Reproduction of the blacktip shark *Carcharhinus limbatus* in the Gulf of Mexico

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Summary

Reproductive and age data were collected for blacktip shark in the Gulf of Mexico from fishery-dependent and –independent sources from 2006-2011. A total of 757 blacktip sharks were sampled for reproductive analysis (399 females, 358 males), of which 742 were aged. Analyses indicate that blacktip sharks are a synchronous, seasonally reproducing species and that females exhibit a biennial ovarian cycle. Male and female reproductive activity (mating, parturition) was relatively truncated, with peaks from March-May. Near-term embryos averaged 38 cm FL, and gestation is approximately 12 months. Fecundity was 4.5 pups per female, and fecundity was found to increase with both maternal size and age. Length at 50% maturity for males and females was estimated to be 105.8 and 119.2 cm FL, respectively, while age at 50% maturity was calculated at 4.8 and 6.3 years. This represents the first comprehensive reproductive study for blacktip sharks in the Gulf of Mexico.

Introduction

Reproductive parameters, such as fecundity, size and age at maturity, 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. 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.

The blacktip shark *Carcharhinus limbatus* is a common coastal species that occupies tropical and sub-tropical waters world-wide (Compagno, et al., 2005). In US 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; Carlson, et al., 2006; Killam and Parsons, 1989). These studies have estimated sizes at maturity for blacktip sharks in the Gulf of Mexico ranging from 103-110 and 117-132 cm FL for males and females, respectively. Ages at maturity in these studies were back calculated from the sizes at maturity at 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. The most comprehensive reproductive study for blacktip sharks in the eastern US was in the 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 was estimated to occur in May and June, which agreed with previous limited observations of reproduction of blacktip sharks in the same area (Bigelow and Schroeder, 1948; Clark and von Schmidt, 1965; Springer, 1940).

Tagging evidence and genetic information indicates that the western North Atlantic and Gulf of Mexico populations of blacktip sharks are separate (Bethea, et al., 2012; Keeney, et al., 2003; Keeney, et al., 2005), with little mixing occurring between the two basins. As such, the blacktip shark is managed as two stocks: Gulf of Mexico and Atlantic Ocean. The objectives of this study are to provide detailed reproductive analysis for the blacktip shark in the Gulf of Mexico for stock assessment.

Methods

Sampling

Blacktip sharks were sampled for ageing and reproductive analysis in the Gulf of Mexico from 2006-2011. The majority of reproductive samples were obtained by fisheries observers aboard commercial longline vessels in the Gulf of Mexico. Additional samples were also collected from a fishery-independent gillnet survey in order to obtain juvenile blacktip sharks not captured by commercial vessels (Fig. 1).

Fisheries observers sampled gonads and vertebral samples from blacktip sharks in an opportunistic fashion when fishing activity and sea conditions were favorable. All blacktip sharks were measured for fork length (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 removed, while for males the claspers, both testes, both epididymedes and the seminal vessicle were sampled. Blacktip sharks sampled were commercial products, therefore vertebral samples were removed from the discarded portion in the cervical region of the spine. Reproductive and vertebral samples were either frozen or kept on ice and then shipped to the National Marine Fisheries Service Panama City Laboratory for processing. Reproductive samples were processed immediately, and vertebrae were catalogued and frozen until they were cleaned and sectioned. For vertebrae preparation and ageing methods see Passerotti and Baremore (2012).

Reproductive analysis

Ovary length, width (mm), and weight (g) were measured for females, along with the width of the oviducal glands and one uterine branch. Clasper length (mm) and calcification state (Y/N) were recorded for males, as were testis length, width, and weight (g), and the width of the epididymis. The seminal vesicle was examined for the presence and characterization of semen.

All blacktip sharks were assigned a stage based on reproductive characteristics. Stages were assigned based on measurements of the gonads along with qualitative examination (Table 1).

Female blacktip sharks were staged from 1-7, with the following definitions: 1) juvenile, no development; 2) juvenile, developing; 3) mature, resting; 4) sperm present in uterus; 5) ovulating; 6) pregnant; 7) post-partum. Males were staged from 1-3: 1) juvenile; 2) mature; 3) mature, running ripe. Females staged 3 or greater were considered mature, while males staged 2 or greater were mature.

To assess the reproductive seasonality and synchrony of blacktip shark in the Gulf of Mexico, plots of gonad measurements were assessed. Females' average ovary weight, maximum follicle diameter (MFD), and oviducal width (OW) were plotted by month, and males' average testis weight and epididymis width were also examined by month. The percentage of mature males and females in each reproductive stage was plotted by month to further assess the seasonality of reproduction. Individual MFD's for Stage 3 females during spring months (January-June) were also plotted to help determine the periodicity of the ovarian cycle.

Fecundity and gestational characteristics of blacktip sharks in the Gulf of Mexico were examined. Fecundity is defined the average number of pups per female for all Stage 6 females that were sampled with intact uteri. The sex ratio of pups *in utero* was tested for significant difference from 1:1 using χ^2 analysis, as was the number of pups in the each uterine branch. Regressions of the number of pups by maternal size and age were used to determine whether fecundity increased with the size and age of females. Stretch total lengths (STL cm) of embryos were plotted by month to determine size at birth and length of gestation.

Size and age at maturity was calculated for males and females separately using a logistic regression:

$$y = \left(\frac{1}{1 + \exp\left(-\left(a + b * (FL)\right)\right)}\right)$$

with binomial maturity data (0=juvenile, 1=mature). Size (L_{50}) and age (A_{50}) at which 50% of individuals were mature was calculated as ($y = -\frac{a}{b}$), and standard errors (SE) were calculated. 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 in maternal condition). Maternal condition was defined as those females classified as Stage 6 which were concurrently pregnant during one year's cycle.

Additional length, age, and maturity data from Carlson et al. (2006) were obtained, and maturity ogives were calculated using the methods described previously. A χ^2 test of likelihood ratios was used to test for differences between L_{50} and A_{50} values for males and females separately between the two studies. Differences among the models from the current and previous studies were minimal ($L_{50females}$ p=0.40, $L_{50males}$ p=0.06, $A_{50females}$ p=0.24, $A_{50males}$ p=0.31), therefore maturity data were combined. The results from the current study, Carlson et al. (2006) data, and from the combined data analyses are presented.

Results

A total of 757 (399 females, 358 males) blacktip sharks were sampled for reproductive analysis, of which 742 (392 females, 350 males) were also aged (Fig. 2). Overall, 169 females examined and 183 of the males examined were classified as mature.

Average MFD, ovary weight, uterus width, and OW values showed distinct differences among stages for females (Fig. 3, Table 1), and testis weight, width, and epididymis width values were unique for the three male stages (Fig 4, Table 2). Though some overlap in the values among stages occurred, these measurements can be used to accurately assign reproductive state when considered in concert and with qualitative observations. For example, Stage 2 and 3 females can often be difficult to distinguish qualitatively because Stage 3 females are resting and their reproductive organs are inactive and reduced in size. However, the average values given in Table 1 help to determine maturity state quantitatively.

