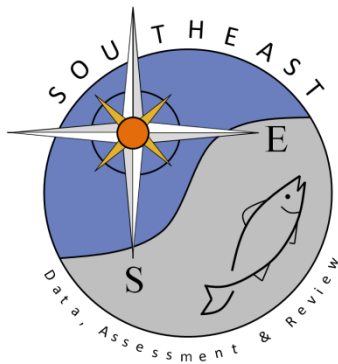


Preliminary data on the reproductive biology of the bonnethead
(*Sphyrna tiburo*) from the southeast U.S. Atlantic coast

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Preliminary data on the reproductive biology of the bonnethead (*Sphyrna tiburo*) from the southeast U.S. Atlantic coast

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Summary

Although several previous studies have examined the reproductive biology of the bonnethead (*Sphyrna tiburo*) from Gulf of Mexico (Parsons, 1993; Manire et al., 1995; Manire and Rasmussen, 1997; Lombardi-Carlson et al., 2003) no published study has provided data on their counterparts from the southeastern U.S. Atlantic coast. Because of this, the goal of this study was to provide preliminary data on the reproductive biology of bonnethead populations from the southeastern U.S. Atlantic coast, making use of archived data from the South Carolina Department of Natural Resources (SCDNR) and new data from a currently ongoing, NOAA Fisheries-supported study on bonnethead reproduction being conducted by SCDNR, Georgia Department of Natural Resources (GADNR), and the University of North Florida (UNF). Our specific objectives were to determine size and age at maturity for male and female Atlantic bonnetheads, characterize reproductive seasonality and periodicity, and determine fecundity.

Methods

Bonnetheads were collected via both fishery-dependent and fishery-independent sampling using various gear types (primarily bottom longline and gillnet) from coastal areas between Winyah Bay, SC to Cape Canaveral, FL between 2002 and 2013. Following capture, sharks were measured to pre-caudal (PCL), fork length (FL) and stretch-total length (STL), sexed, and weighed. Sharks were euthanized to obtain morphological measurements of male (clasper length and degree of calcification, testis width and length, epididymis width) and female (maximum follicle diameter, oviducal gland width, uterus width) reproductive organs. Sub-samples of these organs were also obtained from 2012-2013 animals for use in histological analyses, which focused on validating evidence for spermatogenesis in the male testis and sperm storage in the female oviducal gland. Samples obtained for histology were fixed in 10% formalin prepared in seawater or elasmobranch-modified phosphate buffered saline for 48 h, then rinsed and stored in 70% until processed using routine paraffin histology. When female sharks were determined to be recently ovulated or pregnant, the number of eggs or number, gender, and size of embryos was determined. Other observations made on study animals included the

presence of mating wounds on females and the occurrence of semen in the male reproductive tract.

Data on clasper size and calcification were used to determine size at maturity in males. Data on size and condition of the ovary, oviducal gland, and uterus were used to determine size at maturity in females. Age at maturity was determined based on these data and vertebral band estimates of age obtained in Frazier et al (2013). Logistic models were used to develop maturity ogives based on these parameters, and maturity schedules were calculated based on ogives. Monthly changes in testis size and the occurrence of semen in males, maximum follicle diameter, oviducal gland width, the occurrence of uterine contents, and the presence of mating wounds in females, and embryo size were used to assess reproductive seasonality. The number of embryos was used to determine fecundity, and the relationship between maternal size and fecundity was examined via correlation and quantified via linear regression. The percentage of pregnant females during the gestation period was used to determine reproductive periodicity.

Results

Based on clasper growth and calcification, Atlantic male bonnethead matured at slightly greater than 600 mm fork length (FL) (Fig. 1). Using vertebral band estimates of age from Frazier et al (2013), this corresponded to an age of maturity of approximately 4 years of age. Logistic regression analysis indicated that approximately 50% of males were mature at 617 mm FL (Fig 2) and 3.9 years of age (Fig. 3).

