


2015

The Reproductive Biology of the Finetooth Shark, *Carcharhinus isodon*, in the Northwest Atlantic Ocean

Amanda N. Brown

University of North Florida, n00856608@ospreys.unf.edu

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The Reproductive Biology of the Finetooth Shark, *Carcharhinus isodon*, in the Northwest
Atlantic Ocean

by

Amanda Nevada Brown

A thesis submitted to the Department of Biology
in partial fulfillment of the requirements for the degree of

Master of Science, Biology

UNIVERSITY OF NORTH FLORIDA

COLLEGE OF ARTS AND SCIENCES

May, 2015

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This Thesis titled The Reproductive Biology of the Finetooth Shark, *Carcharhinus isodon*, in the Northwest Atlantic Ocean is approved:

Jim Gelsleichter
Committee Chair

J. Marcus Drymon

Julie Avery

Accepted for the Department of Biology:

Dan Moon
Chair, Department of Biology

Accepted for the College of Arts and Sciences:

Barbara Hetrick
Dean, College of Arts and Sciences

Accepted for the University:

Dr. John Kantner
Dean of the Graduate School

DEDICATION

This thesis is dedicated in loving memory to my mother, Lauren Nevada Brown, for her constant love, support and encouragement. You are my best friend, now and always. And to my father, Tom Brown for picking up where she left off.

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ACKNOWLEDGEMENTS

I would like to first thank the NOAA Cooperative Research Program for providing the funding for this research. A huge thank you to the University of North Florida Graduate program for providing me with a graduate teaching assistantship for 5 semesters, thereby preventing my starvation and homelessness. My greatest thanks to my major advisor and committee chair, Dr. Jim Gelsleichter for being my mentor, for his patience and for sporadically serving as my psychiatrist. You have been an amazing and outstanding advisor and friend. I would like to thank my committee members, Dr. Julie Avery and Dr. Marcus Drymon for taking the time out of their busy lives to assist me through this process and for helping to mold my project into what it became. A deep appreciation goes to my co-author Bryan Frazer at SCDNR for assisting in field sampling and for being an overall badass. I would like to acknowledge the commercial fishermen who were key to my Florida sampling efforts, Jay and Sara Bauman, for being incredible shark fishermen and the most entertaining collaborators I've worked with to date. Waking up to go fishing at 4am with you was never a chore. Also thank you to my lab mates and many volunteers who assisted in field work, especially the UNF TLO classes. And to my wonderful and amazing friends and fellow grad students who have been integral to my sanity and happiness, especially Dean Grubbs, my best friend, partner in crime and unofficial committee member. And most of all to my family. To my brother, an incredible fisherman in his own right (and much better than me), for growing up side by side me, making my childhood epic and learning to love the ocean and fishing with me. To my father, you are one of the main reasons I have accomplished this. Your unwavering and constant support have meant more to me than you can know. I feign to think of my life without you these past 3 years. And most of all, to my mother. This thesis is dedicated to your memory, there are not words to describe what you mean to me. I appreciate everything you have taught me, all the encouragement you have given and the sacrifices you have made. You are my best friend, inspiration and mentor. I hope this makes you proud, as I am proud to be your daughter.

ABSTRACT

Shark fisheries are a multimillion dollar industry in the United States and have significant contributions both recreationally and commercially. In order to maintain this industry, fisheries must be properly managed. An understanding of life history and reproduction is crucial in order to adequately manage shark fisheries. The finetooth shark, *Carcharhinus isodon*, is a member of the small coastal shark (SCS) fishery complex. It is found in Atlantic waters from South Carolina to Florida and throughout the Gulf of Mexico. This species has recently come under increased fishing pressure and has previously been overfished. New data is needed in order to properly assess the reproductive capacity of this species so that healthy populations can be sustained. This study evaluated the overall reproductive cycle of northwest Atlantic populations of *C. isodon* using composite variables of morphology, histology and endocrinology. Atlantic *C. isodon* were found to display seasonal reproduction with biennial reproductive periodicity with a 12 month gestation period and litters of 2-6 pups. E_2 and T displayed similar patterns and were highest during gametogenesis. P4 did not show any discernible patterns. Estrogen receptors were found in developing follicles of gravid and non-gravid females, in the oviducal glands of non-gravid and early pregnant females and in the uterus of pre-ovulatory females. P4 was found in the oviducal glands in all reproductive stages and in the uterus of pre-ovulatory females. Androgen receptors were found in Sertoli cells and mature spermatozoa as well as epididymal epithelial cells.