Reproduction was seasonal and synchronous, with the peak in reproductive activity 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 (Fig. 5). Stage 2 and 3 males had the largest testis weight in May, while epididymis width peaked in June (Fig. 6). Plots of female stages by month showed Stage 5 (ovulating) and Stage 6 (sperm present) occurred as early as March, though ovulation most frequently occurred during June (Fig. 7A). No pregnant or ovulating females were observed in June, indicating that pupping occurred in May and most newly pregnant females were gravid by July. Stage 3 (running ripe) males first occurred in February, and no Stage 3 males were observed after June (Fig. 7B).

Of the 169 females that were classified as mature, 51% (86) were pregnant. Gestation is approximately 12 months in duration, with the fist embryos observed in June and the largest near-term pups occurring in May (Fig. 8). Size at birth is approximately 50 cm STL (~38 cm FL). Fecundity averaged 4.5 (±1.22 SD) pups per female. Fecundity increased with both size and age, though the relationships were weakly correlated (Fig. 9). The sex ratio was not significantly different from a 1:1 ratio overall ($P>0.05$), the sex ratio of pups in each uterine branch was not significantly different ($P>0.05$).

Size and age at maturity estimates are presented in Table 3. When data from the current study were combined with that from Carlson et al. (2006), L_{50} values were 119.2 cm FL and 105.8 cm FL for females and males, respectively (Fig. 10A). Combined A_{50} values were 6.3 years and 4.8 years for males and females, respectively (Fig. 10B, Tables 4-5). Maternal size and age were only available from the current study: L_{50} was calculated as 137.6 cm FL and A_{50} was 10.1 years (Fig. 10A, B). The maternity ogive was multiplied by 0.5 to account for the biennial reproductive cycle. Sizes and ages at maturity were significantly different among sexes for all data combinations.

Literature cited

Bethea, D. M., Carlson J. K., and Grace M. 2012. Tag and recapture data for blacktip shark, Carcharhinus limbatus, in the Gulf of Mexico: 1999-2010. SEDAR29-WP-07.

Bigelow, H. B., and Schroeder W. C. 1948. Fishes of the western North Atlantic, Sears Foundation for Marine Research.

Branstetter, S. 1987. Age and growth estimates for the blacktip, *Carcharhinus limbatus*, spinner, *C. brevipinna*, sharks from the northwestern Gulf of Mexico. Copeia 4:964-974.

Carlson, J. K., Sulikowski J. A., and Baremore I. E. 2006. Do differences in life history exist for blacktip sharks, *Carcharhinus limbatus*, from the United States South Atlantic Bight and Eastern Gulf of Mexico? Environ. Biol. Fishes 77:279-292.

Castro, J. I. 1996. Biology of the Blacktip Shark, *Carcharhinus limbatus*, off the southeastern United States. Bull. Mar. Sci. 59:508-522.

Clark, E., and von Schmidt K. 1965. Sharks of the central Gulf coast of Florida. Bull. Mar. Sci. 15:13-83.

Compagno, L. J. V., Dando M., and Fowler S. L. 2005. A field guide to the sharks of the world, Harper Collins.

Keeney, D., Heupel M., Hueter R., and Heist E. 2003. Genetic heterogeneity among blacktip shark, *Carcharhinus limbatus*, continental nurseries along the US Atlantic and Gulf of Mexico. Mar. Biol. 143:1039-1046.

Keeney, D., Heupel M., Hueter R., and Heist E. 2005. Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (Carcharhinus limbatus) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. Mol. Ecol. 14:1911-1923.

Killam, K. A., and Parsons G. K. 1989. Age and growth of the blacktip shark, *Carcharhinus limbatus*, near Tampa Bay, Florida. Fish Bull 87:845-857.

McEachran, J. D., and Fechhelm J. D. 1998. Fishes of the Gulf of Mexico, University of Texas Press, Austin, TX.

Springer, S. 1940. The sex ratio and seasonal distribution of some Florida sharks. Copeia 1940:188-194.

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

Walker, T. I. 2005. Reproduction in fisheries science. *In* Reproductive Biology and Phylogeny of Chondrichthyes: Sharks, Batoids, and Chimaeras (W. C. Hamlett, ed.), p. 81-127. Science Publishers, Inc., Enfield, New Hampshire.

Table 1. Average values and standard deviations (STDEV) for maximum follicle diameter (MFD), ovary weight, uterus width, and oviducal width (OW) for each female reproductive stage.

Stage	MFD (mm)	STDEV	Ovary weight (g)	STDEV	Uterus width (mm)	STDEV	OW (mm)	STDEV
1	6.97	3.33	15.75	8.42	6.80	5.08	14.85	9.69
2	10.52	3.77	23.60	10.6	20.25	8.79	22.49	5.77
3	16.96	8.72	70.51	41.1	38.74	17.65	30.27	4.96
4	25.87	10.71	79.33	30.1	45.50	15.60	32.80	7.32
5	36.65	7.66	114.16	38	68.06	14.99	42.67	8.60
6	13.53	7.79	61.80	35.5	122.70	28.50	29.70	4.39
7	13.60	7.79	49.33	39	42.40	26.47	26.00	9.19

Table 2. Average testis weight, width, and epididymis width along with standard deviations (STDEV) for blacktip sharks in the Gulf of Mexico.

Stage	Testis weight (g)	STDEV	Testis width (mm)	STDEV	Epididymis width (mm)	STDEV
1	46.73	25.96	27.04	8.46	16.86	5.30
2	114.65	46.50	40.47	8.25	25.49	5.04
3	123.46	48.99	41.46	6.99	31.05	5.19

Table 3. Length and age at 50% maturity for blacktip sharks in the Gulf of Mexico. Data are separated by data source.

	L50 (cm FL)	a	b	SE	A50 (years)	a	b	SE
Current study								
Females	119.3	-25.406	0.213	3.44	6.5	-6.612	1.024	0.83
Males	106.6	-21.937	0.206	3.19	4.8	-5.978	1.237	0.83
Maternity	137.6	-10.030	0.073	1.28	10.1	-3.892	0.385	0.41
Carlson et al. 2006								
Females	117.3	-46.115	0.393	12.84	5.9	-6.846	1.161	1.13
Males	103.4	-85.014	0.082	32.85	4.4	-13.44	3.018	4.35
Combined								
Females	119.2	-28.095	0.236	3.17	6.3	-6.464	1.020	0.62
Males	105.8	-24.010	0.227	3.02	4.8	-6.649	1.393	0.78

Table 4. Age maturity schedule for female and male blacktip sharks in the Gulf of Mexico using data from the current study.

