# Reproductive cycle of sandbar sharks in the northwestern Atlantic Ocean and Gulf of Mexico

Andrew Piercy
Florida Museum of Natural History
University of Florida
Gainesville, FL. 32611

**Summary**

The goal of this study was to gather contemporary data on the reproduction of the sandbar shark in the northwestern Atlantic Ocean and Gulf of Mexico. Specific objectives were to determine the size of maturity for male and female sandbar sharks, determine the timing of reproductive events (e.g. sperm production, vitellogenesis, ovulation, mating, and gestation), and determine if regional variations exist in reproductive parameters.

Male sharks exhibited sizes at 50% and 100% maturity of 140cm FL and 170cm FL respectively. Female sharks exhibited sizes at 50% and 100% maturity of 148 cm FL and 165 cm FL respectively. Both male and female sharks have a defined reproductive cycle. Male reproductive tracts were active from January to June. Mature female sharks exhibited a 3 year reproductive cycle. Egg development occurs from January/February to June. The gestation period for shark embryos is approximately 12 months, with the placental stage beginning in late September after approximately 3 months of development, and parturition occurring in late June. A mean litter size of 9.65 embryos was recorded and no relationship between maternal size and litter size was observed. No variation in reproductive cycles was seen between sharks caught in the Gulf of Mexico and those in the northwestern Atlantic.

## Methods

### Sandbar Shark Collections

Sandbar sharks were captured throughout the year from January 2003 to November 2007 by personnel in the Commercial Shark Fishery Observer Program (CSFOP) sampling the catch in the bottom-longline shark fishery. This sampling covered commercial shark fishing boats from Louisiana east to Florida and north to North Carolina (Burgess and Morgan, 2005) (N=598 sharks sampled). In addition, sharks were also sampled in the Florida Program for Shark Research's (FPSR) fishery-independent sampling program in both the northwestern Atlantic and eastern Gulf of Mexico (NSRC, 2007) (N=143 sharks sampled). Bottom-longline shark fishing vessels typically use between 8 to 16 km (5 to 10 mi) of mainline (monofilament or cable) with gangions consisting of a large circle hook (typically 14/0 to 18/0 in size), short steal leader (61cm) and a longer section of monofilament (1.8 m) attached to the main line with a clip. Hooks are baited with a plethora of bait types, including various teleosts (mullet, *Mugil cephalus*, is common) and smaller sized elasmobranchs. Baited longlines are soaked overnight, usually 8 to 12 hours, and the catch is then hauled in. The CSFOP placed trained scientists on bottom-longline commercial shark fishing vessels to monitor the catch and sample biological materials from a subset of shark specimens, including shark vertebrae, jaws, livers, and reproductive tracts. During times of the year that the bottom-longline commercial shark fishing season was closed, samples were obtained from the FPSR fishery-independent sampling program, thus resulting in near year-round sampling. Fishery-independent sampling was conducted through leasing of several commercial bottom-longline shark fishing vessels utilizing gear and fishing techniques that mirror those used during the commercial season, with the exception of shorter soak times (4-6 hours) to minimize mortality to sharks and any bycatch.

**Sampling for Male Reproductive Biology**

Upon capture, sandbar shark gender was determined and measurements of FL (straight line from the tip of the snout to fork of caudal fin) and total length (TL) (straight line from tip of snout to a perpendicular line drawn from the tip of the upper caudal lobe when in a natural position) were taken. Outer clasper (paired intromittent organs) length was measured for males (Figure 1). Reproductive organs and tissue samples were immediately removed from a subset of male sharks (N=303) and placed in seawater buffered 10% formalin solution. Reproductive samples included the right testis (whole), the right epididymis, and a section of the seminal vesicles.

Size at maturity of male sandbar sharks was assessed through examination of clasper length as a proportion of shark FL, and epididymal width as a function of FL. Both parameters have been correlated with the onset of maturity (Jensen et al., 2002; Driggers et al., 2004), which is visualized by a marked increase in the sizes of the claspers and epididymides as FL increases. The calcification of claspers also corresponds with maturity in male sharks. While calcification data were not available for specimens in this study, comparison of clasper size in proportion to body size and related calcification data from a previous study on sandbar sharks in Australia (McAuley et al., 2007) were used as a proxy to assess maturity of specimens in the present study (claspers were fully calcified at clasper lengths $\geq$ 8% FL). A logistic model was used to develop a maturity ogive based on the above parameters. Epididymal width data were analyzed to assess sizes of maturity and seasonal variation in the activity of epididymides. A testis gonadosomatic index (GSI) was developed by dividing the testis weight (g) by FL (mm) * 100. Fork length was used in the GSI calculation instead of body mass because sharks are not weighed on the vessels. A Kruskal-Wallis test was used to assess statistical significances of any monthly variations in GSI.

Tissue samples from a subset (N= 32) of mature male reproductive tracts (maturity was determined based on the parameters listed above) were analyzed using standard histology techniques at the University of New Hampshire Histology Lab. Briefly, transverse sections of the testis, epididymis, and seminal vesicle (~2-3 mm) were dehydrated in a graded series of alcohol (80-100% ethanol), cleared in a limonene-based solvent, infiltrated in molten paraffin, and processed for routine paraffin histology. Tissue sections (5 μm thick) for use in histological analyses were prepared using a rotary microtome, adhered to poly-L-lysine-coated slides, and stained with Harris hematoxylin and counterstained with eosin. Observations of the prepared slides were conducted with a Leica DM LB2 compound scope.

An index of mature spermatocysts present in the testis was calculated by dividing the area of the testis occupied by late stage spermatocysts with the total area of the testis in cross-section (Figure 2). Stages of spermatogenesis followed those proposed by Maruska et al. (1996). Area measurements of testes were calculated using Image Pro Express imaging software (Media Cybernetics Inc., Bethesda, MD).

## Sampling for Female Reproduction

Upon capture, female sharks were measured for FL and TL and reproductive organs and tissues were removed from a subset (N= 438) and stored on ice or fixed in seawater buffered 10% formalin. Female reproductive samples included the right ovary (only the right ovary is functional), right nidamental gland (shell gland), and a mid-section of the right uterus. If the female shark was pregnant, then the contents of both uteri were placed on ice and stored at –29 °C. Uterine contents were examined later in the laboratory and embryos were sexed, measured (FL and stretch TL) and liver samples dissected and stored at –29 °C. After 2 weeks in the formalin solution, reproductive samples were rinsed in water for 24 hours, dehydrated in 50% ethanol for 1 hour, and stored in 70% ethanol.

For females, the diameter of a single haphazardly selected ovarian egg and nidamental gland (shell gland) width were measured using digital calipers (Fowler Inc., Newton, MA). A marked increase in nidamental gland width has been shown to indicate maturity of carcharhinid sharks (e.g. Driggers et al., 2004). Maturity assessments were made based on a marked increase in the size of the nidamental gland in relation to female shark size and the presence of uterine contents.

The timing of reproductive events and the seasonality of the reproductive cycle were determined through analysis of temporal changes in the morphology of the genital ducts of mature (based on the parameters listed above) sandbar sharks, i.e., changes in oocyte diameter, nidamental gland width, and uterine contents. Tissue samples of nidamental glands (N= 60) from mature sandbar sharks were also prepared using the same standard histological techniques described previously but were processed at Mote Marine Laboratory, FL. Cross-sections of nidamental glands were examined under a compound scope for evidence of sperm storage in the lumen and connective ducts.

## Regional Comparison

Potential differences in reproduction between sandbar sharks in the northwestern Atlantic and Gulf of Mexico were assessed through qualitative analysis. Possible variation in the reproductive cycle was determined through analysis of the timing of reproductive events in the two regions. Regional variations in fecundity were examined by comparing litter sizes with t-tests statistical analysis.

# Results

## Male Reproduction

Maximum proportional outer clasper length occurs at fork lengths greater than 130 cm (Figure 3). Using proportional clasper sizes of calcified claspers in McAuley et al. (2007) as a reference, sandbar claspers in the present study were predicted to begin calcifying at 4% FL and be fully calcified at 8% FL, corresponding to shark lengths between 90 and 160 cm FL, respectively. Assuming that a shark with a proportional clasper length of 8% or greater is mature, sizes of projected maturity can be determined. Logistic regression analysis of proxy calcification data indicated approximately 50% of sharks at 140 cm FL are mature ($p < 0.001$; Figure 4). At a length of 150 cm FL, 89% of sharks would be considered mature with the remaining 11% of the sharks classified as maturing. At a length interval of 170 cm FL, 100% of sharks would be considered mature.

Sandbar shark epididymides begin to increase in size around 140 cm FL (Figure 5). Maximum epididymal width was observed in a 150 cm FL shark. The gonadosomatic index (GSI) for male sandbar sharks showed a significant increase beginning in February with a peak in April (Kruskal-Wallis; $F = 37.82$ $p < 0.01$; Figure 6). Testicular width also showed a marked increase in the spring with a peak in April (Figure 7). Histological analysis of testicular tissue showed an increase in the presence of late stage spermatocysts in the spring months with a maximum in May (Figure 8). These data indicated that sperm production occurred in the months of March through May, with maximum gonadal activity for male sandbar sharks occurring in May.

Epididymal activity followed a similar trend with increases in epididymal width seen in the months of March, April, and May, with maximum width in May (Figure 9). Spermatozoa were present in the epididymides during the spring, with a declining presence after the peak in May (Figure 10). Histological architecture of the epididymides showed variation during periods of sperm maturation and inactivity. Epididymal ducts were enlarged with little connective tissue during the spring and early summer months (Figure 10b). As spermatozoa presence decreased in the epididymis, epididymal ducts decreased in size and the amount of inter-duct connective tissue increased (Figure 10c).

Sandbar shark seminal vesicle activity also exhibited a trend of increasing activity during the spring months. Seminal vesicle widths increased in March, April, and May, with a dramatic decrease in width in June (Figure 11). Histological analysis showed that spermatozoa were present in the seminal vesicle in April and May, with declining amounts of spermatozoa present in June, and no spermatozoa present in the following months (Figure 12). During the period of sperm storage, seminal vesicles exhibited less connective tissue and thinner invaginations projecting into the lumen (Figure 12). As sperm storage declined, seminal vesicle invaginations thickened and connective tissue increased.

These data indicate that mature male sandbar sharks produce sperm in the months of March, April, and May, with a peak in sperm production occurring in May. Sperm storage in the seminal vesicle occurs during April and May. The decline in the presence of sperm in the genital ducts of males in June suggests that mating actually occurs in late May or June.

**Female Reproduction**

Female sandbar sharks exhibit marked increases in nidamental gland widths at fork lengths of 140 cm to 160 cm (Figure 13). Also, pregnant sandbar sharks ranged in size from 140 cm to 182 cm FL. To develop a maturity ogive, therefore, females were considered mature if they were pregnant or had large nidamental glands consistent in size as those of pregnant sharks (width $\geq$ 26 mm). Sizes at which 50% and 100% of the population were mature were 148 cm and 165 cm FL, respectively ($p < 0.001$; Figure 14). Of 20 near-term litters examined, litter sizes ranged from 6 to 14 pups with a mean of 9.65. Embryonic sex ratio did not vary significantly from 1:1 (t-test; $t = 0.55$; $p = 0.58$). Litter size was not correlated with maternal FL ($r^2 = 0.017$) (Figure 14).

Mature female sandbar sharks exhibited a seasonal reproductive cycle. The oocytes began vitellogenesis (the accumulation of yolk) in February and showed increasing size and variation in oocyte diameters through the month of June, after which mean oocyte diameter decreased (Figures 15). To understand the large variation in oocyte diameter observed over the months of February to June, individual oocyte diameters were plotted as a function of month, with the data indicating a bimodal distribution in each month (Figure 16). This bimodality in oocyte diameter indicated that two conditions of mature female sharks occur during the months of vitellogenesis: mature but non-egg developing (smaller oocytes) and mature with egg developing (larger oocytes). Pregnant female sharks do not exhibit concurrent vitellogenic activity during gestation.

Marked increases in weight of the nidamental gland occurred in the months of June and July (Figures 17). This increase corresponded with the presence of fertilized eggs in the uteri and indicated that sandbar shark ovulation occurs in late June. The gestation period for sandbar shark embryos is approximately 12 months, with the placental stage beginning in late September after approximately 3 months of development, and parturition occurring in late June (Figure 19). No evidence of sperm storage was seen in any of the sampled nidamental glands (Figure 20).

**Regional Differences**

No difference in the timing of sperm production, as indicated by testis GSI, was observed between male sandbar sharks in the Gulf of Mexico and northwestern Atlantic Ocean (Figure 21). Males from both regions exhibited increases in epididymal widths during the months April and May (Figure 22). However, a more pronounced increase in epididymal width was observed for Atlantic sharks during this time period (Figure 22).

The nidamental gland showed similar patterns of increasing weight in the late winter and spring months for both Gulf of Mexico and northwestern Atlantic Ocean groups (Figure 23). Females from both regions showed a peak in nidamental gland weight in June and July. Oocyte diameter data indicated that female sandbar sharks in both regions exhibited highly variable values in the spring months (Figure 24). Elevated oocyte diameter measurements for the Atlantic region in the month of May are a product of small samples size (N=3). The presence of mature females with small oocytes was noted in both regions (Figure 24). Of the 20 late-term litters examined, no significant difference in litter size was observed between sandbar sharks in the Gulf of Mexico and Atlantic Ocean (t-test: $t = -0.52$, $p = 0.62$).

## Discussion

**Male Reproduction**

Marked increases in proportional clasper lengths of sandbar sharks were observed to begin at 120 cm FL. Merson (1998) reported increases in clasper lengths at slightly larger sizes, beginning at 130 cm FL and reaching an asymptote at 145 cm FL. Clasper length data in the present study, using calcification data from McAuley et al.’s (2007) study as a proxy, suggests that 50% of sharks are mature at a length of 140 cm FL, and 100% of sharks were mature at 165 cm FL. Based on clasper length and calcification, Merson (1998) reported a 50% likelihood of maturity at 147 cm FL. Other studies have consistently reported 50% maturity lengths of 150 cm FL (Casey and Natanson, 1992; Sminkey and Musick, 1995)

The onset of increased epididymal size occurs at slightly longer fork lengths than the onset of increasing clasper growth. However, this 10 cm difference in fork lengths may not be significant. Further, inadequate sampling of epididymides from smaller size classes may hinder analysis of trends in epididymal growth. Also seasonal variation in epididymal width due to sperm production may confound analysis as mature males exhibit small epididymides during reproductively inactive months. These issues suggest that for this study, epididymal width data are not appropriate for assessing maturity.