CHAPTER ONE:

THE REPRODUCTIVE BIOLOGY OF THE FINETOOTH SHARK, *CARCHARHINUS ISODON*, IN THE NORTHWEST ATLANTIC OCEAN

INTRODUCTION

The finetooth shark, *Carcharhinus isodon*, is a small coastal shark that inhabits waters of the northwest Atlantic Ocean and the Gulf of Mexico (Castro, 1993; Drymon et al, 2006). Atlantic ocean populations of finetooth sharks embark on seasonal migrations, moving south beginning in early fall to spend colder winter months in coastal northeast and central Florida waters once temperatures drop below 20 °C. In late spring/early summer, they begin to return north towards the cooler waters of Georgia and South Carolina (Castro, 1993). Based on qualitative data, their diet consists almost exclusively of small teleost fish, such as *Brevoortia tyrannus* and *Leiostomus xanthurus* although they do occasionally consume crustaceans such as *Callinectes sapidus* (Castro, 1993). A more recent study by Bethea et al (2004) has provided quantitative data on diet which agrees with this assessment but also shows consumption of other pelagic and epibenthic teleosts from families Carangidae, Sparidae, and Syngnathidae, among others.

C. isodon is a member of the small coastal shark (SCS) fishery management complex, which is largely composed of fast growing, small bodied, short lived sharks. Other members of this group include the bonnethead (*Sphyrna tiburo*), the Atlantic sharpnose (*Rhizoprionodon terraenovae*),

and the blacknose shark (*Carcharhinus acronotus*). Although species of the large coastal shark (LCS) fishery complex are more coveted by commercial fisherman, management initiatives enacted in the late 1990s restricted commercial catches of this group (NMFS, 1999), shifting some fishing pressure to small coastal sharks. Recreational fishing and commercial by-catch in gillnet fisheries also account for mortality of *C. isodon* (Trent et al, 1997; de Silva et al, 2001). In just 5 years, commercial landings of small coastal sharks increased from 7 metric tons to 35 metric tons and have continued to rise (Carlson et al, 2003). As a result, certain representatives of the SCS complex including *C. isodon* and *C. acronotus* have been determined to be overfished in recent fishery assessments (Cortes, 2002; NMFS, 2007), prompting the need for greater management of these species based on accurate life history and reproductive biological data.

A review of the available life history data provides some insight as to why *C. isodon* is more susceptible to overexploitation than other members of the SCS complex. For example, *C. isodon* is relatively large at sexual maturity, which has been reported to range between 102-127 cm fork length (FL) for females and 102-117 cm FL for males (Carlson et al, 2003, Drymon et al, 2006; Castro, 1993), sizes comparable to those reported for certain members of the LCS complex such as the blacktip shark, *Carcharhinus limbatus*, which has a size at maturity between 117-126 cm FL for females and ~117 cm FL for males in the Atlantic (Carlson et al, 2006). They have also been reported to exhibit a biennial reproductive strategy and small litter size (2-6 pups), traits shared by large coastal sharks such as the recently overfished dusky shark (*C. obscurus*). These similarities could indicate that *C. isodon* requires management initiatives more comparable with those governing the large coastal shark fishery complex.

Notwithstanding, there remains a need for greater information regarding the reproductive biology of *C. isodon*, a point emphasized by fishery managers in recent stock assessments of the SCS complex (NMFS, 2007). For example, discrepancies in the size at maturity between Atlantic Ocean and Gulf of Mexico populations have been reported in literature (Castro, 1993; Carlson et al, 2003; Drymon et al, 2006). In addition, although previous studies have reported that *C. isodon* typically reproduces biennially, individuals exhibiting signs of annual reproduction have recently been observed in Gulf of Mexico populations (Driggers and Hoffmayer, 2009). