Variability in the Reproductive Biology of the Atlantic Sharpnose Shark in the Gulf of Mexico

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ABSTRACT

The reproductive biology of the Atlantic sharpnose shark *Rhizoprionodon terraenovae* in the Gulf of Mexico was investigated by examining 1,306 specimens (693 females, 613 males) collected from the Florida Keys to waters off Brownsville, Texas USA. The results of this study confirm the annual reproductive cycle established for this species; however, there was a significant amount of variability present within the cycle. Ovulatory and post-ovulatory females were present from March to October, indicating that mating and ovulation (e.g. May to July) were occurring over a more protracted period than previously described. The occurrence of post-partum females from April to September, the varying sizes of the embryos across several months, and the occurrence of mature spermatozoa in the testes of adults from March to November also corroborates the evidence of reproductive plasticity in this species. This observed variability in the reproductive cycle indicates that the Gulf of Mexico Atlantic sharpnose shark population is not completely synchronous in regards to parturition, mating, and ovulation, as a portion of the population is demonstrating reproductive asynchrony. Although the cause of this asynchrony remains unclear, it may be related to the environmental conditions of the Gulf of Mexico, which could provide water temperatures optimal for reproduction of this species through much of the year (e.g. March to October), resulting in a protracted reproductive cycle. Given the results of the current study, reproductive cycles in other carcharhinid species in this region should be examined in more detail to determine if asynchrony is also present, as this phenomenon could impact future management strategies.

Keywords: asynchrony, Carcharhiniformes, protacted mating, reproductive plasticity

INTRODUCTION

Important intraspecific differences in the reproductive biology of some carcharhinid shark species in the western North Atlantic Ocean have been noted (Loefer and Sedberry, 2003; Driggers et al., 2004; Sulikowski et al. 2007; Driggers and Hoffmayer 2009). For example, Driggers et al. (2004) determined that blacknose sharks, *Carcharhinus acronotus*, reproduce biennially in the Atlantic, whereas Sulikowski et al. (2007) found the reproductive periodicity of this species to be annual in the Gulf of Mexico. Driggers and Hoffmayer (2009) provided the first evidence that plasticity in elasmobranch reproductive cycles can exist within a discrete region, as the typically biennially reproductive finetooth sharks, *C. isodon*, in the Gulf of Mexico were found to also exhibit an annual reproductive cycle. In addition, Loefer and Sedberry (2003) compared their data to those of Branstetter (1987) and Parsons (1983) and reported that female Atlantic sharpnose sharks, *Rhizoprionodon terraenovae*, mature at a smaller size and higher age in the Atlantic than in the Gulf of Mexico. Although studies examining the reproductive biology of sharks in the western North Atlantic Ocean are limited, the fact that differences in important reproductive characteristics have been documented for several carcharhinid species suggests that this phenomenon could be more widespread among sharks, especially tropical species (Mattos et al. 2001; Castro 2009), than currently recognized.

The Atlantic sharpnose shark occurs in the coastal waters of the western North Atlantic Ocean from Canada to Mexico (Compagno 1984), and is the most abundant shark species throughout most of its range, including the Gulf of Mexico (Branstetter 1990). Its close proximity to shore and high abundance have made this shark an ideal subject for many ecological and biological studies (e.g. Parsons and Hoffmayer 2005; Hoffmayer et al. 2006; Hoffmayer et al. 2010). Similar to previously documented intraspecific reproductive differences,

several discrepancies in life history parameters have been identified for specimens collected from the same geographic area. For example, Parsons (1983) found that male gonadosomatic index (GSI) for Gulf of Mexico caught Atlantic sharpnose sharks peaked from June to August, while Hoffmayer et al. (2010) reported the male GSI peaked from March to May, suggesting either a temporal shift in the reproductive cycle or a protracted mating season. In addition, Carlson and Baremore (2003) reported that Atlantic sharpnose sharks sampled in the Gulf of Mexico from 1998 to 2001 were maturing at a smaller size and younger age than they were twenty years prior (1979–1980; Parsons 1983).

In addition to the discrepancies identified by Carlson and Baremore (2003) and Hoffmayer et al. (2010), several recent observations of females mating and ovulating outside the known mating season for Atlantic sharpnose sharks in the Gulf of Mexico (Hoffmayer unpublished data) suggest that this species could be exhibiting reproductive plasticity. Understanding the reproductive biology of elasmobranchs is required for successful management, as several reproductive parameters are required for current stock assessment models and changes in these biological parameters could significantly alter the outcome of these assessments (Walker 2005). The objective of the current study was to examine the reproductive biology of the Atlantic sharpnose shark over a large spatial scale in the Gulf of Mexico, develop updated reproductive parameter estimates for stock assessment models, and describe their reproductive cyclicity.

MATERIALS AND METHODS

Sample collection

Atlantic sharpnose sharks were collected in the Gulf of Mexico, from the Florida Keys to the waters off Brownsville, Texas (Figure 1), between March 2008 and February 2012, during fishery independent research surveys or commercial fishing operations. The majority of the specimens were provided by the National Marine Fisheries Service's (NMFS) Supplemental Congressional Appropriation for Expanded Stock Assessment FY2011 (48.6%), followed by the University of Southern Mississippi's Gulf Coast Research Laboratory shark surveys (34.4%), NMFS bottom longline and bottom trawl surveys (10.5%), and from commercial fishers (6.5%) (Table 1). Few reproductive samples were obtained during winter (December, January, and February) as none of the fishery independent surveys were conducted during this time and severe weather conditions and management closures prevented sample collection by commercial fishers.

For all retained specimens sex was determined, and the pre-caudal length (PCL, from the tip of the snout to the anterior margin of the pre-caudal pit), fork length (FL, from the tip of the snout to the posterior notch of the caudal fin), total length (TL, from the tip of the snout to the posterior tip of the caudal fin while in its natural position), and stretch total length (STL, from the tip of the snout to the posterior tip of the fully extended caudal fin) were measured to the nearest millimeter, and a weight was recorded to the nearest 0.1 kg. All measurements were taken on a straight line along the axis of the body. Specimens were then frozen whole or stored on ice (up to 24 hrs) prior to further processing.

Males

Maturity in males was determined by the presence of calcified claspers that rotated 180° relative to normal position and had a freely opening rhipidion (e.g. Clark and von Schmidt 1965). Clasper length was measured from the cloacal apex to the tip of the apopyle. To conduct gross

examinations of internal reproductive tissues, an incision was made from the cloacal origin to the pectoral girdle. Once exposed, the right testis was excised from the epigonal organ and the length, width, and weight were measured. A 2–3 mm thick cross section was removed from the medial section of the right testis, placed in a tissue cassette and fixed in 10% buffered formalin. The sample was dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin following the protocol of Sulikowski et al. (2004, 2005). Prepared slides were examined to assess spermatogenic development based on criteria outlined by Maruska et al. (1996). Specifically, the mean proportion of the testes that were occupied by mature spermatocysts along a straight line distance across the medial section of the right testis was determined. Histologically, mature spermatocysts were identified by the organization of spermatozoa into tightly shaped packets that were arranged spirally along the periphery of the spermatocysts. Once exposed, the condition of the epididymides, ductus deferentes and seminal vesicles was noted as turgid or regressed. In addition, the seminal vesicles were inspected for the presence of seminal fluid.

Females

Females were considered sexually mature if gravid or if they possessed developed oviducal glands, uteri, and vitellogenic follicles. An incision was made from the cloacal origin to the pectoral girdle to expose the reproductive organs. Widths of the right oviducal gland and right uterus (only in non-gravid females) were measured. The left ovary, the only functional ovary, was excised, weighed, and the diameters of all exposed follicles were measured to the nearest millimeter. The stage of each exposed follicle was classified as undeveloped, developing, vitellogenic, or atretic. The uteri were dissected to determine if embryos or fertilized oocytes were present. Embryos were counted and the mass, length (stretch total), and

sex were recorded for each. Mature females were further divided into five reproductive stages including nulliparous, ovulatory, post ovulatory, gravid, and post-partum. Nulliparous females included non-gravid individuals that were close to the size of maturity. Ovulatory females included individuals with fertilized uterine oocytes and large (> 20 mm) vitellogenic follicles. Post ovulatory females were characterized by possessing fertilized uterine oocytes and small (< 10 mm) non-vitellogenic follicles. Gravid females possessed macroscopically visible embryos (> 4.0 mm), while post-partum females had empty uteri with stretched, vascularized walls (width > 15 mm) and distinct placental scarring.

Statistical analysis

A variety of analyses were conducted to gain a better understanding of the reproductive biology of this species. Gonadosomatic indices (GSI) were calculated to estimate the timing of vitellogenesis and ovulation in females and spermatogenesis in males. The GSI for each shark was calculated using the following equation: GSI = 100 x [gonad mass / (mass of animal – gonad mass)]. Linear regression relationships of PCL, TL and STL on FL were derived to facilitate comparison with other studies. To determine size at which 50% of the population was mature, a logistic model, $Y=(1+e^{-(a+bx)})^{-1}$, was fitted to binomial maturity data using a least squares non-linear regression. Median fork length at maturity was determined as $-ab^{-1}$ (Mollet et al. 2000). A one-way analysis of variance (ANOVA) followed by a Tukey's post hoc test (Zar, 1999) was used to determine if there were significant differences in reproductive variables (i.e. testes length, testes width, male and female GSI, maximum follicle diameter, and embryo size) by month. If the assumptions of normality or equal variances were not met, the data were transformed. If the assumptions were still violated, then the non-parametric Kruskal-Wallis ANOVA followed by a Tukey's post-hoc test was performed (Zar 1999). Regional and inter-

annual variability was investigated as potential factors influencing the protracted mating period observed in this study. The Gulf of Mexico was divided into three regions: east (83 to 88°W), central (88 to 92°W), and west (92 to 97°W), and monthly occurrence of ovulatory and post-ovulatory females were compared across regions. In addition, since the largest number of samples was collected during 2009 and 2011, monthly occurrence of ovulatory and post-ovulatory females were compared across these two years. The relationship between maternal fork length and brood size was compared using a linear regression analysis. Numbers of developing embryos occurring in the left and right uteri were compared with a Mann–Whitney *U*-test, given that the samples were not normally distributed. The sex ratio of the embryos was calculated and compared using a Chi-square test with Yates correction. The results are presented as a mean ± SE. All statistical tests were completed using SigmaStat 3.5 and considered significant at α=0.05.