As with other elasmobranchs, mature male sandbar sharks have a defined reproductive cycle and show seasonal variation in the size and activity of their gonads and accessory sex organs (Garnier et al., 1999; Conrath and Musick, 2002; Piercy et al., 2003; Sulikowski et al., 2007). Sandbar sharks, like other carcharhinid sharks, show elevated testicular weight and activity just prior to mating (Sulikowski et al., 2007). However, peak sperm production was not correlated with peak testis width or weight, the latter peaking a month before peak sperm production. This temporal disconnect between peak sperm production and testis size, while short in time span, has been previously reported in other elasmobranch fishes (Maruska et al., 1996; Piercy et al., 2003). The seasonal variation in gonad size is likely an effort to conserve energy through reducing gonad size during times of inactivity. Energy conservation through reduced gonad size is a common strategy not limited to fish species and has been reported in other taxa including birds (e.g. Wikelski et al., 2003).

The timing of peak epididymal and seminal vesicle activity also occurs after peak gonad size. As these organs are involved in sperm maturation and transport, their activity should increase as sperm production peaks. This lagging of peak genital duct activity after peak testis size has also been reported in a recent study on sandbar sharks (Merson, 1998) and other elasmobranch fishes (e.g. Piercy et al. 2003). No evidence of long-term sperm storage was observed in sandbar shark seminal vesicles. The absence of sperm in the lower genital ducts of sandbar sharks caught after the month of July, coupled with a decrease in seminal vesicle width in June, would indicate that mating likely occurs in June. Springer (1960) also reported that mating occurred in June for this species.

Seasonal changes in the histological architecture of genital ducts of male sandbar sharks were similar to those reported for the Atlantic stingray (Piercy et al., 2003). Both species show decreases in the amount of connective tissue present in epididymal and seminal vesicle cross sections as the epithelium grows during periods of sperm production. Further, as seen in the Atlantic stingray (Piercy et al. 2003), seminal vesicle invaginations in mature sandbar sharks decrease in thickness as sperm presence increases.

**Female Reproduction**

In the present study, the marked increase in nidamental gland width at lengths of 140 to 160 cm FL in female sandbar sharks was similar to that noted by Merson (1998) (140 cm to 150 cm). The smallest pregnant shark in the present study was 140 cm FL. Merson (1998) reported the smallest pregnant sandbar shark to be 156 cm FL. Collectively, these data indicate that female sandbar sharks are maturing at sizes of 140 cm to 160 cm FL. A maturity ogive indicates that at a size of 148 cm 50% of females are mature. This size of maturity for females was consistent with previously reported maturity lengths of 150 cm from other sandbar shark studies (Casey and Natanson, 1992; Sminkey and Musick, 1995; Merson, 1998).

As oocyte development has been observed in immature sharks with undeveloped uteri (e.g. White et al., 2002), oocyte diameter was not utilized as a sole marker for maturity in this study. Oocytes may develop without being ovulated, resulting in the resorbtion of the yolk protein by the shark. While other indicators of maturity can be assessed (e.g. presence of hymen, mating scars, presence of sperm in genital ducts) the accuracy of these indicators can be problematic depending on the reproductive behavior of the species. Many indicators may only reveal the occurrence of mating, not maturity. If sandbar shark mating is coercive and initiated by males, as suggested by Portnoy et al. (2006), then females that have mated may not necessarily be mature. Alternatively, if sandbar shark mating is not coerced by males, but initiated by females, then these markers may hold true. This presents a circular argument in that the validity of many proposed markers of maturity is dependent on the belief that only mature females mate. Further, the ability to accurately categorize a mating wound as fresh, or detecting the presence of sperm or hymen, may be hindered by the use of more than one person sampling the sharks and the available training.

Female sandbar sharks have a defined seasonal reproductive cycle. Oocyte diameter data for mature non-pregnant females indicate that vitellogenesis, the accumulation of yolk, occurs between February and June. Merson (1998) reported similar timing of yolk development for sandbar sharks. No concurrent vitellogenesis with gestation was observed in any specimen in the present study. In combination with a protracted gestation period, this suggests that sandbar sharks have a reproductive cycle that is longer than one year. Springer (1960) and Merson (1998) have also previously reported that the reproductive cycle for female sandbar sharks was longer than one year.

The bimodality in oocyte size of mature female sandbar sharks indicated that a portion of the mature population may exhibit a “resting” phase, whereby they are neither increasing their oocyte size through vitellogenesis nor are they pregnant. This results in three reproductive conditions present during the spring: pregnant, mature vitellogenic sharks, and mature “resting” sharks. This indicates the potential for an entire reproductive cycle for female sandbar sharks in this region to be greater than 2 years. While Springer (1960) proposed a 2 year reproductive cycle for sandbar sharks, Merson (1998) also noted bimodal oocyte data indicating a 3 year reproductive cycle.

Reproductive cycles greater than 2 years have been reported in other fishes. Dusky sharks, *Carcharhinus obscurus*, are believed to have a 3 year reproductive cycle, based on the presence of a protracted gestation period (Musick et al., 1993; Dudley et al., 2005). Further, green sturgeon *Acipenser medirostris*, which have growth characteristics similar to sharks, have been shown to skip-spawn with intervals of 2, 3, and 4 years in between reproductive events (Erickson and Webb, 2007). The potential reduction in sandbar shark reproductive output from a more protracted reproductive schedule may impact population growth. Assessment models utilizing biennial reproductive data may be over estimating sandbar shark reproductive output. This reinforces the need for assessing how variation in reproductive parameters can affect potential population growth.

An alternative explanation for the presence of “resting” females is that these sharks were not really mature. However, the size range of females in this resting mode was 146 to 172 cm FL, well within the range of maturity. Further, the widths of the nidamental glands from these females fell well within the range of nidamental widths of pregnant females, which further suggests that these females were indeed mature. While uterine width measurements can be used as another indicator for maturity (Whitney and Crow, 2007), reliable measurements of uterine widths were not available for the specimens in this study.

Another alternative explanation for these bimodal data was that the “resting” females were pregnant, but gave birth early or aborted during capture. No evidence of abortion (e.g., presence of partial litters *in utero*, partial birth) due to stress of capture was noted during the sampling in this study. Further, the time period of parturition was short based on embryonic data in this study, and the presence of free swimming neonates (Mote Marine Lab survey, Tyminski pers. comm.) Observations of neonates in other studies of sandbar sharks are largely restricted to the months of June to August (Nichols and Murphy, 1916; Breeder, 1925; Bigelow and Schroeder, 1948; Osarno, 1992; Merson, 1998). Fishery-independent surveys in the northwestern Atlantic and Gulf of Mexico have reported small numbers of neonate sandbar sharks captured earlier than June: NMFS Panama City reports two in May (Baremore, pers. comm.); and Mote Marine Laboratory's survey reports one undersized in April (Tyminski, pers. comm.). Additionally, while the Virginia Institute of Marine Science fishery-independent surveys report 42 neonates captured in the month of May, this number is far less than the 143 captured in the month of June (Romine, pers. comm.). These studies and data support the observation in the present study that parturition for the majority of sandbar harks occurs in June, but there is some variability in this timing.

A mean litter size of 9.6 pups was observed in the present study, a value similar to litter sizes reported in previous studies. Springer (1960) reported a mean litter size of ten ($\pm$ 2) for sandbar sharks caught in the Atlantic and Gulf of Mexico. Further, Springer (1960) reported no relationship between sandbar shark litter size and maternal size. In contrast, Joung and Chen (1995) reported increasing sandbar shark litter sizes with maternal size for sandbar sharks in Taiwanese waters, albeit it was a very weak relationship ($r = 0.41$).

No evidence of sperm storage by females was observed in this study. While Pratt (1993) found sperm in some female sandbar shark nidamental glands, his samples were only from months just after mating. Furthermore, Pratt (1993) reported that this presence of sperm was likely not long-term storage and that the long reproductive cycle of the sandbar shark would require sperm to be stored for a protracted period of time that may be unfeasible.

**Regional Comparison**

While regional and latitudinal variation in age, growth, and reproduction has been noted for other elasmobranch species (e.g. Tricas et al., 2000; Lombardi et al., 2003; Sulikowski et al., 2007), no regional variation in the timing of reproductive events was observed for sandbar sharks in the Gulf of Mexico and northwest Atlantic Ocean. As little genetic variation is seen between sandbar sharks in these two regions (Heist et al., 1995; Heist and Gold, 1999), the highly migratory nature of sandbar sharks may inhibit regional variation in population genetics, which in turn may conserve reproductive traits. Indeed, a tagging study has shown that sandbar sharks migrate between the Gulf of Mexico and the Atlantic Ocean (Casey and Kohler, 1991).

**Summary**

While this study report slightly smaller sizes of maturity for males, the sizes of maturing female sandbar sharks is similar to lengths of maturity reported in previous studies. The potential impact that small variations in maturity sizes can have on recruitment levels may be minor depending on the age structure. Contrary to some previous studies, the reproductive cycle of female sandbar sharks may be more protracted than conventionally believed. The data presented in this study indicate a 3 year reproductive cycle as opposed to the previously reported 2 year reproductive cycle. This difference in reproductive timing may impact sandbar shark stock assessments.

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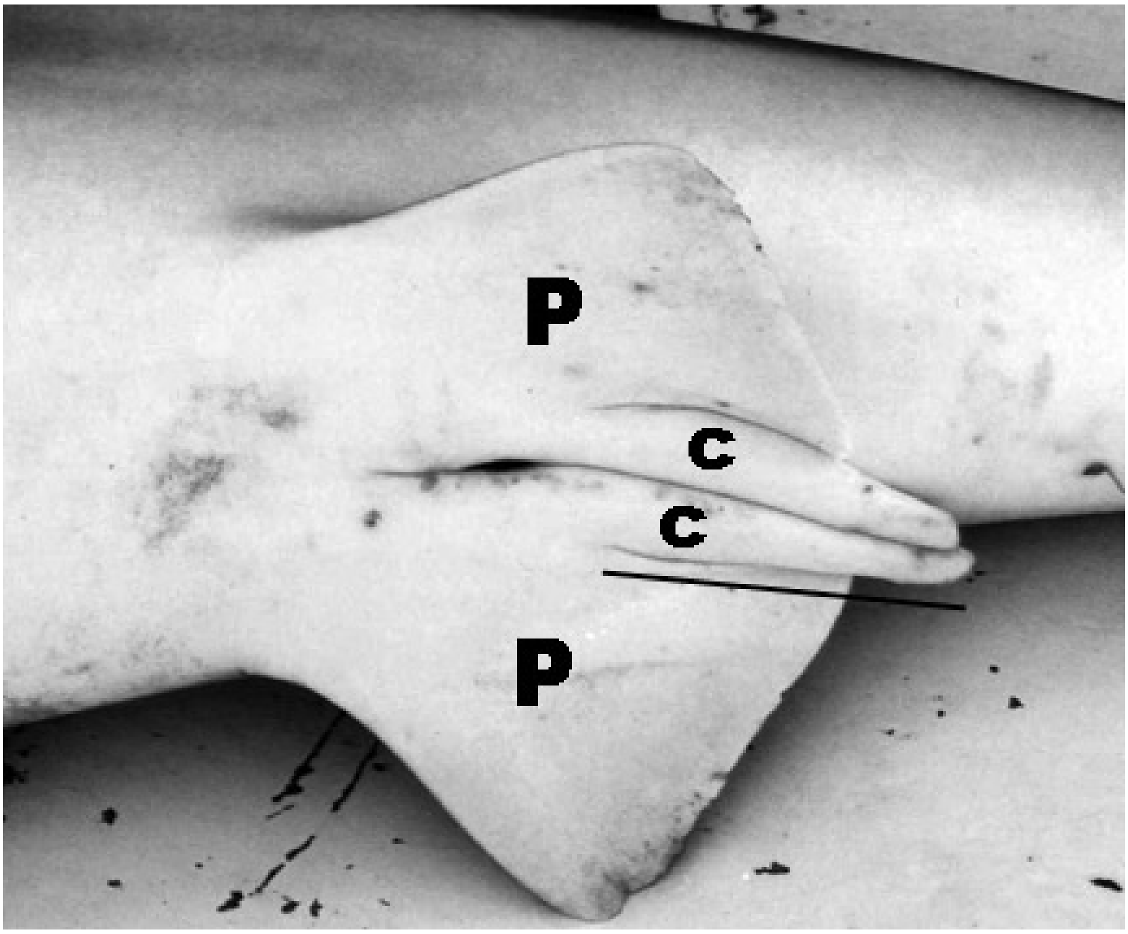


Figure 1. Measurement of outer clasper length in sandbar shark. Measurement is taken from the margin of the pelvic fin (P) to the tip of the clasper ( c). Line represents the plane of measurement.