	Females	-6.612	1.024			Males	-5.978	1.237			Maternity	-3.8919	0.3848	
Age=Bands-1.5	Avg pct mat	SE a	SE b	n		Avg pct mat	SE a	SE b	n		Avg pct mat	SE a	SE b	n
0	0.001342	0.8289	0.1191	58		0.002527	0.8324	0.1565	54		0.019998	0.40755	0.04606	58
0.5	0.002238			40		0.004681			38		0.024139			40
1.5	0.006206			31		0.015946			27		0.035070			31
2.5	0.017090			19		0.052875			10		0.050695			19
3.5	0.046177			10		0.161312			13		0.072756			10
4.5	0.118784			16		0.398553			24		0.103373			16
5.5	0.272892			24		0.695403			24		0.144860			24
6.5	0.510998			20		0.887205			38		0.199296			20
7.5	0.744216			28		0.964412			28		0.267783			28
8.5	0.890123			34		0.989403			22		0.349531			33
9.5	0.957548			34		0.996901			20		0.441198			33
10.5	0.984327			20		0.999098			12		0.537057			20
11.5	0.994314			20		0.999738			7		0.630252			18
12.5	0.997950			8		0.999924			3		0.714655			8
13.5	0.999263			8		0.999978			3		0.786323			8
14.5	0.999735			1		0.999994			1		0.843921			1
15.5	0.999905			2		0.999998			1		0.888201			2
16.5	0.999966			4					0		0.921094			3
17.5	0.999988			1					0		0.944909			1
18.5	0.999996			1					0		0.961834			1
19.5				0					0					
20.5				0					0					
21.5				0					0					
22.5				0					0					
23.5				0		1.000000			1					

Table 5. Maturity schedule for female and male blacktip sharks in the Gulf of Mexico using data from Carlson et al. (2006).

	Females	-6.846	1.161			Males	-13.44	3.018	
Age=Bands-1.5	Avg pct mat	SE a	SE b	n		Avg pct mat	SE a	SE b	n
0	0.001063	1.1354	0.2119	46		0.000001	4.3514	0.9716	51
0.5	0.001897			27		0.000007			32
1.5	0.006033			34		0.000135			26
2.5	0.019012			28		0.002746			10
3.5	0.058277			19		0.053302			10
4.5	0.164998			11		0.535192			9
5.5	0.386867			10		0.959262			9
6.5	0.668299			5		0.997928			8
7.5	0.865472			5		0.999898			2
8.5	0.953580			3		0.999995			1
9.5	0.984984			8		1.000000			1
10.5	0.995248			3		1.000000			1
11.5	0.998507			5		1.000000			1
12.5	0.999532			3					0

Table 6. Maturity schedule for female and male blacktip sharks in the Gulf of Mexico using data from the current study combined with Carlson et al. (2006).

	Females	-6.464	1.02			Males	-6.649	1.393	
Age=Bands-1.5	Avg pct mat	SE a	SE b	n		Avg pct mat	SE a	SE b	n
0	0.001556	0.6195	0.0942	104		0.001294	0.7768	0.0.1536	105
0.5	0.002589			67		0.002593			70
1.5	0.007146			65		0.010359			53
2.5	0.019570			47		0.040446			20
3.5	0.052451			29		0.145108			23
4.5	0.133080			27		0.406006			33
5.5	0.298594			34		0.733509			33
6.5	0.541405			25		0.917246			46
7.5	0.766025			33		0.978087			30
8.5	0.900787			37		0.994467			23
9.5	0.961801			42		0.998620			21
10.5	0.985881			23		0.999657			13
11.5	0.994862			25		0.999915			7
12.5	0.998141			11		0.999979			3
13.5	0.999329			8		0.999995			3
14.5	0.999758			1		0.999999			1
15.5	0.999913			2		1.000000			1
16.5	0.999969			4					0
17.5	0.999989			1					0
18.5	0.999996			1					0
19.5				0					0
20.5				0					0
21.5				0					0
22.5				0					0
23.5				0		1.000000			1

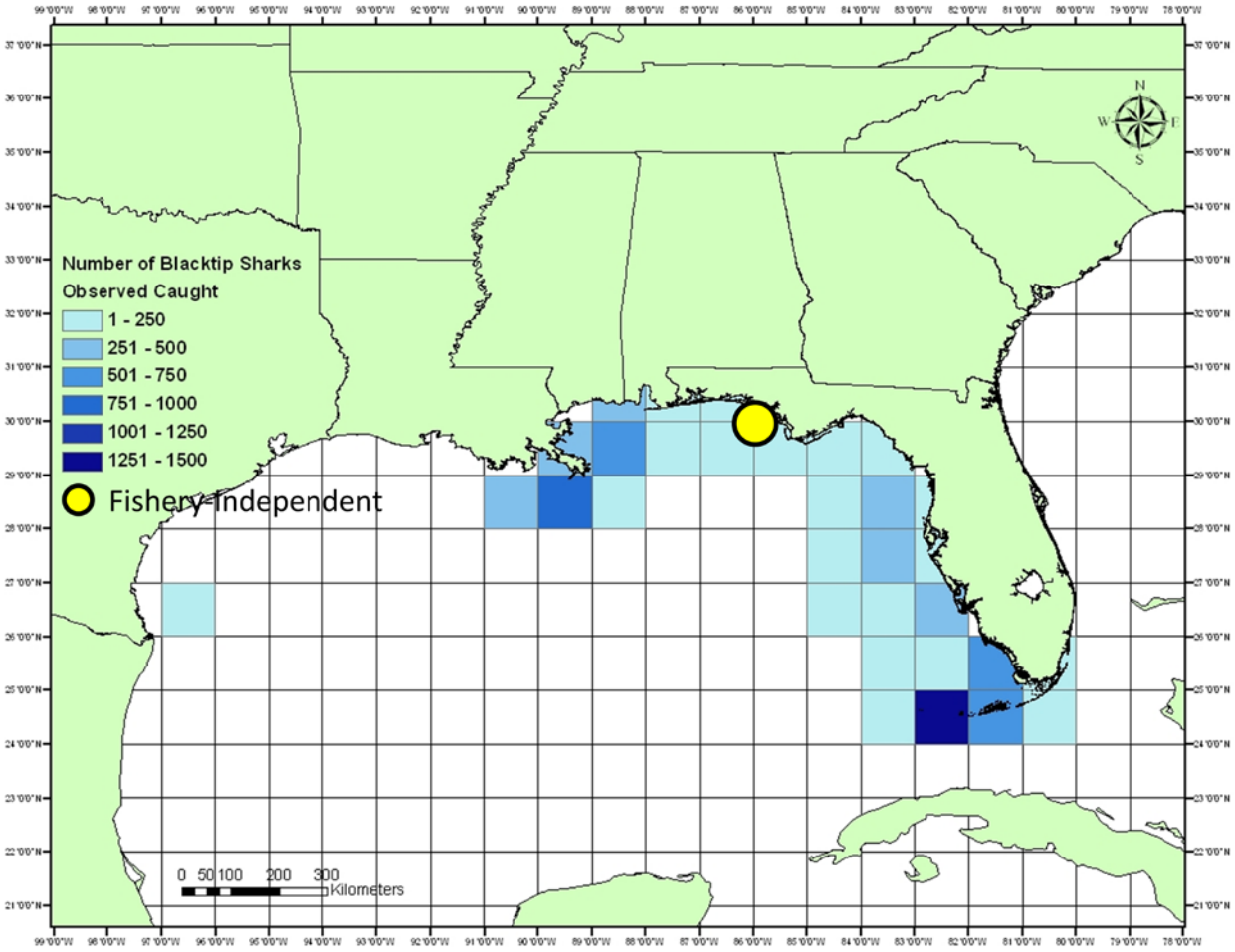


Figure 1. Sampling locations for blacktip sharks collected from 2006-2011.

