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Reproductive Biology of the Spinner Shark Carcharhinus Brevipinna, Off the Southeast U.S. Coast

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REPRODUCTIVE BIOLOGY OF THE SPINNER SHARK, *CARCHARHINUS BREVIPINNA*, OFF THE SOUTHEAST U.S. COAST

By

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CERTIFICATE OF APPROVAL

The thesis “Reproductive biology of the spinner shark, *Carcharhinus brevipinna*, off the southeast U.S. coast” submitted by Kristin Palmrose is approved by the thesis committee.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The spinner shark, *Carcharhinus brevipinna*, is a large coastal shark species that is common on the U.S. southeast coast and caught in both commercial and recreational fisheries. Little research has been conducted on the life history of *C. brevipinna* in the Northwestern Atlantic, presenting challenges for fishery management. This is especially true for reproductive biology, warranting a need to determine how rapidly individuals are reproducing and contributing to the population. This study aimed to characterize reproduction in *C. brevipinna* by determining size-at-maturity, reproductive seasonality, periodicity, and fecundity. This was accomplished by analyzing changes in reproductive tract morphology and histology, and plasma concentrations of gonadal steroid hormones. This was accomplished through two study components, one using archived data and the other using newly collected data. Results from both components were consistent and examined collectively. Size-at-maturity was determined to be 130 and 140 cm FL for males and females, respectively. Marked increases in testis width, epididymis width, and presence of mature spermatozoa from April - July in mature males suggest a seasonal pattern with copulation occurring during this period. Marked increases in maximum follicle diameter (MFD), oviducal gland width, and presence of enlarged vitellogenic follicles in mature non-gravid females was observed during this same period, with newly pregnant females observed in August, suggesting concurrent reproductive cycles between sexes. Pregnant females with full-term embryos observed in June indicate parturition to occur June - July following a 12-month gestation. Individual MFD of non-gravid females collected spring - summer showed at least two cohorts, as well as no vitellogenesis not occurring in gravid females suggests a non-annual periodicity. Litter size was significantly correlated with maternal length and ranged from 4 – 8 pups. This study provides new up-to-date information on the reproductive potential and biology of *C. brevipinna* aiding in stock assessment and management.

INTRODUCTION

The spinner shark *Carcharhinus brevipinna* (Müller and Henle, 1839) is a large coastal shark species that is commonly found in the Atlantic, Pacific, and Mediterranean Oceans in regions with tropical and subtropical waters (Compagno, 1984). *C. brevipinna* is known to inhabit coastal waters within the western Atlantic Ocean from Cape Cod, Massachusetts to southern Brazil (Castro, 1983; Kohler and Turner, 2019). *C. brevipinna* is considered to be a highly migratory species based on results from long-term tagging studies. Kohler and Turner (2019) found *C. brevipinna* to be present during most seasons in regions south of Cape Hatteras, NC, excluding the northern region of North Carolina's coast during winter, likely due to the cooler water temperatures at that time. The study was based on a total of 1,723 spinner sharks, which were recorded being captured in all seasons (spring, summer, fall, and winter) south of Cape Hatteras and in the Gulf of Mexico (GOM). Kohler and Turner (2019) suggested that spinner sharks are most abundant in more northerly portions of their range during summer and fall, and further south during the cooler winter and spring months. The study also found the maximum distance traveled for one individual *C. brevipinna* was 861 nmi moving between where it was tagged in South Carolina to Cape Coral, Florida in the Gulf of Mexico. This animal provided evidence of *C. brevipinna* migrating between the Atlantic Ocean and GOM and supports a single population management approach for this species on the U.S. southeastern region. Federal regulations regarding management and harvest of *C. brevipinna* are based on the assessment of a single population within the NWA and GOM (SEDAR 2006; 2020; Gulak, Enzenauer, & Carlson, 2013). As of the most recently publicized reports and regulations for *C. brevipinna*, there is no distinction between the "Atlantic" or "Gulf of Mexico" for neither state nor federal law.

As a part of a species' range, nursery habitats are described as making up a discrete portion of the range where parturition occurs and/or neonates and juveniles spend the early stages of their lives (Bonfil, 1997; Heupel et al., 2007; Springer, 1967). Castro (1987; 1993) suggested that *C. brevipinna* use geographically discrete nurseries on the U.S east coast. While the location of parturition for spinner sharks was not determined, Castro (1993) suggested that pupping occurs between May and June. Following parturition, juveniles were found inhabiting shallow coastal waters until fall or later. While studying shark populations along the coast of northeast Florida, McCallister et al. (2013) also observed neonate *C. brevipinna* in shallow nearshore waters during late summer months. Studies in the GOM found spinner sharks to use nursery habitats as neonates and juveniles (Bethea et al., 2008; Drymon et al., 2010; Kohler and Turner, 2019; Plumlee et al., 2018). These studies implied that the movement patterns of the species involve both inshore-offshore migrations, as well as latitudinal movements due to both changes in temperature and life stage.

The migratory behavior and relative commonality of *C. brevipinna* in coastal waters lends to the species being caught in numerous fisheries (Belcher and Jennings, 2011; SEDAR 11, 2006). Because of this, the spinner shark is managed under the Highly Migratory Species aggregated Large Coastal Sharks (LCS) complex. However, catch data on *C. brevipinna* has historically been challenging to assess due to frequent misidentification with other large coastal shark species, specifically the blacktip shark *Carcharhinus limbatus* (Branstetter, 1982). For example, conflicting reports were found through Springer (1963) who reported that *C. brevipinna* exhibit a spring run along central Florida's coastline and Dodrill (1977) who found no increase in the abundance of the species during the spring months in a hook-and-line survey conducted from 1974-1977. Further incidents of conflicting reports concerning misidentification

were made evident by Dodrill (1977), where he found that during times of positive sightings of *C. limbatus*, *C. brevipinna* were being caught in the same area but when large numbers of "spinning sharks" were reported in November, only *C. limbatus* were caught. These conflicting sightings, catches, and reports highlight the early misidentification issue between these two similarly appearing species. More recently, a study conducted by Gibson et al. (2019) reported the spinner shark to be the most frequently misidentified species by anglers and most commonly confused with *C. limbatus*. Therefore, obtaining accurate catch data on *C. brevipinna* is often complicated by its similarity to *C. limbatus*, making it more common for fishers to misidentify this species (Branstetter, 1982; Gibson et al., 2019).

Notwithstanding the challenges in proper identification of *C. brevipinna*, species-specific assessment of its catch in southeastern U.S. fisheries show that it can make up a modest, but still not an insignificant portion of large coastal shark landings (SEDAR, 2006). A stock assessment conducted in 2006 (SEDAR 11) reported that large coastal sharks made up 3.5 – 9.5% of commercial landings in the U.S. from 1995-2004 and, of those catches, 0.4 – 13% were *C. brevipinna*. The SEDAR 11 summary report suggested that adult *C. brevipinna* were mainly caught as bycatch on pelagic longlines and both adults and juveniles were commonly caught in nearshore waters. A report conducted by National Oceanic and Atmospheric Administration (NOAA), that aimed to characterize shark and reef fish in bottom longline fisheries within the NWA including the GOM, found *C. brevipinna* to be commonly caught by a variety of different fisheries (Gulak et al., 2013). NOAA's stock assessment and fisheries evaluation (SAFE) reports on the commercial fishery of LCS determined *C. brevipinna* made up 1.31 – 33.75% and 4.79 – 32.8% of LCS landings in the NWA and GOM regions, respectively, between 2010-2019 (NOAA Fisheries, 2016;2020). In the NWA commercial fishery, the proportion of landed *C.*

brevipinna to the total catch of LCS species has shown a gradual increase over the past decade. *C. brevipinna* made up 0.08 – 15.22% and 2.82 – 19.53% of the U.S. Atlantic and Gulf of Mexico recreational fishery, respectively, based on LCS harvests of fish per species from 2011-2019 (NOAA Fisheries, 2016;2020). Recreational harvest trends for the Atlantic region indicate potential decreases, with the most recent year being the lowest in almost a decade, whereas in the GOM harvest rates have shown fluctuations over time, with an increase in most recent years.

Despite its frequent capture and importance to fisheries, very little is known about life history traits of *C. brevipinna* in U.S. waters. This is especially true for information on reproductive biology, which is critical to understand for determining rates of population increase. To date, only limited data are available for age-at-maturity and reproductive biology in southeast U.S. populations of *C. brevipinna*. This includes one of the earliest mentions of a pregnant *C. brevipinna* carrying 10 pups along the coast of Florida, with an estimated fork length (FL) of 158 cm (based on the conversion of a total length (TL) of 190 cm using the FL-TL relationship reported in Carlson and Baremore, 2005) (Bigelow and Schroeder, 1948). For the purpose of consistency, all measurements, henceforth, are presented in FL and data from past studies not initially reported in FL were converted using the FL-TL relationship reported by Carlson and Baremore (2005). Nearly two decades later, Clark and von Schmidt (1965) reported minimum size-at maturity for male *C. brevipinna* at 156 cm FL based on clasper length analysis of 20 individuals from the central west coast of Florida. Additional information on reproduction was provided by Dodrill (1977), who sampled four *C. brevipinna* ranging from 50-177 cm FL off of Melbourne Beach, FL during late winter and summer months between 1974 to 1977. The largest individual (177 cm) sampled in this study was caught in April and found to be a pregnant female, carrying 8 pups (5 female, 3 male) ranging in size of 53.5-56 cm TL. The smallest *C. brevipinna*

(50 cm FL) was caught in June with an umbilical scar still present. Based on these limited observations, Dodrill (1977) suggested that parturition occurred between the months of April and May and hypothesized that periods of mating (late spring to early summer) and gestation (9-12 months) were comparable to other LCS in the region. Dodrill (1977) reported size at birth for *C. brevipinna* to be similar to the previously reported range of 47-60 cm FL in the western Atlantic by Springer (1960). A later study by Branstetter (1987), reported size at maturity of 140 cm and 149 cm FL for males and females, respectively. Branstetter (1980) also provided limited information on the reproductive seasonality and fecundity, reporting that copulation occurred in *C. brevipinna* from the north central Gulf of Mexico in June – July. Correspondingly, parturition was estimated to occur between May – June with a fecundity of 6 – 12 pups, suggesting an 11- to 12-month gestation period. Castro (1993) also suggested parturition occurs during summer months along South Carolina's coast with pups ranging from 47-60 cm FL. However, reproductive periodicity of *C. brevipinna* has not been confirmed for populations inhabiting the NWA. These studies provide some data on the reproductive biology of *C. brevipinna*, but they were limited by small sample sizes of 20 individuals or fewer and to our knowledge, no up-to-date study has been conducted in the NWA region in nearly three decades.

Although little data are available on reproduction for U.S. populations of *C. brevipinna*, aspects of its reproductive biology have been more thoroughly investigated in other regions. For example, Allen and Cliff (2000) studied *C. brevipinna* along the eastern coast of South Africa and found size at maturity for males and females to be 163 cm and 168 cm FL, respectively, and that mature females exhibit a biennial reproductive cycle with a gestation period of 13 –18 months. In the Mediterranean Sea, Capape et al. (2003) suggested a slightly smaller size at maturity of 142 cm FL for males and 163 cm FL for females, with a similar gestation period of

13 – 14 months. Like South Africa populations, Joung et al. (2005) determined that female *C. brevipinna* near Taiwan exhibit a biennial reproductive cycle with a slightly shorter gestation period of 10-12 months. Size at maturity for this population was estimated to be 185 cm FL and 183 cm FL for females and males, respectively. In different regions along the eastern coast of Australia, Sumpton et al. (2010) estimated size at maturity of females to occur around 166 cm FL, whereas Geraghty et al. (2015) found size at maturity to be 174 and 187 cm FL for males and females. *C. brevipinna* gestation period along eastern Australia was determined to be 12 months or longer (Geraghty et al., 2015). Litter size in all the regions varied widely between 3 – 17 pups (Geraghty et al., 2015; Joung et al., 2005; Sumpton et al., 2010). Data from these studies (i.e., reproductive seasonality, period of gestation, and litter size) seemingly agree with the reproductive parameters determined for the U.S. population of *C. brevipinna* and indicate the species may reproduce biennially. However, there are variations in size at maturity for *C. brevipinna* among studies conducted within and outside of the NWA. U.S. studies found males to mature at 140 and 156 cm FL and 149 cm FL for females, whereas the studies conducted outside of the U.S. found larger sizes for maturity at 142-183 cm FL for males and 163-187 cm FL for females. Because reproductive traits can vary among populations, it is critical to assess the reproductive biology of *C. brevipinna* within the NWA to determine how rapidly individuals are reproducing and contributing offspring to the population. In doing so, up-to-date data can facilitate and enhance species-specific management strategies for the U.S. population of *C. brevipinna*. The goal of this study was to characterize reproductive biology of *C. brevipinna* along the southeastern coast of the U.S. and provide data for use in fishery management. Our specific objectives were to determine size at maturity, seasonal patterns in reproduction, reproductive periodicity, and fecundity. This was accomplished by analyzing seasonal changes in

reproductive tract morphology, along with conducting histological examination of gonads to confirm morphological assessments. We also examined changes in plasma concentrations of gonad steroid hormones (i.e., 17-β estradiol (E_2) and testosterone (T) in females and males, respectively), which have been shown to be correlated with sexual maturity and reproductive events in past studies (Awruch, 2013; Gelsleichter and Evans, 2012; Gelsleichter et al., 2002; Manire et al., 1995; Tricas et al., 2005). This was done to determine if reproductive hormone analysis could be useful as a non-lethal approach for assessing reproduction in *C. brevipinna* in future studies. Based on previously described studies, we anticipated that *C. brevipinna* would exhibit maturity ranges of 149-187 cm FL and 140-184 cm FL for females and males, respectively, a biennial reproductive cycle, an 11- to 12-gestation period, and a litter size ranging between 6-12 pups.

METHODOLOGY

Study approach

Because of limited catch of *C. brevipinna* in most fishery-independent shark surveys conducted on the U.S. Atlantic and Gulf coasts, the study examined reproduction in this species through two study components. In the first component, we examined morphology and histology of archived samples of reproductive organs obtained from 65 Gulf and Atlantic *C. brevipinna* (n = 17 males, n = 48 females) collected from 2003 – 2005 by the University of Florida's Directed Shark Fishery Observer Program. This allowed us to generate data on size at maturity and reproductive seasonality in the species, as well as use histological observations to ground-truth results based on changes in gonad morphology. Since NWA and GOM *C. brevipinna* are

currently considered to represent a single population, as previously discussed, all data were grouped with no differentiation between basins.