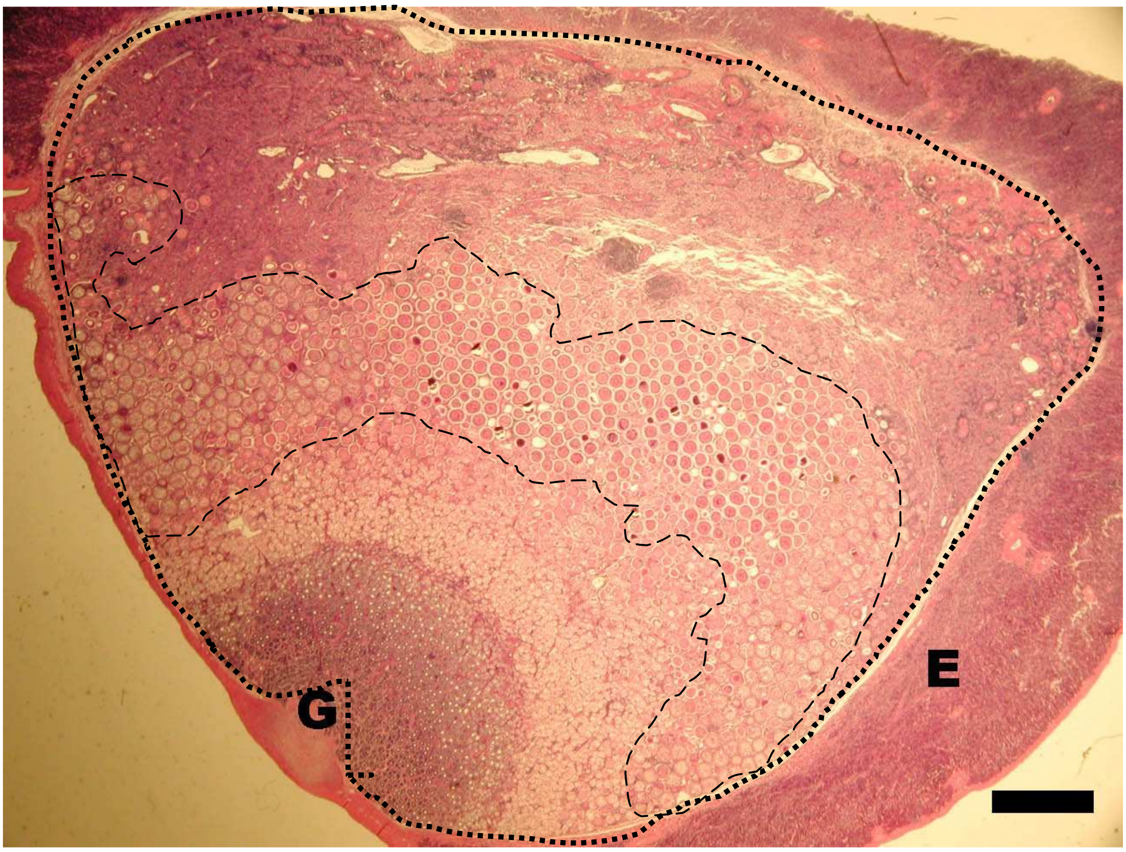


Figure 2. Histological cross-section of sandbar shark testis, showing germinal zone (G) and epigonal tissue (E). Dotted line demarcates the area of testis tissue and dashed line the area occupied by late stage spermatocysts. Bar represents 1mm.

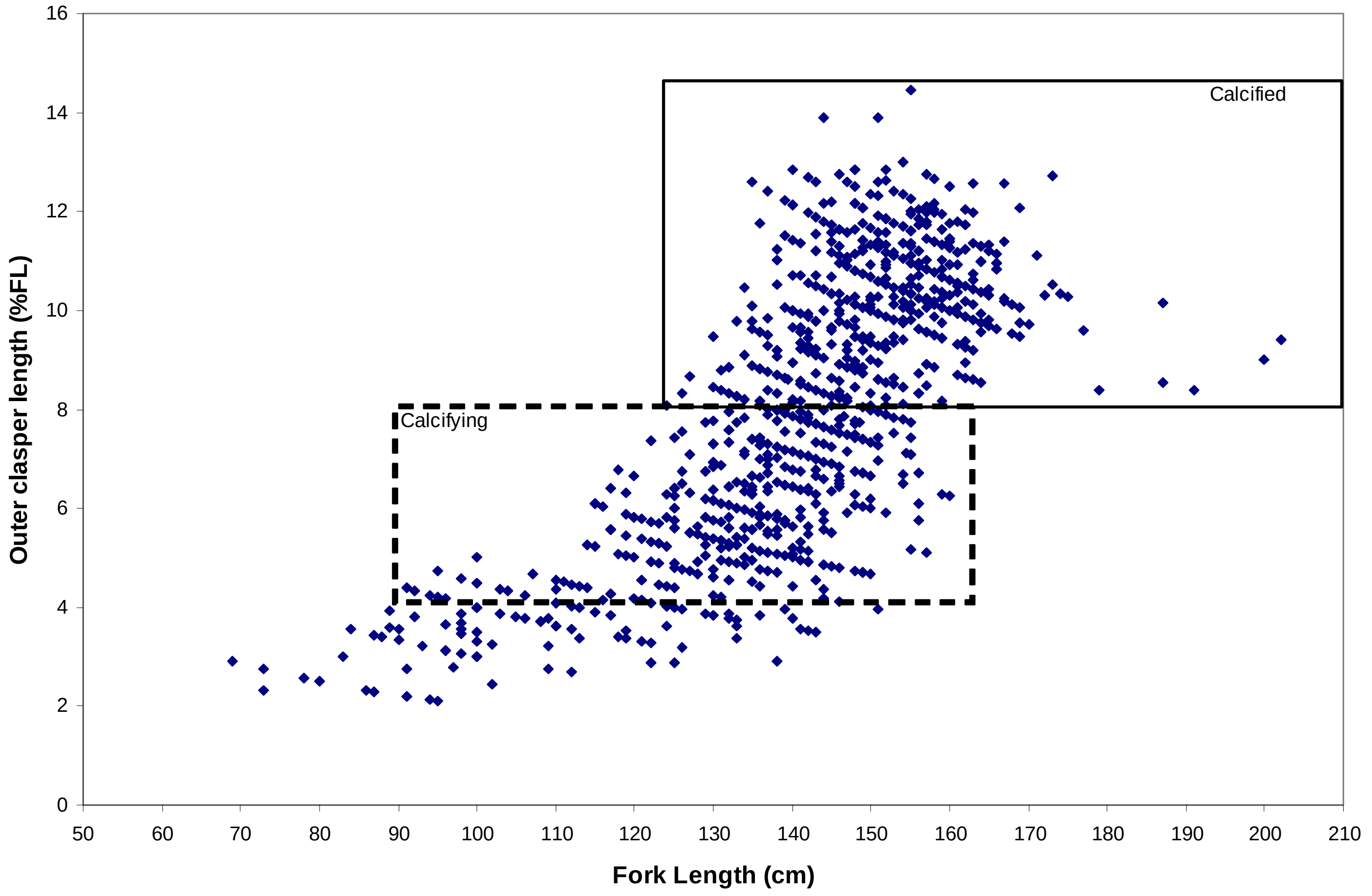


Figure 3. Sandbar shark proportional outer clasper length (mm) as a function of FL (cm) for sharks caught in the Commercial Shark Fishery Observer Program from 2003-2005 (N=1973). Boxes represent specimens that would have "calcifying" and "calcified" claspers based on the proxy clasper data from McAuley et al. (2007).

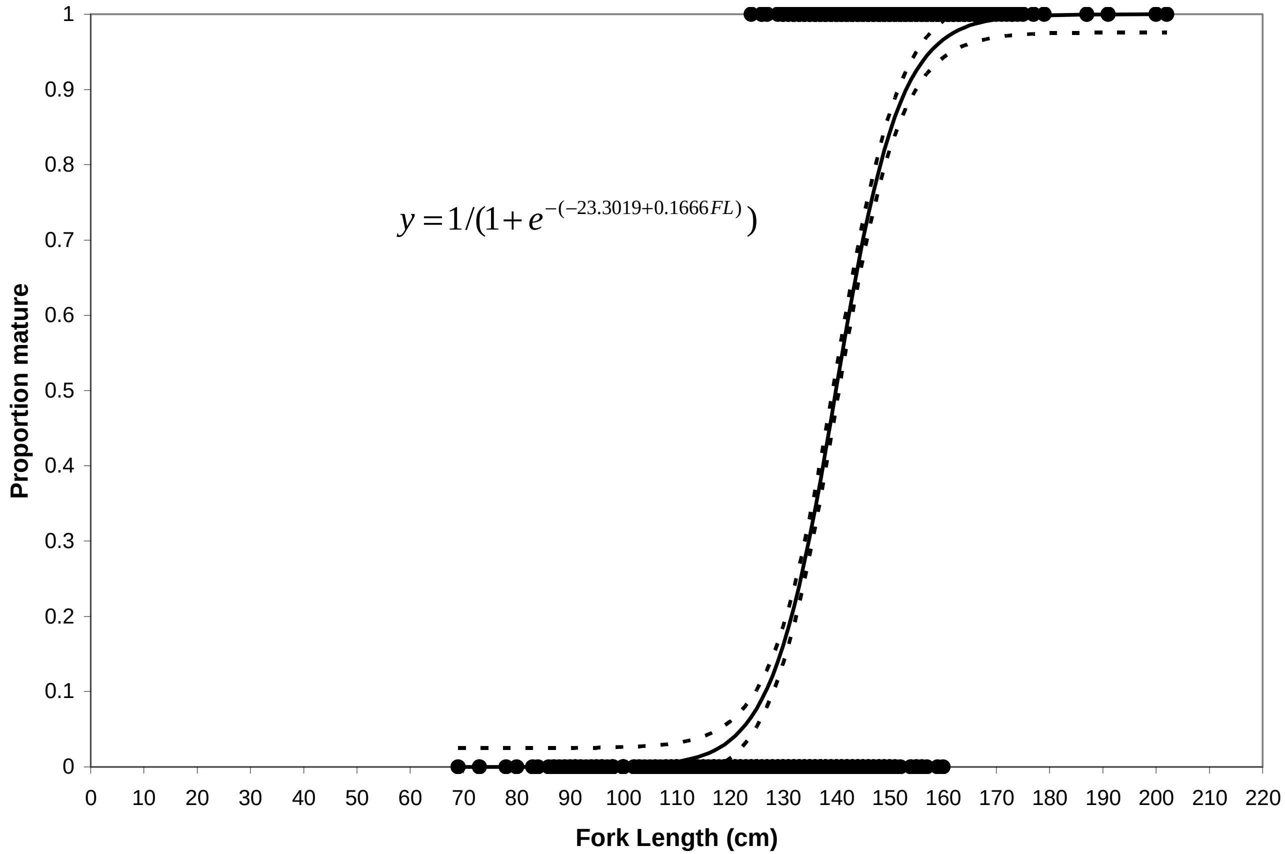


Figure 4. Proportion of male sharks mature based on proxy clasper calcification data. Solid line represents logistic model, dotted lines represent 95% confidence intervals; closed circles represent binary data.

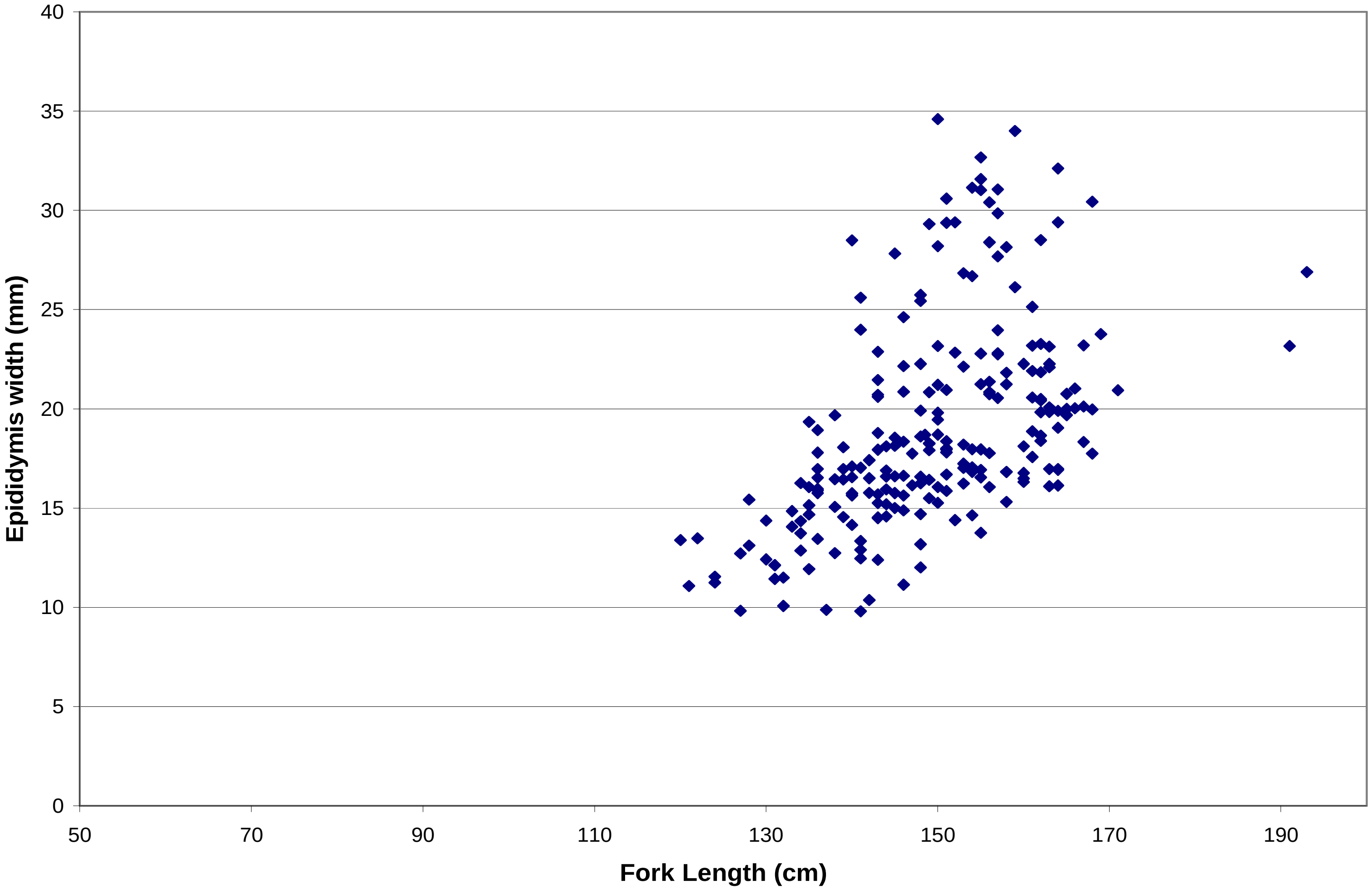


Figure 5. Epididymal width (mm) for sandbar sharks caught in all months.