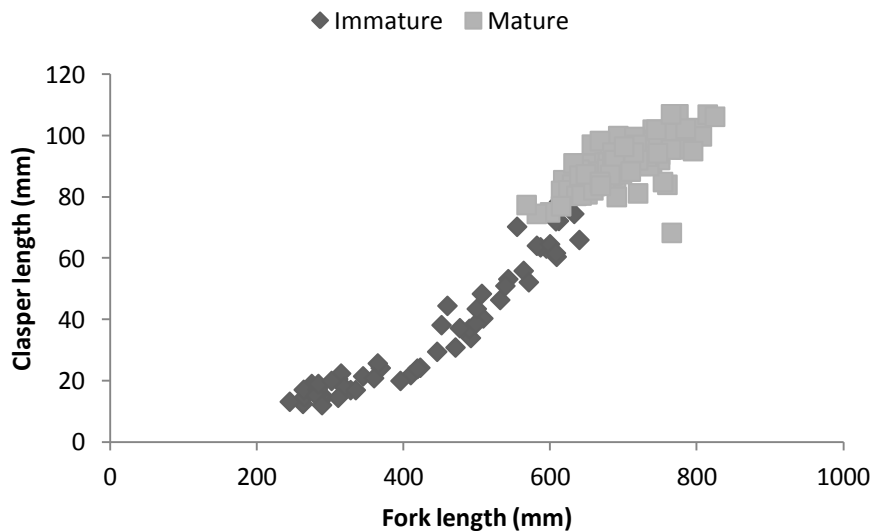


Figure 1. Fork length and clasper length of Atlantic immature and mature male bonnethead sharks (n = 156), illustrating estimated size at maturity.

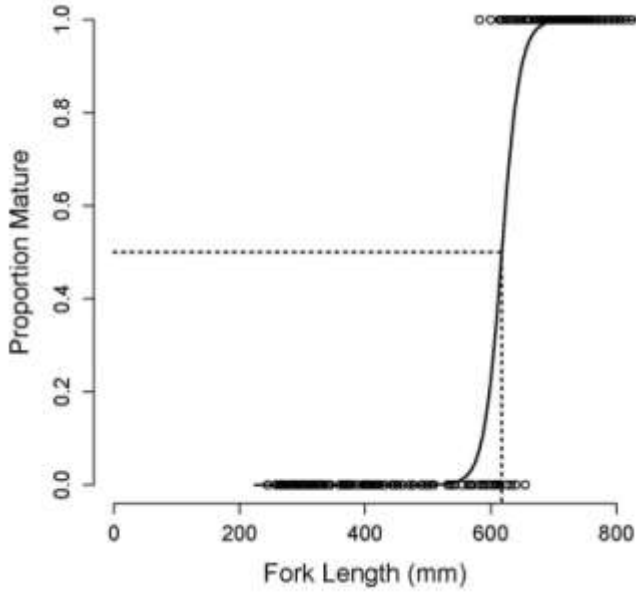


Figure 2. Proportion of male bonnetheads mature based on clasper size and calcification in relation to fork length. Dotted line indicates length at 50% maturity.

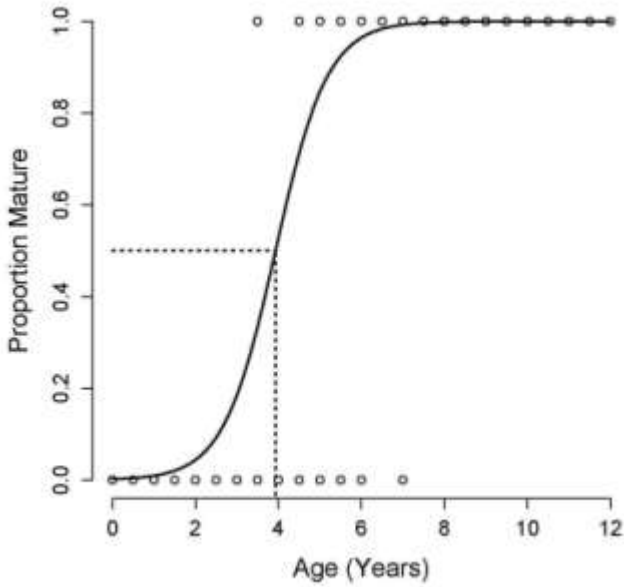


Figure 3. Proportion of male bonnetheads mature based on clasper size and calcification in relation to age. Dotted line indicates age at 50% maturity.