The archival data obtained from the UF observer program lacked certain key metrics needed for a comprehensive analysis of shark reproduction, including clasper size and condition in males and pregnancy status and litter size in females. Therefore, to address these data needs as well as obtain more recent data on spinner shark reproduction, component 2 of the study focused on obtaining data on reproductive tract morphology and histology, as well as plasma concentrations of gonadal steroid hormones (which vary in relation to sexual maturity and reproductive stage), in newly collected samples from fishery-independent surveys and fishery-dependent sampling conducted along the southeast U.S. coast.. Since data from the two study components represented two discrete time periods, they were not combined; however, commonalities as well as differences between their results were characterized.

Component 1

Morphology

As mentioned above, archived gonad and reproductive tract samples obtained from commercial fishery-caught specimens between 2003 and 2005 were used for morphological and histological analysis. Metadata for these samples included the month and location of capture, stretched total length (STL) of individuals (which as previously mentioned were converted to FL using the conversion provided by Carlson and Baremore, 2005), and preserved reproductive organs, which were used to obtain information on size-at-maturity, reproductive seasonality, and periodicity. All archived organs were initially preserved in 10% seawater-buffered formalin and eventually stored in 70% ethanol after the initial dissection of the animal. When present, the

entire reproductive tract and gonads were examined for each specimen. Sexual maturity could not readily be determined in all individuals because of the lack of data on clasper size and calcification in males, and occasional incomplete nature of reproductive tract samples. Nonetheless, when possible, gonad size, condition, and other aspects of reproductive tract morphology (e.g., presence of mature spermatozoa / sperm in the epididymides and seminal vesicles in males, oviducal gland size in females) was used to assess maturity. It is well known in elasmobranchs that marked inflections of oviducal gland width in females and testis width in males indicate a period of maturation. To determine reproductive stage, morphological measurements were collected from all preserved reproductive organs. For component one, reproductive stage was categorized by ovarian morphology and uterine contents / pregnancy. Due to many of the archived samples in component one not including the entire reproductive tract, no uteri, empty uteri, and no data on pregnancy status, all animals were assumedly non-pregnant.

To assess reproductive stage in males, maximum testes width and head epididymis, or the widest portion of the epididymis, was measured (mm). The head epididymis, which is the broadest portion of the epididymis, is known to receive spermatozoa by way of the efferent ducts from the testes and fluctuates in size based on maturity and reproductive stage. Since size of these organs are known to vary significantly in relation to body size in mature males, testis width and epididymis width were expressed as percentages of FL, henceforth described as testis index (TI = testis width/FL × 100) and epididymis index (EI = epididymis head width/FL × 100).

Maximum follicle diameter (MFD) in ovary samples and oviducal gland width (OGW), at the widest portion, were measured (mm) on a straight-line basis in female *C. brevipinna*. Ovarian morphology was evaluated by determining the presence and number of pre-vitellogenic,

vitellogenic and atretic follicles. When uteri were present in the archived samples obtained from UF it was dissected to determine if ovulated ova or developing embryos were present; however it was found that all uteri were empty.

Histology

Sub-samples (~2 mm in tissue width) of archived reproductive tract and gonad samples were obtained for histological analysis. Sub-samples were dehydrated in an ascending, graded series of reagent alcohols (80-100%), cleared in a limonene-based solvent (CitriSolv, Fisher Scientific, Fair Lawn, NJ), and processed for routine paraffin histology following techniques described by Gelsleichter et al. (2002, 2003). 5-µm transverse histological sections were prepared using a rotary microtome. Tissue sections were adhered to poly-L-lysine coated slides and stained using Harris hematoxylin and eosin to analyze the cellular architecture using a compound microscope. Histological sections of testis and epididymis were examined to characterize stages of spermatogenesis in male *C. brevipinna*, as Parsons and Grier (1992) did for male bonnetheads, *Sphyrna tiburo*. For this study, histological sections of testes were categorized into 5 stages: pre-meiotic, meiotic, post-meiotic, evacuated and degenerated spermatocysts. Histological evaluation of the oviducal glands in females was examined to determine if sperm storage occurred.

Data Analysis

Due to limited collection of samples, especially during winter months, morphological and histological data from all years were combined. The primary measurement used for analysis was FL, because it is largely preferred for use in fishery science especially in species with a prominently forked caudal fin, as well as for use in measurements of sharks with damage to the

top lobe of the caudal fin. Morphological data were compared by FL of the corresponding animal to assess size at maturity. Because of the limited amount of data with maturity indicators (i.e., clasper size and condition), supplemental binomial maturity data (0 = immature, 1 = mature, n = 208) was obtained from surveys conducted by the National Marine Fisheries Service, South Carolina Department of Natural Resources (SCDNR), and the University of North Florida (UNF) were used to generate a maturity ogive to further evaluate size at maturity for males. The collaborative collection effort among these institutions led to a total of 208 individual males being assessed for maturity. Due to limitations of a smaller sample size, a logistic curve could not be fitted to female maturity data; therefore, size at maturity was further assessed by comparison to similar carcharhinid species. *Carcharhinus plumbeus* (sandbar shark), C. *limbatus*, *Carcharhinus isodon* (finetooth shark), and *Carcharhinus falciformis* (silky shark), which inhabit the same region as this study, were comparatively examined to determine which species most closely resembles our current knowledge on the life history and biology of *C. brevipinna*. Based on life history similarities and numerous studies, an average size at maturity from *C. plumbeus* and *C. limbatus* was determined to be 130.7 and 142 cm FL for males and females, respectively (Branstetter, 1987; Baremore and Hale, 2012; Castro, 1983; Castro, 1996; Carlson et al., 2006; Piercy et al., 2016). The MFD and OGW in females and TI and EI in males were compared by month to examine reproductive seasonality. Although seasonal changes in MFD and OGW are generally assessed separately for gravid and non-gravid females, since there were no data on pregnancy status for individuals without the uteri present all mature individuals were assessed together.

All data sets were assessed for normality and equal variances, and non-parametric alternative were used if datasets failed assumptions for use of parametric statistical tests.

Variations in mean TI and EI were analyzed using one-way Analysis of Variance (ANOVA) test followed by the Tukey *b* test. Variations in mean monthly MFD were analyzed using the Kruskal–Wallis test followed by Dunn's post hoc test. Variations in mean monthly OGW were analyzed using one-way ANOVA followed by the Tukey *b* test.

Histological analysis was conducted using light microscopy as described in prior studies (Gelsleichter et al., 2003). The testicular stages described by Parsons and Grier (1992) were used as a guide to establish similar criteria for *C. brevipinna* to qualify temporal changes in testicular structure and spermatogenesis. Spermatogenesis was also characterized in a semi-quantitative fashion by determining the proportion of the testes undergoing different stages of spermatogenesis (Pre-Meiotic, Meiotic, Post-Meiotic, Evacuated, Degenerating) along a straight line extending from the germinal zone to the efferent tubules, as conducted by Maruska et al., 1996. The male head epididymis was qualitatively analyzed for the presence of spermatozoa. Histological data were used to provide further insight into what reproductive events were occurring and when they were occurring based on time and size of capture.

Component 2

Animal collection

Samples for the second study component were obtained within the U.S. Atlantic southeastern region of Cape Hatteras, North Carolina and Jacksonville Beach, Florida between the years of 2014 to 2020. These newly collected samples were obtained from fishery-independent surveys or fishery-dependent sources; the use of experimental animals complied with U.S. animal welfare laws, policies and guidelines, and were approved by the University of North Florida's Institutional Animals Use and Care committee (IACUC). Sharks were collected

using bottom longline by UNF and SCDNR as a part of fishery-independent surveys, and commercial gillnet and bottom longline fishers in North Carolina. This collaborative fishing effort allowed for increase in sample size, as well as obtaining samples in regions and periods where *C. brevipinna* have been historically difficult to collect (Branstetter, 1980; 1987; Castro, 1993; Clark and von Schmidt, 1965; Dodrill, 1977). Each shark that was captured was sexed and straight-line measurements of precaudal length (PCL), FL, and STL were recorded in centimeters (cm). Precaudal length was measured from the tip of the rostrum to the precaudal pit, fork length was measured from the tip of the rostrum to the fork of the caudal fin, and stretched total length was measured from the tip of the rostrum to the posterior tip of the fully extended top lobe of the caudal fin. External features, such as presence of mating wounds and calcification of claspers, were also assessed. Afterwards, most sharks were euthanized following approved protocols for dissection and evaluation of reproductive organs; however, most sharks from fishery-independent surveys were tagged-and-released. All life stages were used to assess size at maturity for *C, brevipinna*, and mature individuals were used to determine reproductive seasonality, periodicity, and fecundity.

Euthanized sharks were dissected to obtain measurements of reproductive organs and sub-samples for histological analysis; however, most samples were obtained from fishery-dependent sources and stored frozen, making them less suitable for histological evaluation. Maturity in males was determined by the presence of freely rotating (180°) calcified claspers with a fully functional rhipidion, as previously described by Clark and von Schmidt (1965). Female maturity for component two was determined based on ovarian and reproductive duct condition including the presence, coloration, and size of enlarged follicles, oviducal gland, uterus, and presence of yolk sacs and/or embryos.

Morphology

After assessment of maturity, morphological examination and measurements closely followed component 1, with two additions: 1) new male samples were also examined for presence of semen in the seminal vesicles and 2) female ovarian stage was determined based on the presence, condition, and, when present, number of atretic and vitellogenic follicles. For gravid females, the size, stage of development, and sex of each embryo was recorded. Female reproductive stage for component two was categorized by morphological condition of the ovary and pregnancy status. Gravid females were determined early or late based on embryo development, with either an early yolk nutrient source or the latter placental matrotrophy. When of discernable size, pre-caudal length (PCL), fork length (FL), and total length (TL) of embryos were measured on a straight-line basis and sex was determined if claspers were visible by eye or through the use of a dissecting microscope. Litter size and sex ratio per uteri were recorded and then combined for a total per pregnant female.

Hormone measurements

When possible (i.e., mainly during fishery-independent surveys), blood was obtained from sharks via caudal venipuncture (3- to 5 ml) using sterile syringes and needles and stored on ice until returned to the laboratory. Blood was centrifuged at 1300g for 5 minutes to obtain plasma, which was stored frozen (-18°C) until used for reproductive hormone analysis. Plasma samples were used to analyze circulating gonadal steroid hormone concentrations of E_2 and T for females and males, respectively. Chemiluminescence immunoassays (AccuLite CLIA, Monobind Inc., Lake Forest, CA) for both E_2 and T were used to conduct hormone analysis following the manufacturer's instructions with slight modifications. To increase antibody binding and improve specificity of binding, the microwell plate was left overnight in a controlled

temperature of 4˚C after the appropriate tracer reagent was added to all wells. Following this, luminescence in the microwells was determined using Synergy HT Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT, USA). Validation of E_2 and T kits for use with *C. brevipinna* plasma was determined using parallelism and measurements of percent recovery. Parallelism was used to determine if unknown analytes in a serial dilution of pooled samples (1/1 – 1/20 and 1/1 – 1/32 for females and males, respectively) interacted with antibodies in a manner similar to that of assay standards. Percent recovery was determined using "cold spikes", where known amounts of hormone standard were added to each pooled sample dilution and recovery was calculated. The results from these validation tests determined the ideal dilution of *C. brevipinna* plasma to use to avoid matrix effects. For each sample, hormone concentrations were calculated by multiplying by the dilution factor (1/5 for E_2 and 1/10 for T).

Data analysis

Morphological, histological, and endocrinological data from all years were combined, as was performed in component 1, because of limitations in sample size. Morphological data and hormone concentrations were compared by FL of the corresponding animal to assess size at maturity. The MFD and OGW in females and TI and EI in males were compared by month to examine reproductive seasonality. Seasonal changes in MFD and OGW were assessed separately in non-pregnant and pregnant females because they exhibited different patterns of follicular development.

All data sets were assessed for normality and equal variances, and non-parametric alternative were used if datasets failed assumptions for use of parametric statistical tests. Variations in mean TI and EI were analyzed using one-way ANOVA followed by the Tukey *b* test. Variations in mean monthly MFD and OGW for non-gravid females were analyzed using

the Kruskal–Wallis test followed by Dunn's post hoc test. Variations in mean monthly MFD and OGW for gravid females were analyzed using one-way ANOVA followed by the Tukey *b* test. Correlations between FL of pregnant females and litter size were examined using the Spearman rank-order correlation coefficient. Embryo size (TL) by month of collection was analyzed using one-way ANOVA followed by the Tukey *b* test. For embryos in which sex was determined, sex ratio was tested for significant difference with a 1:1 ratio using the chi-square ($\chi 2$) test.

Because of limited samples, E_2 and T could not be statistically evaluated by month to assess reproductive seasonality. Instead, plasma hormone measurements were compared by FL to observe how concentration levels varied with size and reflect sexual maturity. Variations between T concentrations of immature and mature males were analyzed using an independent Student *t* -test. Females determined pregnant by dissection or ultrasonography were compared to non-pregnant females to determine if concentrations differed. Variations of E_2 concentrations in females by size, maturity, and reproductive status were analyzed using a one-way ANOVA followed by the Tukey *b* test.

RESULTS

Component 1

Morphology

A total of 65 archived samples (17 males and 48 females) were examined in the first component of this study. Males ranged in size from 124 – 197 cm FL and females from 96 – 187 cm FL. Of the 17 males, 16 were determined to be mature based on the maturity ogive (Size at 50% maturity = 130 cm FL, $P < 0.0001$, n = 208, Figure 1). Examination of OGW for all females by FL in component 1, depicted an inflection in OGW between 138 – 140 cm FL (Figure 2).