RESULTS

A total of 1,306 Atlantic sharpnose sharks were collected during this study, ranging from 316 to 935 mm FL and 0.2 to 7.5 kg (Figure 2). Relationships among the three length measures and TL vs Wgt are reported in Table 2.

Males

A total of 613 male (143 immature, 470 mature) Atlantic sharpnose sharks (316 to 875 mm FL; 0.22 to 6.8 kg) were sampled for reproductive analyses (Figure 2). Mature males were collected during each month of the study except for December, January, and February. Clasper length exhibited a sigmoidal relationship with FL and was best described by the following equation: CL = exp(6.28204 - 127.77/FL) (Figure 3). Claspers grew gradually in sharks < 550

mm FL, followed by a rapid growth until 650 mm FL, which is the onset of maturity. Mean clasper length was 12.7 ± 0.1 % of FL once maturity was reached (n = 470), and claspers were fully calcified, able to rotate, and the rhipidions were fully functional. The length at 50% maturity for male Atlantic sharpnose sharks was 629 mm FL (a= -104.559, b = 0.166, r^2= 0.81; Figure 4). The smallest fully mature male was 595 mm FL, and the largest immature male examined was 663 mm FL.

Testicular cycle

Monthly mean male GSI exhibited a prominent peak (April) during the reproductive cycle (Figure 5a), and was significantly higher (H_8 = 241, p < 0.001) during spring (March-May, 0.3–0.4%) as compared to summer and fall (June-November, 0.15-0.2%). Testis length did not significantly change over the annual cycle (F_{448} = 0.99, p = 0.441; Figure 5b); however, testis width followed a similar trend to GSI with significantly higher values being observed during spring (13–16 mm) as compared to summer and fall (11–12 mm; H_8 = 114.1, p < 0.001; Figure 5b). Histological analysis revealed that mature spermatozoa were present in male Atlantic sharpnose shark testes from March to November (Figure 6a). Based on GSI, histology, and testis width data, March through May is the peak time for spermatogenesis. Epididymides, ductus deferentes, and seminal vesicles remained turgid and full of seminal fluid after testicular regression began (Figure 6b). In addition, seminal fluid was present in 99% of the mature males examined from March to November.

Females

A total of 693 female (114 immature, 580 mature) Atlantic sharpnose sharks (384 to 935 mm FL; 0.25 to 7.2 kg) were sampled for reproductive analyses (Figure 2). Mature females were collected during each month of the study except for December and January. The length at 50%

maturity for female sharks was 632 mm FL (a= -156.274, b = 0.247, r^2= 0.71; Figure 4). At approximately 550 mm FL, the oviducal gland began to rapidly increase in size (Figure 7) from a mean width of 8.6 ± 0.3 mm to 15.4 ± 0.1 mm for the newly mature females. The smallest mature female was 581 mm FL, and the largest immature female was 665 mm FL.

Ovarian cycle

Monthly mean GSI for mature females changed significantly throughout the reproductive cycle (ANOVA: $F_{9,566} = 32.8$, $p < 0.001$) with two significant peaks observed; a primary peak occurring in May and a secondary peak occurring in September (Figure 8a). However, a scatter plot of GSI by month revealed a considerable amount of variability from April to October, with the largest variability occurring during June (0.07–1.0%; Figure 8b). Gonadosomatic index values were variable and ranged from 0.03 to 0.73% for gravid, 0.02 to 0.61% for post ovulatory, 0.10 to 0.82% for ovulatory, and 0.17 to 1.0% for post-partum females (Figure 8b). Maximum follicle diameter ranged from 1.8–30.8 mm, and ovulation occurred when follicles were between 25 and 30 mm. Similar to GSI, monthly maximum follicle diameter changed significantly throughout the reproductive cycle (ANOVA: $F_{9,571} = 16.1$, $p < 0.001$) with peaks occurring in May and September (Figure 9a). A scatter plot of maximum follicle diameter by month revealed a large amount of variability from March to October with diameters ranging from 1.6 to 25 mm monthly during this time (Figure 9b).

Of the 580 mature females examined, 19 (3.3%) were nulliparous, 56 (9.7%) were ovulatory, 110 (19.0%) were post-ovulatory, 368 (63.4%) were gravid, and 27 (4.7%) were post-partum. Gravid females were encountered during each month and were numerically dominant, except in June (Figure 10). Almost half (44%) of the post-partum females were encountered outside the previously documented time of parturition for this species (Parsons 1983; Loefer and

Sedberry 2003; Figure 10). Ovulatory and post-ovulatory females were encountered from March to November and ranged from 5 to 83% of the females encountered by month (Figure 10). When data were analyzed by region (east, central, west) and year (2009 and 2011) it was still apparent that a large percentage (25-59%) of the females in mating condition were encountered outside the known mating season (Parsons 1983); however, due to the small and inconsistent sample sizes across regions and years, no spatiotemporal correlations could be determined. Of the 94 ovulatory and post-ovulatory females encountered outside the known mating window, most were thought to be nulliparous females; however, the majority (60%) was larger than the size at 50% maturity. Three post-ovulatory females from March 2009 had mating scars, recently fertilized uterine oocytes, and no vitellogenic follicles (Figure 11a). In addition, several ovulatory and post-ovulatory females from October 2009 were examined, in particular, one specimen that had fresh mating scars and two fertilized oocytes transiting between the oviducal gland and the uterine horns (Figure 11b).

Brood size

A total of 1658 embryos (711 males, 755 females, 192 undetermined) from 368 broods were analyzed. Brood size ranged from one to nine individuals, and significantly increased with maternal FL (F_{382}= 484.15, $p < 0.001$, $r^2 = 0.56$; y=0.0221x-12.887; Figure 12). Mean brood size was 4.5 ± 0.1 embryos, and significantly more embryos were found in the left uterus (Left: 56%; 2.4 ± 0.06 embryos; Right: 44%; 1.9 ± 0.05 embryos; Mann-Whitney, $U_{382} = 42136.5$, $p < 0.001$). The ratio of male to female embryos was 1:1.06, which was not significantly different from 1:1 (Chi-square, $\chi^2 = 1.229$, $p = 0.268$). Unfertilized oocytes were present in 9.8% of the gravid females.

Embryos ranged from 4.4 to 380 mm STL (0.1 to 250 g). By late September, the yolk sac and stalk had differentiated into the placenta and umbilical cord for most of the embryos. Starting in July, uterine growth was rapid until November, but then slowed from February to June (Figure 13). Given that the majority of the embryos reached maximum size in May and June, parturition was assumed to primarily occur in late May and early June (Figure 13). The mean size of embryos close to parturition was 329 ± 3 mm STL and 154 ± 7 g. The growth rate of the embryos observed in this study suggests a 10-11 month gestation period. Similar to the variability observed with the timing of mating and ovulation in the females, a large amount of variability was found in monthly embryo length (Figure 13). For example, six gravid females sampled over a 10-day period in September 2009 had embryos ranging in size from 80 to 150 mm STL, along with fertilized oocytes (Figure 14).

DISCUSSION

It has been accepted as dogma that most carcharhinid and sphyrnid sharks exhibit a synchronous cycle where parturition, mating, and ovulation occur over a short period of time (Wourms and Demski 1993; Hamlett and Koob 1999). This short opportunistic window has been speculated to evolve to maximize the reproductive success of these species by increasing the survival of the young (Castro 2009). Despite this predominant reproductive strategy among the carcharhinids, this information has been obtained from only a few species, largely from temperate waters of the western North Atlantic Ocean including Atlantic sharpnose (Parsons 1983; Loefer and Sedberry 2003), blacktip, *Carcharhinus limbatus* (Castro 1996), finetooth, (Castro 1993), blacknose (Driggers et al. 2004; Sulikowski et al. 2007), sandbar, *C. plumbeus* (Baremore and Hale 2012), and bonnethead, *Sphyrna tiburo* (Parsons 1993) sharks. In addition,

several of these studies have lacked adequate sample sizes and intervals to fully assess potential reproductive patterns and/or anomalies that could exist within a population. The variability observed in the current study could be due, in part, to some of these shortcomings in previous studies on the reproductive biology of carcharhinid sharks.