Based on the condition of the reproductive tract, females matured at slightly greater than 800 mm FL and at an estimated age of approximately 7 years. Logistic regression analysis indicated that approximately 50% of females were mature at 818 mm FL (Fig 4) and 6.7 years of age (Fig. 5).

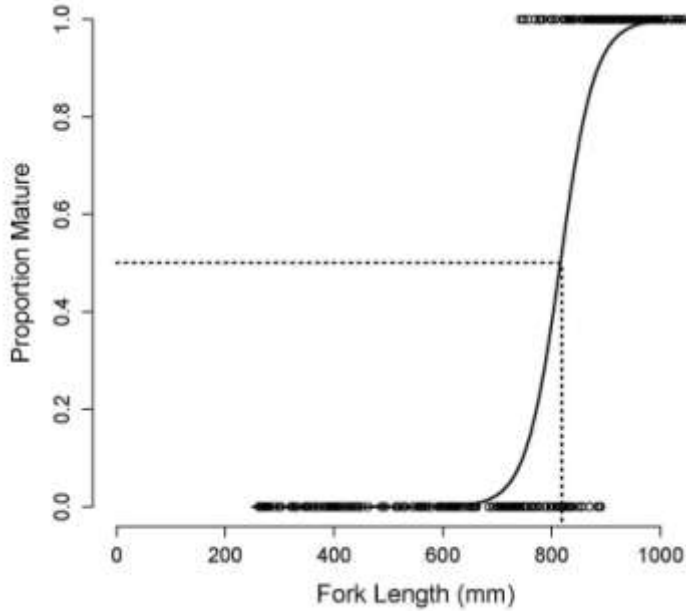


Figure 4. Proportion of female bonnetheads mature based on reproductive tract condition in relation to fork length. Dotted line indicates length at 50% maturity.

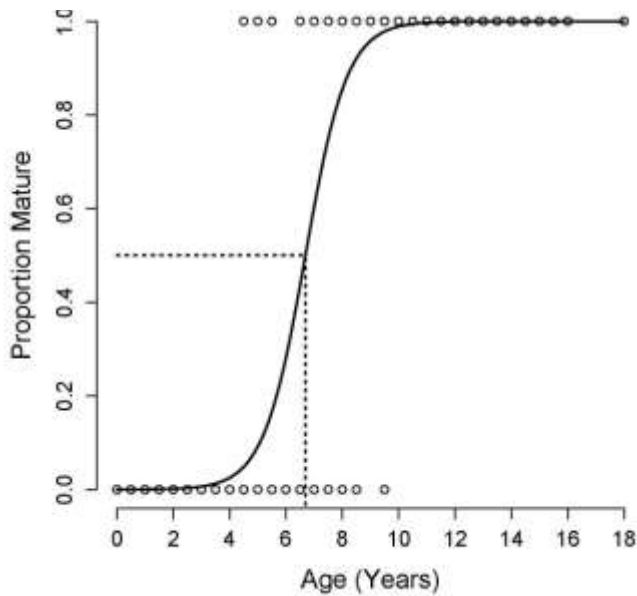


Figure 5. Proportion of female bonnetheads mature based on reproductive tract condition in relation to age. Dotted line indicates age at 50% maturity.

Maturity schedules for male and female bonnetheads from Atlantic waters are provided in Table 1. Maturity schedules for Gulf of Mexico bonnetheads based on data from Lombardi-Carlson (pers. comm.) and combined Gulf and Atlantic schedules are provided in Tables 2 and 3, respectively.