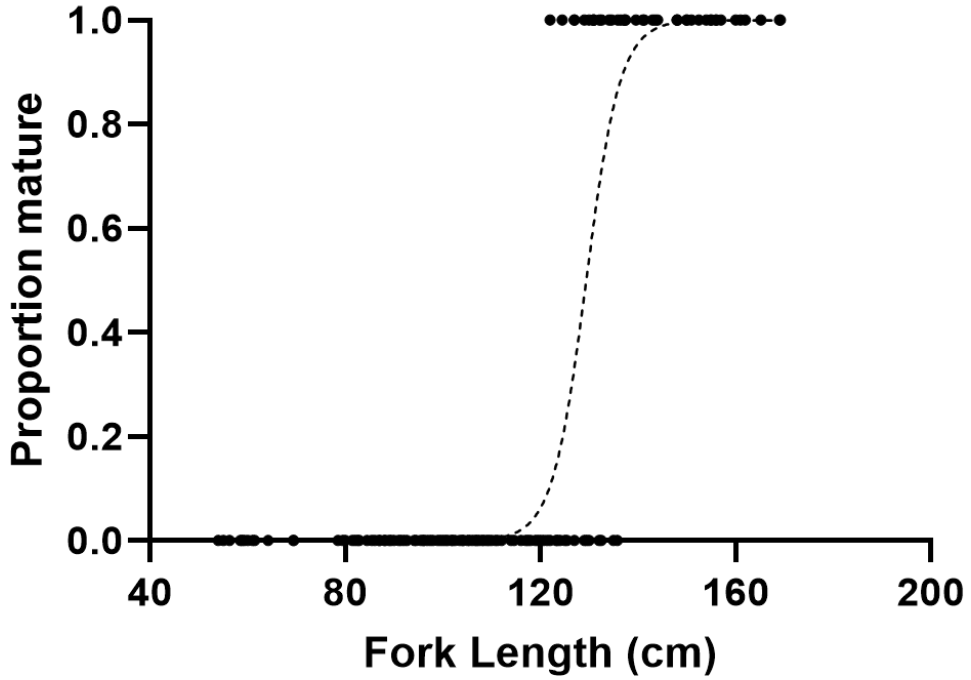

Figure 1. Length at maturity ogive of male spinner sharks, *Carcharhinus brevipinna*, in the Northwestern Atlantic (X @ 50% = 130 cm FL). Size ranged from 54 – 169.2 cm FL (n = 208).

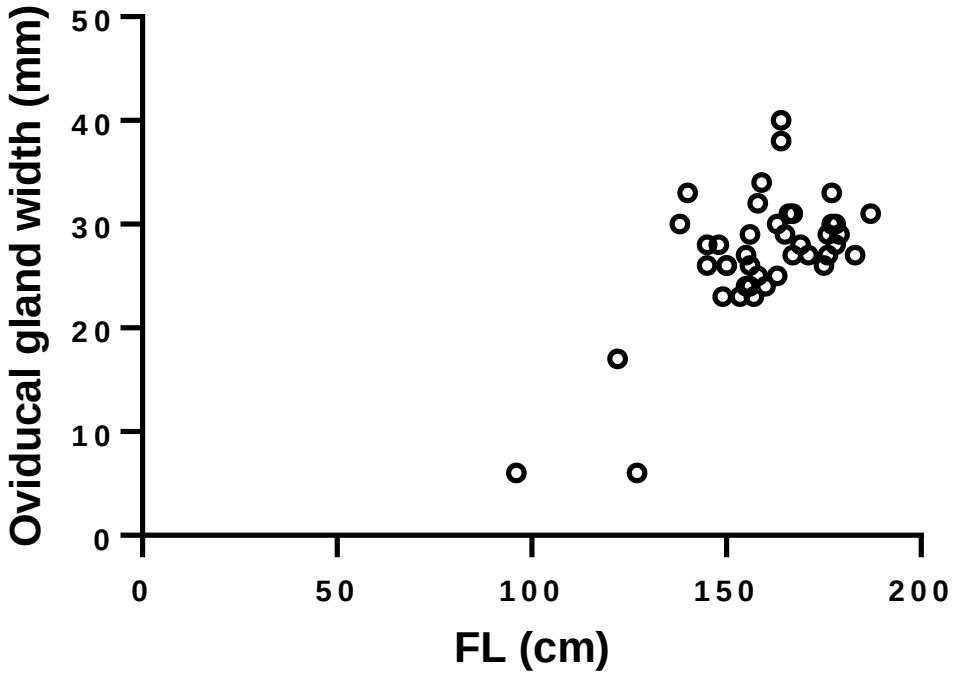

Figure 2. Oviducal gland width of female spinner sharks, *Carcharhinus brevipinna*, by fork length. Points represent individual animals, both immature and mature, from the archived samples. Sizes ranged from 96 – 187 cm FL, with an inflection occurring at 138 cm FL, indicating size at maturity (n = 42).

Females were determined to be mature at 140 cm FL, based on comparison to similar carcharhinid species, as previously mentioned, and changes in OGW in relation to size. Because of this, 42 females were determined to be mature.

TI was calculated for 13 of the 16 mature males determined to be mature, showing testes size was highest in April and March, followed by decreased size in June and July (Figure 3). Significant differences were observed between measurements of TI compared by month (one-way ANOVA, $F_{4,8} = 6.715$, $P = 0.011$, Figure 3). Mature males caught in January showed significantly smaller testis size compared to April – May, with similar TI to animals caught during the summer months. Although there were no significant differences in male EI (n=16) by month, EI exhibited a similar temporal pattern, with peak sizes occurring in April and May followed by smaller sizes in January, June, and July (one-way ANOVA, $F_{4,11} = 1.809$, $P = 0.1974$, Figure 4).

There was no evidence of pregnancy or recorded pregnancy status for any of the archived samples; therefore all mature females were analyzed together as assumed non-gravid individuals. Follicle diameter gradually increased from January until peaking in June (MFD = 40mm), followed by a significant decrease occurring July – October (Kruskal-Wallis $H = 20.63$, $P = 0.0144$, $df = 9$, Figure 5). Females collected in November showed increasing follicle diameters, indicating follicular development may begin occurring around this time, gradually increasing and peaking in early summer. Individual MFD by month of collection, for mature females, depicted at least two cohorts of animals during spring and summer months, suggesting reproductively inactive females during the period which mating is likely occurring (Figure 6). However, it was also possible that these ovaries came from pregnant individuals, for which uterine samples were not obtained. OGW differed significantly by month of collection, where seasonal changes in

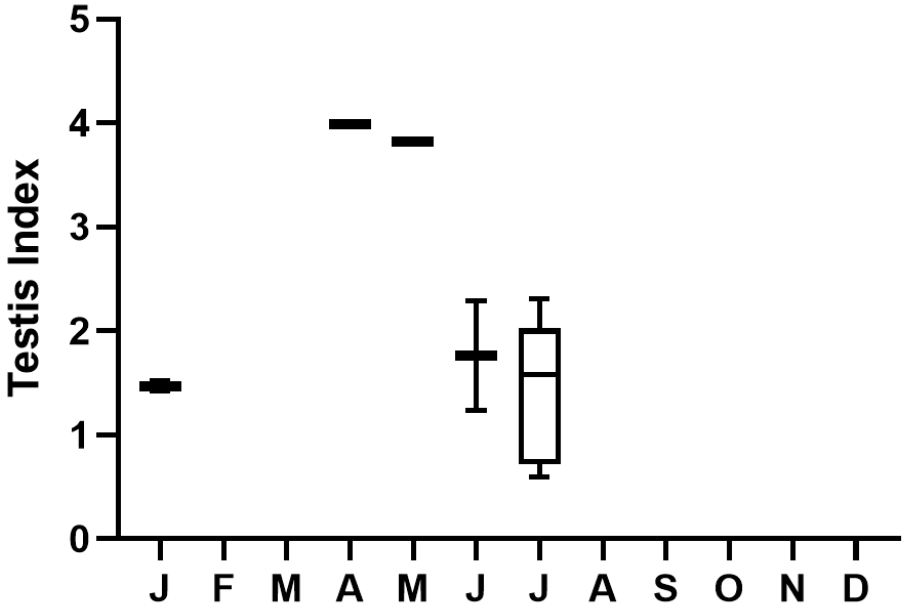

Figure 3. Testis index of mature male spinner sharks, *Carcharhinus brevipinna*, by month of collection. Box plots extend from the 25th to the 75th percentile, with the line at the median. Whiskers indicate the 10th and 90th percentiles. Sample size shown above month of collection.

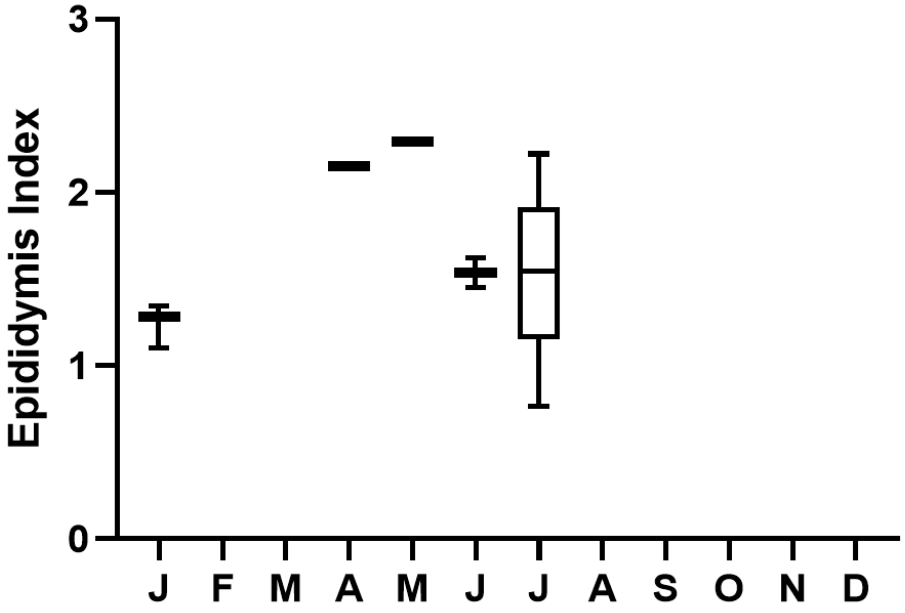

Figure 4. Epididymis index of male spinner sharks, *Carcharhinus brevipinna*, by month of collection. Box plots extend from the 25th to the 75th percentile, with the line at the median. Whiskers indicate the 10th and 90th percentiles. Sample size shown above month of collection.

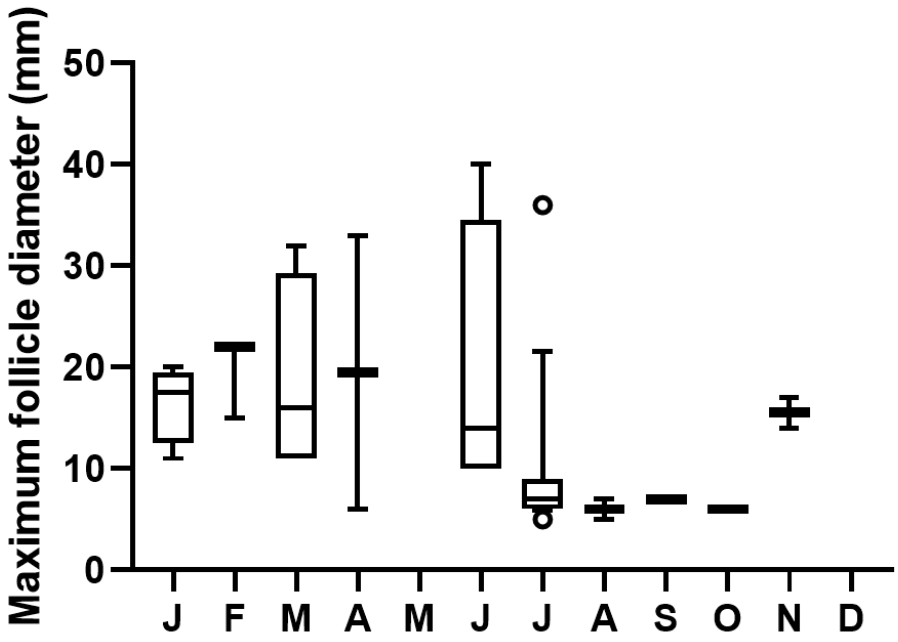

Figure 5. Maximum follicle diameter of female spinner sharks, *Carcharhinus brevipinna*, by month of collection. Box plots extend from the 25th to the 75th percentile, with the line at the median. Whiskers indicate the 10th and 90th percentiles, with individual points beyond this are represented as open circles. Sample size shown above month of collection.

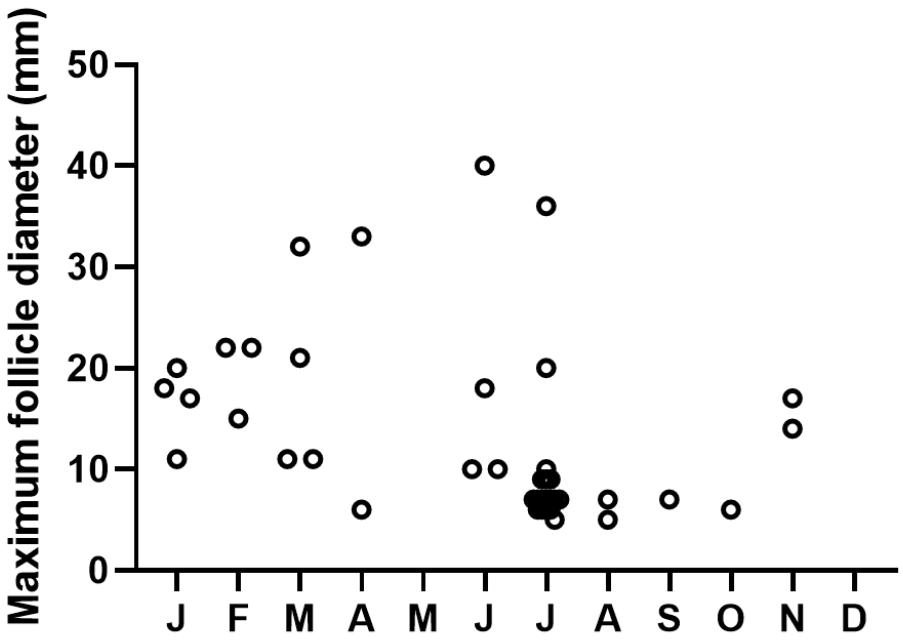

Figure 6. Maximum follicle diameter of mature female spinner sharks, *Carcharhinus brevipinna*, by month of collection. Points represent individual animals from the archived samples obtained through the University of Florida's directed shark fishery observer program from 2003-2005 (n = 44).

OGW followed concurrently with the patterns of follicle growth (one-way ANOVA, $F_{9,28}$ = 2.921, P = 0.014, Figure 7). Similar to follicular development, OGW began to enlarge in November and continued to increase in size until peaking in mid-spring, followed by decreasing sizes June – October (Figure 7).

Histology

Histological samples were collected during the months of January, April, and July, with all years combined for analysis as previously mentioned. As previously mentioned, spermatocysts present in histological sections of testes were categorized into 5 stages: pre-meiotic, meiotic, post-meiotic, evacuated and degenerated spermatocysts. Histological examination of 12 mature male testes by month of collection showed temporal patterns of spermatogenesis (Figure 8). Testis collected in January had markedly increased proportions of spermatocysts containing germs cells in pre-meiotic and meiotic stages than animals collected in June and July (Figure 8a). Male testis collected in June and July had markedly increased proportions of post-meiotic spermatocysts, containing elongating spermatids and mature spermatozoa, and evacuated spermatocysts in comparison to the winter samples (Figure 8b and 8c). Head epididymis collected from males in January (Figure 9a and 9b) exhibited low amounts of residual spermatozoa within regressed ducts. Animals collected in April (Figure 9c) – mid-July (Figure 9d) exhibited larger head epididymis widths, enlarged ductus deferens, and high amounts of spermatozoa, while individuals collected late-July (Figure 9e) exhibited residual spermatozoa. Differences in the proportion of males exhibiting presence of spermatozoa in histological sections of the epididymis were observed between January, April – June, and July (Figure 10). Female oviducal glands that were histologically evaluated showed no presence of

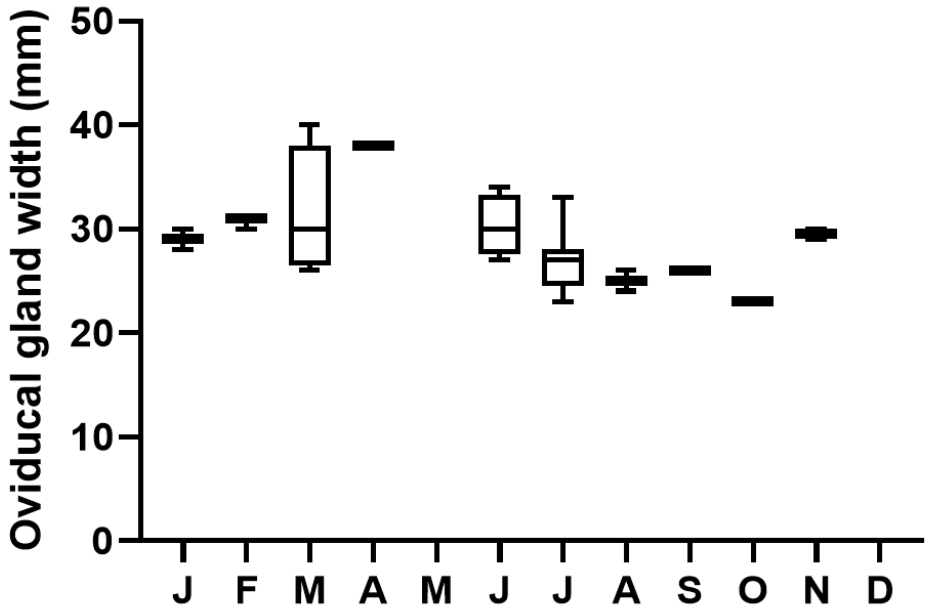

Figure 7. Oviducal gland width of female spinner sharks, *Carcharhinus brevipinna*, by month of collection. Box plots extend from the 25th to the 75th percentile, with the line at the median. Whiskers indicate the 10th and 90th percentiles. Sample size shown above month of collection.

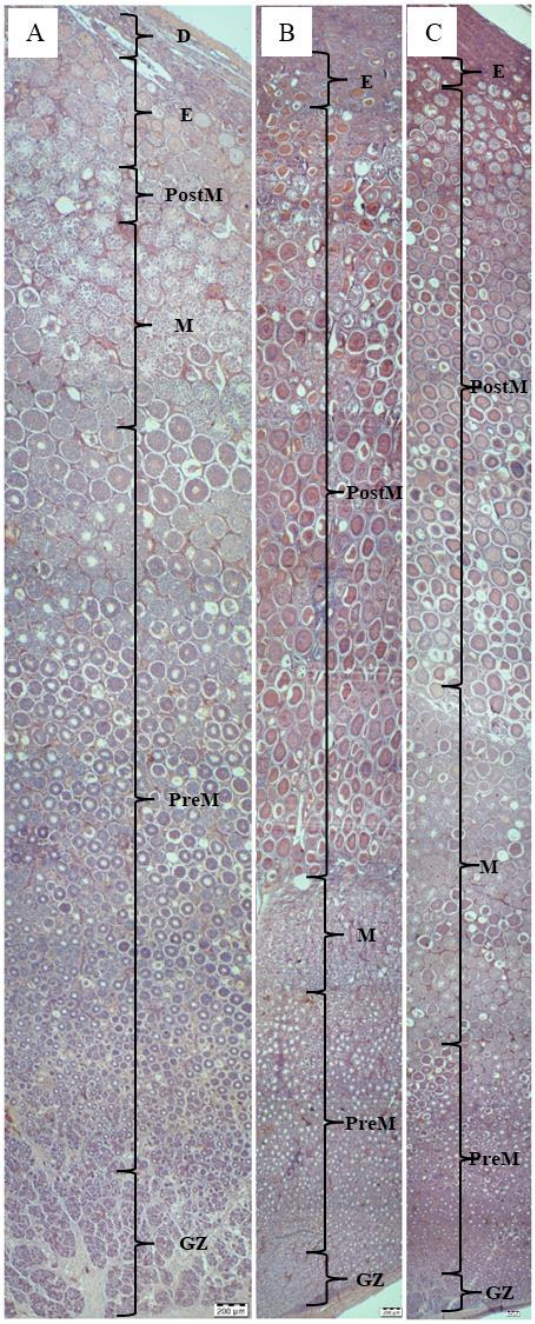

Figure 8. Histological architecture of mature male testis from spinner sharks, *Carcharhinus brevipinna*, collected during (a) January, (b) June, and (c) July. Varying months of collection demonstrate temporal changes in reproductive stage. a) testis from a male collected in January, depicting a large portion of spermatocysts containing germs cells in pre-meiotic and meiotic stages. b) testis from a male collected in June, depicting a large portion of post-meiotic spermatocysts, containing elongating spermatids and mature spermatozoa with a few evacuated spermatocysts. c) testis from a male collected in July, depicting similar proportions of spermatogenesis stages as b) with an overall increase in testis cross section length. Images show the full cross section of the testis with the corresponding scale bar (200 μm) in the lower right of each image. GZ – germinal zone, PreM – pre-meiotic spermatocysts, M – meiotic spermatocysts, PostM – post-meiotic spermatocysts, E – evacuated spermatocysts, and D – degenerated spermatocysts.

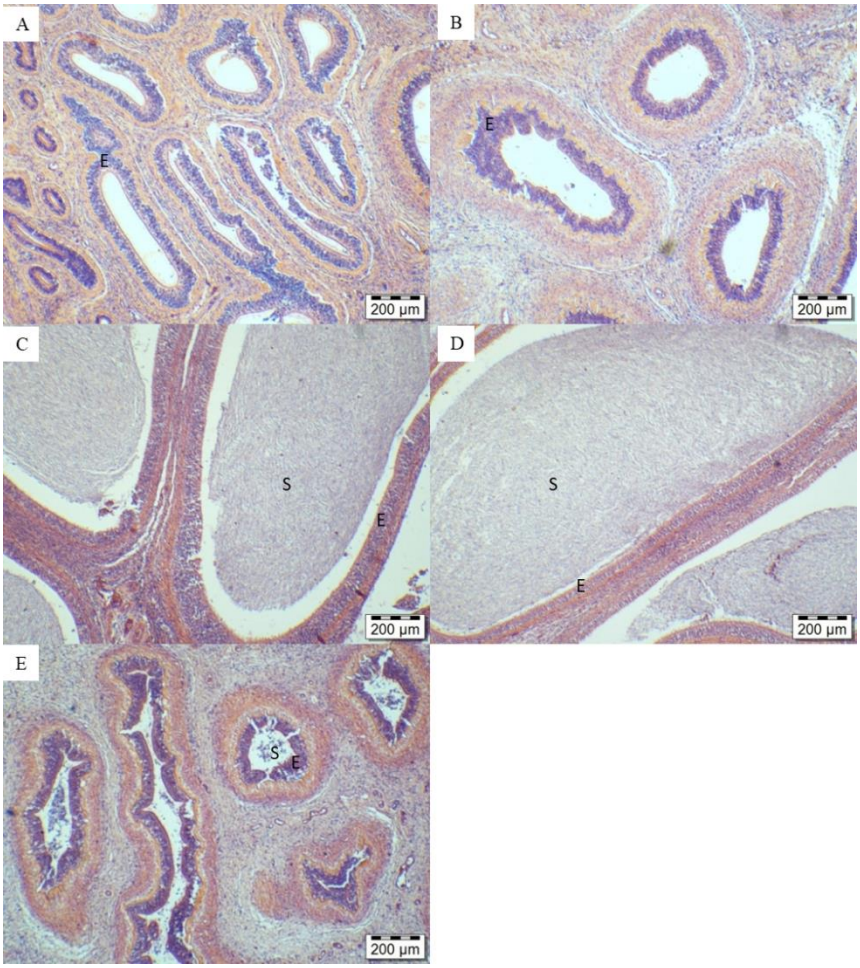

Figure 9. Histological architecture of male head epididymis in mature male spinner sharks, *Carcharhinus brevipinna*, illustrating temporal changes in reproductive stage. (a & b) epididymis of mature males collected in January demonstrating regressed ducts with absence of spermatozoa, (c) epididymis of a mature male collected in April demonstrating presence of spermatozoa and enlarged ducts, d) epididymis of a mature male collected in July demonstrating large amounts of spermatozoa and enlarged ducts, and e) epididymis of a mature male collected in late July, demonstrating residual spermatozoa and regressed ducts. Scale bar is shown on the lower right of each image. E – epithelium and S – spermatozoa.

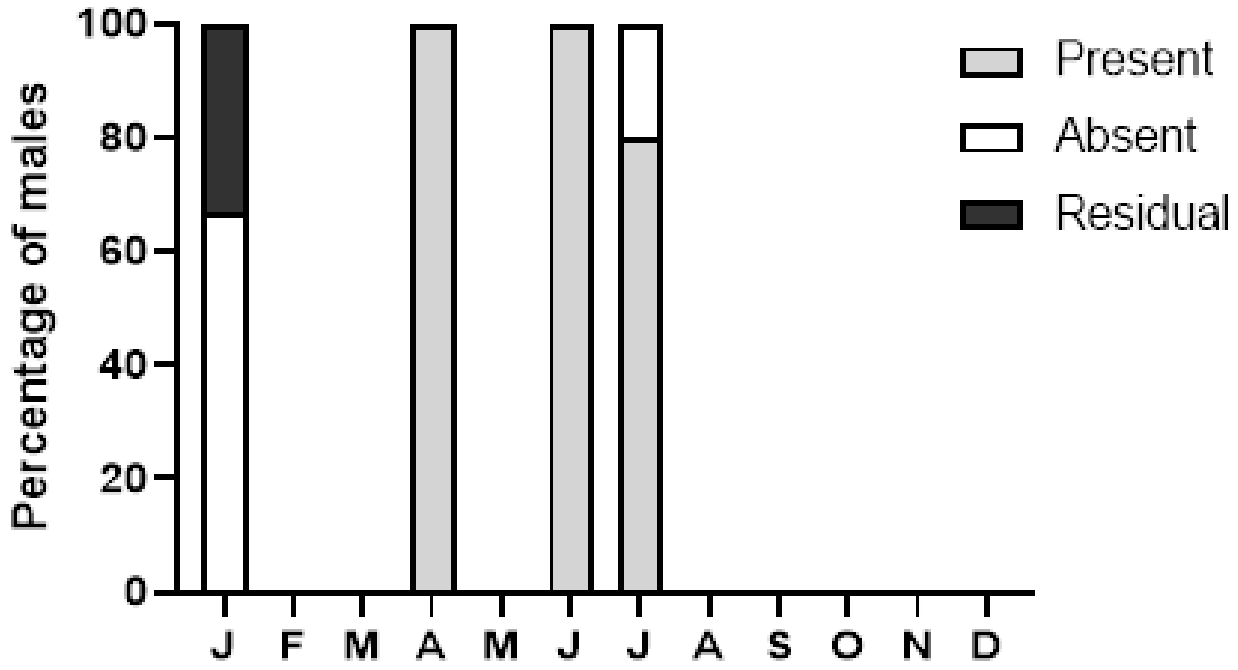

Figure 10. Percentage of mature male spinner sharks, *Carcharhinus brevipinna*, with spermatozoa in the lower reproductive tract by month of collection. Legend illustrates presence, absence, or residual spermatozoa. Sample size shown above month of collection.

spermatozoa within the luminal epithelium or tubules of the oviducal gland, suggesting sperm storage in females was not occurring.

Quantitative analysis of the formerly mentioned 12 mature male testes further supported temporal patterns of spermatogenesis. Males collected in January consisted of all stages of spermatogenesis but, on average, were predominantly composed of the germinal zone with pre-meiotic spermatocysts containing primary and secondary spermatogonia (51.73%) and the meiotic stage containing spermatocysts with primary and secondary spermatocytes (35.78%) (Figure 11). On average, the individuals collected in June exhibited all stages of spermatogenesis with pre-meiotic (24.68%), meiotic (26.48%), post-meiotic (20.12%), and a degenerative zone (27.50%) making up similar proportions of the testis (Figure 11). However, when examining these individuals separately, one testis was primarily made up of meiotic and post-meiotic spermatocytes (Figure 11), whereas the other individual sampled during this time had spermatocysts with primary and secondary spermatocytes, followed directly by a large degenerative zone (55%). Testis sampled in July had the highest average of post-meiotic (32.06%) and evacuated (9.33%) spermatocysts, suggestive of recent spermiation (Figure 11).