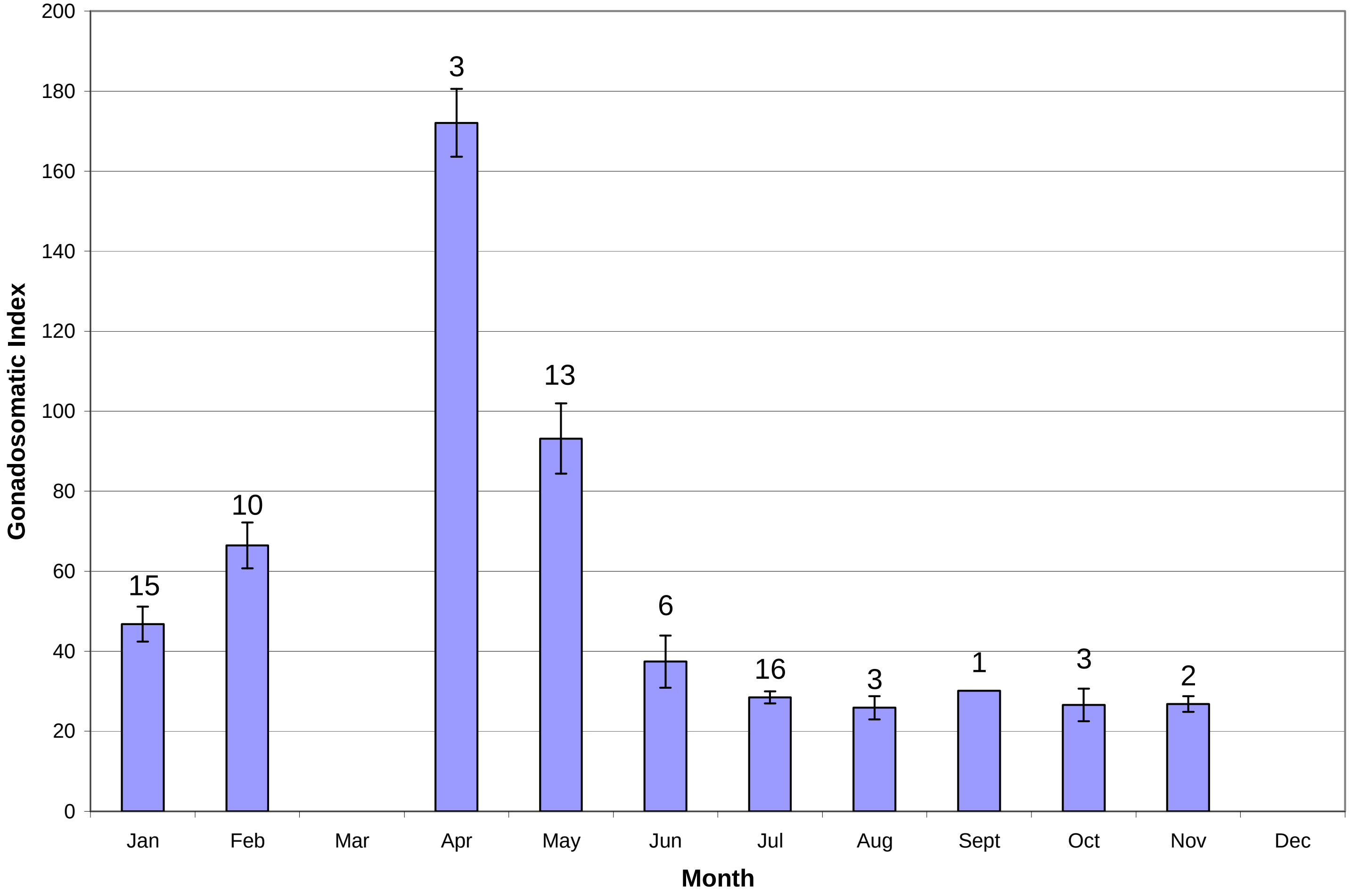


Figure 6. Mean gonadosomatic index for male sandbar sharks by month. Error bars representstandard error, numbers represent sample size.

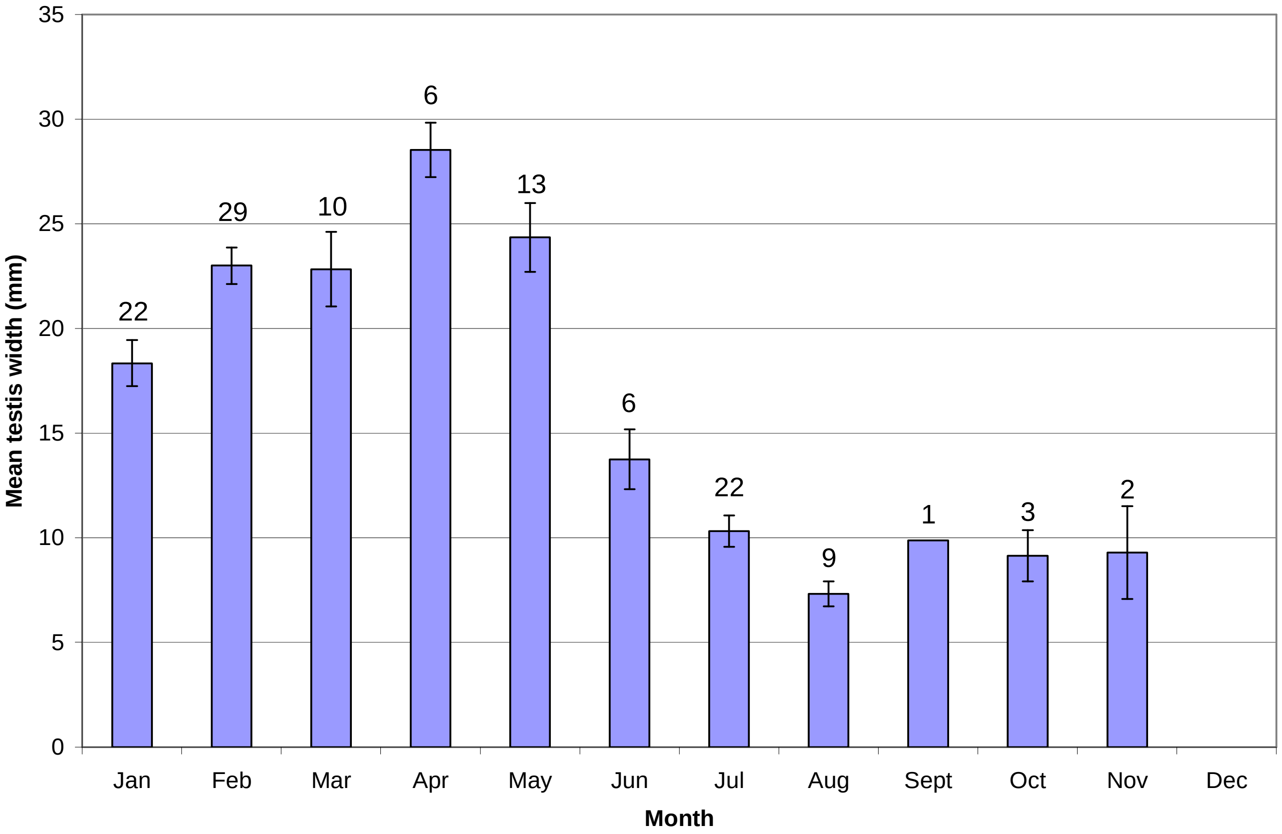


Figure 7. Mean testis width (mm) for sandbar sharks by month; error bars represent standard error, numbers represent sample size.

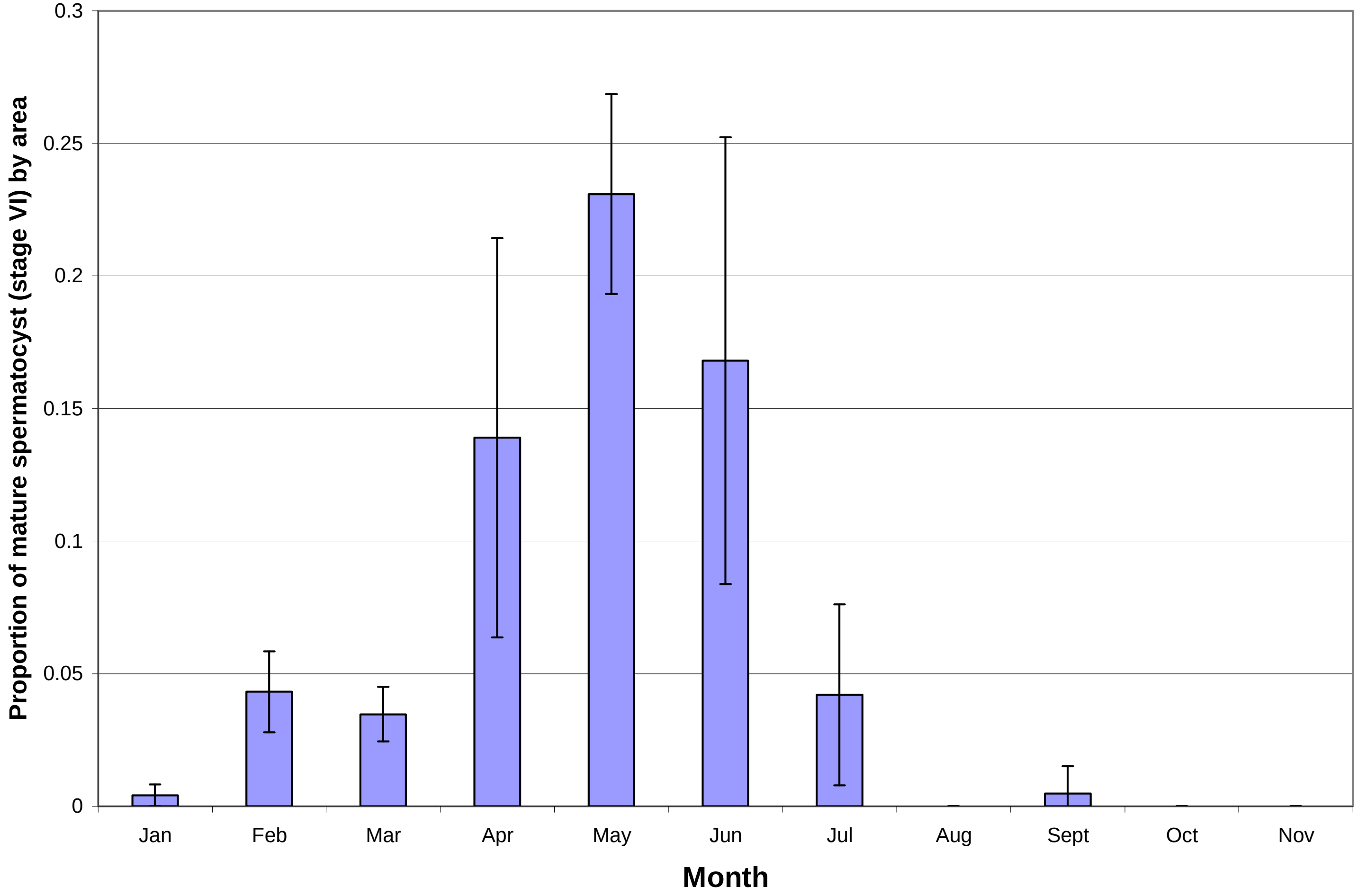


Figure 8. Proportion of mature spermatocysts (stage VI) in sandbar shark testis by month (N=3 samples per month) error bars represent ±SE.

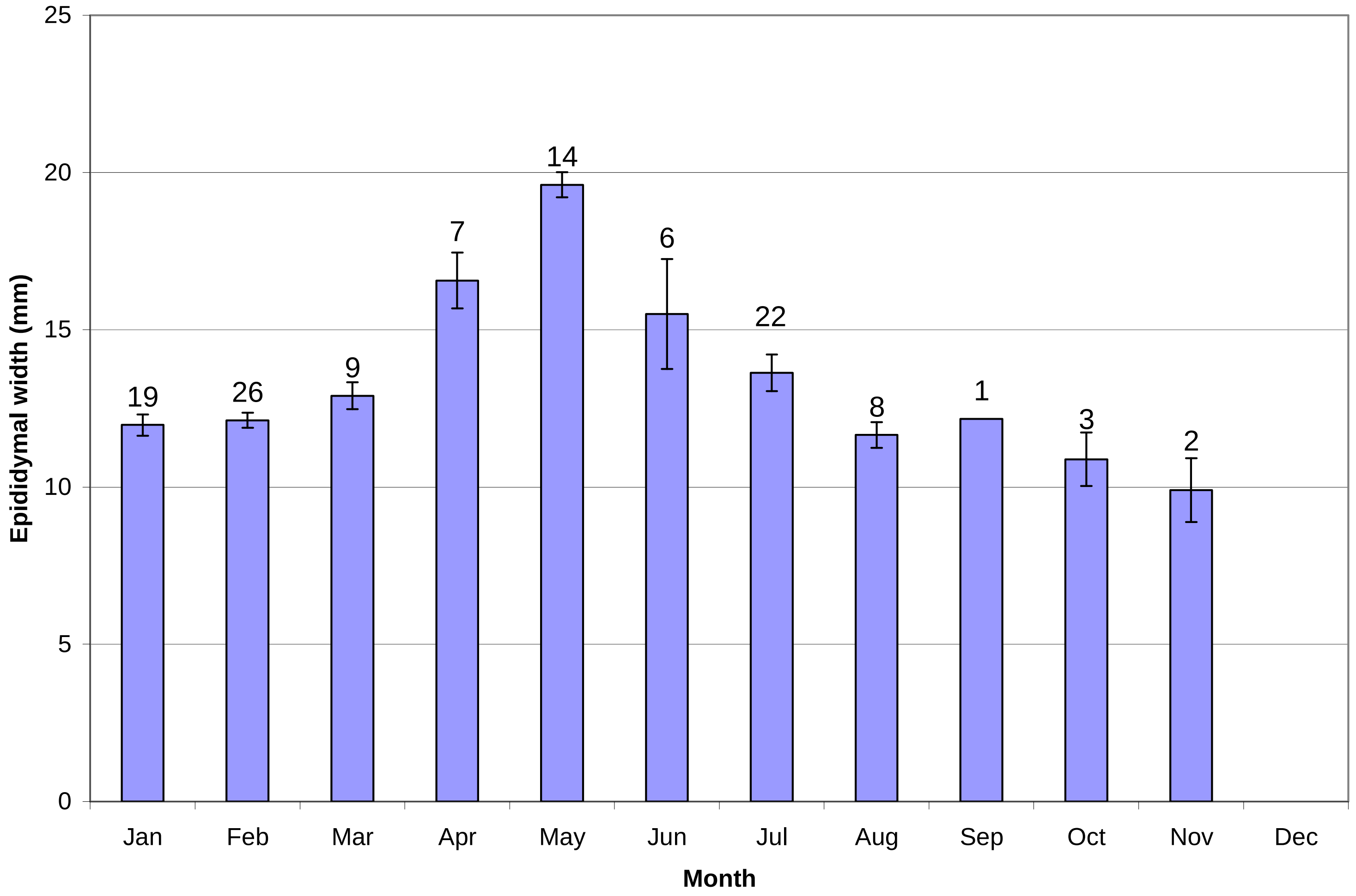


Figure 9. Sandbar shark mean (± SE) epididymal width (mm) by month for mature sharks. Numbers above bars indicate sample size.