Parsons (1983) first described the reproductive biology of Atlantic sharpnose sharks in the Gulf of Mexico and documented an annual, synchronous reproductive cycle where a clearly defined timing of mating, ovulation and parturition were observed. However, this study was limited by a small sample size (mature male: n=33, mature female n=30) and discrete spatial scale; all sharks were collected in coastal and offshore waters off Alabama. Based on the broad spatial coverage and large sample sizes, our results to date represent the most comprehensive reproductive analysis for Atlantic sharpnose sharks in the Gulf of Mexico. Similar to Parsons (1983), the current study reports that females simultaneously carry term embryos and vitellogenic follicles which confirm the proposed annual cycle; however, it is clear from the current data that some degree of asynchrony also exists within a portion of the population. For example, ovulatory and post-ovulatory females, which would only be expected to occur from May to July in a synchronous population (Parsons 1983; Loefer and Sedberry 2003), were observed in high numbers nearly year round. In addition, this asynchrony was also observed with maximum follicle diameter, as ovulatory females, with large vitellogenic follicles, were collected during September and October, two to three months after the known timing of ovulation for this species. The cumulative results of these observations was the documentation of two peaks in mean female GSI values, one in May and another in September, suggesting that a significant portion of the population was ready to mate and ovulate outside the previously described reproductive period (Parsons 1983).

311 Asynchrony in elasmobranch reproductive cycles can also be defined by the presence of
312 embryos at various stages of development, with no coordinated pattern of growth among months
313 (Castro 2009). For example, this developmental pattern has been observed in the embryos of
314 Caribbean sharpnose sharks, *Rhizoprionodon porosus*, collected in waters off northern Brazil
315 which resulted in the presence of full term embryos over a protracted period (Mattos et al. 2001).
316 Although the current study found a general increasing trend in embryo length from July to the
317 following June, a significant amount of variability was observed among embryos. For example,
318 embryos between 40 and 60 mm STL were found in gravid females during June, July, and
319 August. In addition, gravid females collected in September possessed embryos at various stages
320 of development from recently fertilized oocytes to 150 mm STL embryos (Figure 14). Previous
321 studies suggest that embryos of this size would range between 40 and 120 days old (Parsons
322 1983; Loefer and Sedberry 2003), suggesting a protracted mating season occurring between
323 April and July. Interestingly, mature spermatozoa were present in the testes and semen was
324 present in the seminal vesicles nearly year round (March to November). This is in contrast to
325 previous studies that have shown that Atlantic sharpnose sharks only have semen present in the
326 male reproductive tract during a few months following peak GSI (Parsons 1983; Loefer and
327 Sedberry 2003; Castro 2011). Thus, based on the present findings of spermatogenesis occurring
328 in the testes throughout most of the year, male Atlantic sharpnose sharks in the Gulf of Mexico
329 appear to have the ability to mate throughout most of the year, which is in agreement with the
330 protracted mating season observed with the females.

331 Although variability in the reproductive cycle of sharks has been documented in the past,
332 it has been limited to a few of studies. For example, Walker (2007) found that gummy sharks,
333 *Mustelus antarcticus*, off southern Australia showed a high degree of synchrony in their

reproductive cycle; however, several individual females were out of phase by up to three months. Female great hammerheads, *Sphyrna mokarran*, in northern Australian waters, exhibited a relatively synchronous reproductive cycle; however, variability was observed in the timing of mating and ovulation, suggesting that ovulation could take place over an extended period (~6 months) (Stevens and Lyle 1989). Baremore and Hale (2012) reported variability in the reproductive cycle of the sandbar shark by documenting post-partum females from April to September and females with sperm present in their uteri from April to August. Thus, in conjunction with the current findings, the variability in the reproductive cycle of carcharhinid sharks may be more common than previously documented; however the source of this variability needs further investigation.

It is unclear why a significant amount of variability is present in the reproductive cycle of Atlantic sharpnose sharks in the Gulf of Mexico; however, nulliparous females could account for some of this variability. Castro (2009) reported that nulliparous female Atlantic sharpnose sharks in waters off South Carolina would mate two to three weeks prior to the larger females that have completed at least one reproductive cycle. Motta et al. (2007) suggested a similar protracted mating season for the Brazilian sharpnose shark, *R. lalandii*, where mating takes place between April and June for nulliparous females, and between July and September for post-partum females. This phenomenon is most likely occurring in the Atlantic sharpnose shark population in the Gulf of Mexico and could, in part, help explain the more protracted mating season observed in the current study. Based on the aforementioned studies, it was anticipated that the majority of the ovulatory and post-ovulatory females collected outside the known mating season would be nulliparous females; however, this group only accounted for approximately 40% of the females in the current study, suggesting that some other phenomenon was responsible for the observed

reproductive variability. We believe this variability in the reproductive cycle of Atlantic sharpnose sharks is real because 70% of the ovulatory and post-ovulatory females collected from August to November were larger than 650 mm FL, which is well above the size at maturity for this species.

Another potential source of this variability could be related to the environmental conditions prevalent in the Gulf of Mexico. In more stable environments such as tropical and deepwater regions, several species have been shown to display asynchronous reproductive cycles with protracted mating and parturition seasons (Mattos et al. 2001; Verissimo et al. 2003; Braccini et al. 2006; Castro 2009). Environments such as these, with stable conditions and ample food supplies permit the expansion of the narrow windows of mating and parturition because there are no energetically limiting factors (Castro 2009). For example, environmental conditions have been shown to influence the reproductive periodicity in the gummy shark. Walker (2007) reported that the population of gummy sharks east of 138°E displayed an annual cycle, while the population west of 138°E displayed a biennial cycle, and this difference in reproductive cyclicity was explained by environmental differences, primarily water temperature, between the two regions. Additionally, Hoffmayer et al. (2010) suggested that increased sea surface temperatures in the north central Gulf of Mexico from 1979 to 2009, particularly during spring, allowed males to become reproductively active earlier in the year. Since Atlantic sharpnose sharks have such a large distribution in the western North Atlantic Ocean that spans both temperate and tropical regions, it is possible that this species could display signs of both synchrony and asynchrony. The environmental conditions in the Gulf of Mexico, which is located between western North Atlantic Ocean and Caribbean Sea, could provide water temperatures optimal for reproduction of this species through much of the year (e.g. March to October), resulting in a protracted

reproductive cycle observed in this study. Due to varying oceanographic conditions across the eastern, central, and western Gulf of Mexico, it's possible the asynchronous reproductive cycle observed in this study could be accounted for, in part, by spatial variability. However, a detailed study which systematically collects specimens from all three regions of the Gulf of Mexico will be needed to determine if this variability occurs on a finer scale than we observed.

In conclusion, the large amount of variability observed in both female GSI and maximum follicle diameter over an extended temporal period (March to October), as well as presence of mating scars observed throughout this period indicate that mating and ovulation in this species is occurring over a more protracted period than previously described. The occurrence of post-partum females from April to October and the varying sizes of the embryos across several months also support this hypothesis. Finally, the presence of spermatogenesis occurring in the testes of adult male sharks from March to November corroborates the reproductive plasticity observed in this species. Thus, based on the findings presented herein, the observed variability in Atlantic sharpnose shark reproduction is a result of asynchrony in parturition, mating, and ovulation, within a portion of the population.

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409

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Table 1. Summary of Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, specimens collected in the Gulf of Mexico. Operation times indicate when the fishery independent surveys are conducted and when opportunistic samples were obtained from commercial fishers. Gear types include bottom longline (BLL; Driggers et al. 2009) and bottom trawl (BT; Driggers et al. 2010). The sampling areas either include the entire northern Gulf of Mexico (GOM) or the north central GOM. NMFS = National Marine Fisheries Service, USM/GCRL = University of Southern Mississippi's Gulf Coast Research Laboratory, ESA = Expanded Stock Assessment.

Survey	Operation times	Years	Gear types	Sample area
NMFS ESA Project	April-October	2011	1.6 km BLL	northern GOM
USM/GCRL	March-October	2008-2012	1.6 km BLL	north central GOM
NMFS BLL	BLL: August-September	2008-2009	1.6 km BLL	northern GOM
NMFS BT	BT: October-November	2008-2009	12.2 km BT	
Commercial Fishers	November-March	2009-2012	1 km gillnet	north central GOM

Table 2. Length-length relationships for Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, specimens collected in the Gulf of Mexico. All lengths are measured in mm. FL = fork length, PCL = precaudal length, STL = stretch total length.

Conversion	n	Equation	r^2
FL to PCL	1299	= (0.9421 * FL) – 16.673	0.99
FL to TL	846	= (1.1135 * FL) + 45.679	0.96
FL to STL	1279	= (1.167 * FL) + 36.993	0.99
FL to Wgt Males	608	= 1×10^{-8} ($FL^{2.9554}$)	0.97
FL to Wgt Females	693	= 1×10^{-9} ($FL^{3.3071}$)	0.95
FL to Wgt All	1301	= 3×10^{-9} ($FL^{3.1592}$)	0.95

FIGURE CAPTIONS

Figure 1. Locations where Atlantic sharpnose sharks, *Rhizoprionodon terraenovae*, were collected in the Gulf of Mexico from 2008 to 2012.

Figure 2. Length frequency of Atlantic sharpnose sharks, *Rhizoprionodon terraenovae*, caught in the Gulf of Mexico from 2008 to 2012.

Figure 3. Relationship between fork length and clasper length for Atlantic sharpnose sharks, *Rhizoprionodon terraenovae*.

Figure 4. Proportion mature vs fork length (mm) for male (solid line) and female (dashed line) Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Horizontal bold line represents length at which probability of being is 0.50.

Figure 5. Variation in mean a) gonadosomatic index and b) testes length and width for mature Atlantic sharpnose sharks, *Rhizoprionodon terraenovae*, by month. Letters not in common indicate a significant difference at $\alpha = 0.05$. Sample size is indicated. Error bars represent ± 1 SE.