Component 2

Animal Collection

A total of 26 males and 37 females were sampled in the second component of this study. Sample size of males and females varied per reproductive analysis (i.e., morphology, histology, and endocrinology) based on the range of samples taken at the time of collection. Males were collected in the months of May, June, and September, ranging in size from 58 – 169.2 cm FL. Females were collected March through October, ranging in size from 57 – 195.6 cm FL.

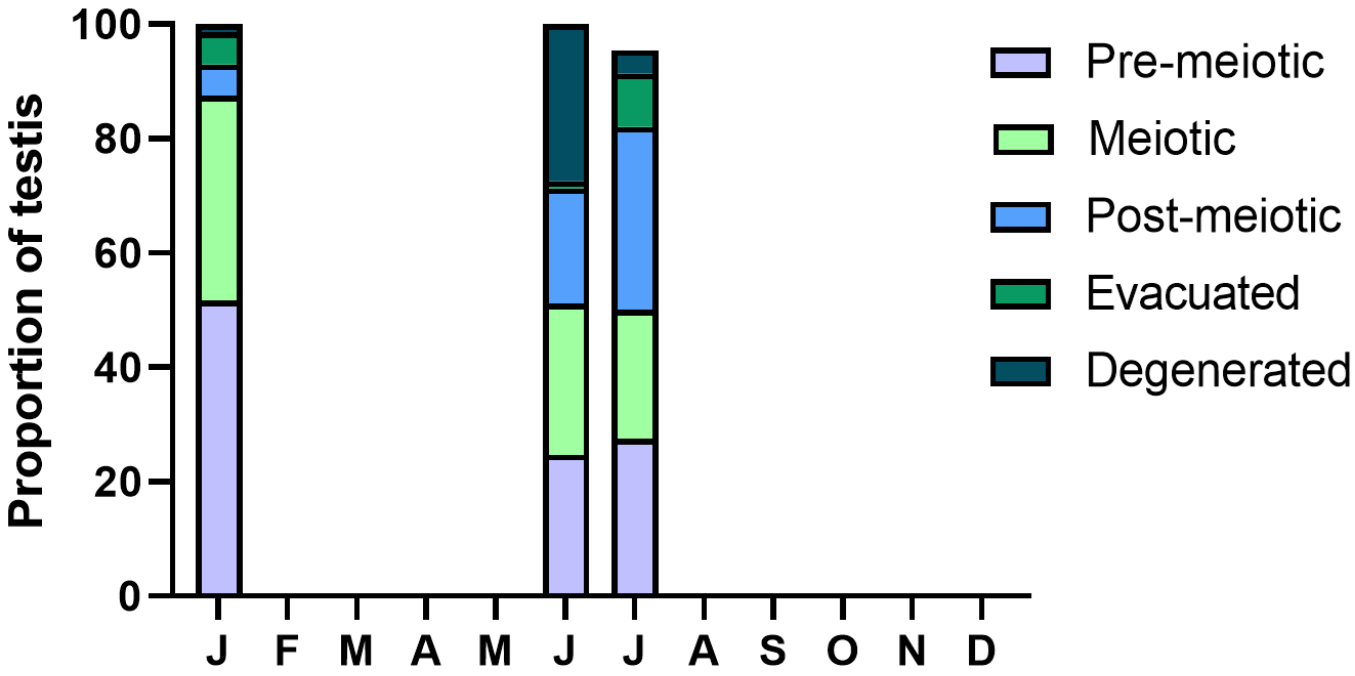

Figure 11. Seasonal changes in the proportion of different stages of spermatogenesis in the testes of mature male spinner sharks, *Carcharhinus brevipinna*. Legend illustrates the stages of spermatogenesis used in this study. Sample size shown above month of collection.

Morphology

Reproductive morphology was examined in 19 males for the second component of this study, 18 of which were determined to be mature. Based on reproductive morphology and examination of clasper condition as previously described, all males ≥ 129.5 cm FL were mature. Due to limitation of collection, a seasonal pattern could not be observed in TI of mature males; however, testes size was shown to be largest in May followed by a significant decrease in June and September (one-way ANOVA, $F_{2,15} = 4.140$, $P = 0.037$, Figure 12). EI was measured in 17 mature males, where EI was highest in May followed by markedly decreased sizes in June and September (Figure 13). There were significant differences of EI among all three months of collection (one-way ANOVA, $F_{2,14} = 12.650$, $P = 0.001$, Figure 13). Semen with high opacity was observed in the lower reproductive tract (i.e., ductus deferens) of males collected in April and May, whereas males collected in September had no semen present.

Reproductive morphology was examined in 28 females for the second component of this study, 22 of which were determined to be mature based on reproductive morphology. Like component 1, an inflection in OGW was observed around 140 cm FL, indicating the onset of maturity (Figure 14). MFD in non-gravid females did not vary significantly by month of collection. MFD exhibited increased sizes in March and June, with follicle diameter peaking (35 mm) in June (Kruskal-Wallis $H = 1.309$, $P = 0.832$, $df = 4$, Figure 15). Several different follicular observations were found in the month of June: enlarged vitellogenic follicles, small pre-vitellogenic follicles, and slightly enlarged atretic follicles. One individual collected during June exhibited enlarged (35 mm) vitellogenic follicles, indicating a period just prior to ovulation and mating. Enlarged viteolligenic follicles present in the ovary indicate the period just prior to fertilization, occurring by copulatory activity, for which viable follicles will become fertilized by

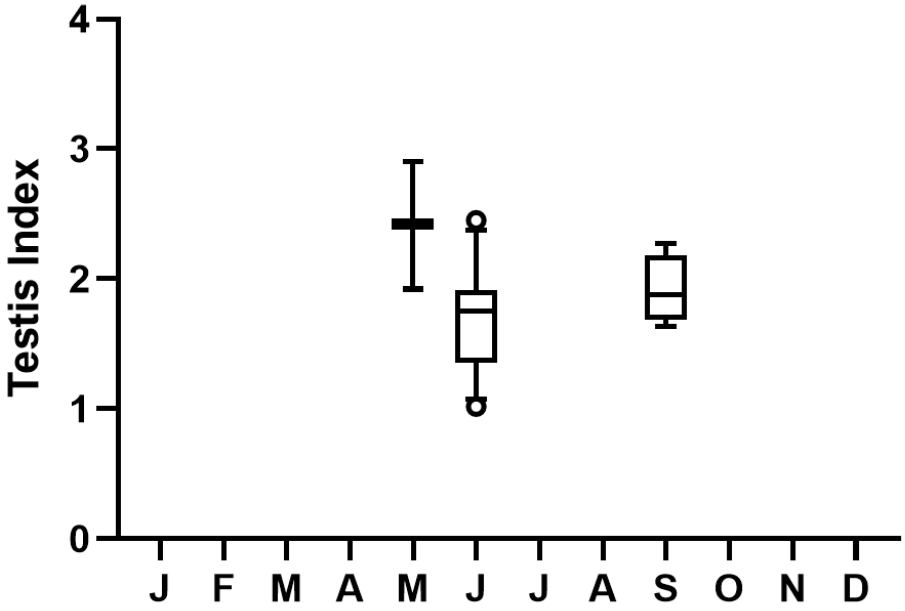

Figure 12. Testis index of male spinner sharks, *Carcharhinus brevipinna*, by month of collection. Box plots extend from the 25th to the 75th percentile, with the line at the median. Whiskers indicate the 10th and 90th percentiles, with individual points beyond this are represented as open circles. Sample size shown above month of collection.

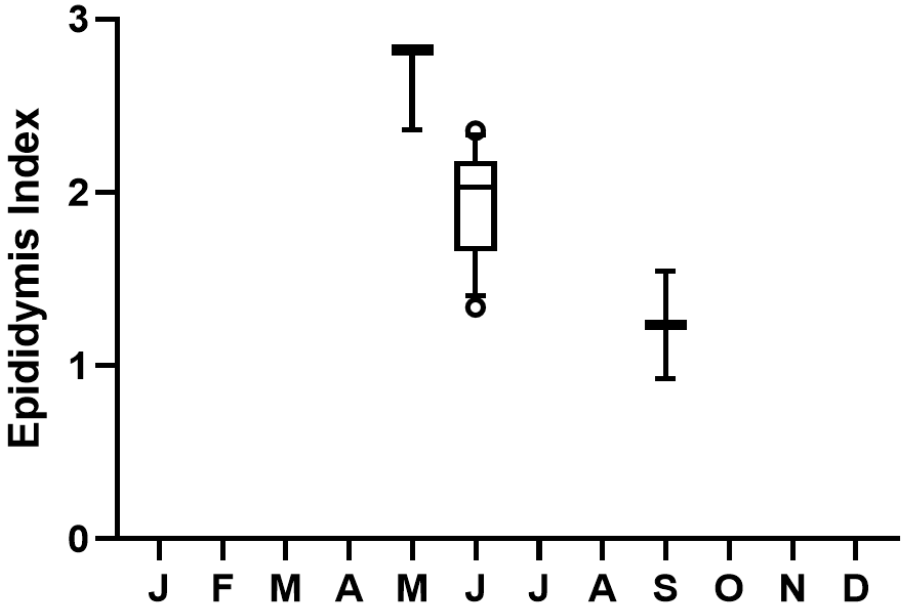

Figure 13. Epididymis index of male spinner sharks, *Carcharhinus brevipinna*, by month of collection. Box plots extend from the 25th to the 75th percentile, with the line at the median. Whiskers indicate the 10th and 90th percentiles, with individual points beyond this are represented as open circles. Sample size shown above month of collection.

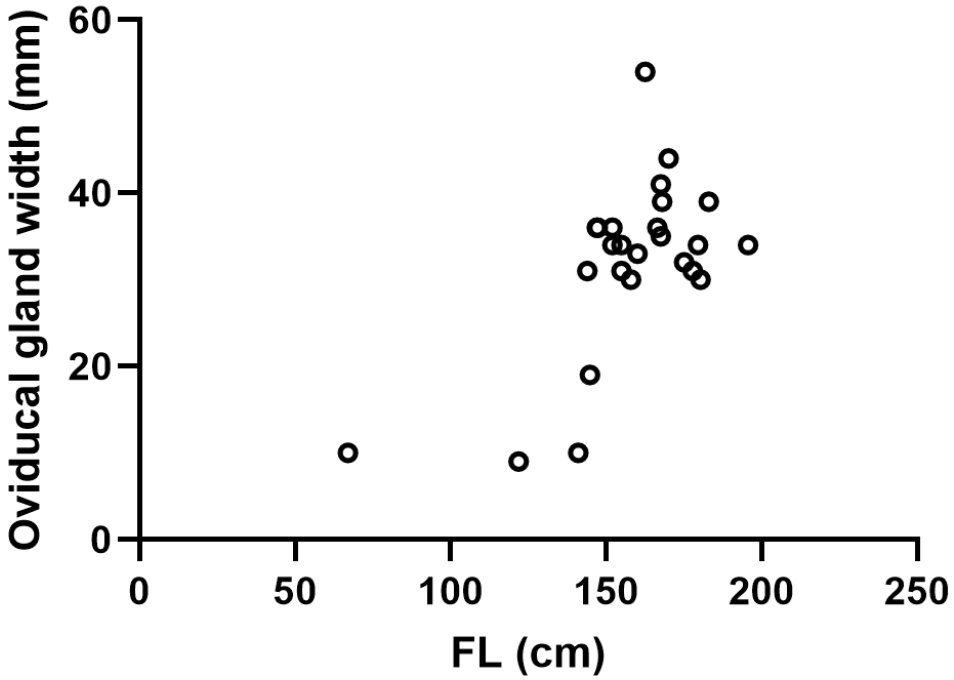

Figure 14. Oviducal gland width for female spinner sharks, *Carcharhinus brevipinna*, by fork length. Points represent individual animals, both immature and mature. Sizes ranged from 67 – 195.6 cm FL, with an inflection occurring at 143 cm FL, indicative of size at maturity (n = 25).

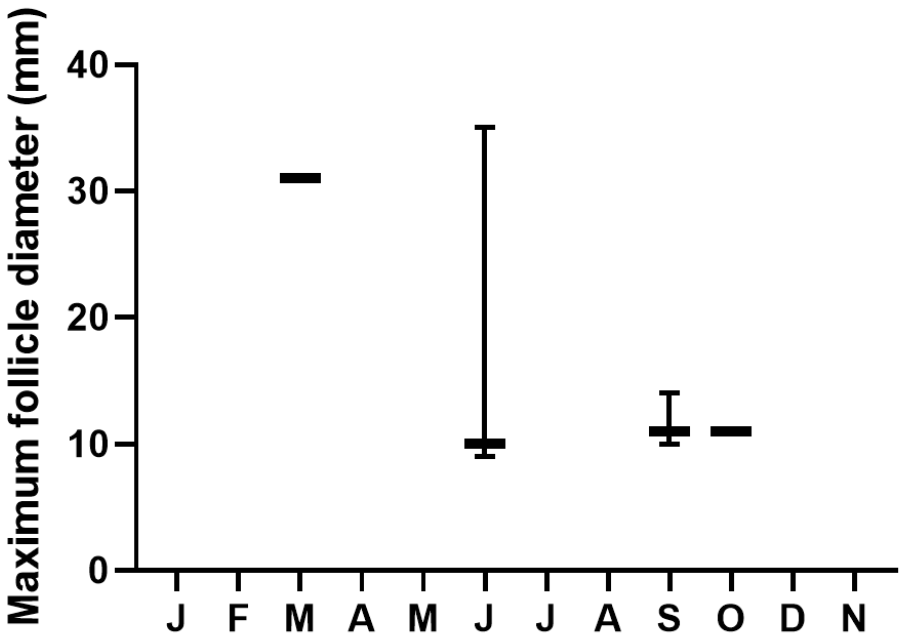

Figure 15. Maximum follicle diameter of mature, non-gravid, female spinner shark, *Carcharhinus brevipinna*, by month of collection. Box plots extend from the 25th to 75th percentile, lines represent the median. Whiskers indicate the 10th and 90th percentiles. Sample size shown above month of collection.