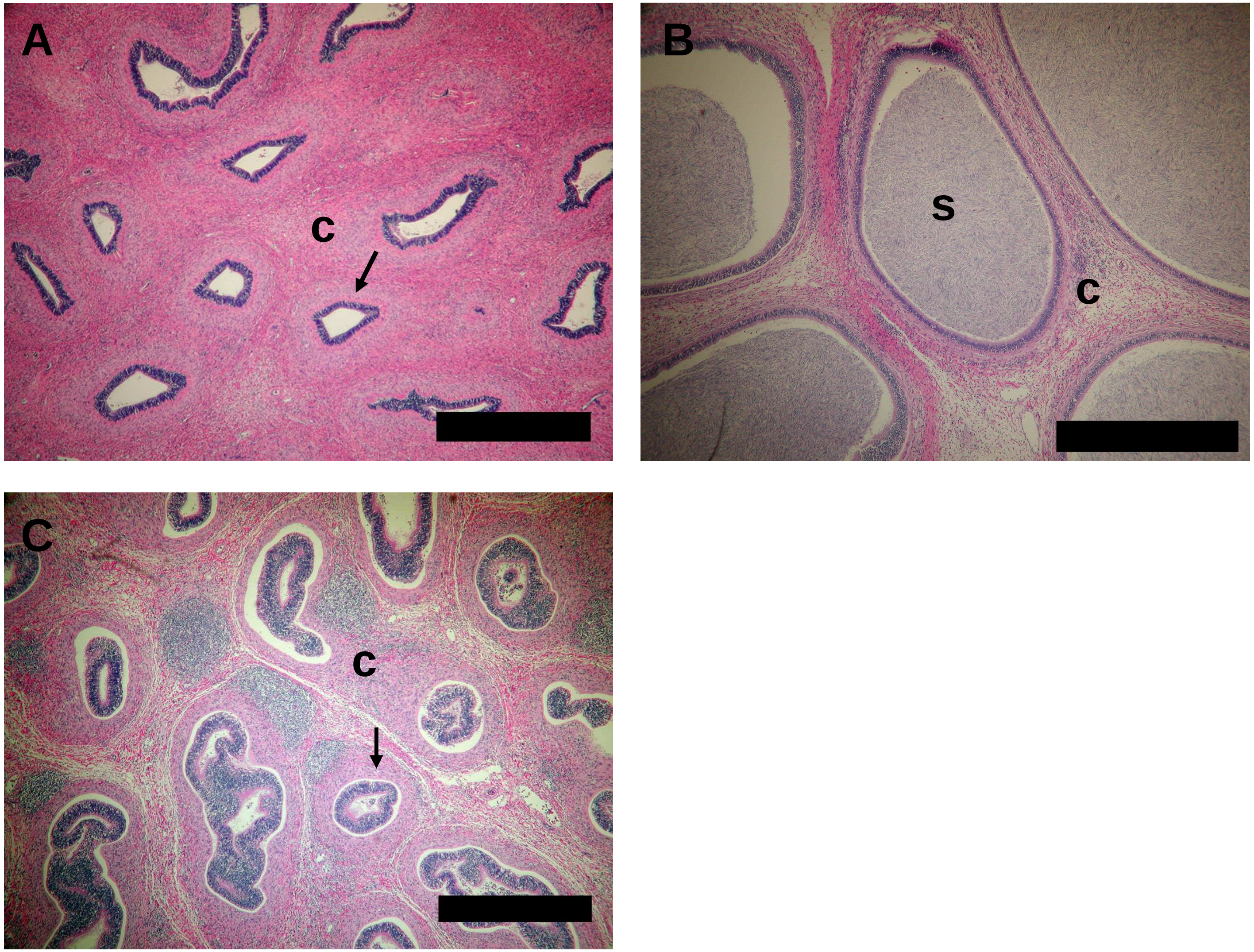


Figure 10. Tissue architecture of the epididymis of sandbar sharks: A) during reproductively inactive months (e.g. November); B) during peak sperm production (May); and C) after sperm production (June); e and arrow: epididymal duct; s: spermatozoa; c: connective tissue. Bar represents 1mm.

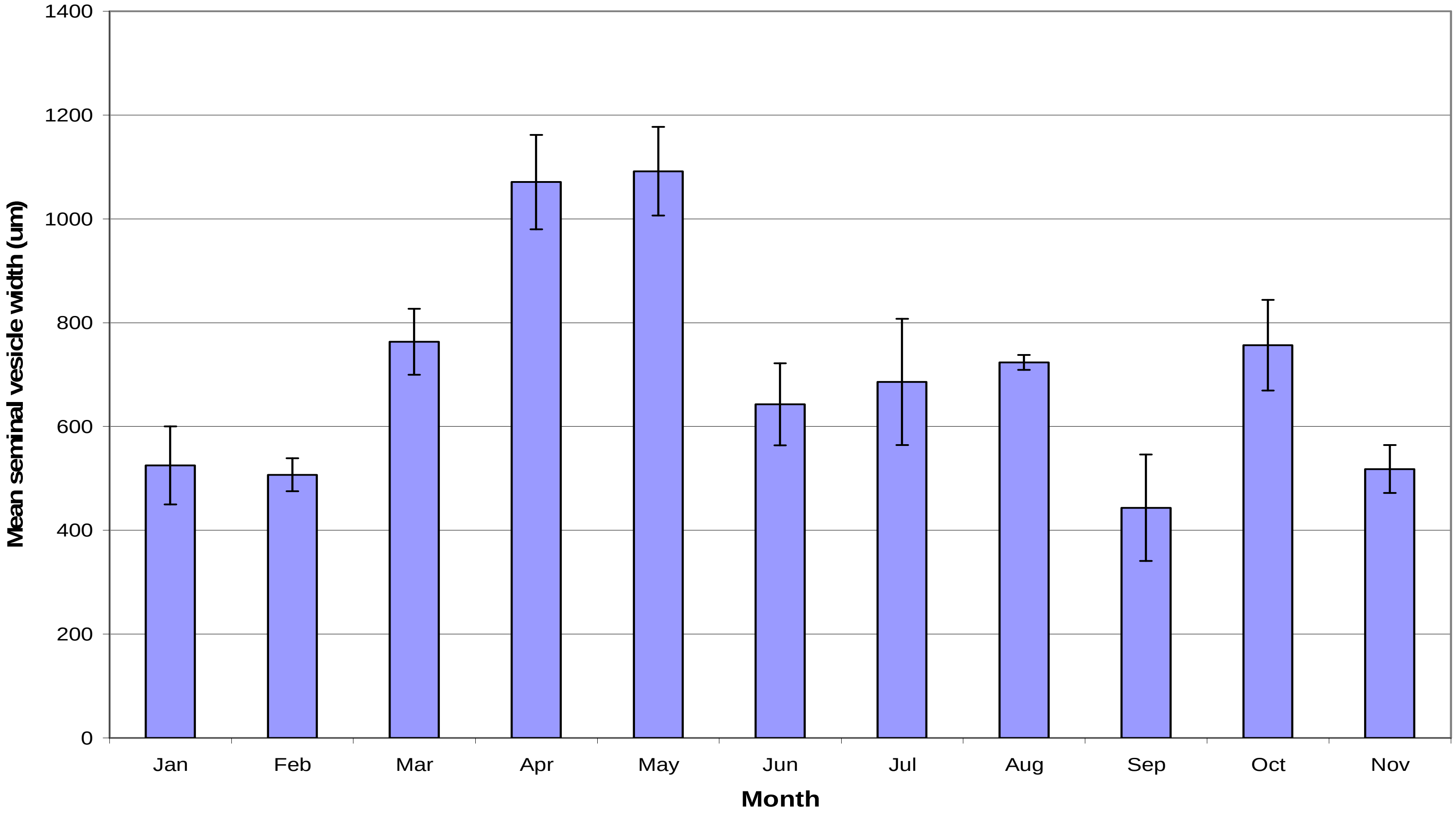


Figure 11. Sandbar shark seminal vesicle width (μm) by month (N=3 samples per month). Error bars represent standard error.

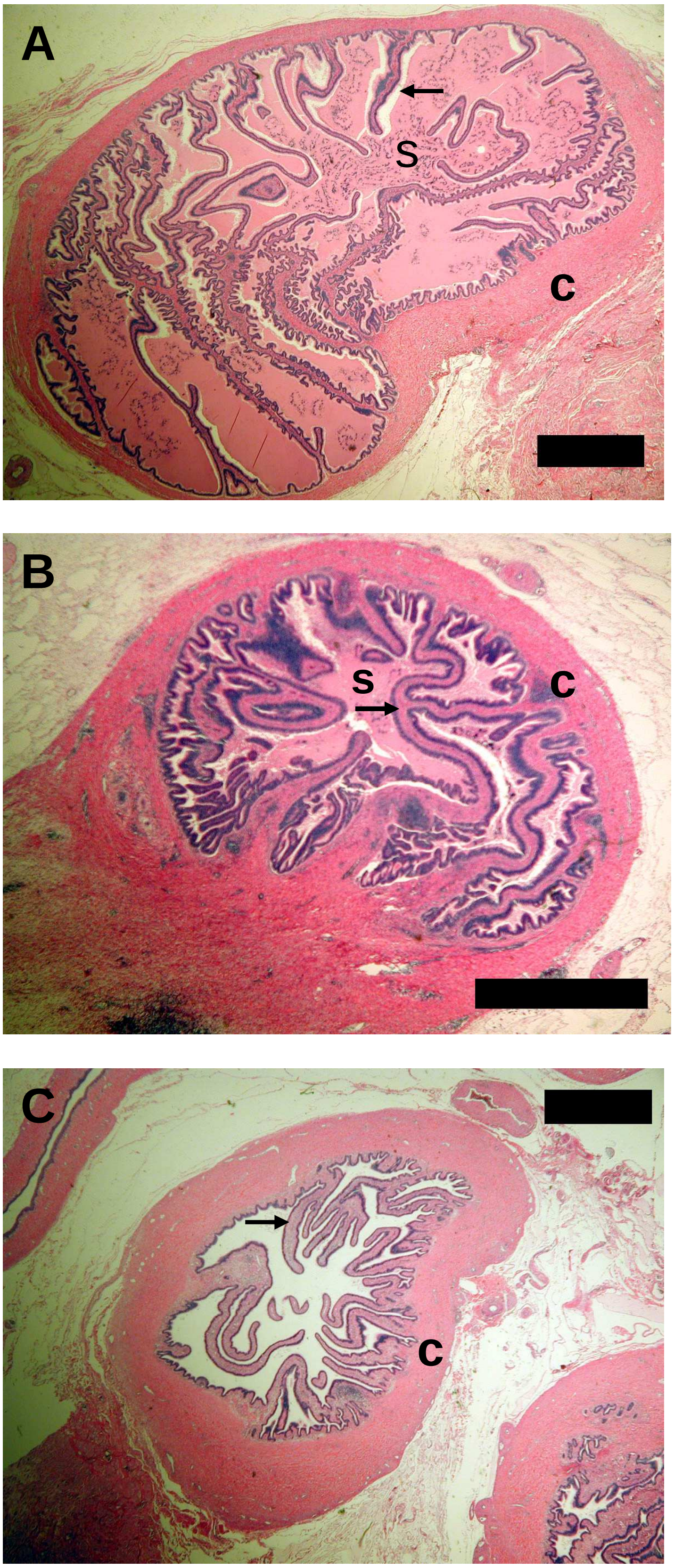
A
S
C
B
S
C
C
C

Figure 12. Seminal vesicles of sandbar sharks: A) tissue architecture during sperm storage (May; magnification 8x); B) tissue architecture during mating period (June; magnification 12.5x); C) tissue architecture during the months following mating (e.g. August; magnification 8x); lower case "c": connective tissue; s: seminal fluid with spermatozoa; arrow: seminal vesicle invaginations. Bar represents 1mm.

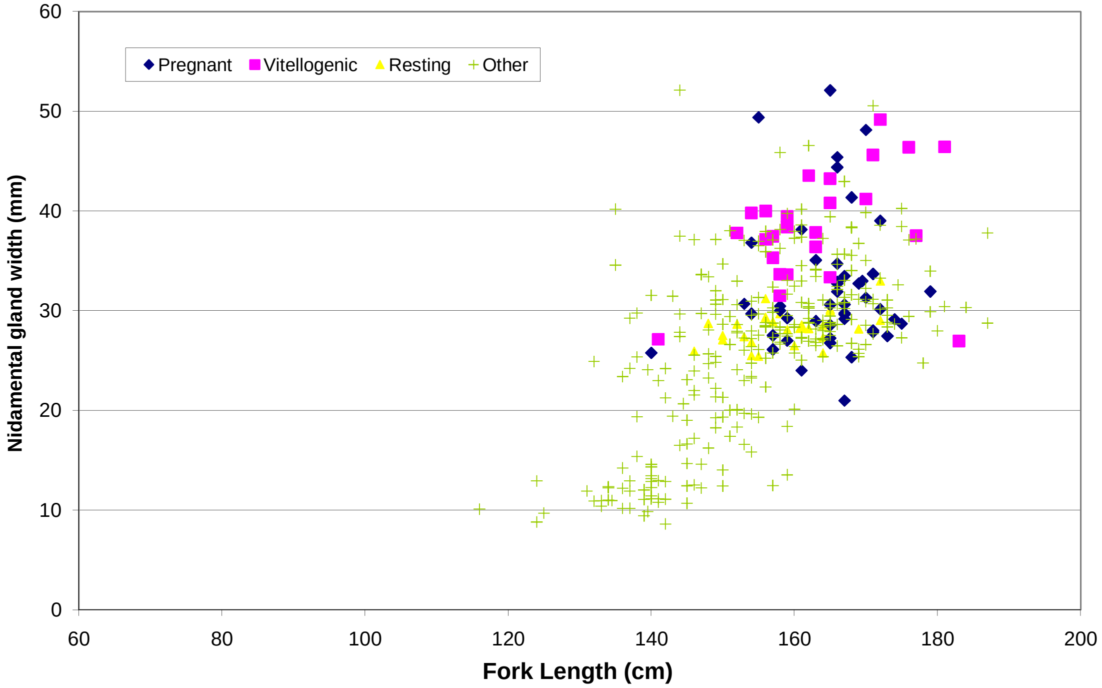


Figure 13. Nidamental gland width of sandbar sharks collected in the northwestern Atlantic Ocean and Gulf of Mexico.

