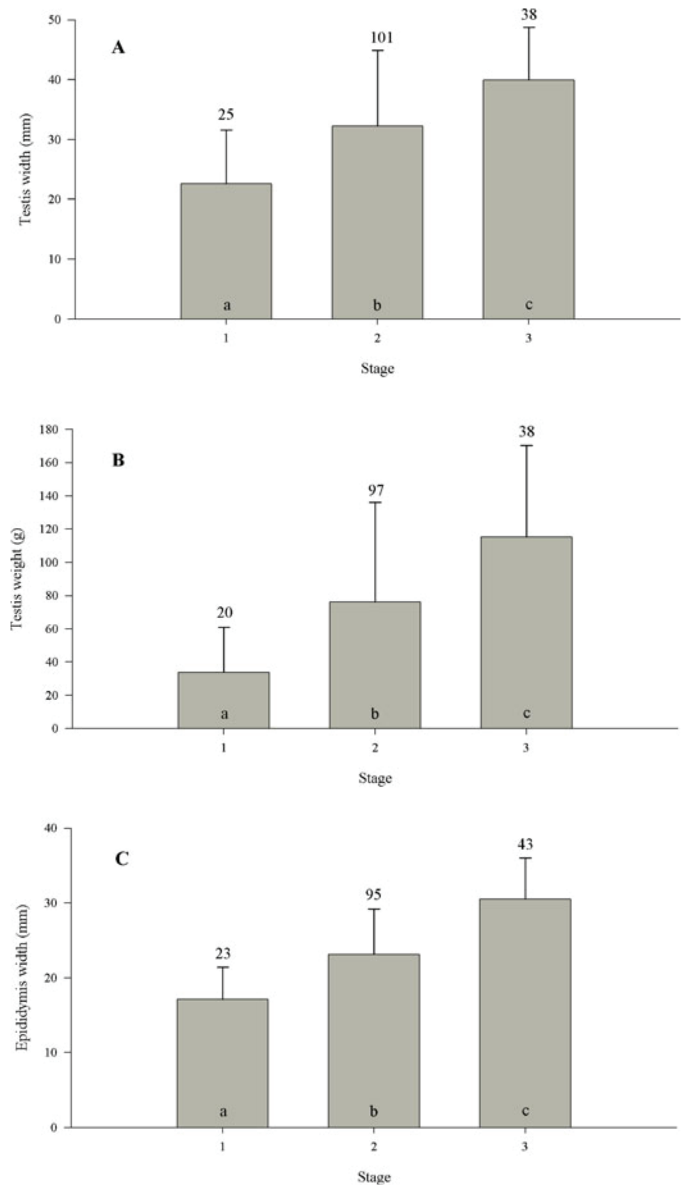

FIGURE 4. Average **(A)** testis width, **(B)** testis weight, and **(C)** epididymis width for male Blacktip Sharks sampled for reproductive analysis, by stage. Stages are as follows: 1 = juvenile; 2 = mature; and 3 = mature, running ripe. Sample sizes are listed above the standard deviation error bars. Values with the same lowercase letters are not significantly different (Wilcoxon rank-sum test: $P > 0.05$).

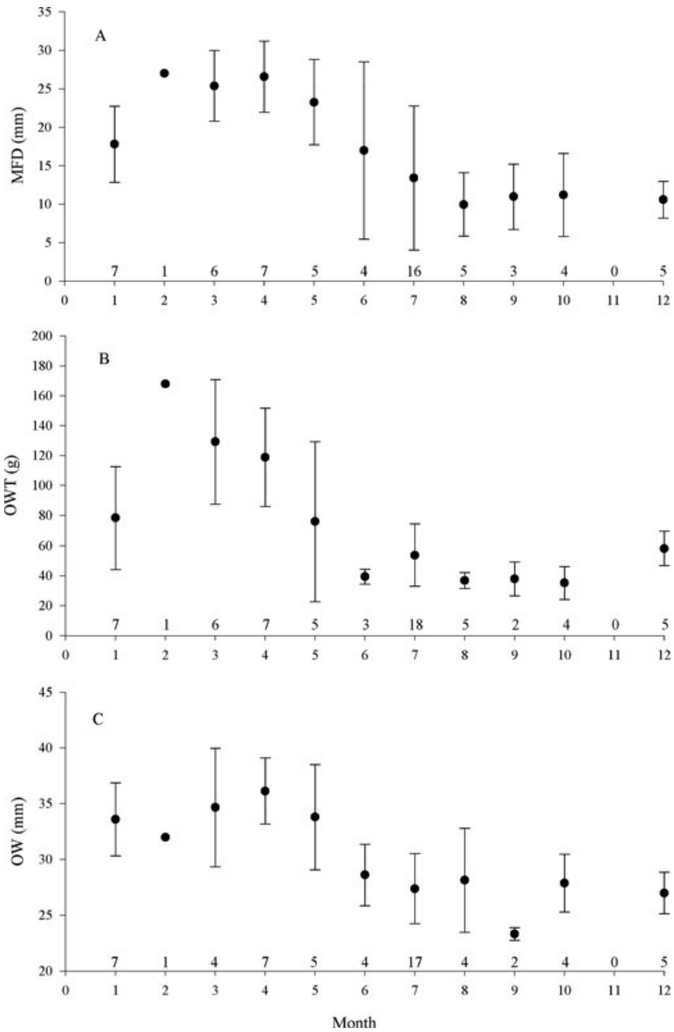

FIGURE 5. Monthly averages of **(A)** maximum follicle diameter (MFD), **(B)** ovary weight (OWT), and **(C)** oviducal width (OW) for stage 3 female Blacktip Sharks in the Gulf of Mexico from 2006 to 2011. The error bars represent SDs, and sample sizes are listed above the months on the *x*-axis.

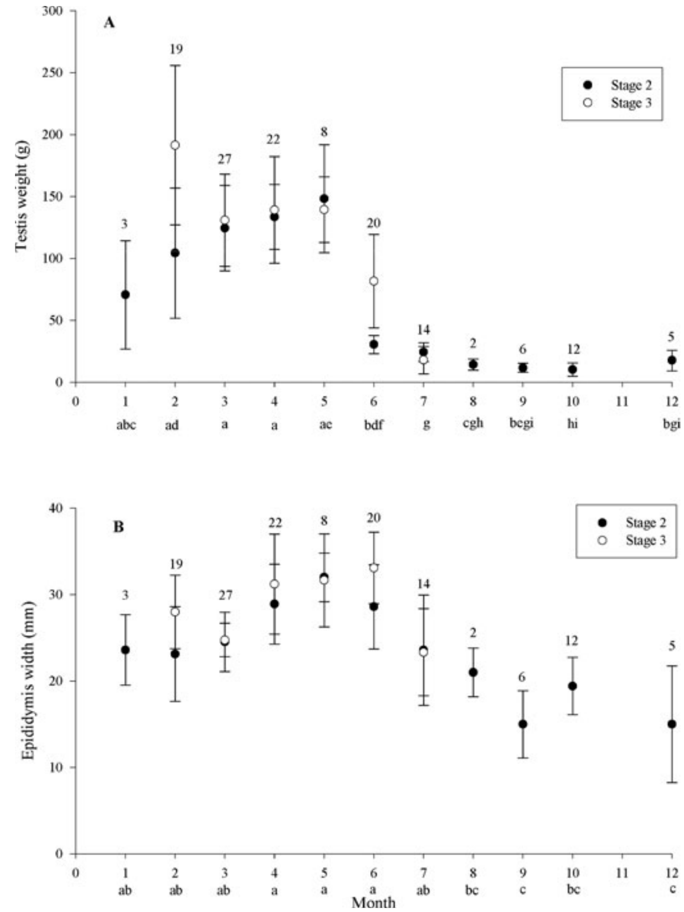

FIGURE 6. Monthly averages of **(A)** testis weight and **(B)** epididymis width for stage 2 and 3 Blacktip Sharks in the Gulf of Mexico from 2006 to 2011. Sample sizes are given above the standard deviation error bars. The letters below the *x*-axis indicate statistical equivalence for combined stage 2 and 3 males at the 0.05 level.

in duration, with the first embryos being observed in June and the largest near-term pups occurring in May (Figure 8). Size at birth was approximately 50 cm STL (~38 cm FL). Fecundity averaged 4.5 (SD, 1.22) pups per female. Fecundity increased significantly ($P < 0.001$) with both size and age, though the relationships were weak (Figure 9). The sex ratio of adults was not significantly different from 1:1 overall ($\chi^2 = 2.47$, $n = 302$, $P > 0.10$), and the sex ratio of pups in each uterus was not significantly different (left: $\chi^2 = 1.31$, $n = 150$, $P > 0.10$; right: $\chi^2 = 1.68$, $n = 152$, $P > 0.10$).