Figure 6. An image of a) a representative histological section of the right testis stained with hematoxylin and eosin, and b) the gross reproductive anatomy of a mature male (73 cm FL) Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, from the Gulf of Mexico. Mature spermatocyst are denoted as "MS" and immature spermatocyst denoted as "IS" in (a). Epididymis (1), ductus deferens (2), seminal vesicle (3), testes (4), and clasper (5) are identified in (b). Photo credits: J. Sulikowski (a), E. Hoffmayer (b).

Figure 7. Relationship between fork length and oviducal width for Atlantic sharpnose sharks, *Rhizoprionodon terraenovae*.

Figure 8. a) Mean gonadosomatic index (GSI) and b) a scatter plot of GSI by reproductive phase for female Atlantic sharpnose sharks, *Rhizoprionodon terraenovae,* plotted by month. Letters not in common indicate a significant difference $\alpha = 0.05$. Numbers below the mean indicate sample size. Error bars represent ± 1 SE.

Figure 9. a) Mean maximum follicle diameter and b) scatter plot of maximum follicle diameter by reproductive phase for Atlantic sharpnose sharks, *Rhizoprionodon terraenovae,* by month. Letters not in common above the mean indicate a significant difference at $p < 0.05$. Numbers below the mean indicate sample size. Error bars represent ± 1 SE.

Figure 10. Percentage of mature female Atlantic sharpnose shark, *Rhizoprionodon terraenovae,* in each reproductive phase by month in the Gulf of Mexico.

Figure 11. Photos of asynchronous Atlantic sharpnose shark, *Rhizoprionodon terraenovae,* females: a) a post ovulatory female, collected on 3/13/2009, showing oocytes in the uteri, b) an ovulatory female collected on 10/2/2009, showing two fertilized oocytes in route to the uterus. Both sharks had numerous mating scars on their bodies. The oviducal gland (1), uterus with fertilized oocytes (2), and a fertilized oocyte between oviducal gland and uterus (3) are identified. Photo credits: E. Hoffmayer.

Figure 12. Scatter plot of relationship between the number of Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, offspring and maternal fork length (mm).

Figure 13. A box and whisker plot of stretch total lengths of Atlantic sharpnose shark, *Rhizoprinodon terraenovae*, embryos plotted by month. The upper and lower boundaries of the gray box represent the 25^{th} and 75^{th} percentiles, and the line within the box marks the median. The error bars above and below the box represent the 90^{th} and 10^{th} percentiles, and the white circles indicate outliers. The black circles on the x-axis represent recently fertilized oocytes

found within post-ovulatory females, which were present from March to November indicating a protracted mating season and most likely an asynchronous cycle. The number above the black circles indicates the number of post-ovulatory females.

Figure 14. An image of five Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, embryos and one fertilized oocyte that were collected from six adult females during a 10-day period in September 2009. The embryos range in size from 80 to 150 mm stretch total length. Photo provided by E. Hoffmayer.

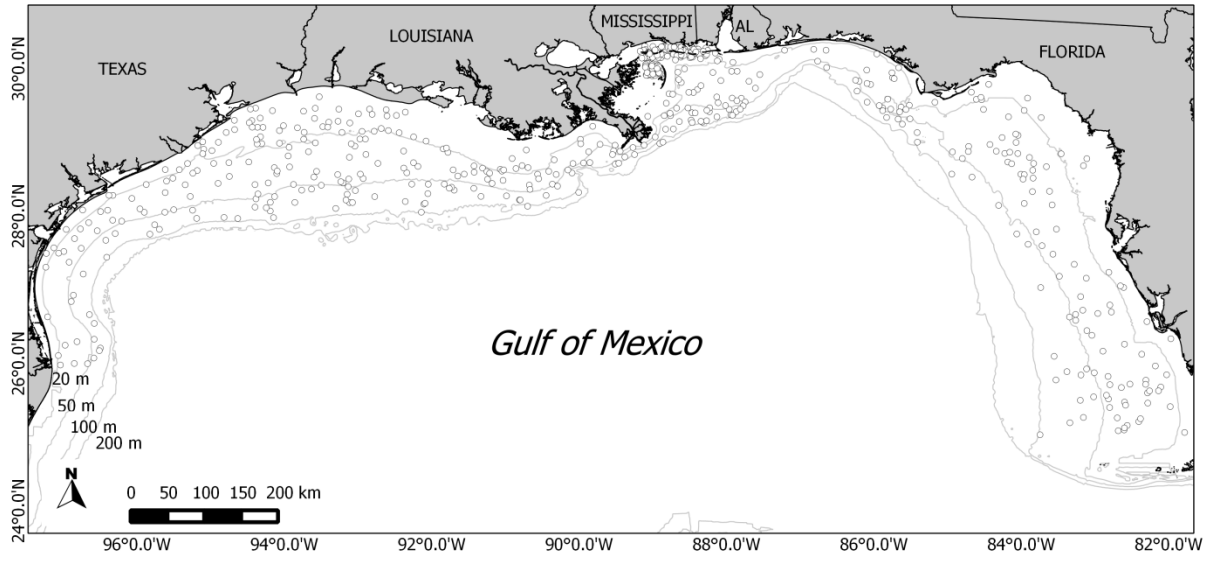
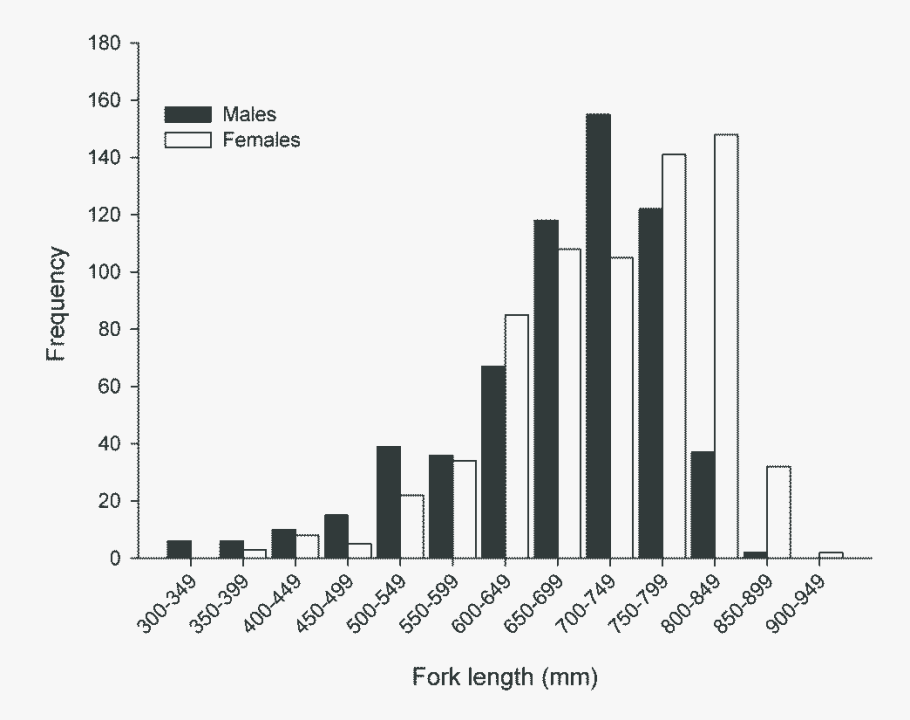

TEXAS
LOUISIANA
MISSISSIPPI
AL
FLORIDA
Gulf of Mexico
20 m
50 m
100 m
200 m
N
0 50 100 150 200 km
30°0.0'N
28°0.0'N
26°0.0'N
24°0.0'N
96°0.0'W
94°0.0'W
92°0.0'W
90°0.0'W
88°0.0'W
86°0.0'W
84°0.0'W
82°0.0'W

Figure 1



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590 Figure 2

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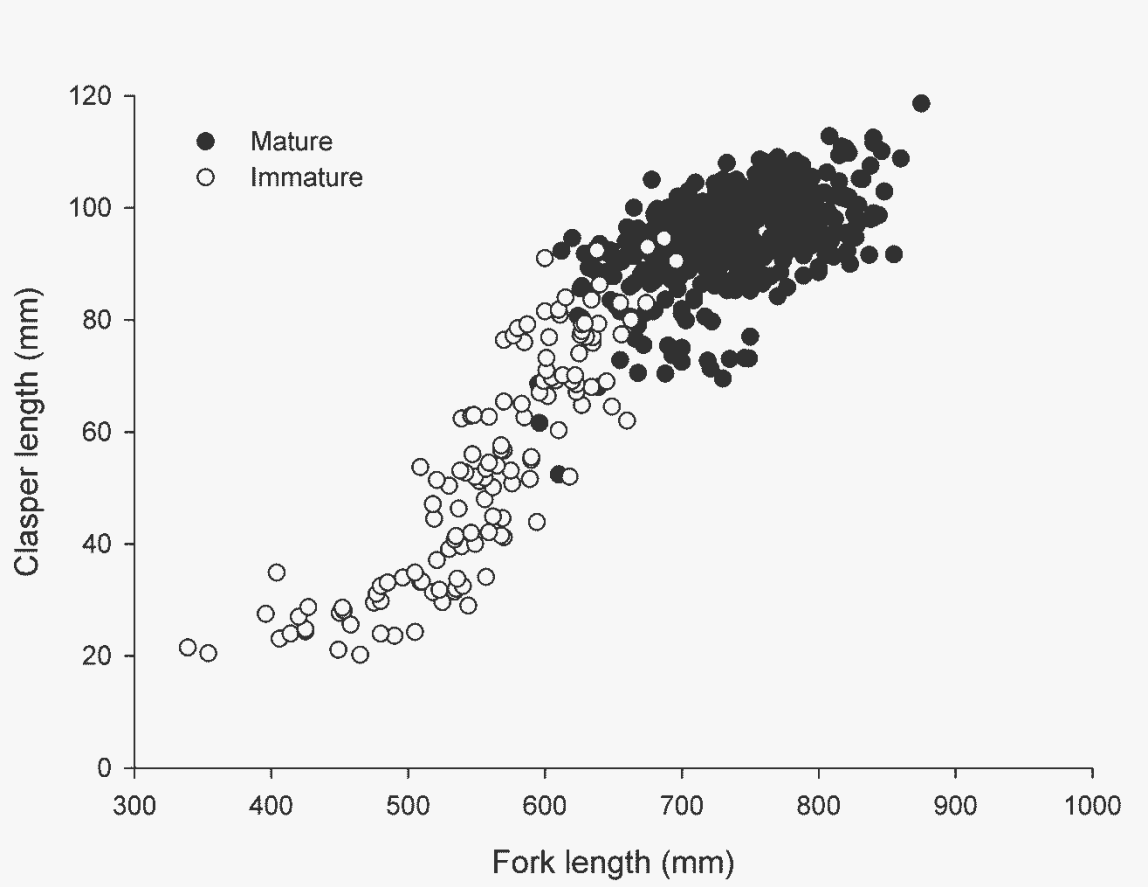