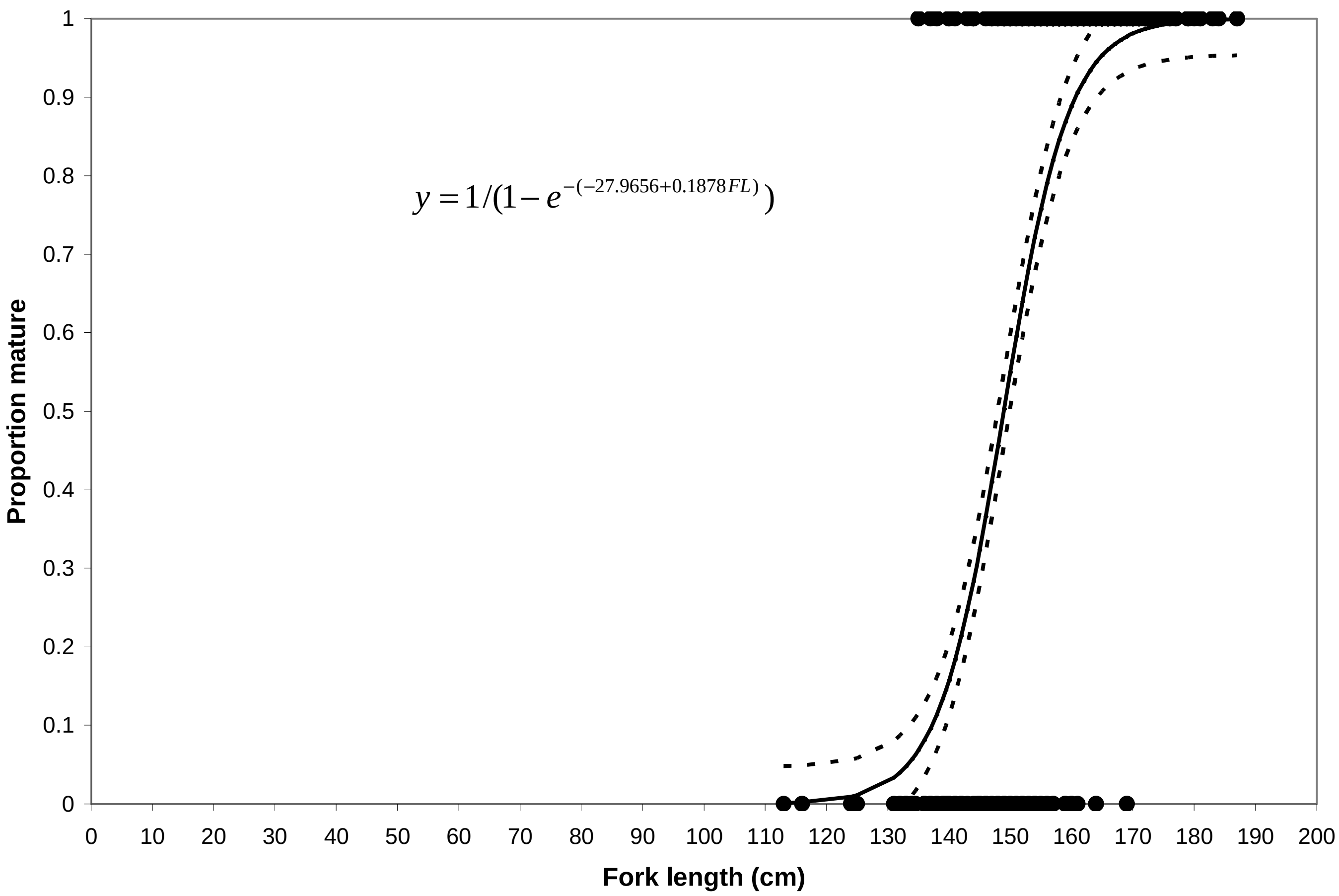


Figure 14: Proportion of female sandbar sharks classified as mature based on presence of uterine contents or large nidamental glands. Solid line represents logistic model, dotted lines represent 95% confidence intervals; closed circles represent binary data.

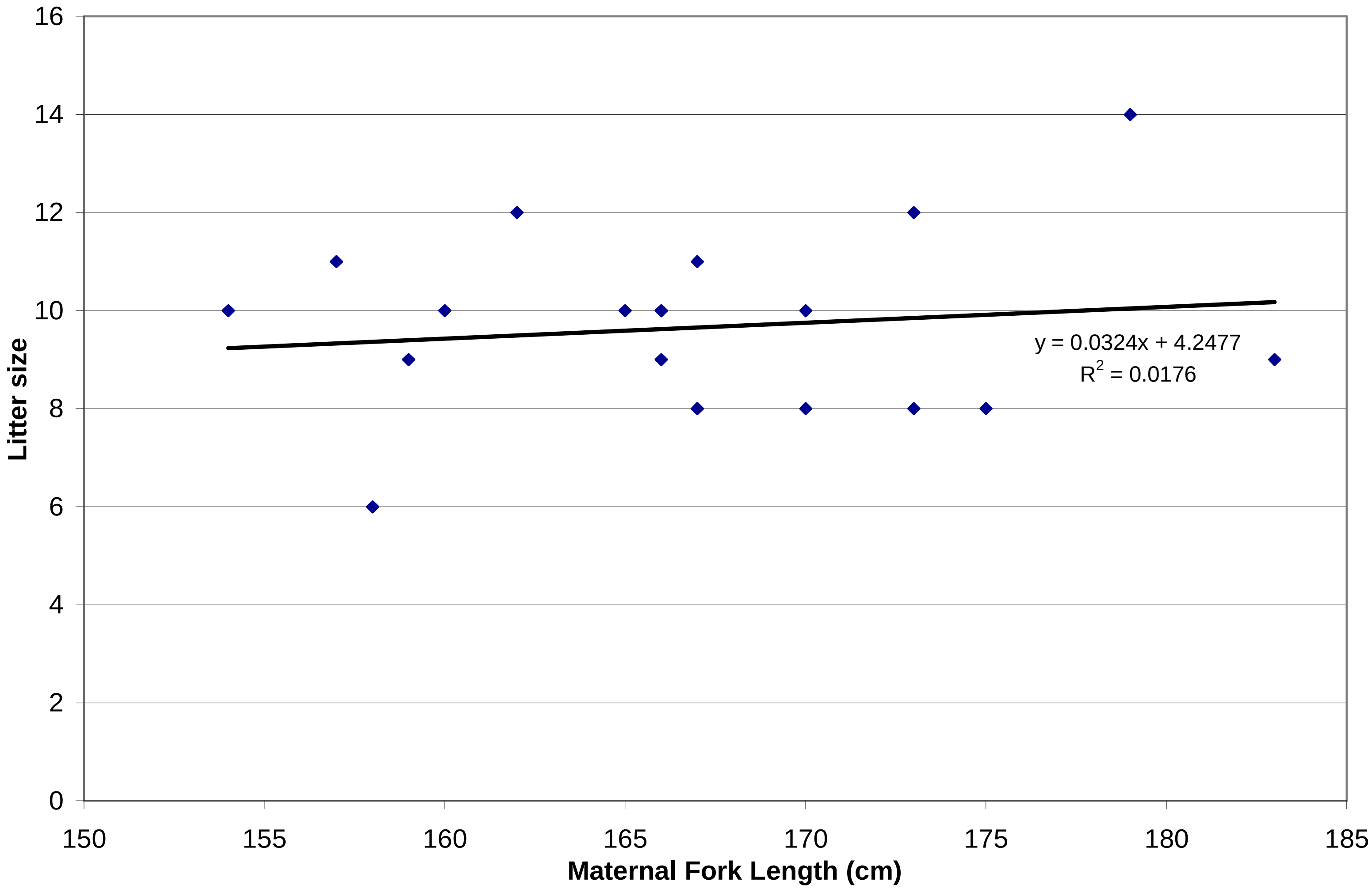


Figure 15. Sandbar shark litter size by fork length (cm) for specimens collected in the northwestern Atlantic Ocean and Gulf of Mexico.

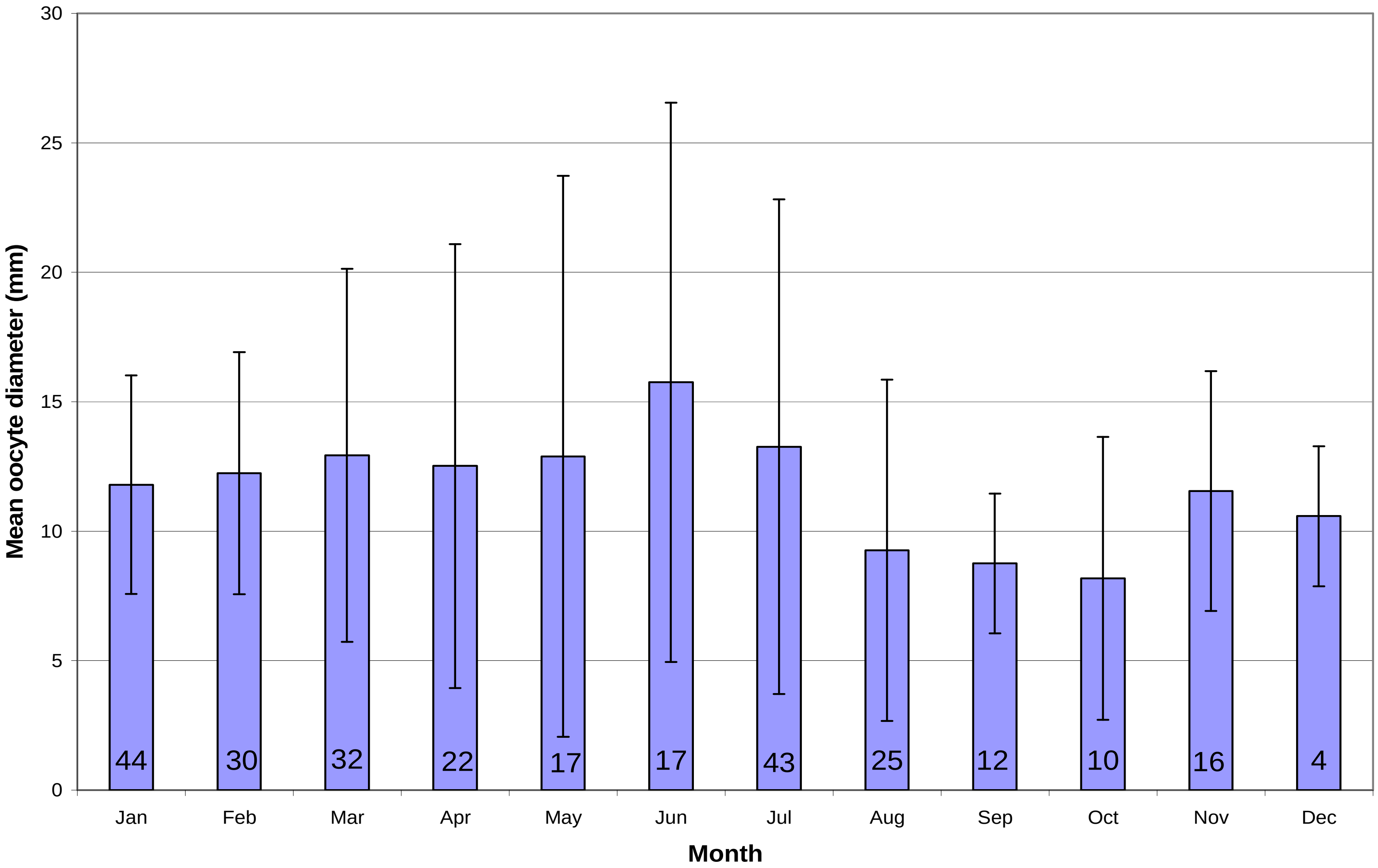


Figure 16. Non-pregnant mature sandbar shark mean oocyte diameter (mm) by month. Error bars represent standard deviation, numbers are sample size.