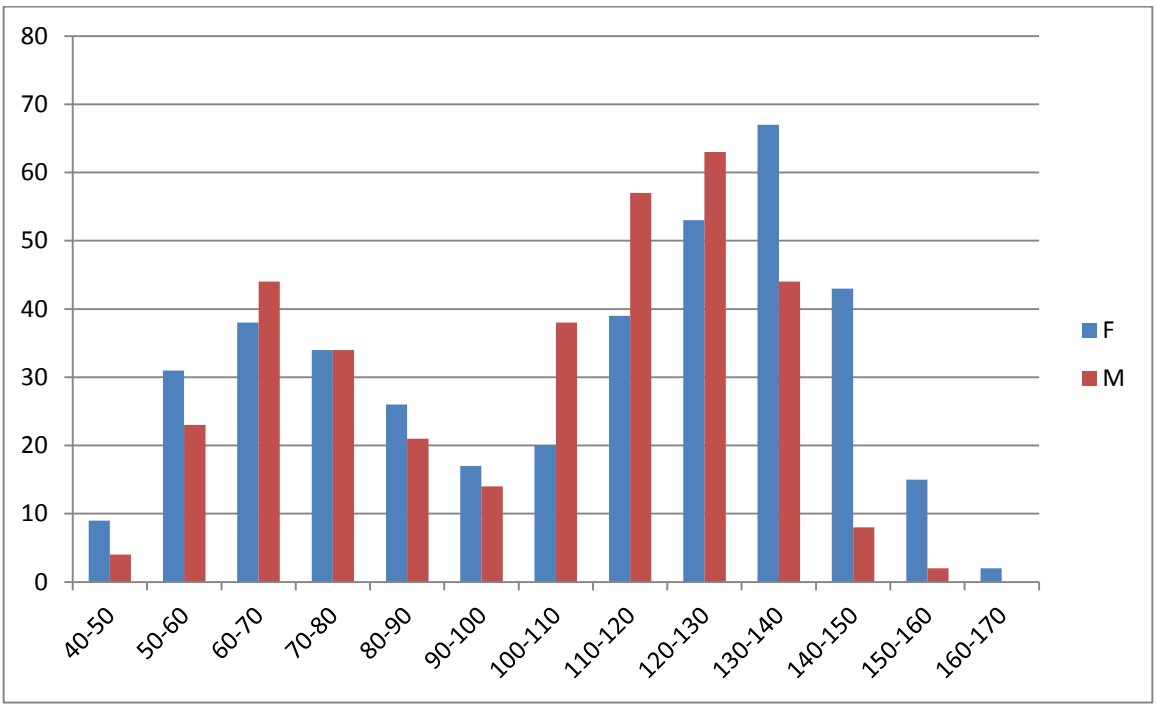


Figure 2. Length frequency of all aged blacktip sharks.

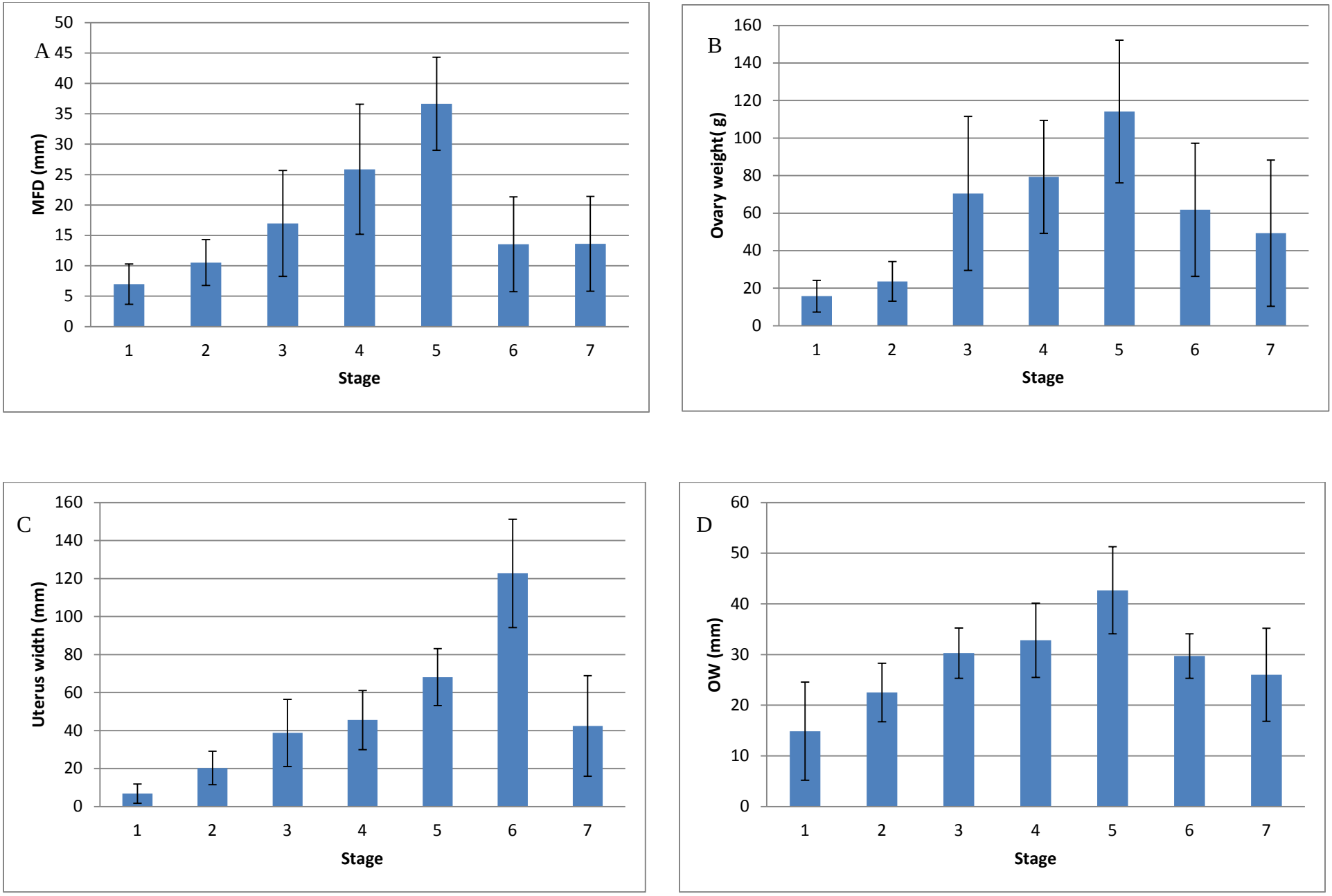
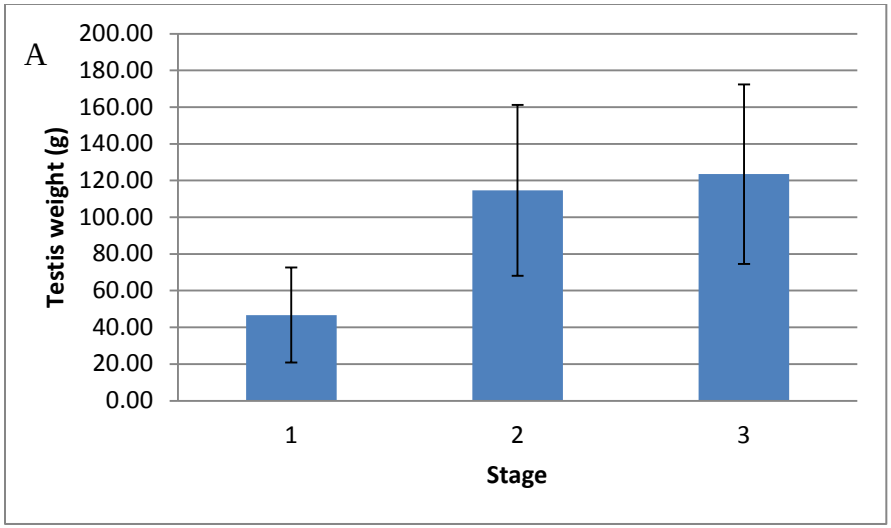
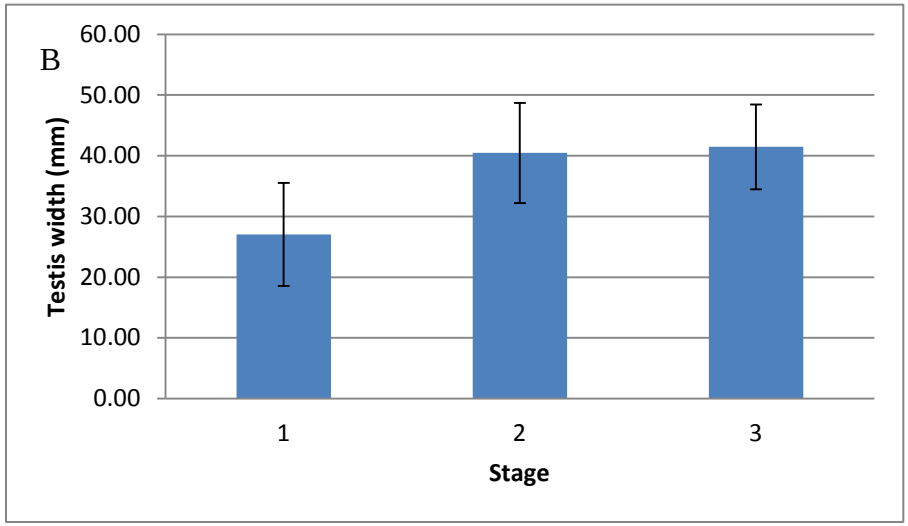