A total of 31 litters of near-term pups were examined between February and May to determine whether reproductive output and quality was affected by maternal size and age. Near-term pups averaged 49.3 cm STL (Figure 8), which was similar to our estimated size at birth. Average pup STL per litter, average pup weight per litter, and the total weight of all pups per litter showed significant increases with increasing maternal FL and weight ($P < 0.05$), though the correlations were weak. The K (condition factor) value for near-term embryos did not change significantly with maternal FL or weight ($P > 0.05$). Only the total weight of pups per litter increased significantly with maternal age ($P < 0.05$); no other relationships (average pup STL, average pup weight, and K) were significant ($P > 0.10$). In terms of RSE, significance levels, and R^2 values, maternal weight was the best predictor of near-term pups' average STL, average weight, and the total weight of the litter (Figure 10). These findings suggest that maternal size—and especially weight—has a greater impact on offspring fitness than maternal age for Blacktip Sharks in the Gulf of Mexico.

Size and age at maturity estimates are presented in Table 3. When data from the current study were combined with those from Carlson et al. (2006), the L50 value was 119.2 cm FL and 105.8 cm FL for females and males, respectively (Figure 11A; Table 3). The corresponding A50 values were 6.3 and 4.8 years (Figure 11B; Table 3). Maternal size and age were