male sperm. Two of the animals collected in June exhibited markedly decreased MFD (9 and 10 mm) with follicles appearing to be pre-vitellogenic, indicating the earliest stages of follicular development. Lastly, one female collected during this same time period exhibited slightly enlarged (14 mm) atretic follicles, likely indicating a maturing or newly matured female that had partial follicular enlargement followed by regression. Nonetheless, similar to the archived samples, non-gravid animals collected in June exhibited multiple cohorts suggesting a non-annual periodicity (Figure 16). Animals collected in September and October exhibited smaller MFD with evident vitellogenic follicles, indicating progression of early-stage follicular development. MFD in gravid females varied significantly by month of collection (one-way ANOVA, $F_{4,6} = 15.060$, $P = 0.003$). Gravid females exhibited smaller MFD measurements in comparison to non-gravid females and decreased in size from April – September for all months except August (Figure 17). The gravid females collected in August had enlarged non-ovulated follicles (21 – 33 mm) all exhibiting degenerative characteristics (i.e., atretic and flaccid), yet during this time the dominant condition of the ovaries were small, atretic follicles. Therefore, the enlarged MFD in these animals should not be confused with follicular development occurring during pregnancy, but rather degenerating non-ovulated follicles. The embryos of these females examined averaged 15.7 cm STL and sex for most of the embryos could not yet be identified. Gravid females, including individuals collected in August, exhibited only non-vitellogenic follicles, whereas vitellogenic follicles were only observed in non-gravid females, suggesting follicular growth and gestation is asynchronous. OGW did not vary significantly in non-gravid or gravid females by month of collection, however, OGW of non-gravid females followed a similar pattern as follicular development. As with MFD of non-gravid females, OGW in non-gravid females had increased sizes in March, peaked in July (54 mm), and decreased marginally in size

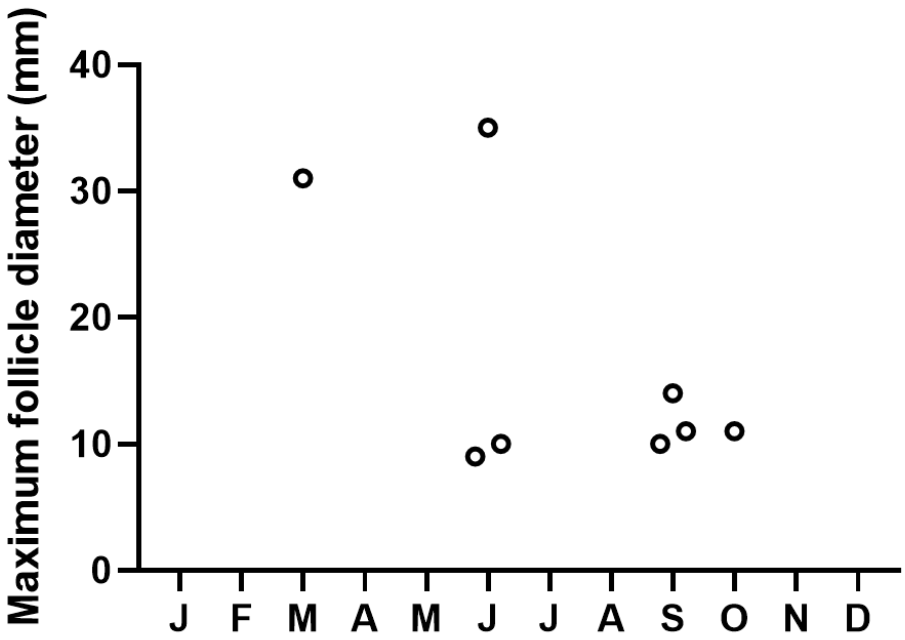

Figure 16. Maximum follicle diameter of pre-vitellogenic or vitellogenic follicles of mature, non-gravid, female spinner sharks, *Carcharhinus brevipinna*, by month of collection. Points represent individual animals (n = 8).

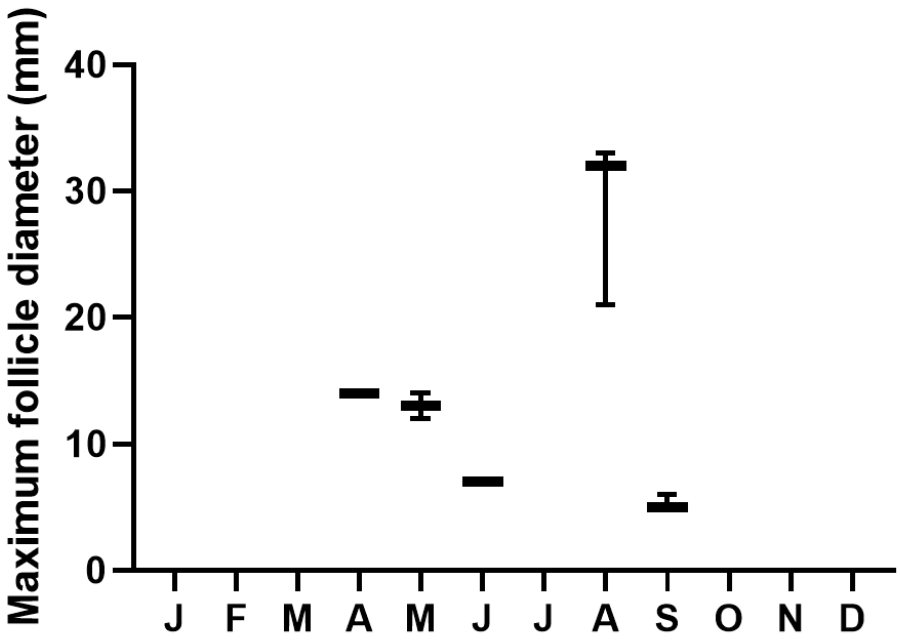

Figure 17. Maximum follicle diameter of gravid female spinner sharks, *Carcharhinus brevipinna*, by month of collection. Box plots extend from the 25th to the 75th percentile, however due to lack of variability in the small sample size, no boxplot is illustrated. The lines represent the median. Whiskers indicate the 10th and 90th percentiles. Sample size shown above month of collection.

September – October (Kruskal-Wallis H = 3.483, P = 0.340, df = 4, Figure 18). OGW in gravid females exhibit similar sizes across all months, with the largest size occurring in April, followed by decreased sizes March – September (one-way ANOVA, $F_{4,7} = 2.315$, P = 0.157, Figure 19).

Of the mature females examined, 16 (53%) were determined pregnant via ultrasound and/or dissection. The smallest and largest pregnant females were 143.9 and 195.9 cm FL. The earliest embryo development was observed in August (4.8 – 5.7 cm FL [based on the conversion of 9.8 – 10.8 cm STL using the FL-TL relationship in Carlson and Baremore, 2005]) for which sex could not yet be determined visually or by a dissecting scope. The latest term pregnant females were observed in June with pups ranging in size from 51.4 – 53.2 cm FL. Remnants of yolk sacs were present in the uteri of females examined in early August, however by mid- to late August all embryos examined had developed 'complete' placental connections. Embryo size within each litter were highly similar, on average ranging 2.28 cm STL between the largest and smallest measured embryo. Even though collection over winter months was limited, a synchronous pattern of embryo growth was observed with significant increases between all months except May and June (one-way ANOVA, $F_{4,61} = 804.8$, P = < 0.0001, Figure 20). Litter size ranged from 4 to 8 pups with a mean ± standard deviation of 5.07 ± 1.32. Sex ratio per litter was determined to not differ significantly from a 1:1 ratio ($\chi 2 = 0.066$, P = 0.796, df = 1, n = 66). Litter size and maternal length were found to significantly correlated ($r_s = 0.5938$, P = 0.0387, n = 66, Figure 21).

Hormone analysis

Circulating plasma gonadal hormone concentrations were examined in 11 females (7 mature) and 5 males (3 mature). Due to limitations of sample collection, seasonal patterns of plasma T and E_2 concentrations could not be analyzed by month of collection. T concentrations

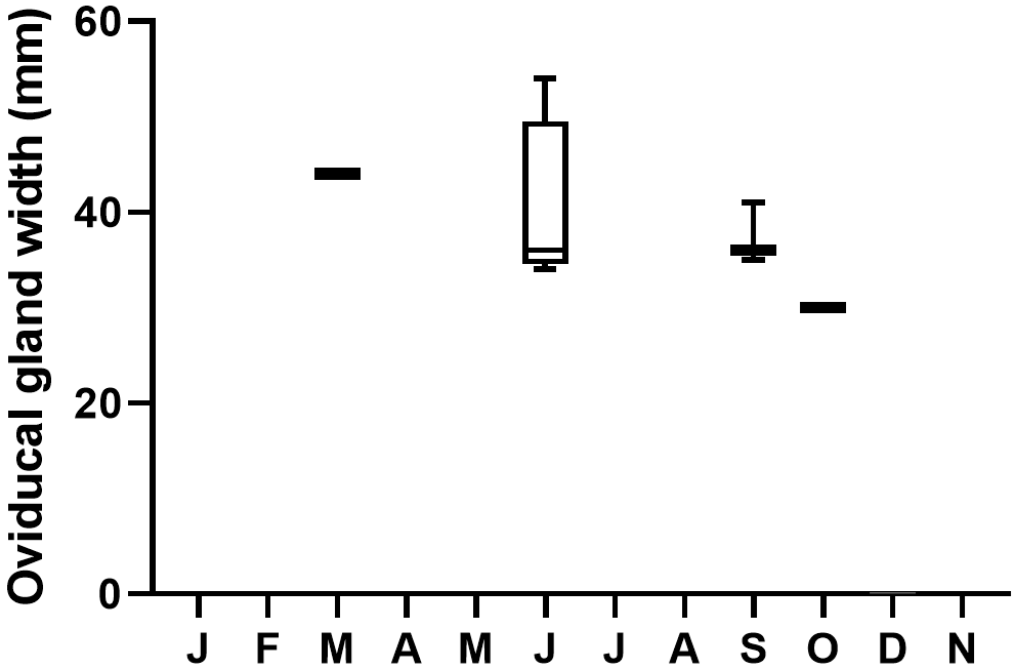

Figure 18. Oviducal gland width for mature non-gravid female spinner sharks, *Carcharhinus brevipinna*, by month of collection. Box plots extend from the 25th to the 75th percentile, with the line at the median. Whiskers indicate the 10th and 90th percentiles. Sample size shown above month of collection.

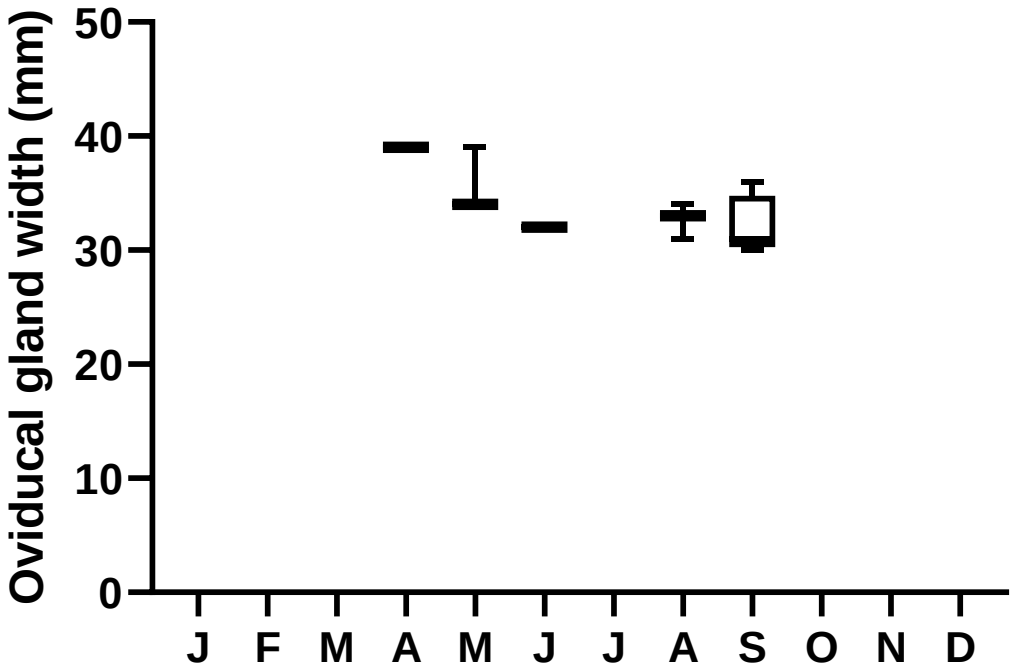

Figure 19. Oviducal gland width for gravid female spinner sharks, *Carcharhinus brevipinna*, by month of collection. Box plots extend from the 25th to the 75th percentile, with the line at the median. Whiskers indicate the 10th and 90th percentiles. Sample size shown above month of collection.

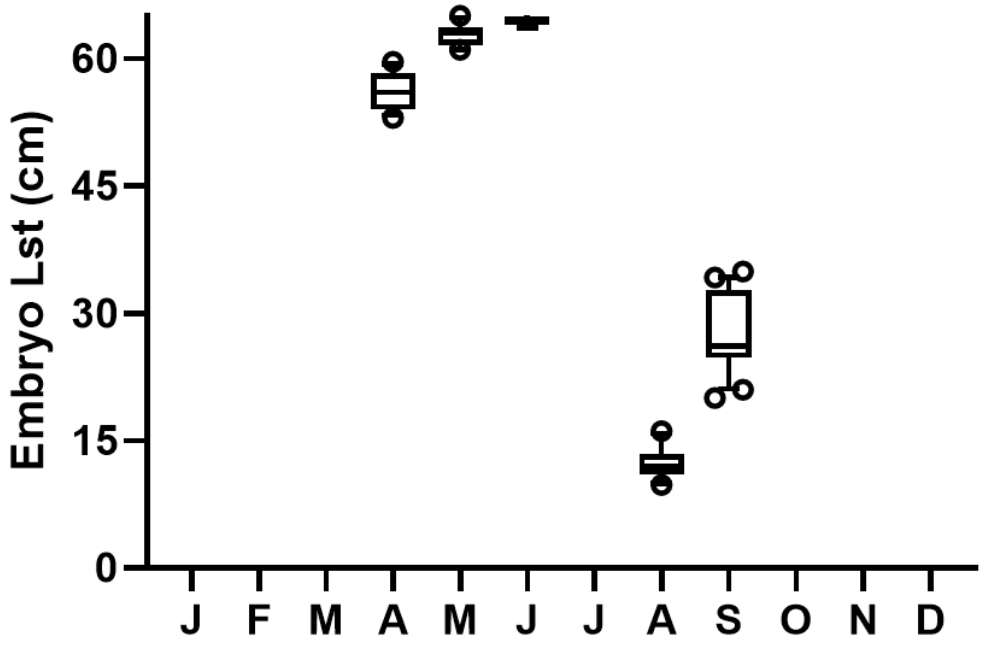

Figure 20. Embryo stretched total length of gravid female spinner sharks, *Carcharhinus brevipinna*, by month of collection, demonstrating monthly changes in embryo size. Box plots extend from the 25th to the 75th percentile, with the line at the median. Whiskers indicate the 10th and 90th percentiles, with individual points beyond this are represented as open circles. Sample size shown above month of collection.