Figure 3. Average A) maximum follicle diameter (MFD), B) ovary weight, C) uterus width, and D) oviducal width (OW) by reproductive stage for female blacktip sharks in the Gulf of Mexico.





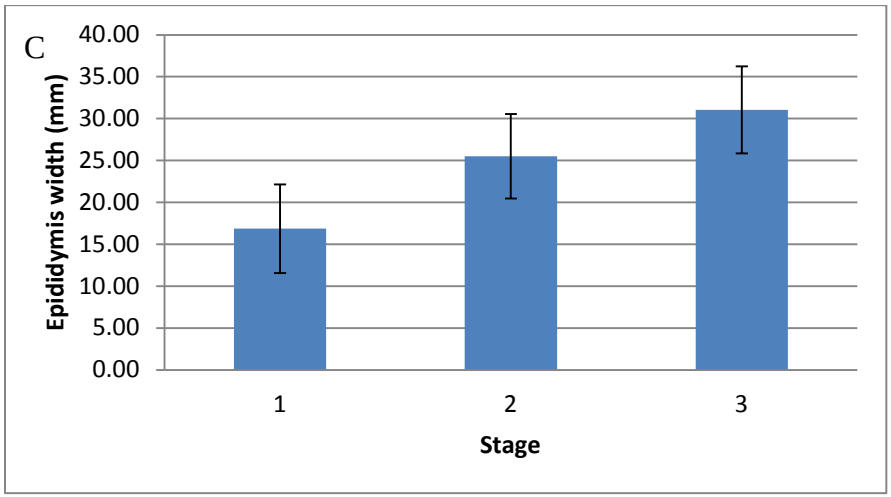


Figure 4. Average A) testis weight, B) testis width, and C) epididymis width by stage for male blacktip sharks in the Gulf of Mexico.