Table 1. South Atlantic Bight Maturity Schedule

Proportion Mature			Proportion Mature		
FL (mm)	Female	Male	Age (years)	Female	Male
200		0.00	0	0.00	0.00
250	0.00	0.00	0.5	0.00	0.00
300	0.00	0.00	1.5	0.00	0.02
350	0.00	0.00	2.5	0.00	0.09
400	0.00	0.00	3.5	0.01	0.33
450	0.00	0.00	4.5	0.05	0.71
500	0.00	0.00	5.5	0.18	0.92
550	0.00	0.01	6.5	0.47	0.98
600	0.00	0.22	7.5	0.78	1.00
650	0.00	0.91	8.5	0.93	1.00
700	0.02	1.00	9.5	0.98	1.00
750	0.09	1.00	10.5	1.00	1.00
800	0.35	1.00	11.5	1.00	1.00
850	0.75		12.5	1.00	1.00
900	0.94		13.5	1.00	
950	0.99		14.5	1.00	
1000	1.00		15.5	1.00	
			16.5	1.00	
			17.5	1.00	

Table 2. Gulf of Mexico maturity schedule.

Proportion Mature			Proportion Mature		
FL (mm)	Female	Male	Age (years)	Female	Male
250		0.00	0	0.00	0.00
300	0.00	0.00	0.5	0.00	0.06
350	0.00	0.00	1.5	0.02	0.38
400	0.00	0.00	2.5	0.26	0.85
450	0.00	0.00	3.5	0.81	0.98
500	0.00	0.05	4.5	0.98	1.00
550	0.00	0.29	5.5	1.00	1.00
600	0.04	0.75	6.5	1.00	
650	0.35	0.96	7.5	1.00	
700	0.87	1.00			
750	0.99	1.00			
800	1.00				
850	1.00				
900	1.00				
950	1.00				

Table 3. Combined South Atlantic Bight and Gulf of Mexico maturity schedule.

Proportion Mature			Proportion Mature		
FL (mm)	Female	Male	Age (years)	Female	Male
200		0.00	0	0.00	0.00
250	0.00	0.00	0.5	0.04	0.10
300	0.00	0.00	1.5	0.10	0.27
350	0.00	0.00	2.5	0.18	0.56
400	0.00	0.00	3.5	0.31	0.82
450	0.00	0.01	4.5	0.48	0.94
500	0.02	0.05	5.5	0.65	0.98
550	0.05	0.23	6.5	0.79	0.99
600	0.11	0.65	7.5	0.89	1.00
650	0.21	0.92	8.5	0.94	1.00
700	0.38	0.99	9.5	0.97	1.00
750	0.59	1.00	10.5	0.99	1.00
800	0.76	1.00	11.5	1.00	1.00
850	0.88		12.5	1.00	1.00
900	0.94		13.5	1.00	
950	0.99		14.5	1.00	
1000	1.00		15.5	1.00	
			16.5	1.00	
			17.5	1.00	

Based on morphological and histological observations on mature males and females (Figure 6), Atlantic bonnetheads were shown to exhibit a seasonal reproductive cycle. Males were shown to undergo spermatogenesis from June to September and mate with females in late September, based on testis morphology and histology and the presence of fresh mating wounds on females. Females were shown to store spermatozoa in the oviducal gland from September to late April/early May based on morphology and histological structure of this organ, and undergo vitellogenesis between January and late April/Early May based on follicle growth. Ovulation occurred between late April-early May based on the presence of ova in both the ovary and uteri, and females were gravid between early May to mid-September. Parturition occurred before late September. Virtually all females (98%, n = 129) examined between early May to mid-September were pregnant, suggesting that reproduction is annual with only occasional exceptions (e.g., at least one of these animals was shown to have uterine obstructions).