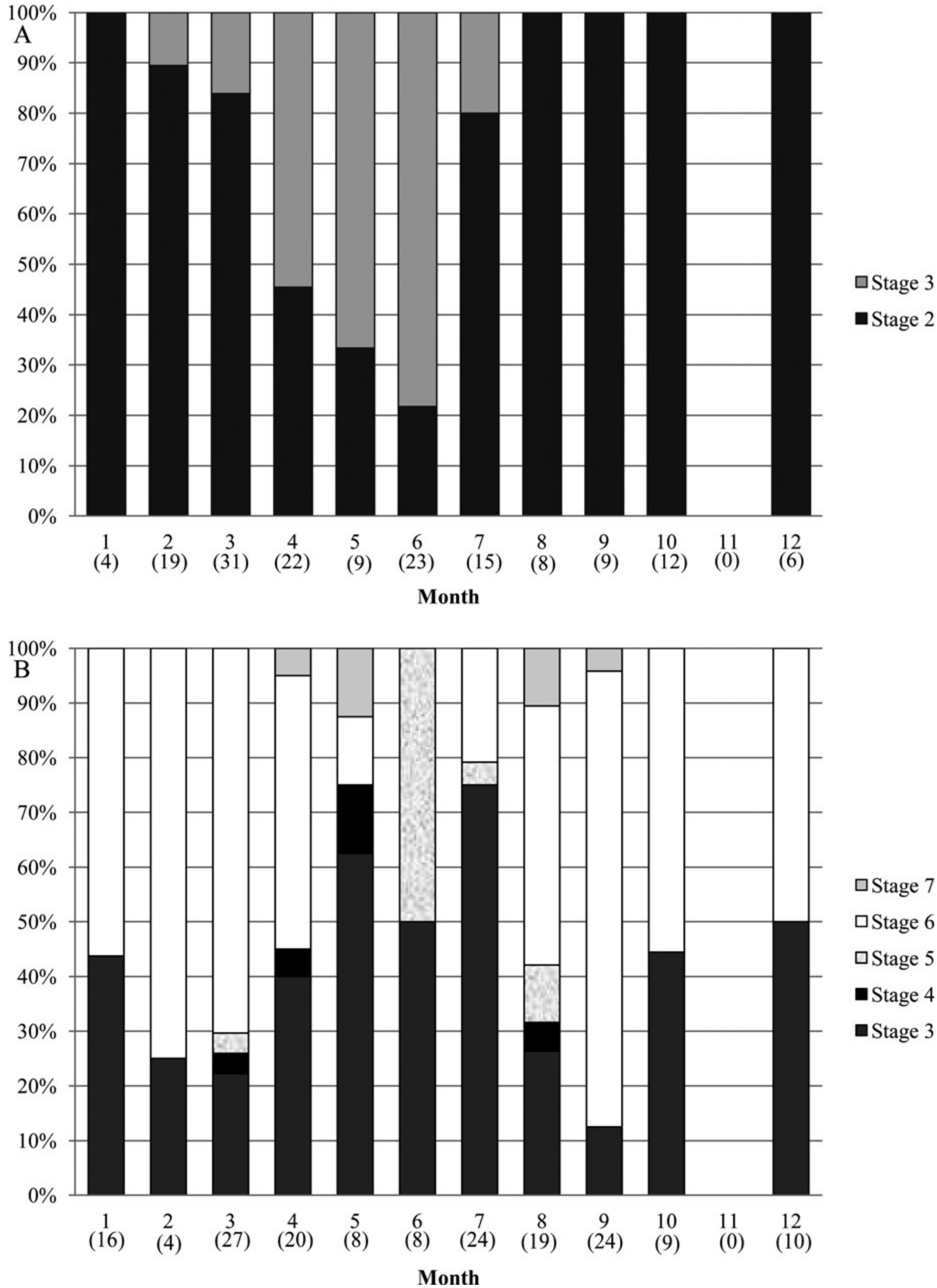

FIGURE 7. Relative frequencies of stages for mature **(A)** female and **(B)** male Blacktip Sharks in the Gulf of Mexico from 2006 to 2011, by month. See Figures 3 and 4 for definitions of the stages. Sample sizes are indicated in parentheses below the *x*-axes.

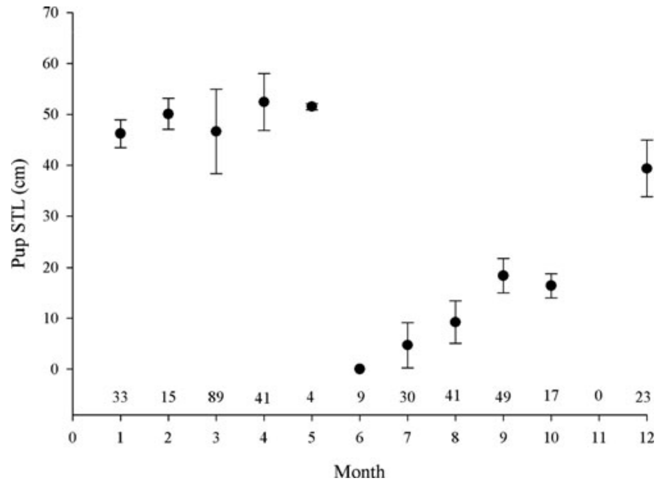

FIGURE 8. Average in utero pup size (stretch total length [STL]) for Blacktip Sharks in the Gulf of Mexico from 2006 to 2011, by month. Sample sizes (number of pups measured) are listed above the months on the *x*-axes; the error bars represent SDs.