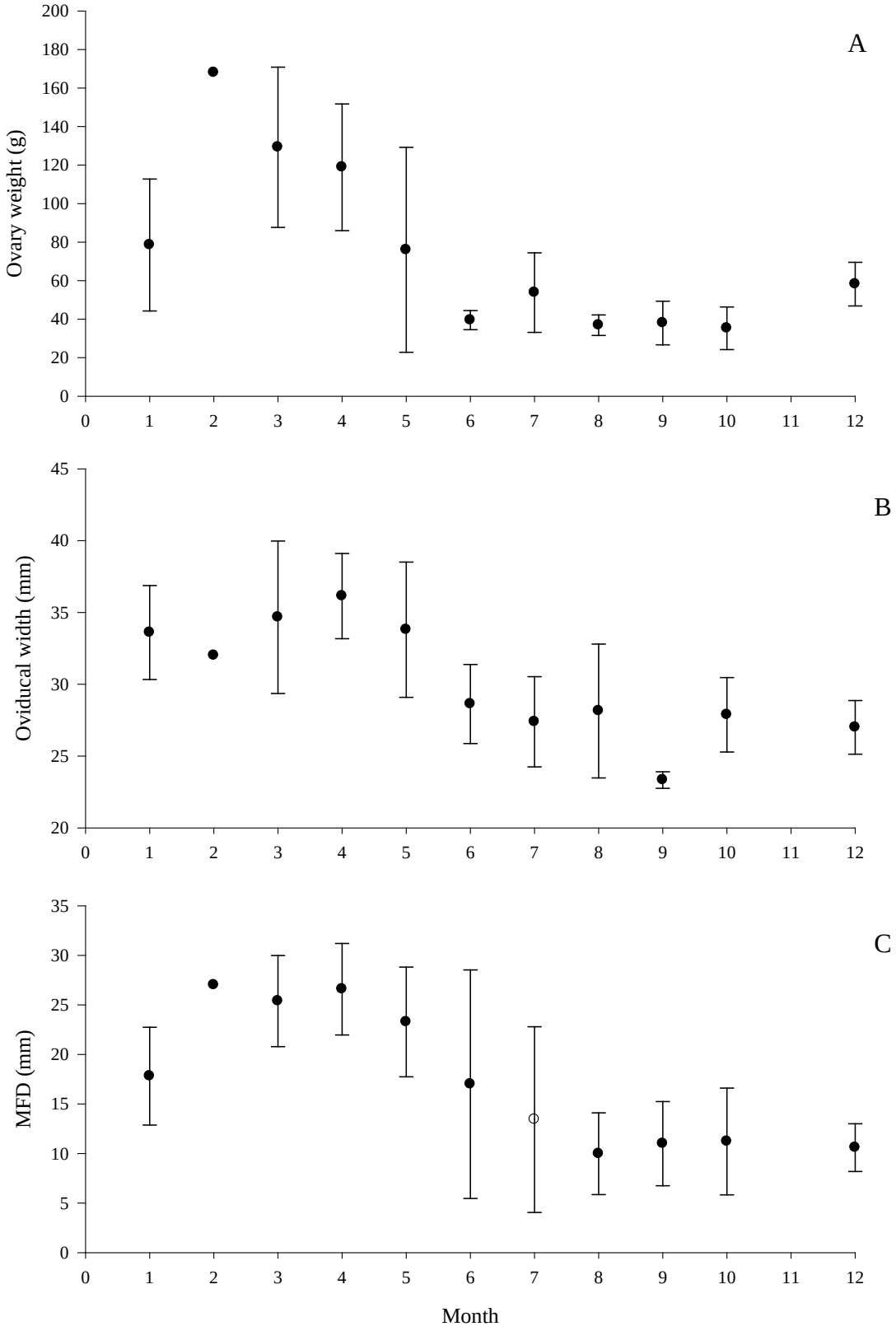


Figure 5. Average A) ovary weight, B) oviducal width, and C) maximum follicle diameter (MFD) by month for female blacktip sharks in the Gulf of Mexico. Error bars represent standard deviation.

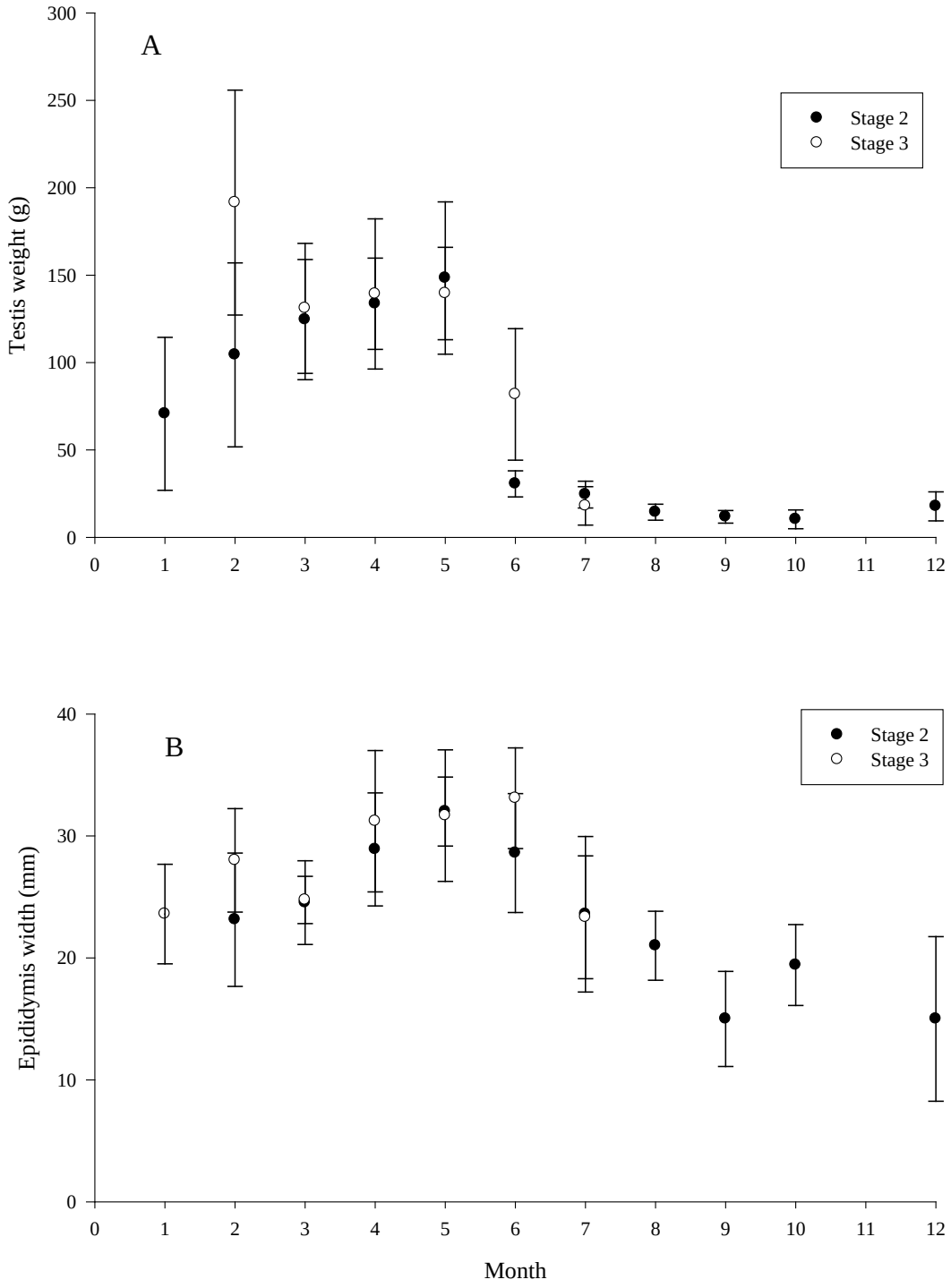


Figure 6. Average A) testis weight and B) epididymis width by month for Stage 2 and Stage 3 male blacktip sharks in the Gulf of Mexico.