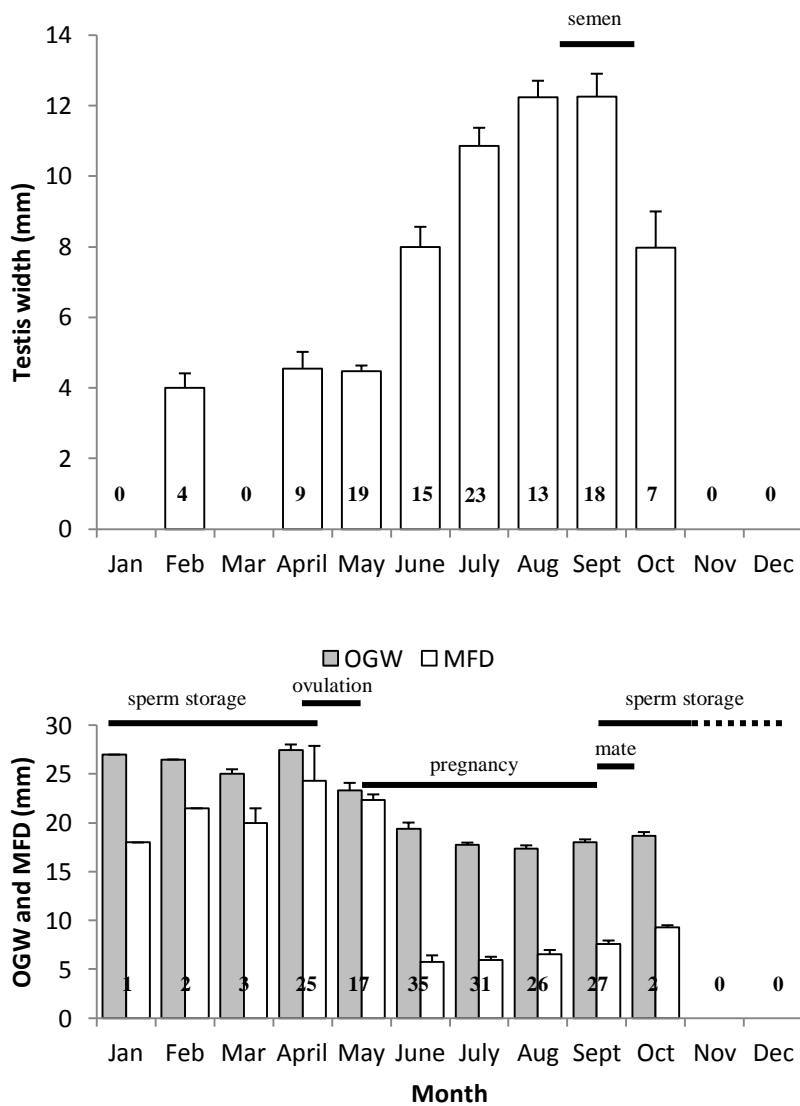


Figure 6. a) Testis width in male and b) oviducal gland width (OGW) and maximum follicle diameter (MFD) in female *S. tiburo*. Values are means \pm SE. n are shown in bars. The periods during which semen was observed in males, presence of mating scars, when spermatozoa was observed in histological sections of oviducal gland, ovulation, and when females were pregnant are shown (sperm storage is presumed in Nov/Dec due to lack of samples during these months).

Litter size ranged from 2 to 14 in pregnant females (mean litter size \pm SD = 8.8 ± 2.39 , n = 83 females), and was significantly correlated with female size (Fig. 7). Sex ratio of embryos was 0.51 (n = 517 embryos), suggesting a 1:1 ratio of female to male offspring. Although mature females ovulated between late April and mid-May, visible signs of embryonic development were not observed until early June when embryos as small as 9 mm were seen. Following this period, embryonic development was rapid (Fig. 8). Embryos were yolk-dependent until mid-late July to early August, when placenta-dependent embryos were observed.