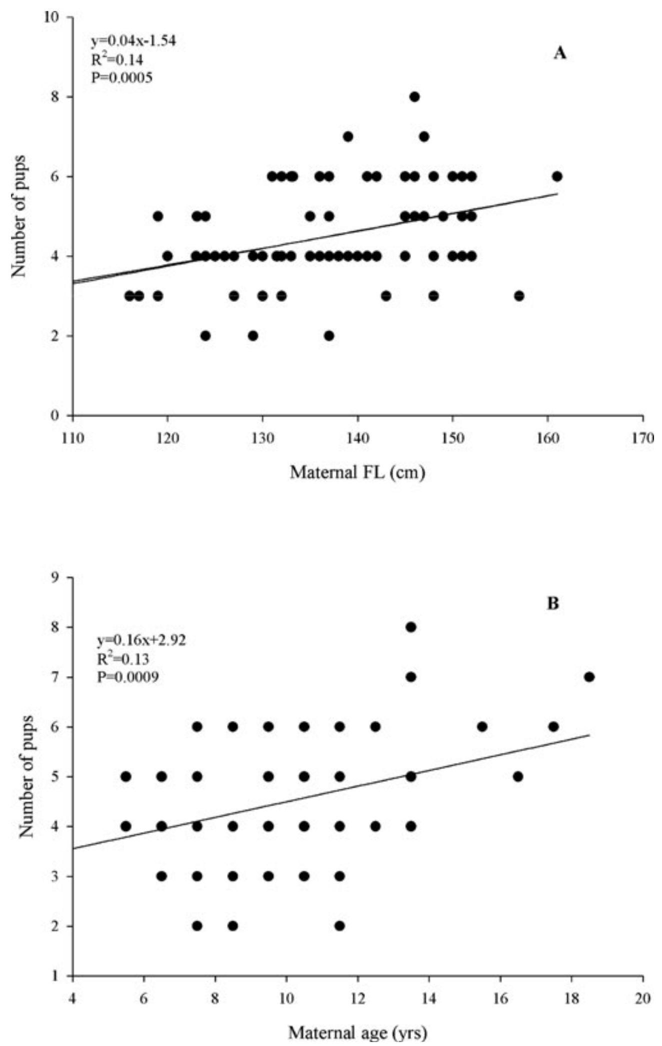

FIGURE 9. Relationships between fecundity and maternal (**A**) FL and (**B**) age for Blacktip Sharks in the Gulf of Mexico from 2006 to 2011.

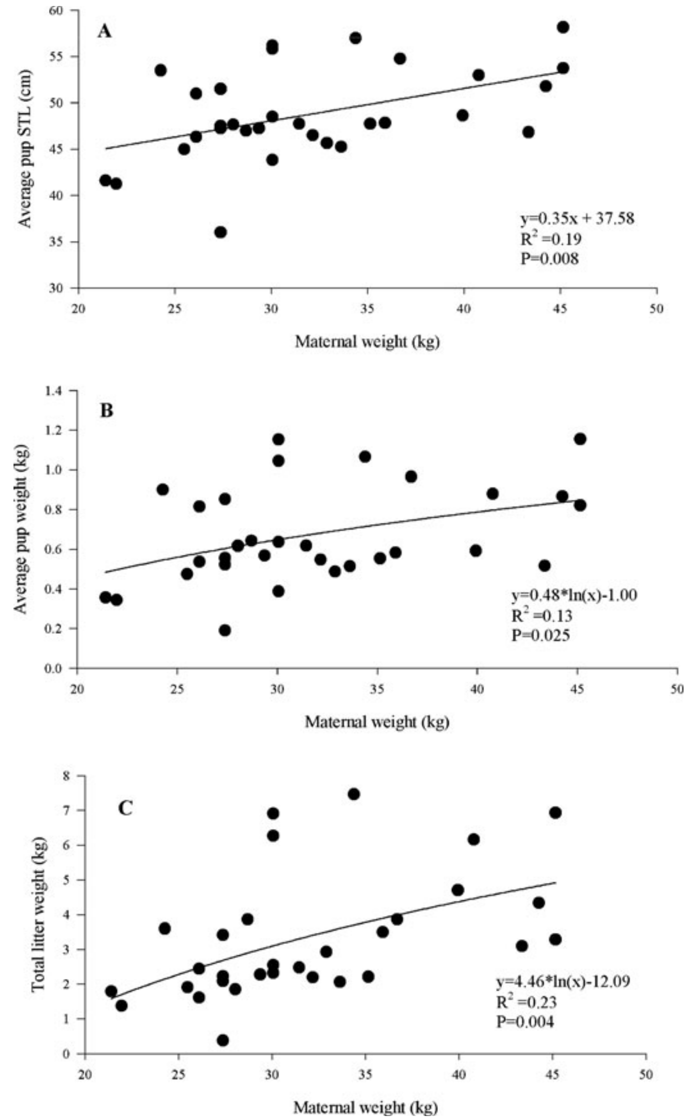

FIGURE 10. Relationships between maternal weight and (**A**) average stretch total length (STL), (**B**) average pup weight, and (**C**) total litter weight of near-term in utero pups for Blacktip Sharks in the Gulf of Mexico from 2006 to 2011.

only available from the current study: L50 was calculated as 137.6 cm FL and A50 was 10.1 years (Figure 11A, B; Table 3). The maternity ogive curve was multiplied by 0.5 to account for the biennial reproductive cycle (Figure 11A, B). Size and age at maturity were significantly different among sexes for all data combinations.