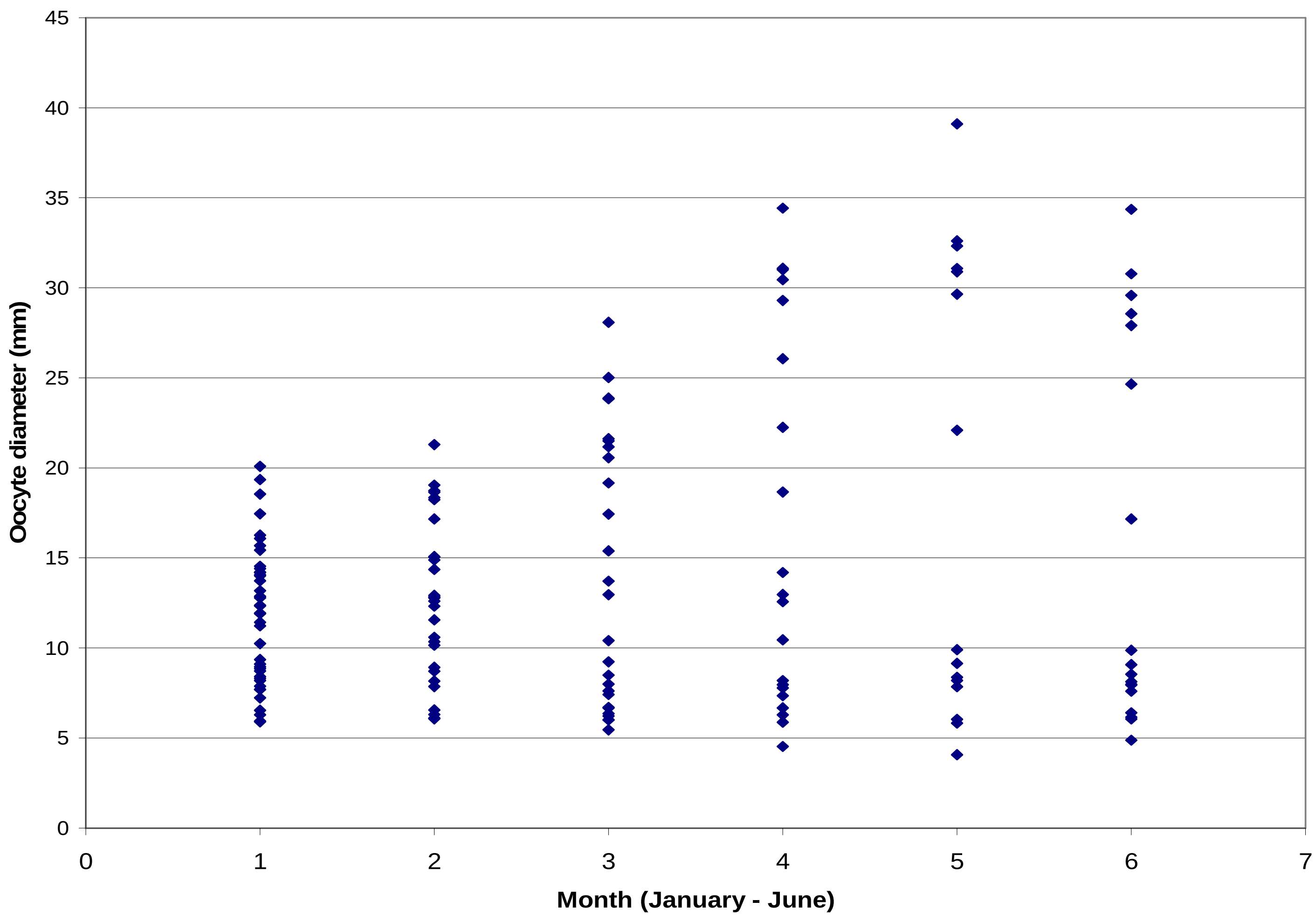


Figure 17. Oocyte diameters (mm) of individual non-pregnant mature sandbar sharks by month showing bimodality in diamters starting in March. Data points represent one measured oocyte for one mature shark.

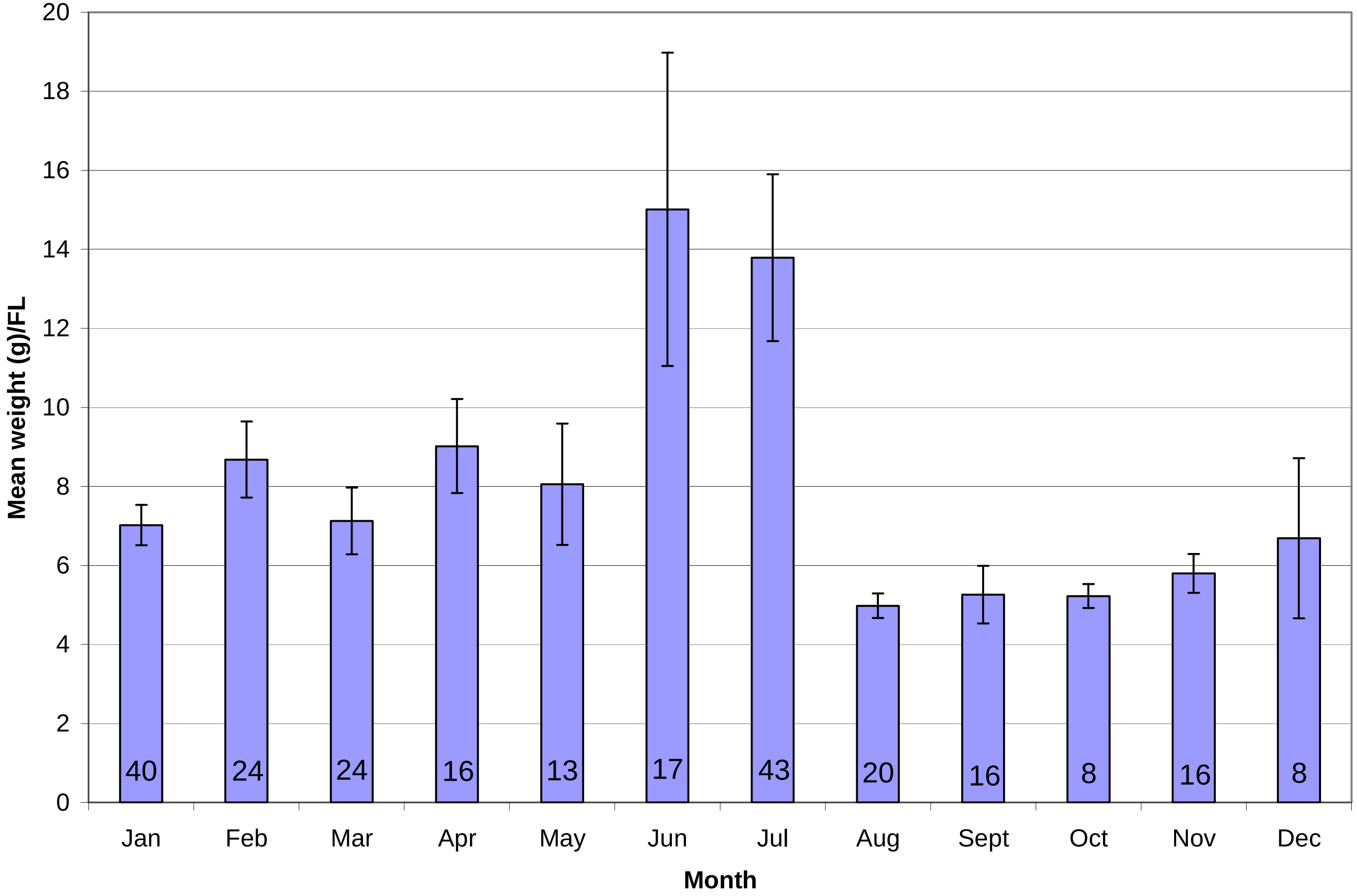


Figure 18. Non-pregnant mature sandbar shark nidamental gland weight (g/FL) by month. Error bars represent standard error, numbers are sample size.

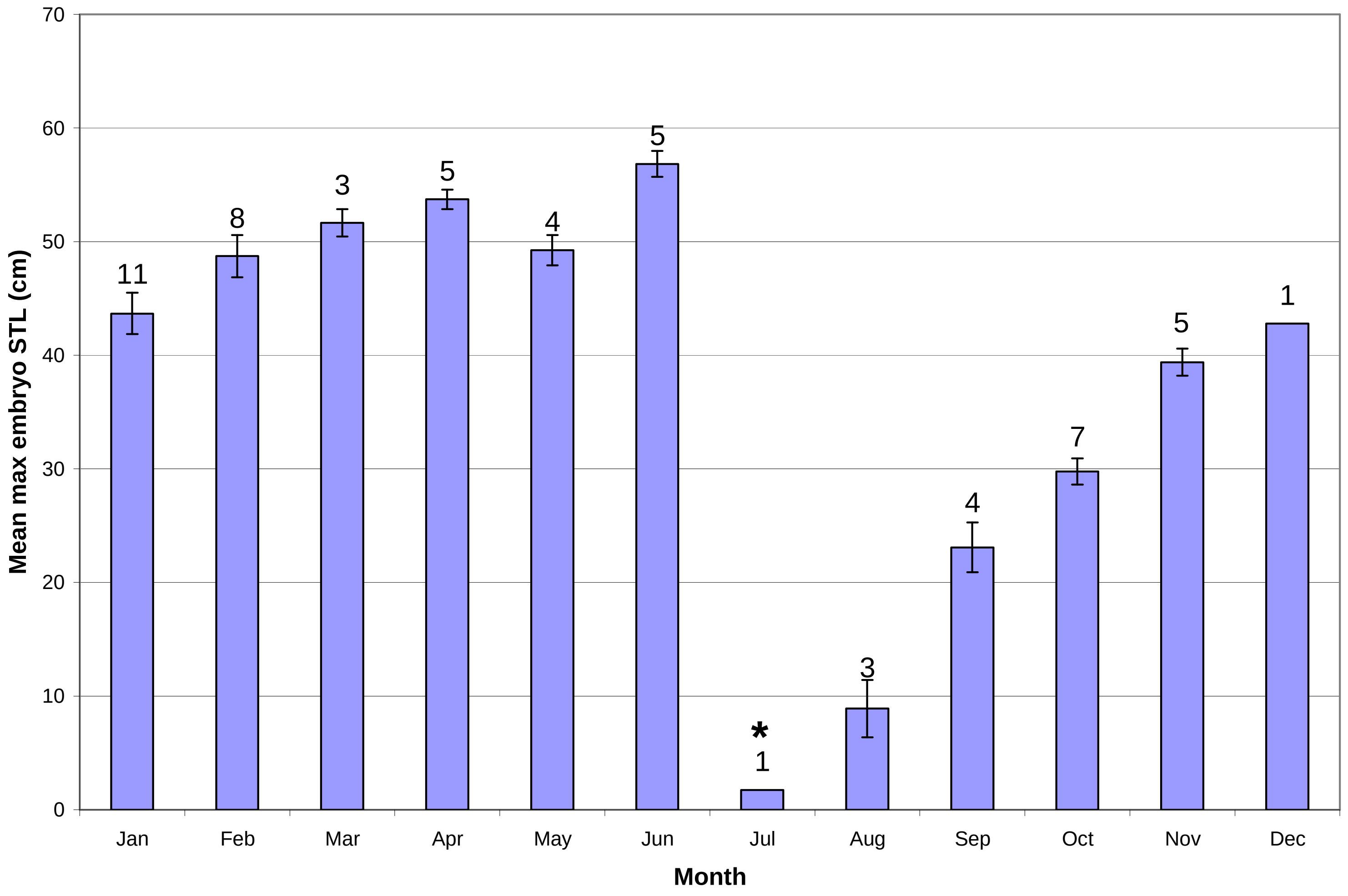


Figure 19. Monthly mean maximum embryo stretch total length of sandbar shark litters. Error bars represent standard error; numbers are sample sizes (numbers of litters examined).

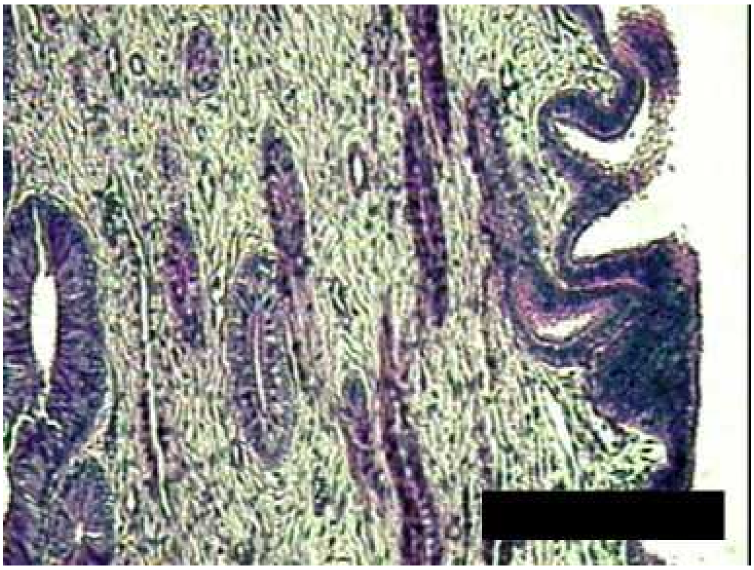

Figure 20. Histological preparation of sandbar shark nidamental gland. Area where sperm would be present if stored shown by the arrow, as well as connective tissue ( c) and outer tunic of the gland (o). Bar represents 10 micrometers.