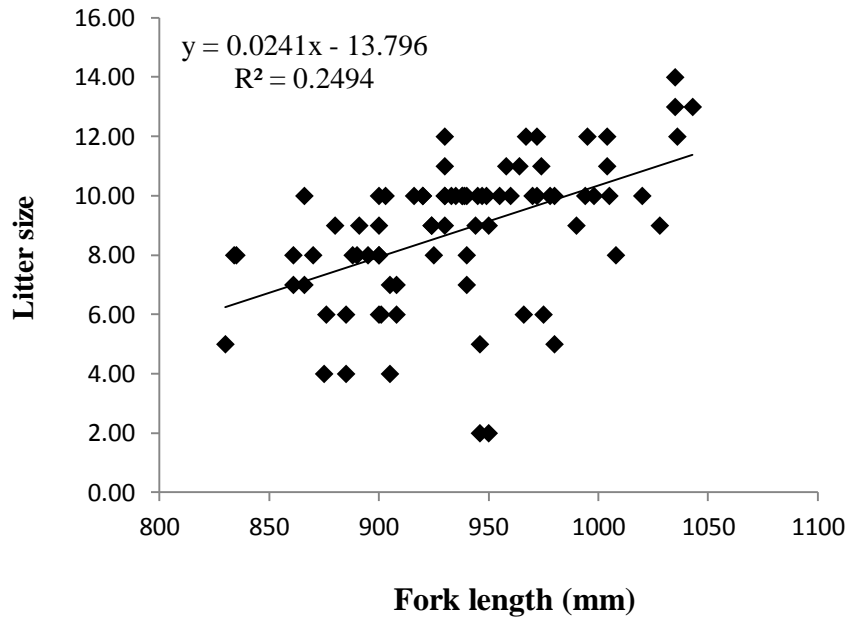


Figure 7. Relationship between maternal fork length (FL) (mm) and litter size in Atlantic bonnethead. Litter size and maternal FL were significantly correlated (Pearson $r = 0.499$, $p < 0.0001$, $n = 83$).

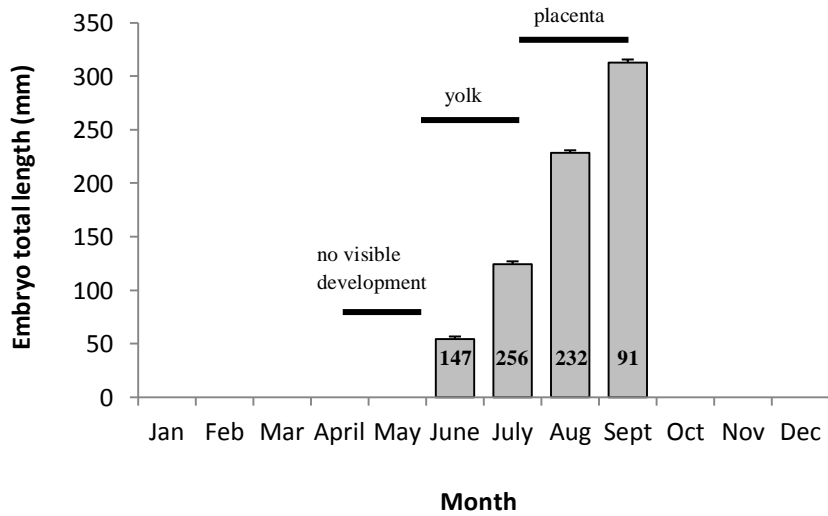


Figure 8. Total length of embryos from pregnant female *S. tiburo*. Values are means \pm SE. n are shown in bars. The post-ovulatory period during which there were no visible signs of embryonic development, and the periods during which yolk or placenta were the primary supplier of embryonic nourishment are indicated.

Discussion

The preliminary results from this study suggest that Atlantic bonnetheads mature at a larger size and older age than their counterparts from the Gulf of Mexico. Previous data suggest that male and female bonnetheads from the Gulf of Mexico mature at 572.4 ± 10.5 and 662.6 ± 10.3 mm FL, respectively, sizes corresponding to 1.7 ± 0.2 and 2.9 ± 0.2 years of age (Lombardi-Carlson, pers. comm.). In contrast, Atlantic male and female bonnetheads mature at 617.8 ± 12.1 and 818.5 ± 14.4 mm FL, respectively, at ages of 3.9 ± 0.3 and 6.7 ± 0.3 . These data, based on logistic analysis of maturity indicators, are presented in Tables 4 and 5 for convenience. Although these differences were considerable (i.e., they indicate that age-at-maturity for Atlantic bonnetheads occurs at over twice the age of their Gulf counterparts), it is important to note that Frazier et al (2013) also reported that longevity of Atlantic bonnetheads is over twice that reported for Gulf populations. Therefore, the overall reproduction contributions of females in Atlantic and Gulf populations are likely comparable.

Table 4. Logistic analysis of size at maturity for Atlantic and Gulf (and combined) populations of *S. tiburo*.