DISCUSSION

This study represents the most comprehensive reproductive analysis for Blacktip Sharks in U.S. waters and is the first to describe the reproductive cycle, ovarian periodicity, and size and age at maturity in the Gulf of Mexico. The reproductive parameters fell within expected ranges when compared with those of previous studies in the western North Atlantic (Clark and von

TABLE 3. Length (L50) and age (A50) at 50% maturity and maternity for Blacktip Sharks in the Gulf of Mexico. The parameters *a* and *b* were estimated by the logistic model and presented with their standard errors. Data are separated by source.

Sex or maternity status	L50 (cm FL)					A50 (years)				
	Value	*a*	SE	*b*	SE	Value	*a*	SE	*b*	SE
Current study										
Females	119.3	−25.41	3.44	0.21	0.03	6.5	−6.61	0.83	1.02	0.12
Males	106.6	−21.94	3.19	0.21	0.03	4.8	−5.98	0.83	1.24	0.16
Maternity	137.6	−10.03	1.28	0.07	0.01	10.1	−3.89	0.41	0.39	0.05
Carlson et al. (2006)										
Females	117.3	−46.12	12.84	0.39	0.11	5.9	−6.85	1.13	1.16	0.21
Males	103.4	−85.01	32.86	0.08	0.32	4.4	−13.44	4.35	3.02	0.97
Combined										
Females	119.2	−28.09	3.17	0.24	0.03	6.3	−6.46	0.62	1.02	0.09
Males	105.8	−24.01	3.02	0.23	0.03	4.8	−6.65	0.78	1.39	0.15

Schmidt 1965; Castro 1996; Carlson et al. 2006): reproduction was synchronous and seasonal, with the peak of mating and parturition occurring mostly during the spring months of May and June, respectively. The female ovarian cycle appears to be biennial, with 1 year of gestation being followed by a year-long "resting" period. Sizes and ages at maturity did not differ significantly from previous estimates in the Gulf of Mexico (Carlson et al. 2006).

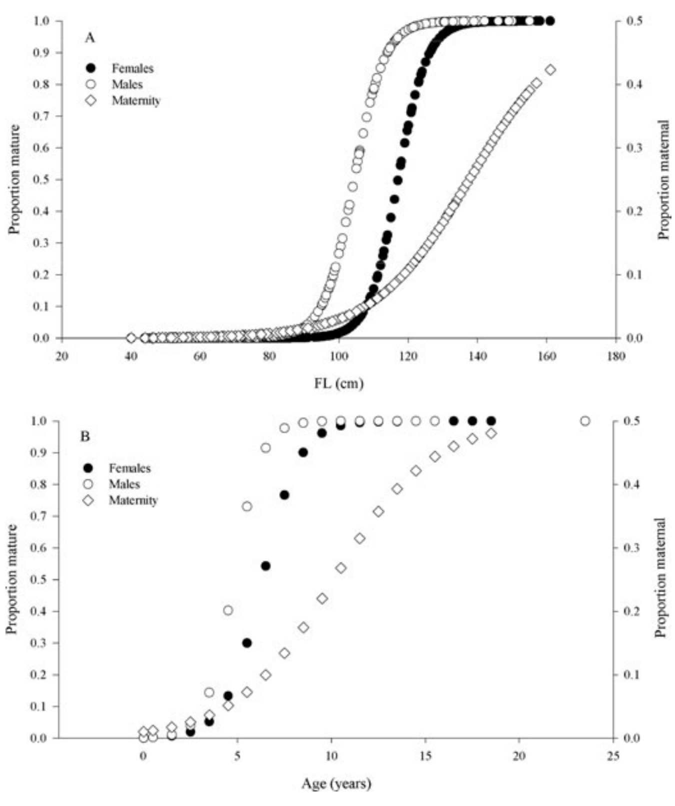

FIGURE 11. Maturity and maternity ogives for **(A)** FL and **(B)** age of male and female Blacktip Sharks in the Gulf of Mexico from combined data sets (1996–2002 and 2006–2011). Maternity ogives were multiplied by 0.5 to account for the biennial reproductive cycle and only contain data from 2006 to 2011.