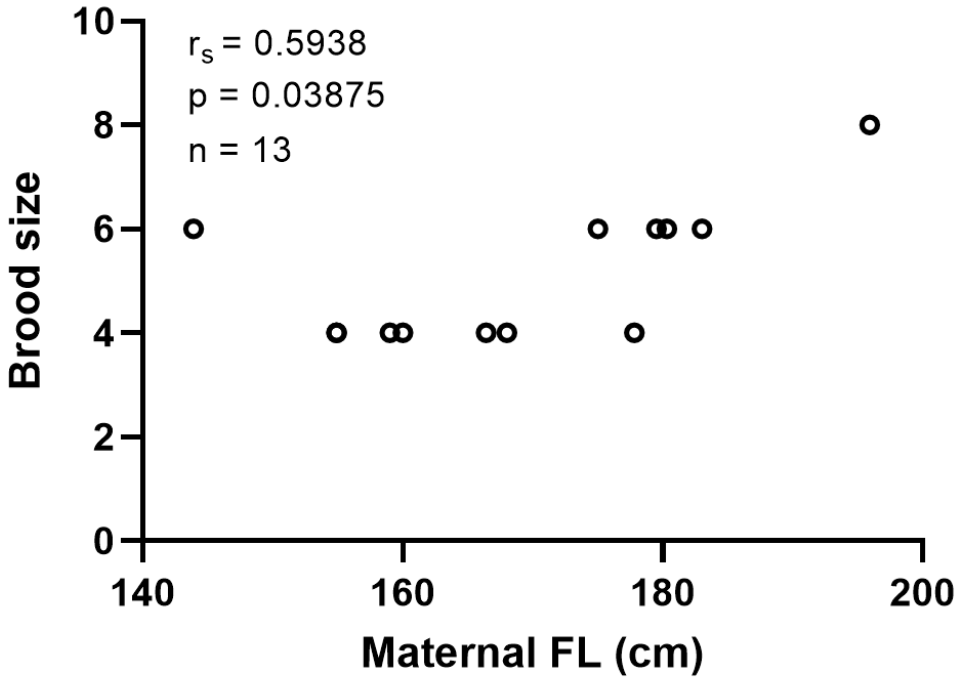

Figure 21. Relationship between the brood size of female spinner sharks, *Carcharhinus brevipinna*, and maternal fork length. Points represent individual animals. Results from the regression analysis and line of best fit are shown at the top left.

in males varied significantly by size; males ≥160 cm FL had significantly elevated concentrations compared to the immature animals (58 and 74 cm FL) that had virtually undetectable levels ($t(3) = 7.886$, P = 0.004, Figure 22). Although E_2 concentrations in females did not vary significantly by size, differences based on maturity and reproductive status was observed (one-way ANOVA, $F_{2,8} = 1.426$, P = 0.284, Figure 23). Immature females (≤ 138.2 cm FL) did not exceed concentrations of 314.15 pg/mL, whereas the highest concentration of mature individuals was 1757.38 pg/mL. Gravid females exhibited lower levels of E_2 concentrations than that of reproductively active, non-gravid females. However, one female sampled in April, during mid-late pregnancy, had an E_2 concentration of 1123.44 pg/mL in comparison to other gravid females caught within the same time period exhibiting concentrations of 156.54 and 221.28 pg/mL. Like the separate cohorts observed with MFD of non-gravid females, E_2 concentrations for mature non-gravid females exhibited relatively low (306.6 pg/mL) and high (1757.38 pg/mL) concentrations during the same time period (Figure 22).

DISCUSSION

This study aimed to characterize reproduction of *C. brevipinna* along the southeastern U.S. coast through two approaches. The first component of this study was composed of archived data obtained from the UF observer program which provided us with 65 samples (17 males and 48 females). The results of the first component found size at maturity to be 130 and 140 cm FL for males and females, respectively, based on a maturity ogive for males and marked inflection of the oviducal gland width at ≥ 138 cm FL, along with literature comparison for females. Male testis and epididymis sizes increased during the early months of the year with peaks occurring concurrently in spring followed by decreasing sizes in June and July. Histological analysis of the male testis showed temporal changes in spermatogenesis, similar and associated with testis size,

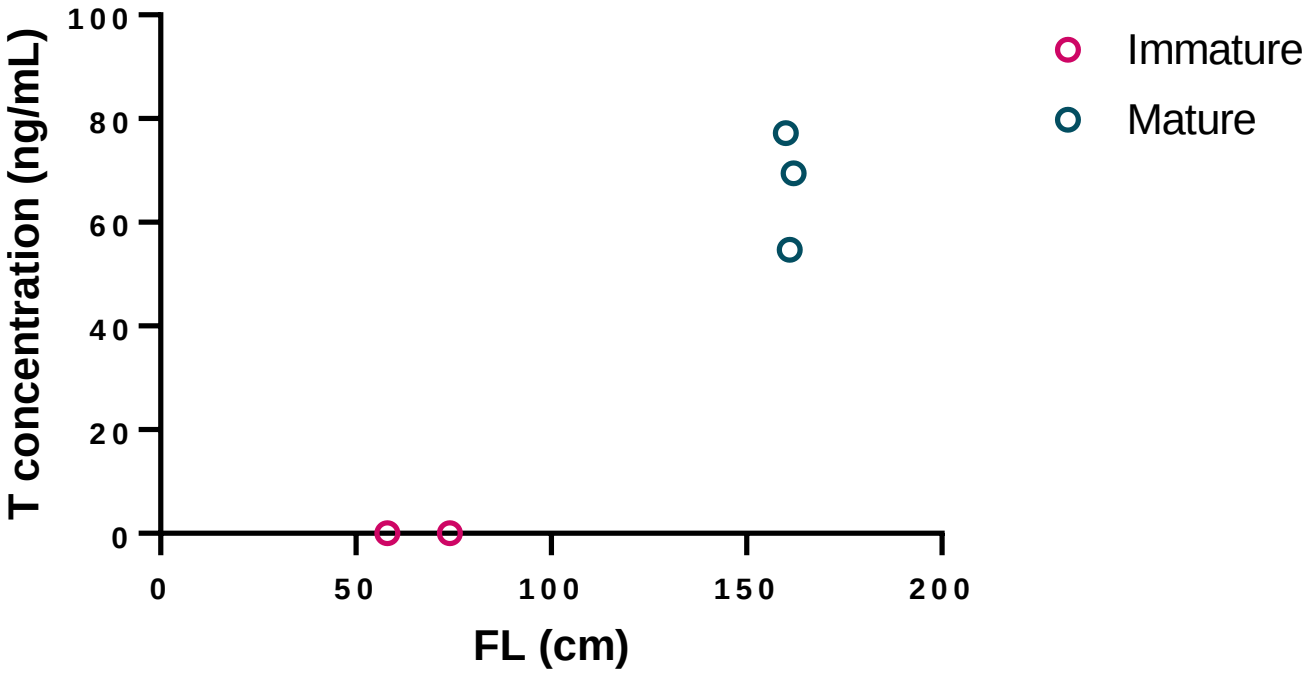

Figure 22. Plasma testosterone concentrations in male spinner sharks, *Carcharhinus brevipinna*, by fork length. Points represent individual animals (n = 5), where immature and mature animals are illustrated in different colors as shown in the legend, to the right.

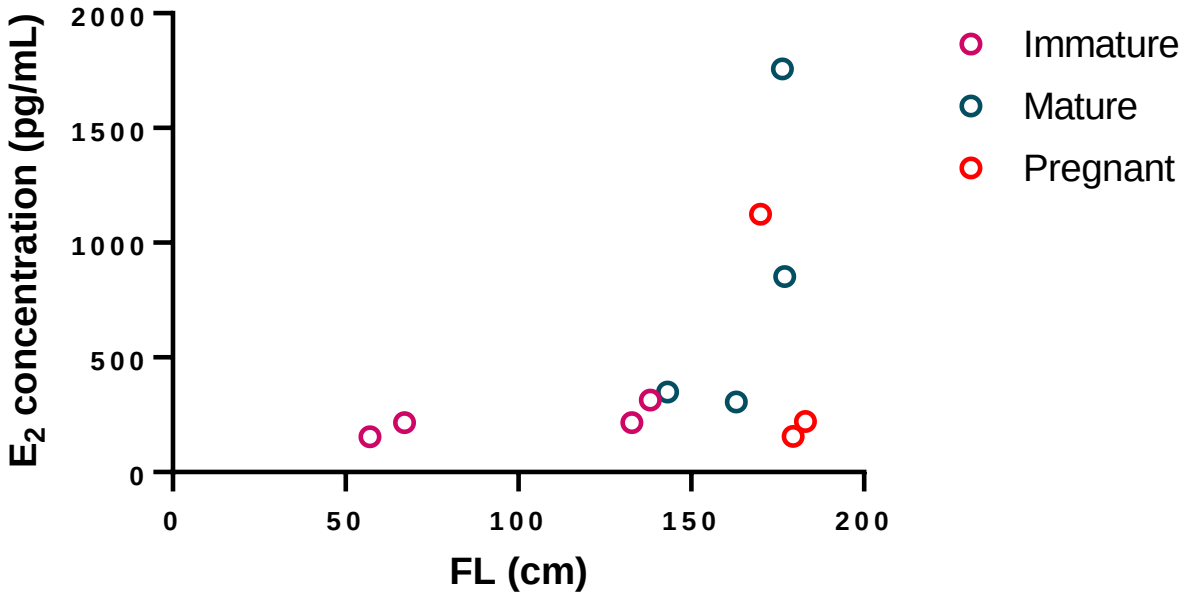

Figure 23. Plasma estradiol concentrations in female spinner sharks, *Carcharhinus brevipinna*, by fork length. Points represent individual animals (n = 11), where immature, mature, and pregnant animals are illustrated in different colors as shown in the legend, to the right.

in which evidence suggests mating to occur during late spring – early summer. Agreeably, histological evaluation of the male head epididymis showed a high abundance of mature spermatozoa in males collected April – July, indicative of mating. The females examined in component one exhibited temporal patterns of follicular development and oviducal gland widths concurrent with male gametogenesis. Both MFD and OGW followed a synchronous pattern where sizes increased up to and peaking in spring months, followed by decreased sizes in July, which remained decreased until November. MFD in mature females exhibited more than one cohort during spring months, which indicates a non-annual periodicity. However due to limitations of the archived data set (e.g., issues with fixation of tissues, lack of reproductive organs, lack of pregnancy status), reproductive periodicity could not be more precisely determined based on these results. The overall results of component one show seasonal, concurrent periods of gametogenesis for male and female *C. brevipinna* with mating likely occurring during late spring / early summer and a non-annual reproductive periodicity (because of variability in MFD in females).

The second approach focused on newly collected data from fishery-dependent and -independent surveys from which 63 samples, 26 males and 37 females, were collected. All *C. brevipinna* over the size of 129.5 and 143.9 cm FL for male and females, respectively, were found to be mature. Markedly increased oviducal gland sizes also demonstrated that females 143.9 cm FL or larger were mature. Component two males showed peak testis and epididymis widths in May, followed by decreasing sizes in June and September. Non-gravid females showed a synchronous pattern of MFD and OGW that increased from March to peak sizes in June, followed by decreased sizes in July with slight increases observed September – October. As observed in component one, individual MFD of mature, non-gravid females by month showed at

least two cohorts during the month of June, further supporting the notion of a non-annual periodicity. Additionally, all gravid females sampled had ovaries composed of atretic follicles with no evidence of follicular development occurring during pregnancy, also indicating a non-annual reproductive periodicity. MFD and OGW of gravid females showed similarly decreasing sizes from April – September and April – October, respectively. Copulation and parturition were shown to occur in June – July with a gestation period of approximately 12 months based on morphological and histological evaluation of the male and female reproductive tract. Embryo growth increased synchronously by month of collection with the smallest embryos observed in August and the largest sampled in June. Litter size ranged from 4 – 8 pups per litter with a median of 4 pups. Gonadal steroid hormones (T and E_2 for males and females, respectively) showed immature animals had virtual absent or lower concentrations than mature individuals. E_2 in gravid females exhibited lower concentrations than non-gravid females, with the exception of one animal. Additionally, some non-gravid mature females exhibited low E_2 concentrations nearing the projected time of ovulation, indicative of non-reproductively active females and strengthening evidence for a non-annual reproductive periodicity. The results from this component validated the findings of component one, based on similarities between the components, and further expanded our knowledge of the reproductive biology of *C. brevipinna*. Results from both components found similar sizes at maturity, concurrent gametogenesis, copulation and parturition likely occurring June – July, and gestation for females lasting approximately 12 months on a likely biennial basis. For these reasons, the following discussion will view the two components of this study collectively.