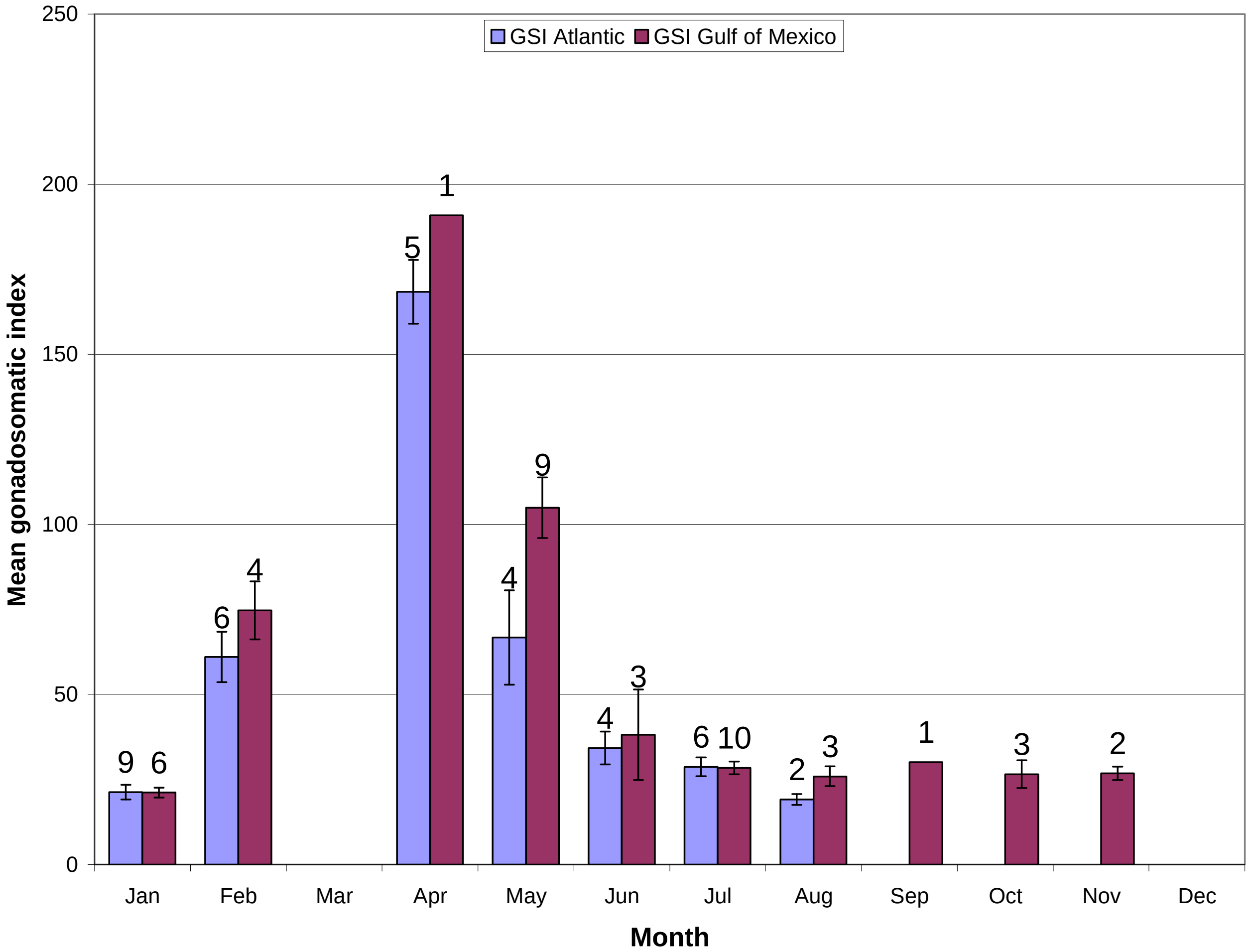


Figure 21. Mean testis gonadosomatic index by month for sandbar sharks sampled in the northwestern Atlantic Ocean and Gulf of Mexico. Error bars represent standard error; numbers are sample size.

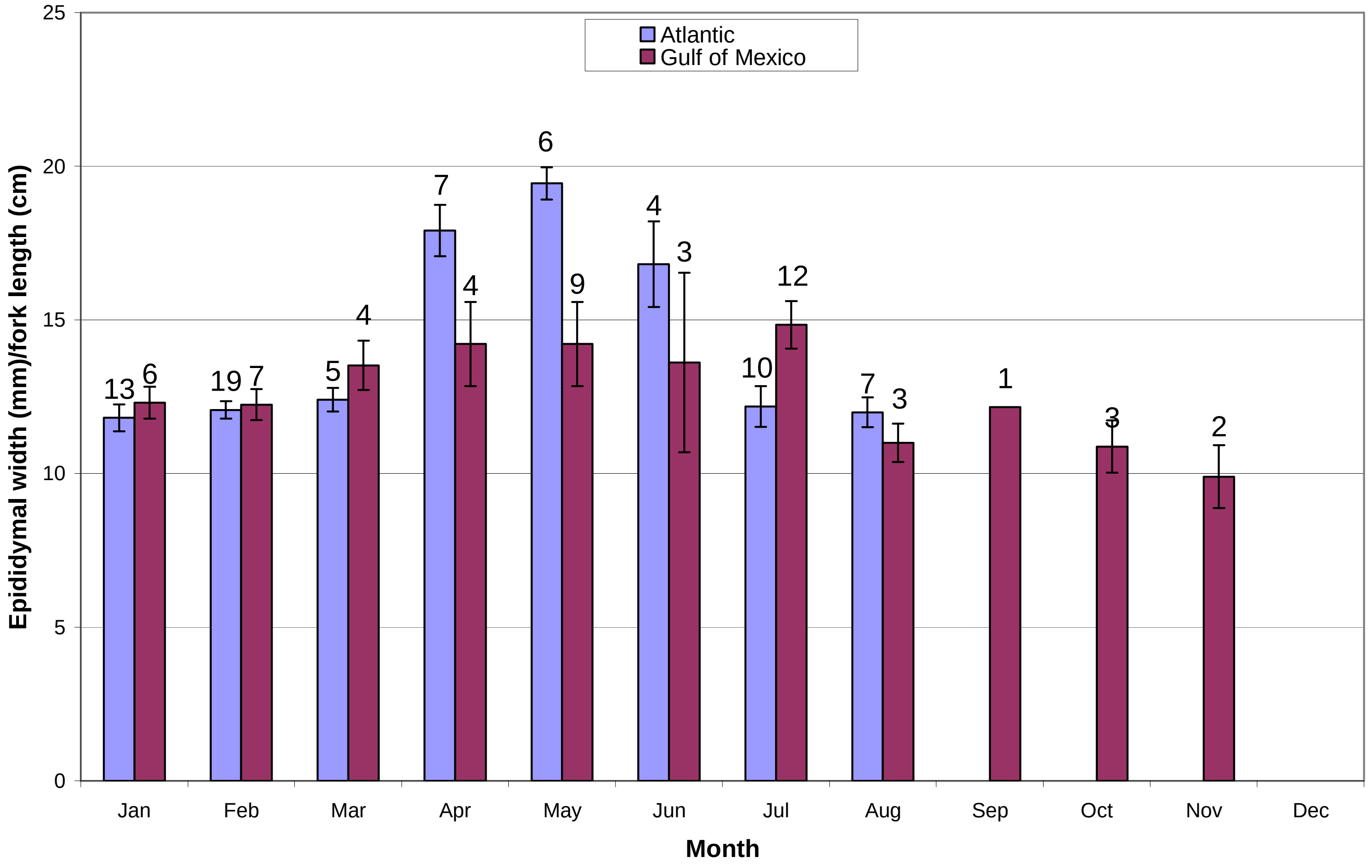


Figure 22. Mean epididymal widths by month comparing northwestern Atlantic Ocean and Gulf of Mexico sandbar sharks. Error bars represent standard error; numbers are sample size.

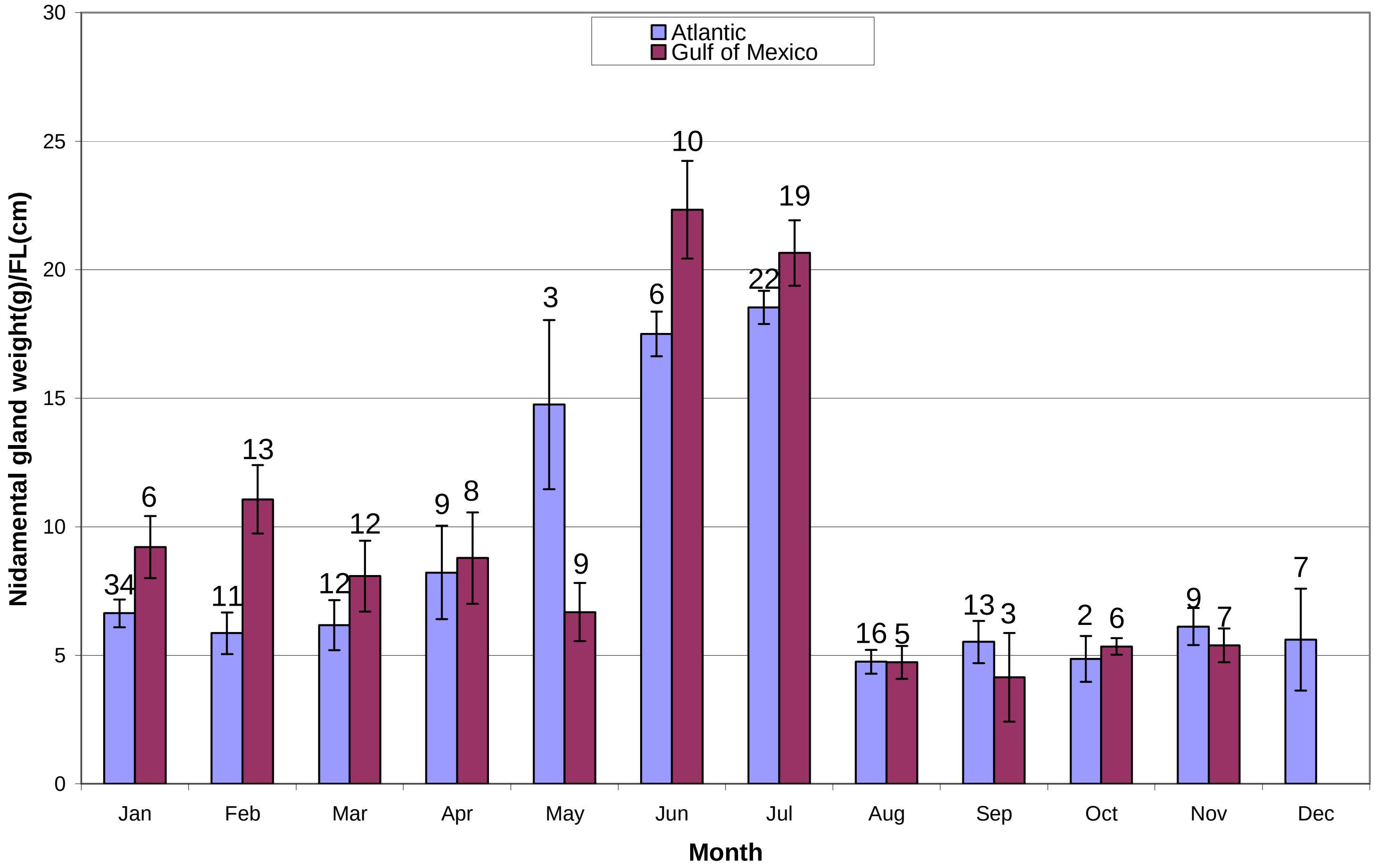


Figure 23. Mean nidamental gland weight (g) by month, comparing northwestern Atlantic Ocean and Gulf of Mexico sandbar sharks. Error bars represent standard error; numbers are sample size.

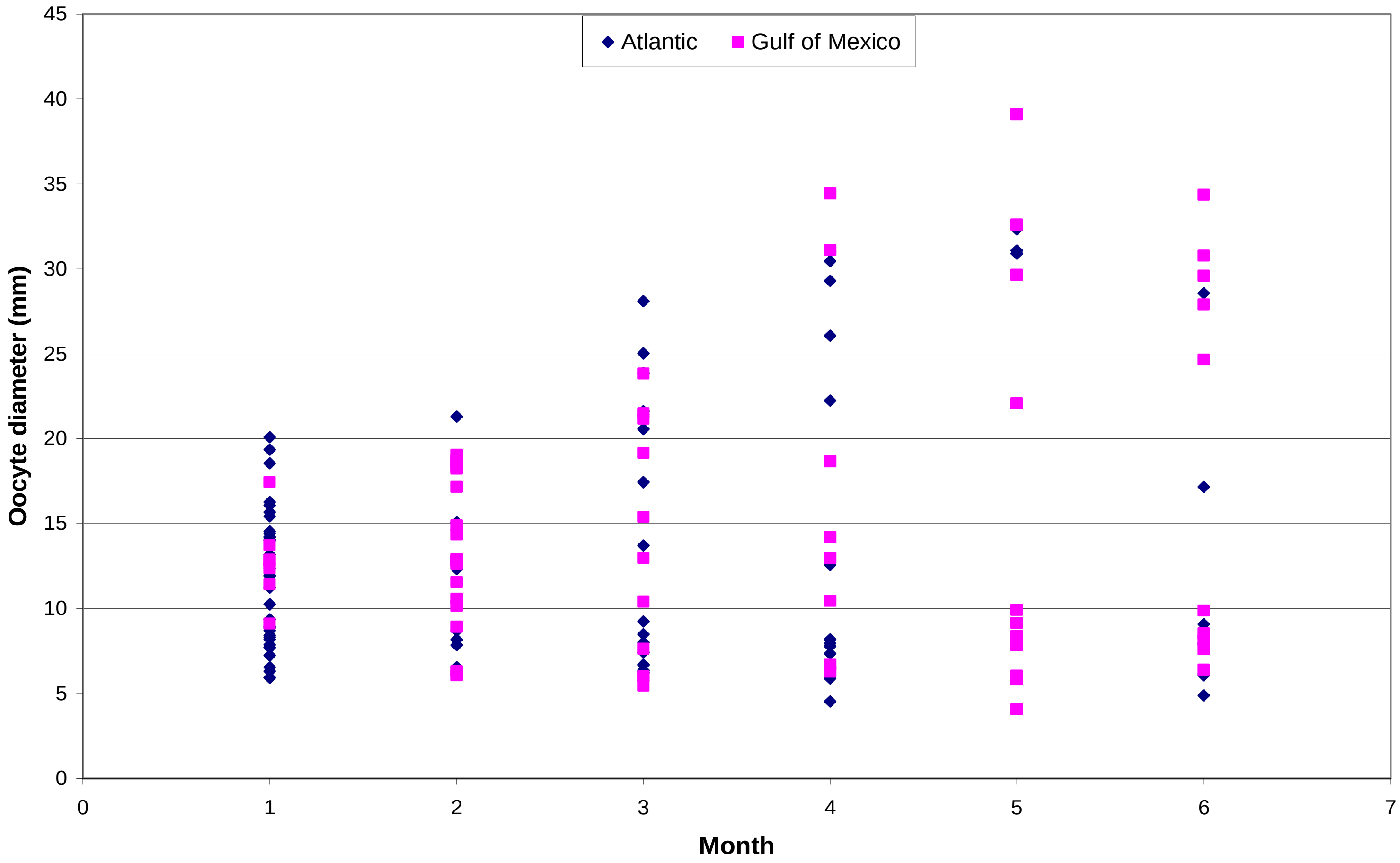


Figure 24. Non-pregnant mature sandbar shark oocyte diameters (mm) by month, comparing northwestern Atlantic Ocean and Gulf of Mexico sandbar sharks.