It is common in the elasmobranch reproductive literature for researchers to define maturity in females in a purely qualitative way; the color of follicles, the shape of the oviducal gland, and differences between the oviducal gland, oviduct, and uterus are described quite often (Driggers et al. 2004; Ebert 2005; Barnett et al. 2009); however, these observations are largely subjective and have not undergone the scrutiny of quantitative analysis. In addition, researchers hoping to use these observations in their own analysis may not be able to determine exactly how maturity was assessed. Developing juvenile females can be very difficult to distinguish from resting mature females because some reproductive organs and ovarian follicles are reduced to nearly their prematurity levels during resting periods. We found that using benchmark measurements along with observational data helped to more easily assign maturity stage. Because all measurements were significantly different between juvenile and mature animals, we feel the estimates are robust.

We found that the fecundity of females (i.e., the average number of pups per litter) increased with both maternal size and age. While the relationships were not strong, they provide more evidence that elasmobranchs become more fit reproductively as they grow and age (Baremore and Hale 2012), assuming that senescence is not a factor. The prevailing theory of elasmobranch reproductive biology is that the increase in body size allows for an increase in fecundity (Conrath and Musick 2001, 2012; Goodwin et al. 2002). However, it stands to reason that, like teleost fishes, older elasmobranchs may be able to devote more energy to reproduction and therefore produce more fit offspring. The trade-off between producing more "fit" (larger) offspring and more numerous offspring has not been fully explored for viviparous shark species.

Size at birth is a major determinant of initial mortality and vulnerability to predation for juvenile sharks (Cortés 1998). A larger size at birth could give neonatal sharks an advantage over their smaller counterparts. We found that maternal weight was the factor most highly correlated with reproductive fitness in terms of embryo size and total litter weight, while maternal age was not significant. Sharks, like most fishes, grow asymptotically in length (Hoenig and Gruber 1990), while continuing to put on mass throughout their life spans. Therefore, heavier females may have more capacity for larger broods than females of similar length (and age) but less mass. In this study weight was regressed on FL, which could have reduced the error in the estimates and therefore biased the results; however, we believe that because the relationship between length and weight is very robust for Blacktip Sharks, these findings are relevant.

This is the first study to produce length- and age-at-maternity estimates for Blacktip Sharks. While useful for stock assessments, this method is not widely applied in elasmobranch life history studies. The A50 estimate of 10.1 years for females in maternal condition was higher than might be predicted based on the A50 of 6.3 years for all females. However, because this is a biennial species, only half of the mature females will be pregnant during a given year. Therefore, for half of the females to be in maternal condition, all of the females must be mature. The A98 for female Blacktip Sharks (the age at which 98% of females are mature) was calculated to be 10.5 years, which is very similar to the A50 for females in maternal condition.

Our estimates of sizes and ages at maturity were not significantly different from those obtained by Carlson et al. (2006). This suggests that density-dependent changes in mortality were not a factor for the Gulf of Mexico population sampled, though the sampling periods may not have been distinct enough for us to detect such differences (Sminkey and Musick 1995). Total landings of Blacktip Sharks in the Gulf of Mexico have declined since the late 1980s, with a marked decrease after 2004 (Cortés and Baremore 2012). In recent years, strict quotas have been established in response to stock assessment results for other large coastal species. These regulations limit the number of large coastal sharks harvested per trip (NMFS 2008). The total mortality of Blacktip Sharks was likely affected, as this species dominates the landings of large coastal sharks in the Gulf of Mexico. Blacktip Sharks have never been assessed as overfished, nor has it been found that overfishing is occurring in the Gulf of Mexico (SEDAR 2006, 2012). The consistent life history parameters over time and positive stock assessments indicate that Blacktip Sharks are being sustainably harvested in the Gulf of Mexico. Reproductive and age analysis should be undertaken periodically, as the stock continues to be affected by fishing mortality.

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