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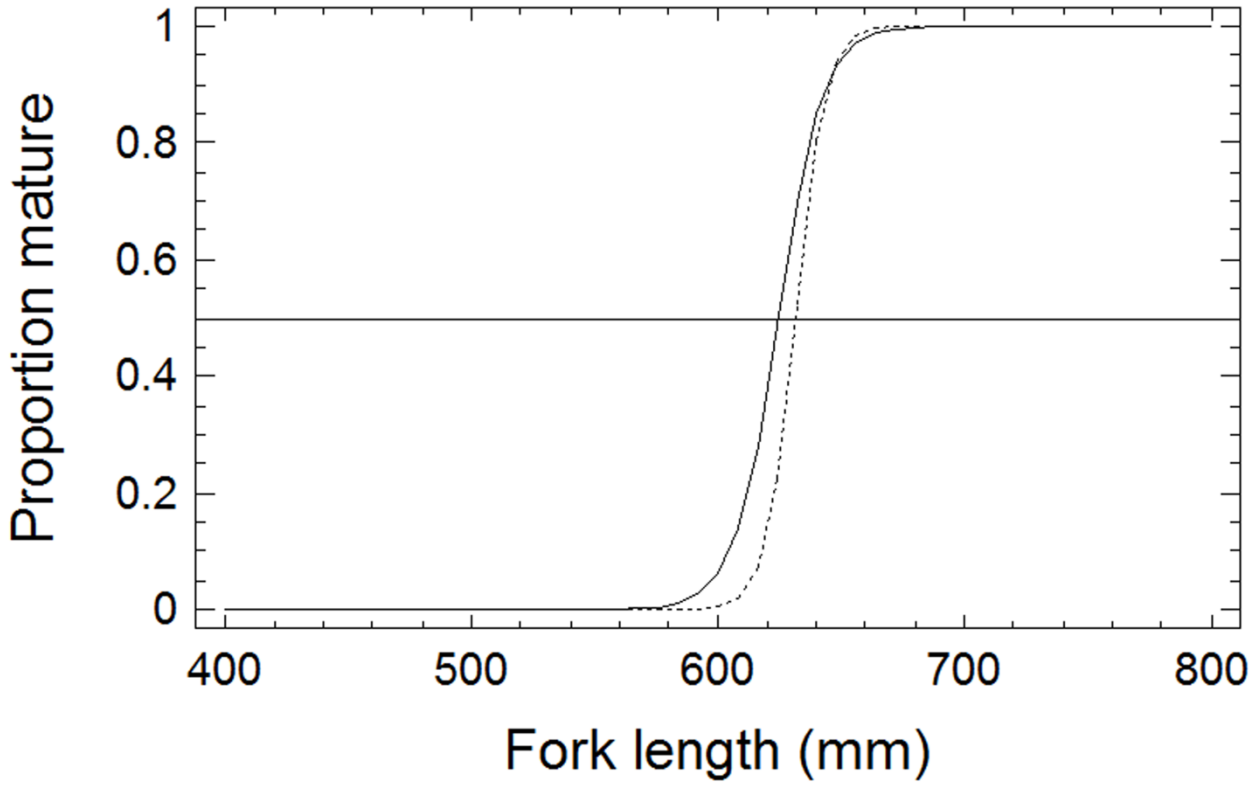

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596 Figure 4

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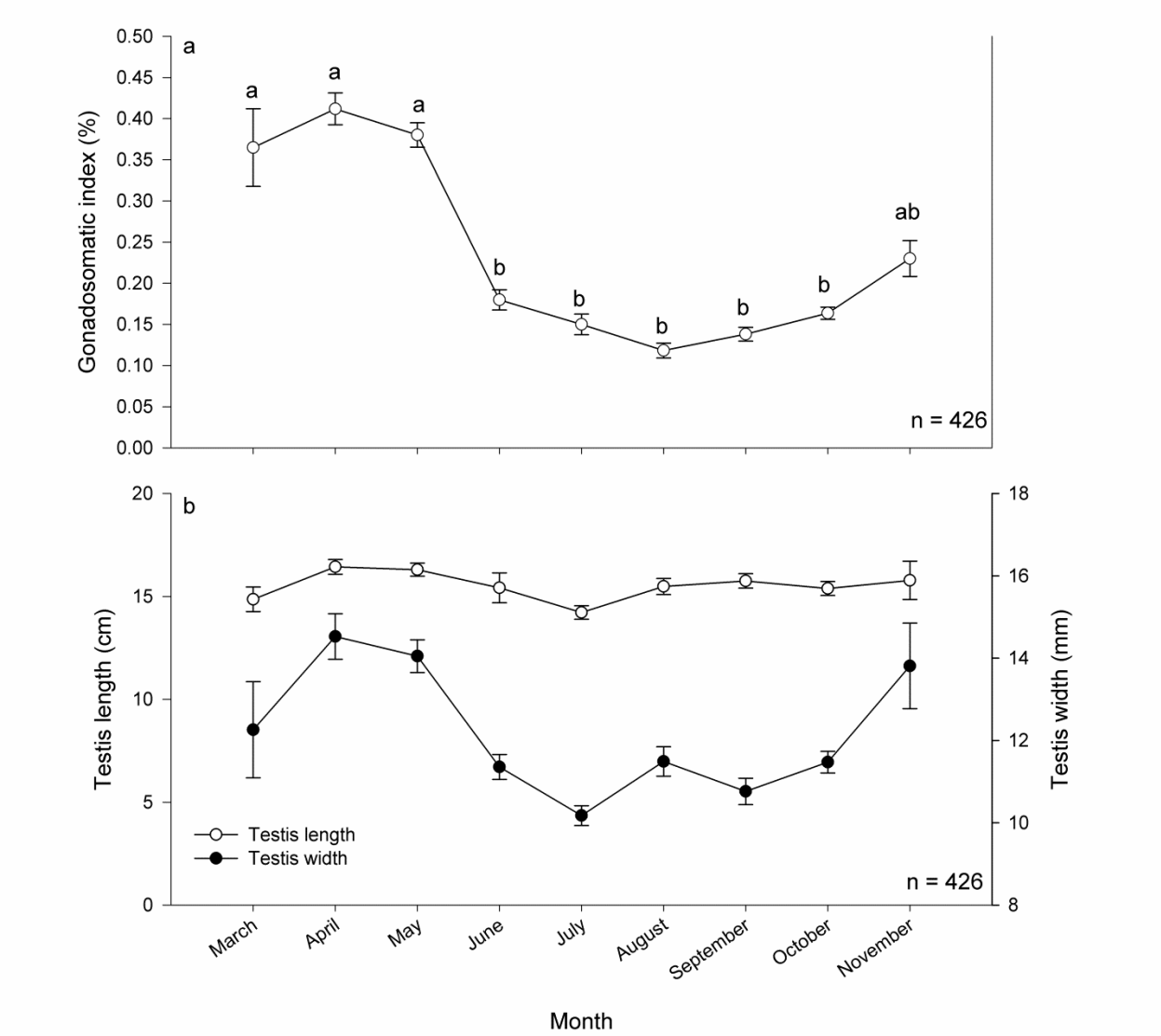