Region	Sex	<i>a</i>	SE (<i>a</i>)	<i>b</i>	SE (<i>b</i>)	n	FL (mm)
South Atlantic Bight	F	-27.89 ± 7.34	3.75	0.034 ± 0.008	0.004	329	818.5 ± 14.4
	M	-43.25 ± 27.69	10.09	0.070 ± 0.045	0.016	216	617.8 ± 12.1
Gulf of Mexico	F	-33.51 ± 13.37	5.25	0.051 ± 0.020	0.008	282	662.6 ± 10.3
	M	-23.19 ± 6.07	3.11	0.041 ± 0.011	0.005	256	572.4 ± 10.5
Combined	F	-12.01 ± 1.49	0.96	0.016 ± 0.002	0.001	611	729.0 ± 16.2
	M	-21.50 ± 3.56	2.20	0.037 ± 0.006	0.004	472	583.1 ± 9.4

Table 1. Logistic analysis of age at maturity for Atlantic and Gulf (and combined) populations of *S. tiburo*.

Region	Sex	<i>a</i>	SE (<i>a</i>)	<i>b</i>	SE (<i>b</i>)	n	Age (Years)
South Atlantic Bight	F	-9.07 ± 2.60	1.22	1.357 ± 0.383	0.179	329	6.7 ± 0.3
	M	-6.27 ± 2.32	1.09	1.595 ± 0.580	0.254	216	3.9 ± 0.3
Gulf of Mexico	F	-7.36 ± 2.29	1.05	2.521 ± 0.762	0.351	248	2.9 ± 0.2
	M	-3.90 ± 0.82	0.50	2.261 ± 0.512	0.275	239	1.7 ± 0.2
Combined	F	-3.32 ± 0.43	0.28	0.716 ± 0.201	0.593	577	4.6 ± 0.4
	M	-2.84 ± 1.07	0.28	1.242 ± 0.266	0.117	455	2.3 ± 0.2

Preliminary data from this study suggest that Atlantic bonnethead sharks exhibit reproductive traits similar to those previously reported for Gulf of Mexico populations with only subtle differences. Both groups undergo a seasonally-timed, annual reproductive cycle, but the timing of certain events differs such as mating, which was observed to occur earlier in Atlantic versus Gulf populations (i.e., September in Atlantic versus November-December in Gulf populations), and ovulation, which occurred later in the Atlantic population compared with Gulf *S. tiburo* (i.e., Mid-April to early May in Atlantic *S. tiburo* versus mid-March to early April in Gulf females). While these differences are not expected to impact the overall reproductive contributions of Atlantic versus Gulf females, it is intriguing to consider if the additional 1.5-3

months of sperm storage has implications for reproduction. For example, we are currently investigating if the shorter length of the sperm storage period (and presumably, greater survival of good quality spermatozoa) in southern Gulf populations of *S. tiburo* explain the lower rate of infertility previously observed in these animals compared to more northerly populations (Parsons, 1993). However, it is important to note that differences in fertility rate in these populations are not necessarily expected to influence population growth rate, i.e., other demographic traits such as growth rate, size/age at maturity, litter size, and longevity have been shown to affect population growth rates in a manner in which even a ten-fold difference in infertility rate does not result in a significant difference in population increase (Manire, unpublished data).

Litter size was slightly lower in Atlantic (mean \pm SD = 8.8 ± 2.39) versus Gulf (10 ± 3) *S. tiburo*. This was unexpected because mature females grow to a larger maximum size in the Atlantic versus the Gulf and size and litter size were positively correlated.

In summary, our preliminary results suggest the following:

- Atlantic bonnethead sharks mature at a larger size and more advanced age than their Gulf counterparts.
- Following sexual maturity, Atlantic bonnethead sharks reproduce following the same seasonally-occurring reproductive cycle that their Gulf counterparts exhibit, but mate earlier and undergo a slightly more protracted sperm storage period.
- Like their Gulf counterparts, Atlantic female bonnetheads reproduce annually.
- Despite their larger size and evidence for a positive relationship between maternal size and fecundity, litter size of Atlantic female *S. tiburo* is slightly lower than that of Gulf females.

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