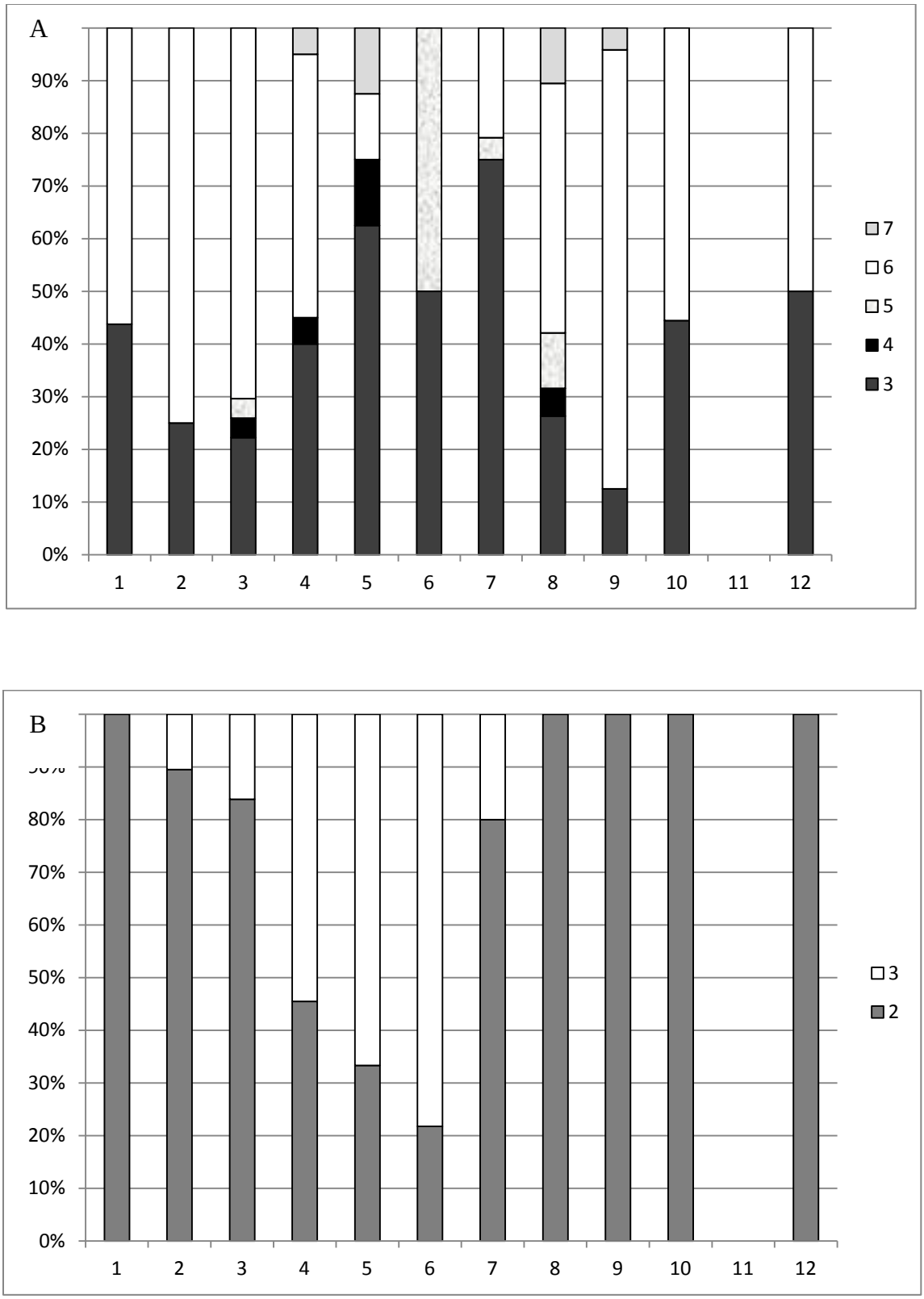


Figure. 7. Percentage of mature blacktip sharks in each reproductive stage by month for A) female and B) male blacktip sharks in the Gulf of Mexico. For plot A: Stage 3=mature, resting, Stage 4=sperm present in uterus, Stage 5=ovulating, Stage 6=pregnant, Stage 7=post-partum. For plot B: Stage 2=mature, Stage 3=mature, running ripe.

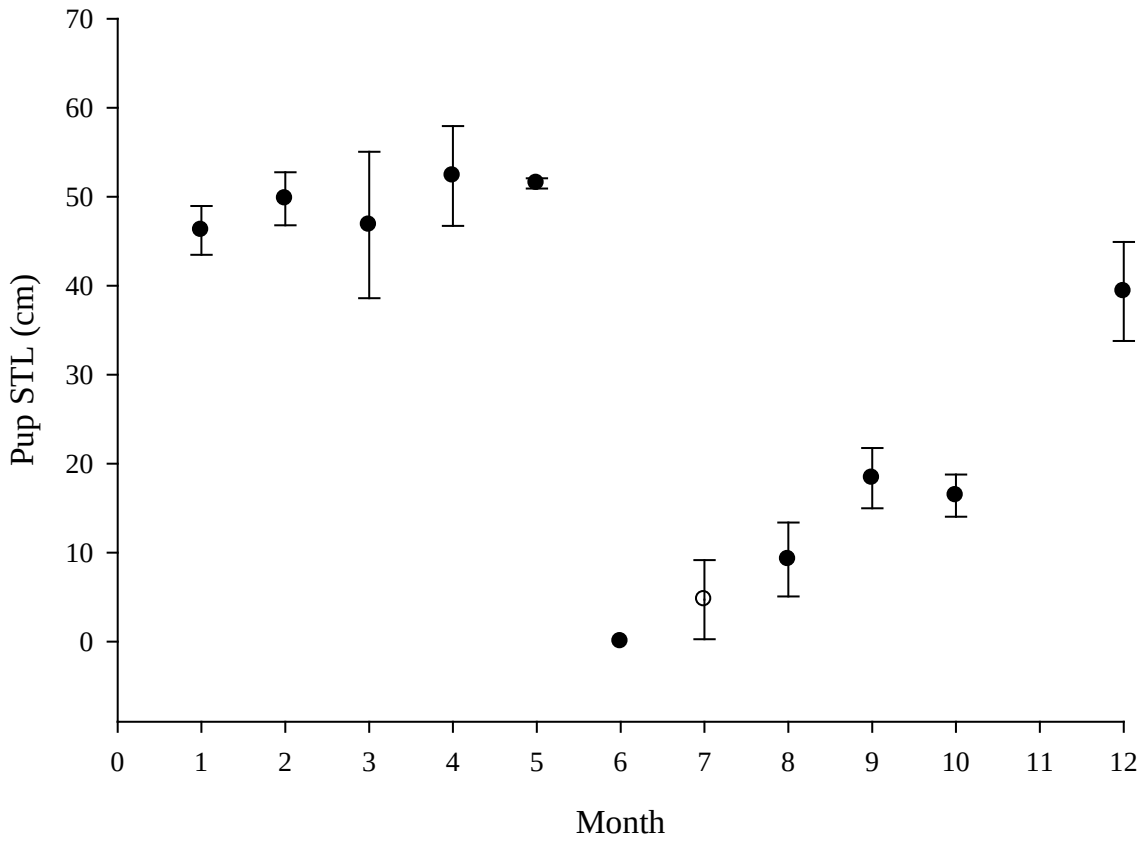


Figure 8. Average stretch total lengths (STL) of blacktip shark pups *in utero* by month. Error bars represent standard deviation.