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599 Figure 5

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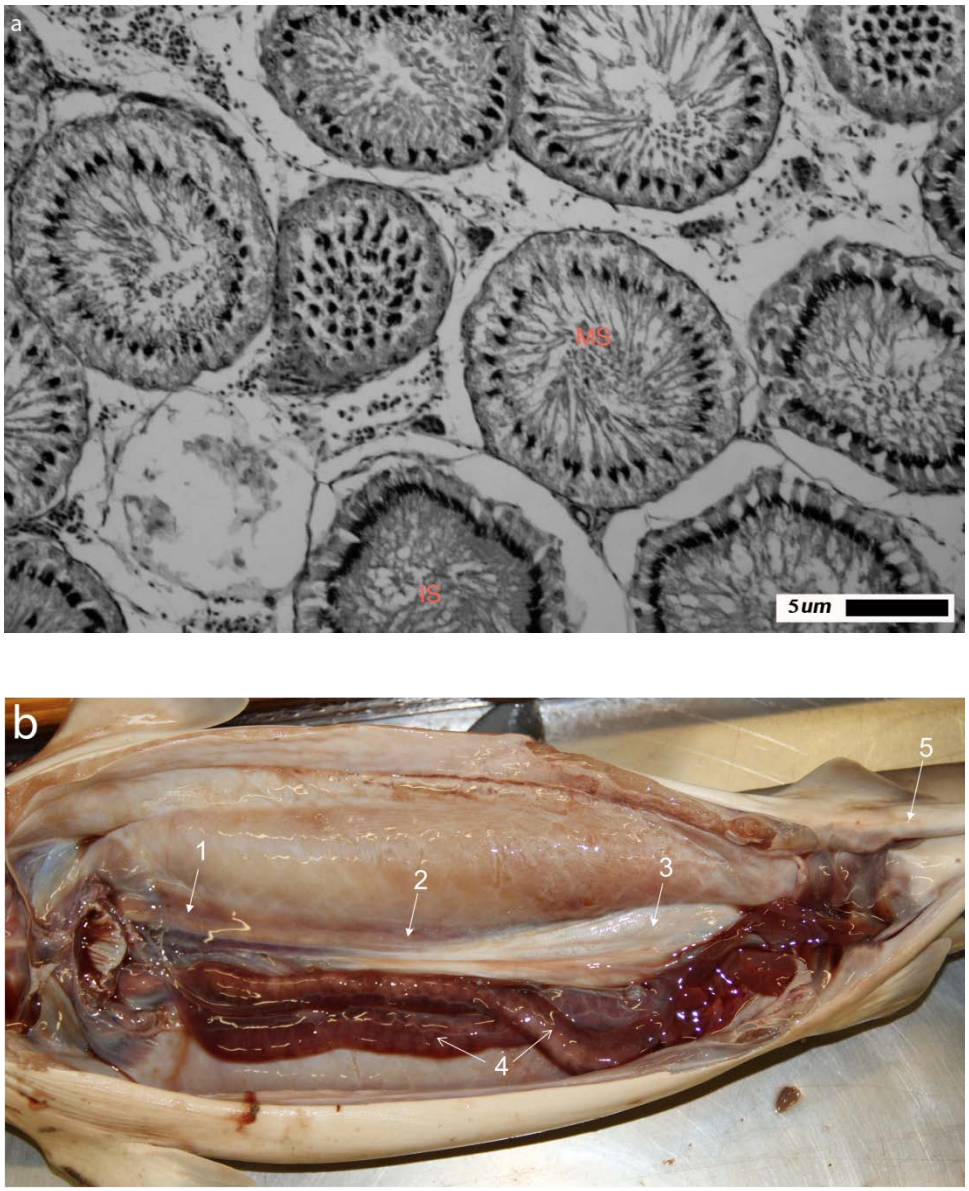

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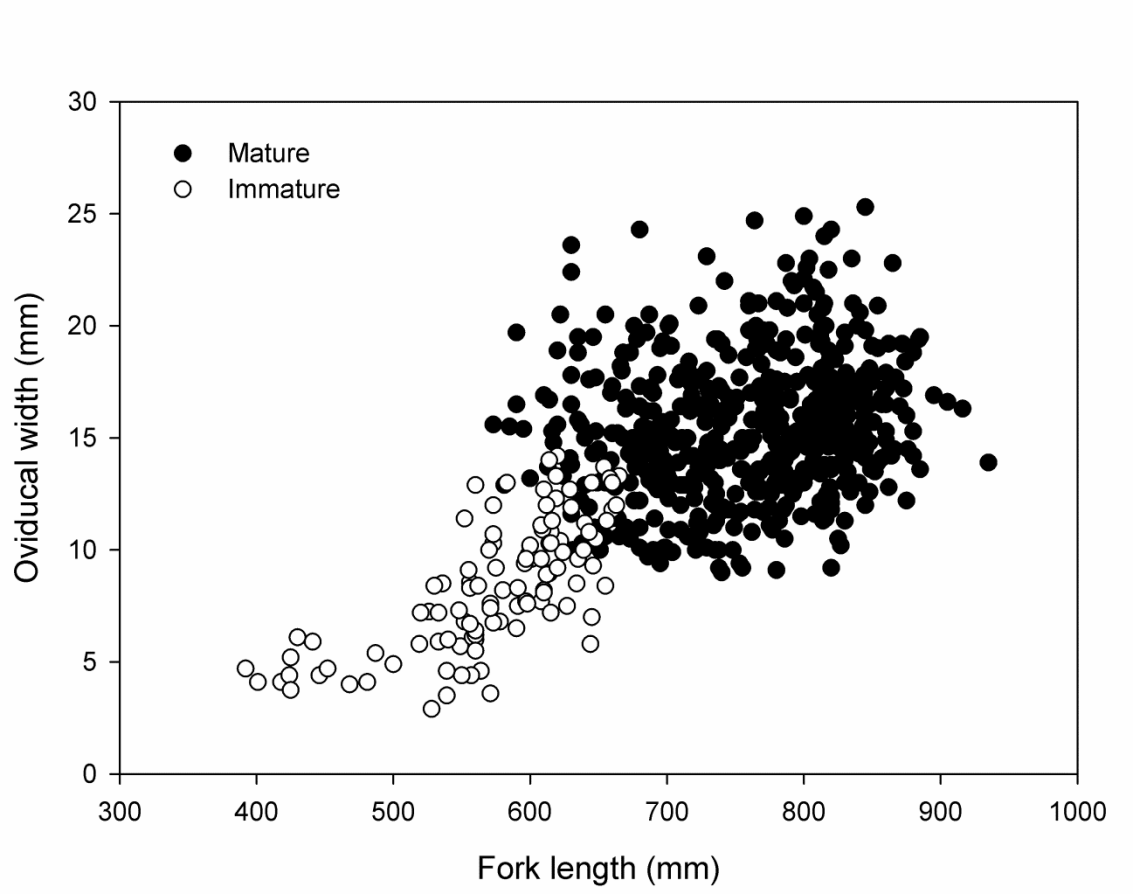

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617 Figure 7

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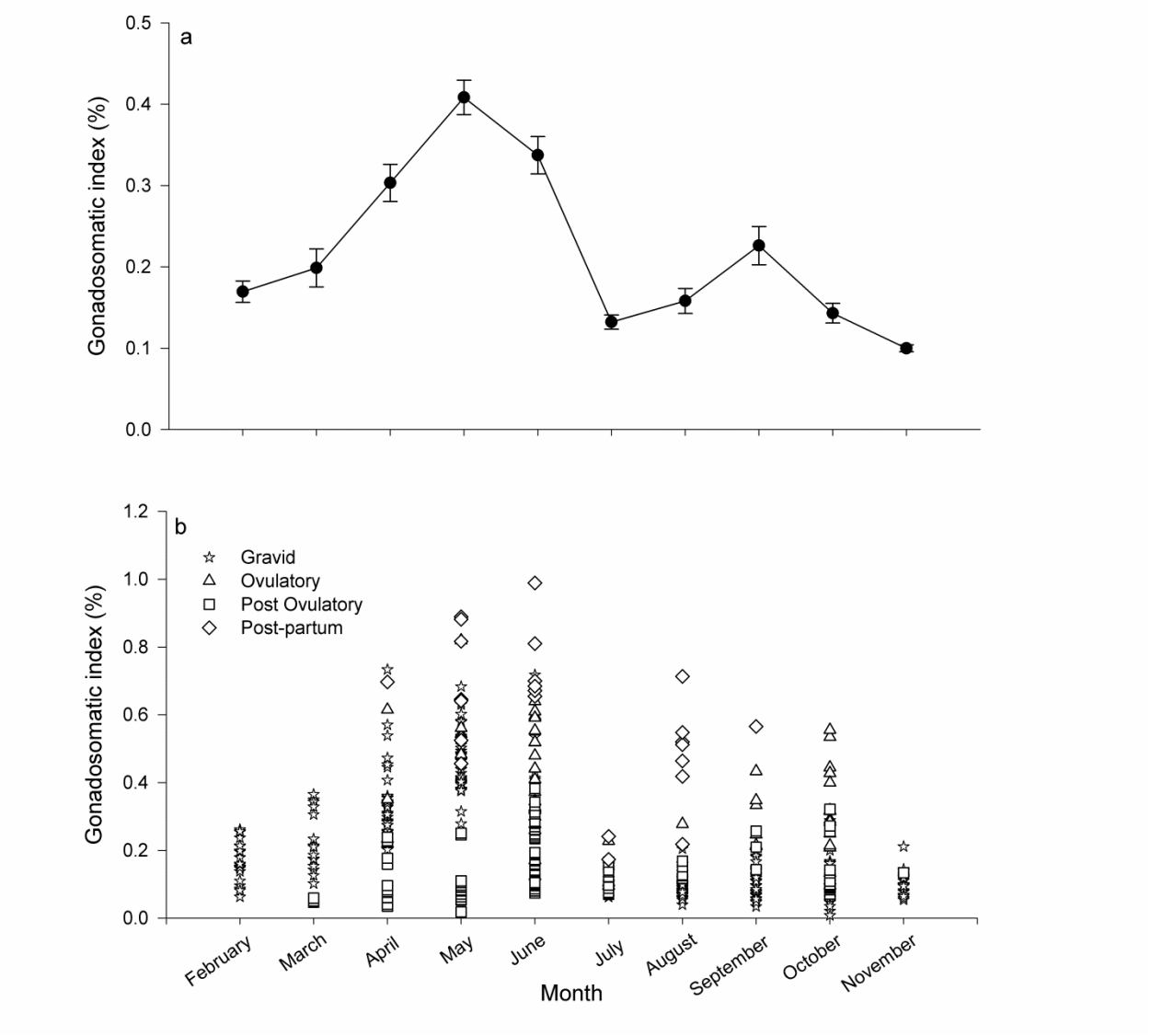