The findings of this study were similar to past studies conducted both within and outside of the United States (Table 1). Previous studies conducted in the NWA found copulation and

	Location	Size-at-maturity (FL cm) (♂, ♀)	Season	Gestation (months)	Litter size	Periodicity	Literature
Inside of the U.S.	Gulf of Mexico	156, NA	NA	NA	NA	NA	Clark & von Schmidt, 1965
	Melbourne Beach, FL	NA, NA	Spring – Summer	9 – 12	8	NA	Dodrill, 1977
	Gulf of Mexico	140, 149	June – July	11 – 12	6 – 12	NA	Branstetter, 1980; 1987
	South Carolina	NA, NA	May – June	NA	NA	NA	Castro, 1993
Outside of the U.S.	NW Taiwan	183, 185	Oct. – Dec.	10 – 12	3 – 14	Biennial	Joung et al., 2005
	Australia-Coral Sea	NA, 166	Spring – Summer	NA	4 – 16	NA	Sumpton et al., 2010
	Australia-Tasman Sea	174, 187	NA	12 +	5 – 14	NA	Geraghty et al., 2015
	South Africa	163, 168	Jan. – Mar.	13 – 18	$\bar{X}$: 9	Biennial	Allen and Cliff, 2000
	Mediterranean Sea	142, 163	Spring – Summer	13 – 14	6 – 10	NA	Capape et al., 2003

Table 1 Results from this study and previous studies on spinner shark, *Carcharhinus brevipinna*, reproduction. NA: not assessed.

parturition to occur in *C. brevipinna* during late spring – early summer (Dodrill, 1977), May – June (Castro, 1993), and June – July (Branstetter, 1980), consistent with our results. A gestation period of approximately 12 months and similar litter sizes for NWA *C. brevipinna* were also reported by Dodrill (1977), Branstetter (1980), and Bigelow and Schroeder (1948). Gestation periods for *C. brevipinna* populations outside of the U.S. were reported to be 12 – 18 months but based on the geographical distance between the populations, variation is anticipated. Studies conducted outside of the U.S. had higher variability of litter sizes; however, the range we found in this study (4-8 pups per litter) fit within the previous reported ranges of 3-17 pups per litter (Geraghty et al., 2015; Joung et al., 2005; Sumpton et al., 2010). Based on our largest embryos measuring 51.4 – 53.2 cm FL and the smallest collected young-of-year *C. brevipinna* measuring 57 – 58 cm FL, size at birth is likely to reflect the sizes reported by Springer (1960) and Castro (1993) of 47 – 60 cm FL.

Although reproductive periodicity had not previously been confirmed for the NWA population of *C. brevipinna*, a biennial cycle was reported by Allen and Cliff (2000) in South Africa and Joung et al. (2005) in NW Taiwan, which was found to be similar to our findings. Although we lacked the ability to thoroughly investigate these observations with the archived data, newly collected samples allowed for greater insight into reproductive periodicity of the NWA *C. brevipinna* population. As observed in both components, individual MFD of mature, non-gravid females by month showed at least two cohorts during the spring and summer months. Newly collected animals that were sampled during the month of June, when mating or parturition is likely occurring, were found to exhibit either enlarged vitellogenic follicles, small pre-vitellogenic follicles, or slightly enlarged atretic follicles. It is possible that the female exhibiting slightly enlarged atretic follicles represent maturing / newly matured female that experienced

partial follicular development followed by follicular regression, rather than undergoing ovulation. Similar observations of marginally enlarged atretic follicles in maturing and newly matured animals was also shown in the spiny dogfish, *Squalus acanthias*, (Gračan et al., 2013) and nurse shark, *Ginglymostoma cirratum* (Rêgo et al., 2019). However, it is also possible that these individuals are in a "resting" phase, perhaps suggesting the occurrence of 3 cohorts of female *C. brevipinna* during this period: pregnant individuals, mature vitellogenic females, and mature, non-vitellogenic females. Nonetheless, these observations suggests that annual periodicity is not occurring in *C. brevipinna*, but rather biennial or possibly triennial periodicity. However, due to lack of sample size over a greater span of months, a more precise reproductive periodicity should not be suggested without a more robust investigation.

Despite the numerous similarities between this study and previous studies, size at maturity differed between this study and previous research conducted in the U.S. and non-U.S. regions. As previously mentioned, studies conducted in the NWA found males to mature at 140 and 156 cm FL and 149 cm FL for females, whereas studies conducted outside of this region found larger sizes of maturity at 142-183 cm FL and 163-187 cm FL for males and females, respectively (Table 1). Although size at maturity determined for this study was most similar to the smaller sizes reported within the NWA, our findings were nearly 10 cm smaller for each sex. This, interestingly, makes our findings the smallest reported sizes at maturity for this species to date. Maturity was determined by maturity ogive data for male size at maturity, in which at any given time 50% of the male population is mature, was found to be 130 cm FL. Likewise, results from component two found all males over 129.5 cm FL to be mature based on freely rotating (180°) calcified claspers with a fully functional rhipidion. Previous studies on reproduction in elasmobranchs have used logistic models to simulate a maturity ogive as a marker for size at

maturity in combination with morphological, histological, and endocrinological analysis (e.g., Chen, 2004; Driggers et al., 2004; Jensen et al., 2002; Baremore and Passerotti; 2013, Frazier et al., 2014; and Piercy et al., 2016). Marked inflections in head epididymis and oviducal gland width as a function of FL, as well as clasper calcification have shown to be correlated with maturity (Baremore and Passerotti, 2013; Driggers et al., 2004; Piercy et al., 2016). Furthermore, circulating gonadal steroid hormones (T and E_2 for males and females, respectively) have been shown to correlate with size at maturity and play an important role in regulating reproduction in carcharhinind species and numerous chondrichthyans (Brown et al., 2020; Huepel et al., 1999; Rasmussen, L. and Gruber, S., 1993). More significant to this study, changes in plasma gonadal steroid hormones (i.e., markedly increased concentrations) with maturity and pubertal development has been shown to be a good indicator of maturity in the bonnethead shark, *Sphyrna tiburo*, (Gelsleichter et al., 2002), the draughtboard shark, *Cephaloscyllium laticeps*, (Awruch et al., 2008), the thorny skate, *Amblyraja radiata*, (Sulikowski et al., 2006), and numerous other elasmobranchs (Becerril-García et al., 2020; Jeffery et al., 2012). It can be concluded that based on these previous studies, the indices of maturity used in our study are consistent with past studies evaluating reproduction in elasmobranchs. Differences observed between sizes at maturity for the NWA population of *C. brevipinna* may be attributed to discrepancies in sample size. Although our study does not encompass a very robust sample size, it is several times that than of the largest sample size prior to this study (i.e., 20 individuals).

The present study showed seasonal patterns in follicular development similarly followed patterns observed in other carcharhinid species (Brown et al., 2020; Piercy et al., 2016; Sulikowski et al., 2007). However, one female from component one exhibited enlarged vitellogenic follicles in July, while all other individuals had markedly decreased sizes during this

time. Driggers and Hoffmayer (2009) found plasticity of reproductive periodicity in *C. isodon*, in which some animals were reproducing annually and others biennially. This observation in comparison to the individual observed in this study exhibiting variability in seasonality, is seemingly 'more drastic' in terms of reproductive variability; therefore, it is highly probable for a few individuals, in our case one, to exhibit plasticity in seasonality. Walker (2007) found gummy sharks, *Mustelus antarcticus*, to have a synchronous reproductive cycle, but a few females were reported to be out of phase for nearly three months. More recently studies conducted by Hoffmayer et al. (2012) and Baremore and Hale (2012) found variability in reproductive cycles in Atlantic sharpnose and sandbar sharks, respectively. Similar to observations reported in Walker (2007), this study found *C. brevipinna* to have synchronous gametogenesis between sexes with the exception of one female sampled in July (Figure 4). Plasticity of reproductive biology may be more common in carcharhinid species than initially reported, however, since only one individual was observed to have a protracted cycle it is unlikely this occurs in a significant number of sharks.

An interesting finding observed during this study was a markedly increased E_2 concentration in a single pregnant female. Moreover, the pregnant female was collected during April, when this individual would be in the late stages of pregnancy. Based on assessment of embryos collected during this study and reproductive morphology of pregnant females, parturition is likely occurring June – July. Therefore, the observed increased levels of E_2 was occurring in a mid to near term pregnant female. Previous studies assessing reproduction in elasmobranchs using gonadal steroid hormones found T and E_2 to play important roles in regulating reproduction, where E_2 in gravid females are shown to have decreased concentrations throughout gestation (e.g., Awruch, 2013; Becerril-García, et al. 2020; Gelsleichter and Evans,

2012; Sulikowski et al., 2004). Although, E_2 in gravid females exhibited lower levels than that of reproductively active females in this study, one female sampled in April, during mid-late pregnancy, exhibited an E_2 concentration 5 to 7 times higher than that of the other gravid females caught within the same time period. Earlier and recent studies have reported increased circulating E_2 concentrations occurring mid-pregnancy in elasmobranchs **(**Brown et al., 2020**;** Snelson Jr. et al. 1997; Tricas et al., 2000). The Atlantic stingray, *Hypanus sabinus*, was reported to experience minor periods of fluctuating E_2 concentrations concurrently with changes in MFD during pregnancy (Johnson and Snelson Jr, 1996**)**. These studies focusing on *H. sabinus* lead authors to suggest that E_2 plays a role in the gestation period of the species, specifically with the change from yolk dependency to histotrophy. Brown et al. (2020) also observed higher than anticipated E_2 concentrations of pregnant *C. isodon*, as well as fluctuating MFD during gestation. However, unlike these studies, there was no evidence discovered during our research that suggests annual periodicity or follicular development / fluctuations during pregnancy. Like the single individual potentially experiencing protracted mating previously discussed, this instance of marked elevated E_2 concentration in a pregnant female should not be causation for suggesting an annual periodicity based on one individual observation. It should however, initiate a deeper investigation into the mechanism and manners reproduction is regulated in *C. brevipinna*. For instance, future work focusing on histological evaluation of folliculogenesis in conjuncture with circulating E_2 concentrations can enhance our understanding on the influences these cycles have on one another and ultimately the reproductive biology of this species.

Although histological results from the present study provide evidence for synchronous gametogenesis of *C. brevipinna,* one male had signs of post mating, testicular remodeling (i.e., regression) occurring during the hypothesized month of mating. Histological evaluation of

testicular architecture conducted on the finetooth (Brown et al., 2020), sandbar (Piercy et al., 2016), and bonnethead shark (Gonzalez De Acevedo et al., 2020) found testicular remodeling, regression, and accumulation of evacuated spermatocysts to occur over one to two months post mating. For example, finetooth shark males exhibited a large proportion of evacuated spermatocysts March – May, suggesting onset of mating (Brown et al., 2020). Evacuated spermatocysts in the finetooth shark was highest in the months of June – July, as well as testicular remodeling and an absence of post-meiotic development. Of the two animals collected during June in this present study, one had a degenerative zone encompassing 55% of the testis. Histological evaluation of this male's head epididymis showed a large epididymis containing a sizeable number of mature spermatozoa, suggesting the animal could have still been reproductively active. Based on the histological evaluation of 12 mature male testes, mating is likely to occur between June – July (Figure 8). Therefore, it is probable that the mature male exhibiting high degeneration in late June may have experienced more rapid testicular remodeling than most individuals in the population. Yet, it is important to highlight the sample size of males collected in June (n = 2) being slightly lower than males collected in July (n = 5). If a larger sample size was examined it may have become more evident what proportion of males experienced late stages of spermatogenesis, indicating mating, while during the same period examining what proportion of males were undergoing testicular regression. Nonetheless, an increased sample size would help to address the potential causality of these observations in males, as well as the single individual discrepancies in females previously mentioned. Expanding collection of individuals over winter months for both sexes, prior to projected mating in males (March – May) and increasing sample size overall could 'ground truth' the data presented in this

study while also obtaining samples during months of no collection to further analyze the reproductive biology of *C. brevipinna* over a complete 12-month period.

Despite our limited sample size, this study is the most robust study on NWA *C. brevipinna* reproduction to date with a total of 128 sharks sampled (43 males and 85 females). Subsequently, the results of this study have contributed to a greater understanding of the reproductive biology of *C. brevipinna* and will provide valuable information to management for the species population in the NWA region. Although reproductive periodicity has been confirmed in distant geographical populations, to our knowledge, this is the first study to provide evidence of biennial periodicity for NWA *C. brevipinna* populations. This study is also one of the few to evaluate reproductive morphology and only to evaluate reproductive histology in the NWA population. Earlier studies conducted on *C. brevipinna* in the NWA by Clark and von Schmidt (1965) and Branstetter (1980) showed size at maturity to be larger than we found and therefore further assessment on size at maturity is warranted, particularly for females as variation in size at maturity could influence recruitment. We anticipated our findings to be similar to reproductive parameters reported in earlier studies, which we did find, yet understood deviations were possible due to limitations of sample size, geographical locations, and overall lack of prior knowledge on *C. brevipinna*. Nevertheless, this study provides new up-to-date information on the reproductive potential and biology of *C. brevipinna* that will aid in assessing current stock status and developing species-specific management policies for the NWA population.

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VITA

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EDUCATION

University of North Florida: 2018 - 2021

Masters of Science in Biology; Coastal and Marine Biology Program

Thesis (current document): Reproductive biology of the spinner shark, *Carcharhinus brevipinna*, off the southeast U.S. coast

Advisor: Dr. Jim Gelsleichter

University of North Florida: 2014 - 2017

Bachelor of Science

Major: Biology; Minor: Environmental Studies

Undergraduate Research: Reproductive endocrinology of the blacktip shark, *Carcharhinus limbatus*, off the Southeastern U.S. coast

Advisor: Dr. Jim Gelsleichter

EMPLOYMENT

Georgia Department of Natural Resources: 2017

Marine Technician

Supervisor: Donna McDowell / Carolyn Belcher

RESEARCH EXPERIENCE / EXPEDITIONS

University of North Florida – COASTSPAN Survey: 2016 - Current

Undergraduate and Graduate Research Assistant

Florida State University: Oct 2019; Oct & Sept 2020; March 2021

Lead Scientist: Dr. Dean Grubbs

Research Team Member

OCEARCH: 2018 - 2020

Research Team Member

Cape Eleuthera Institute and Florida International University: Jan 2019; June-July 2019

Research Team Member

South Carolina Department of Natural Resources: June 2017; May 2019

Lead Scientist: Bryan Frazier

Research Team Member

MAJOR PRESENTATIONS

Joint Meeting of Icthyologists and Herpetologist

July 2019: Snowbird, Utah

July 2017: Austin, Texas

Conference of Florida Graduate Schools

April 2019: Florida International University

Showcase of Osprey Advancements in Research and Scholarship

April 2017 & 2019

SCIENTIFIC OUTREACH

OCEARCH Outreach: 2019-2020

Georgia CoastFest: October 2017

St. Johns Riverkeeper: 2012

SCIENTIFIC MEMBERSHIPS

American Elasmobranch Society