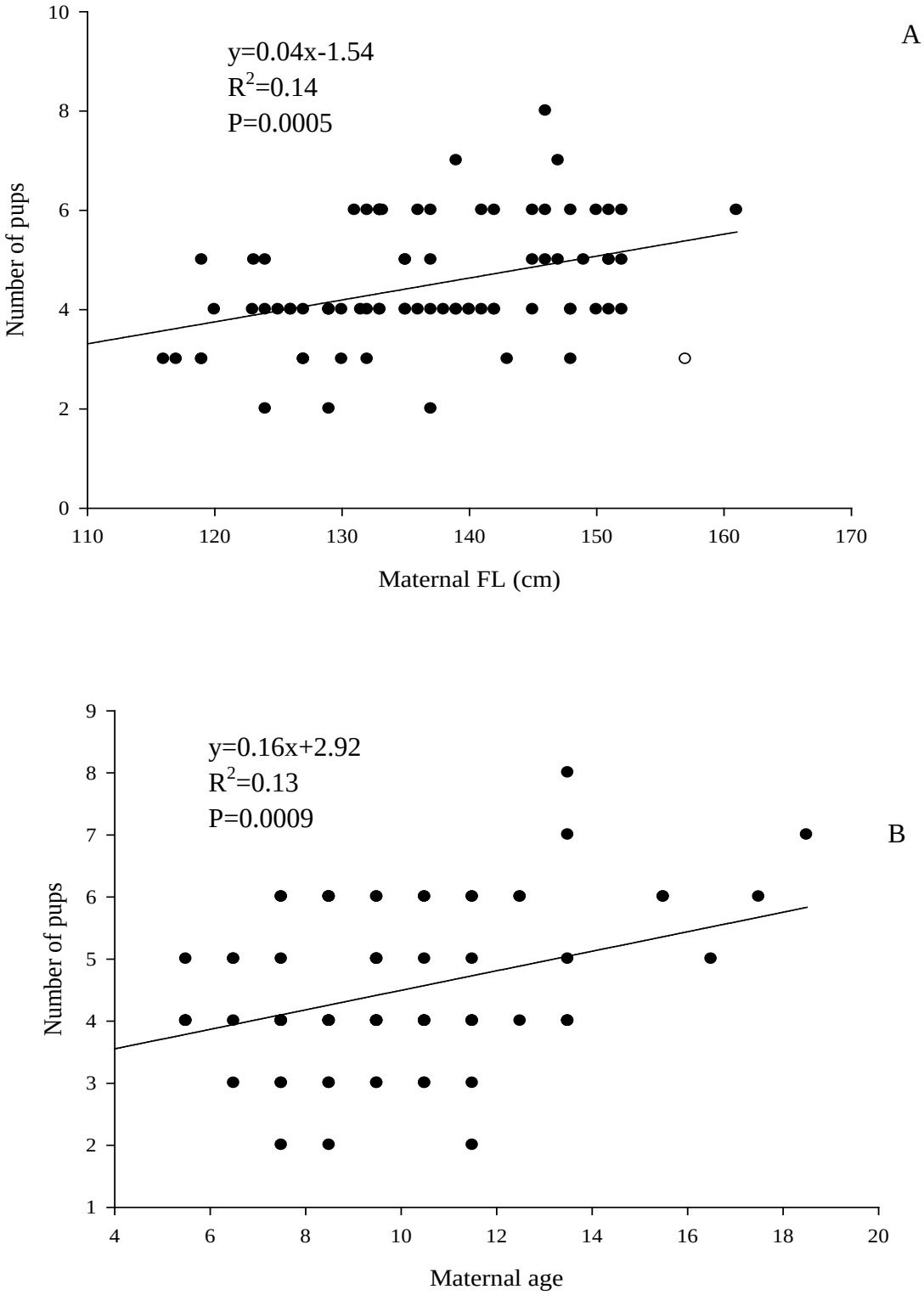


Figure 9. The relationship of the number of pups per female by maternal A) size and B) age showing a significant increase in fecundity with size and age for blacktip sharks in the Gulf of Mexico.

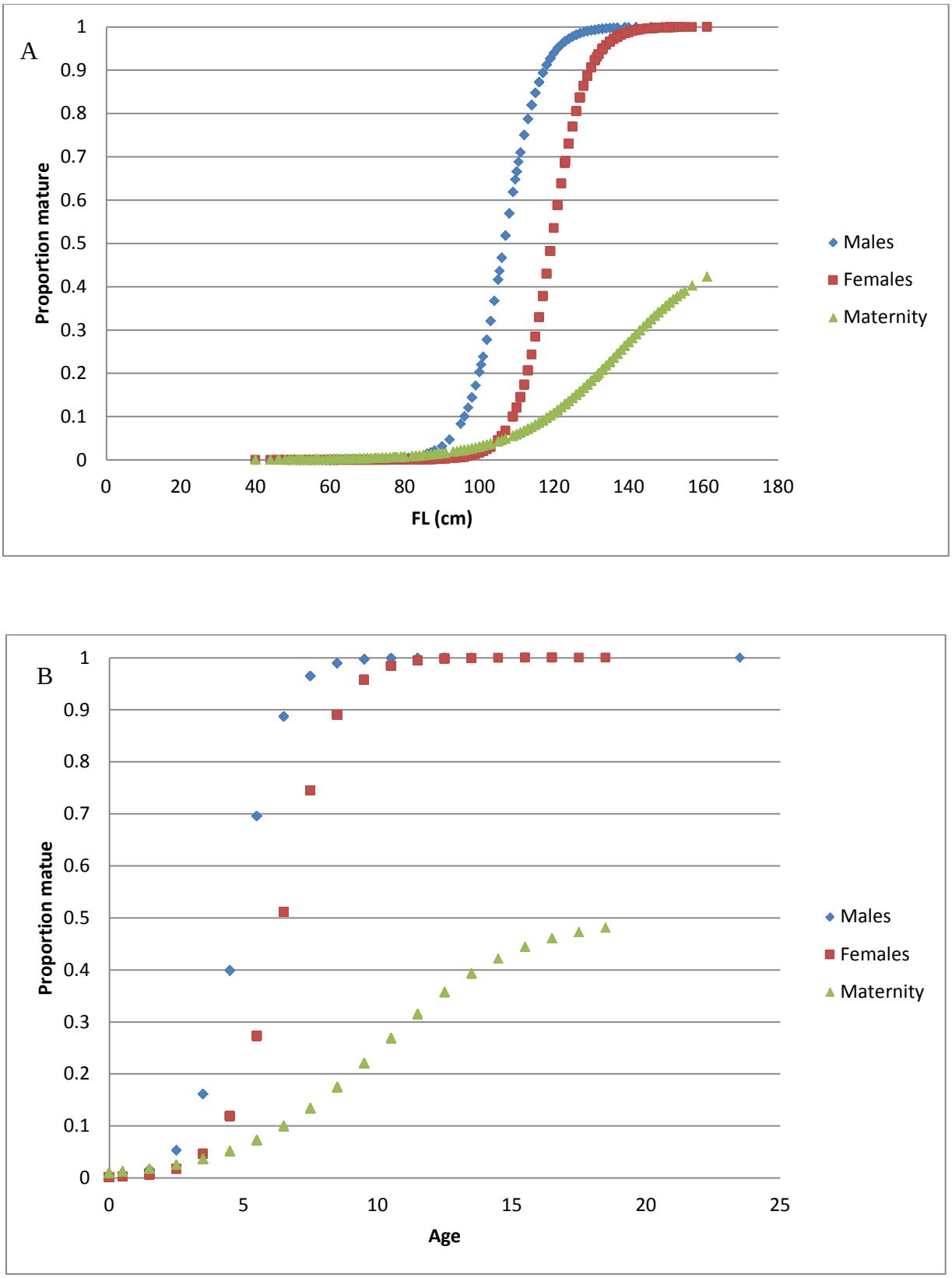


Figure 10. Maturity and maternity ogives for blacktip sharks by A) fork length (FL) and B) age for males, females, and females in maternal condition.