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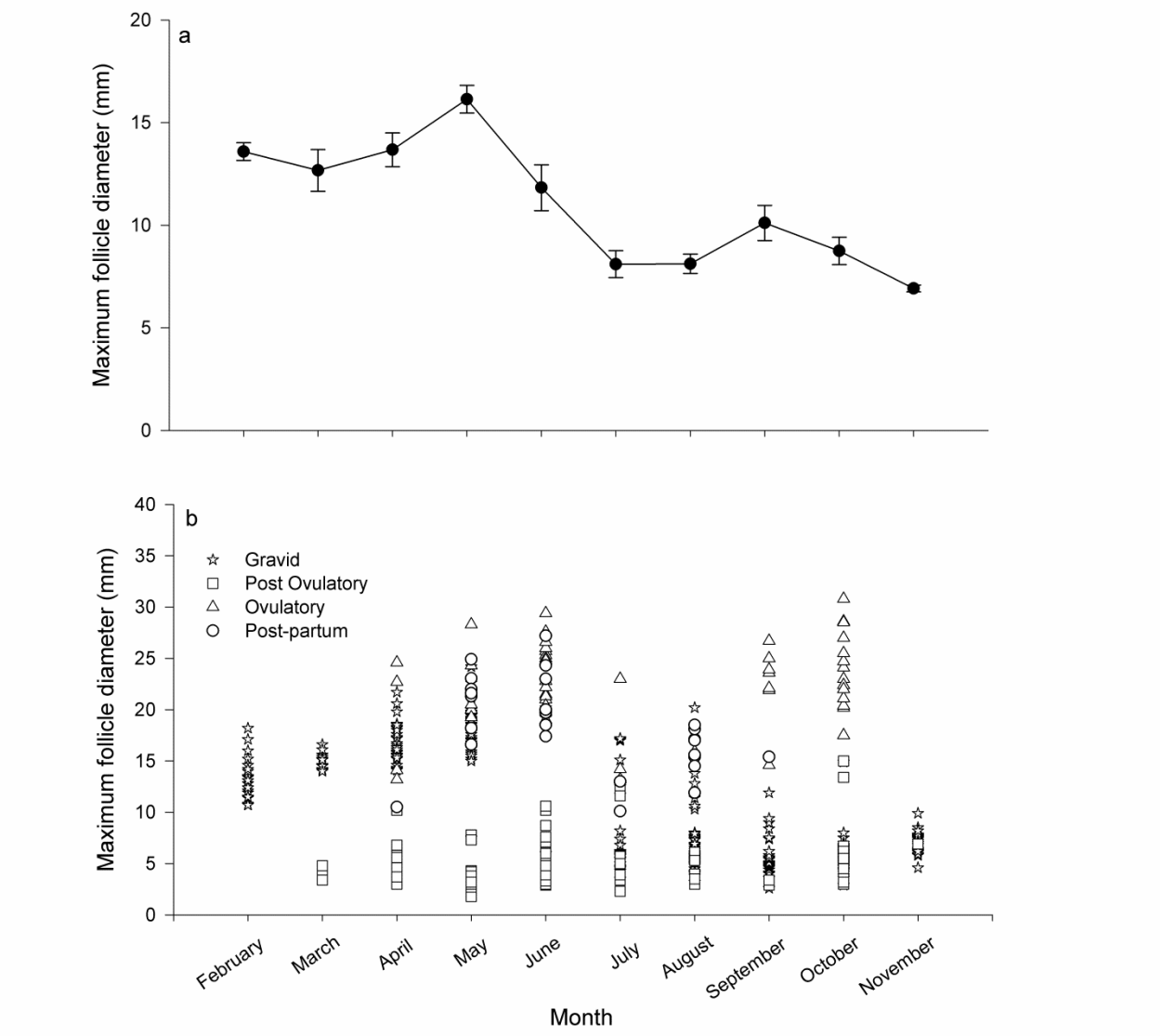

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623 Figure 9

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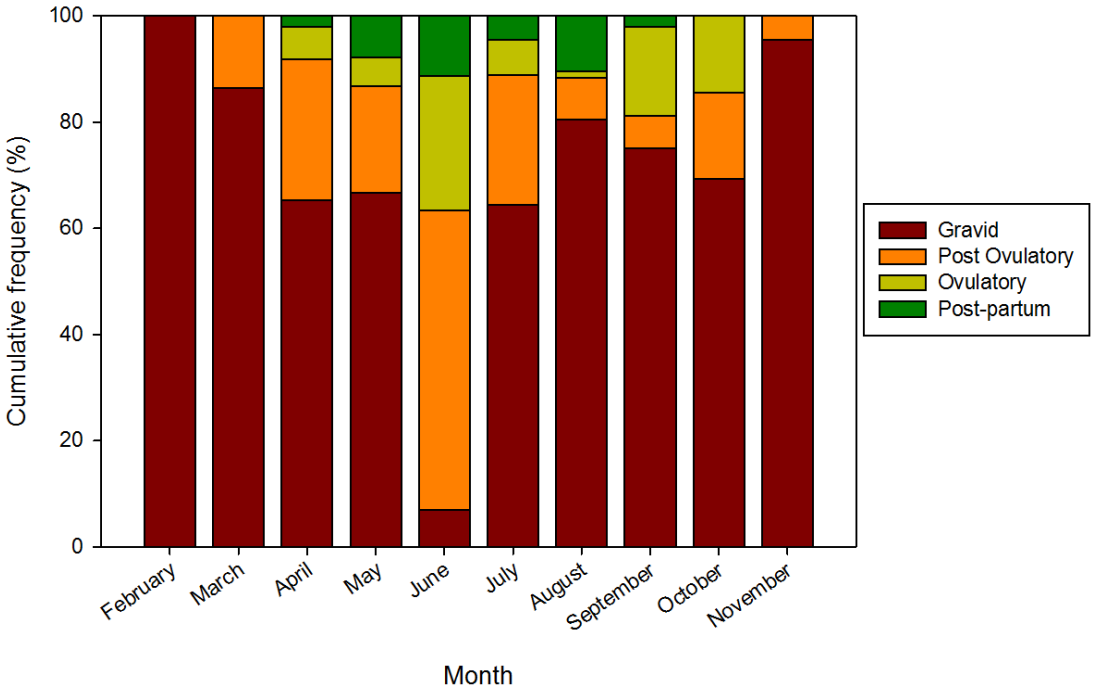

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626 Figure 10

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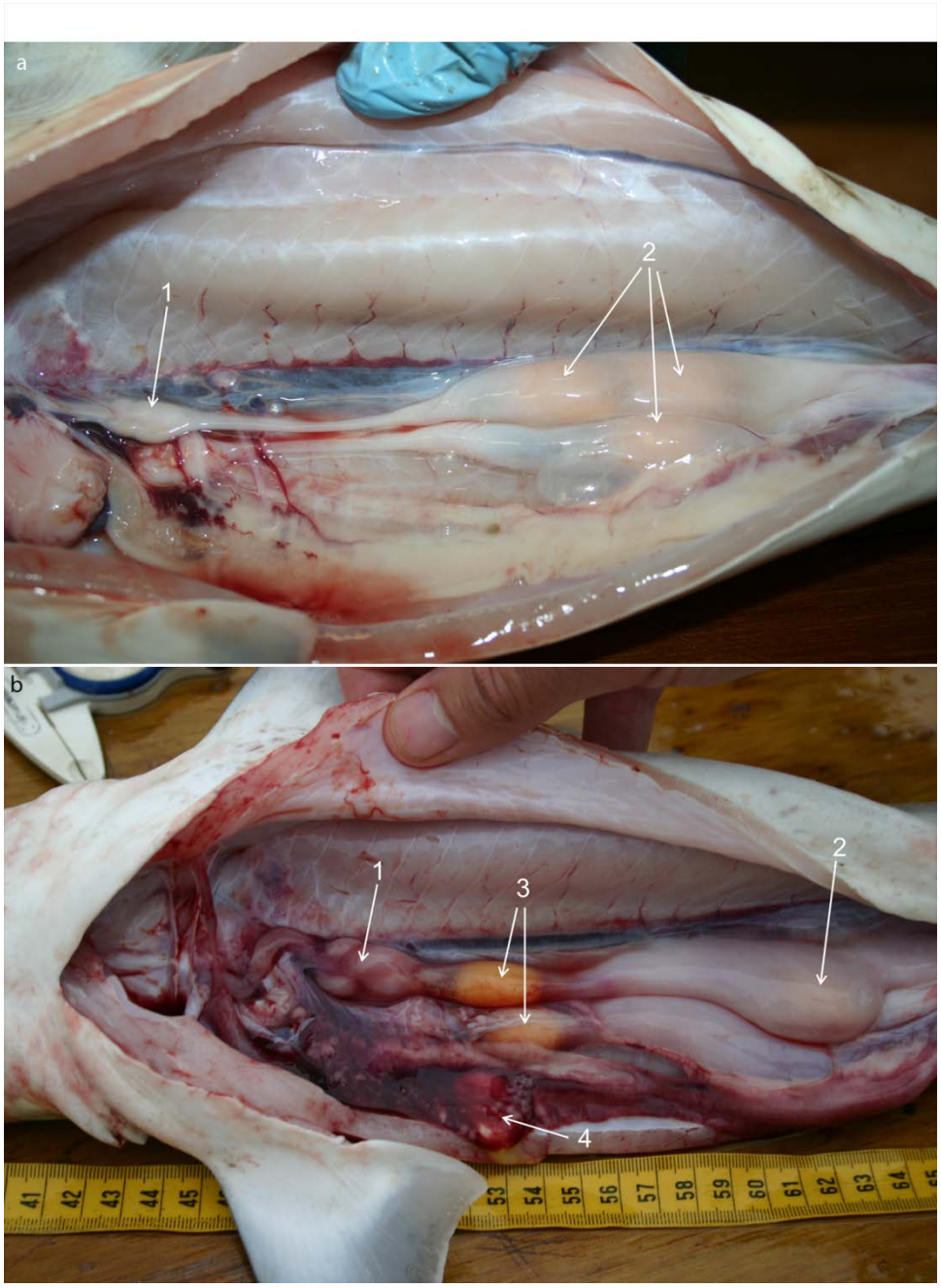

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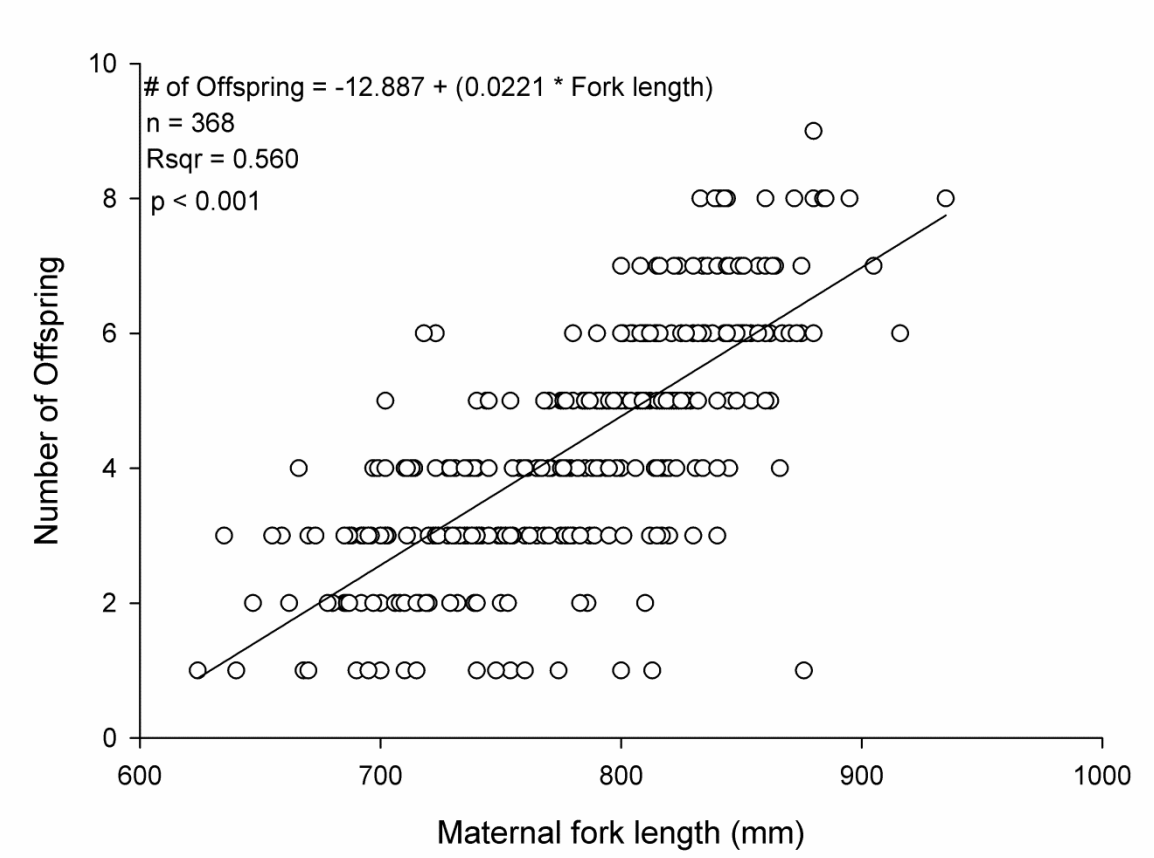

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645 Figure 12

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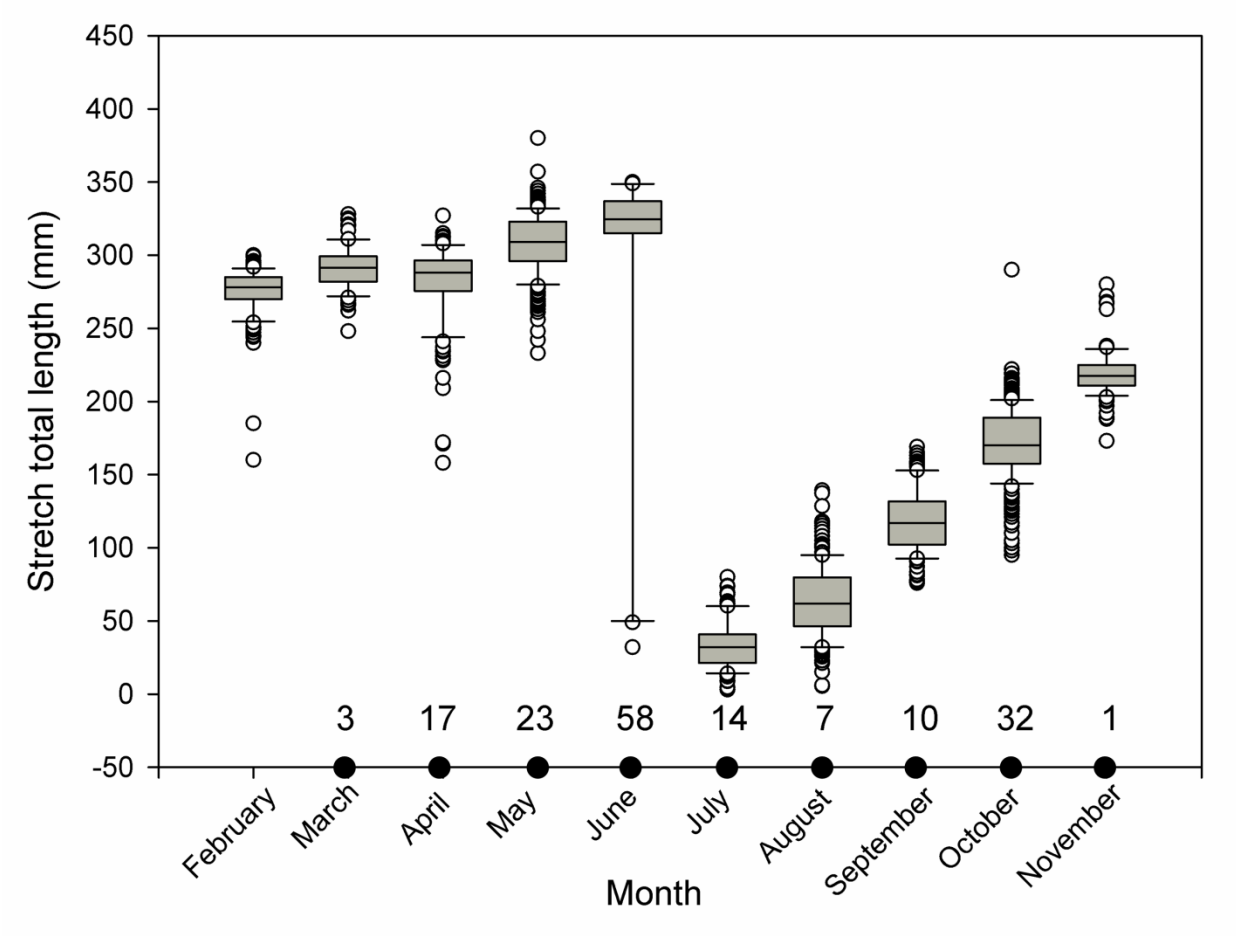

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648 Figure 13

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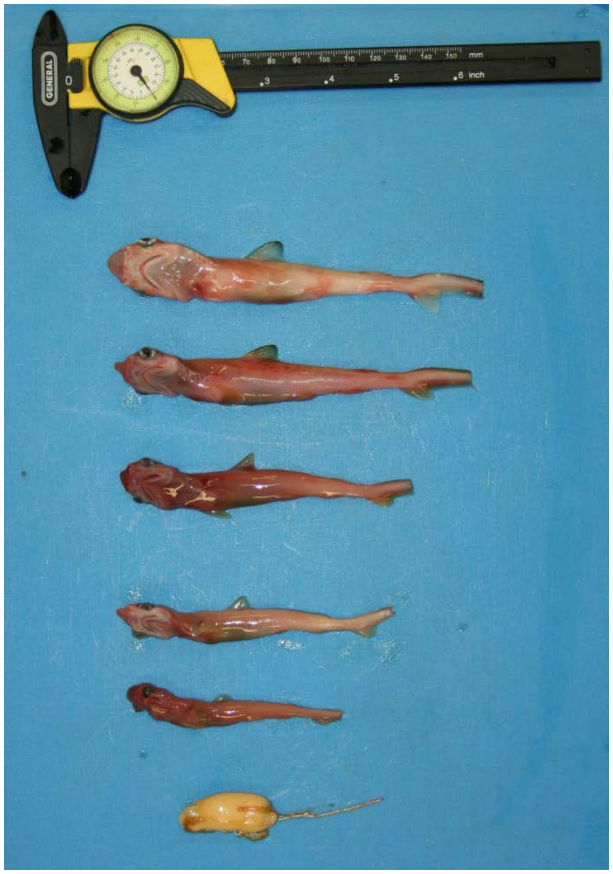

Figure 14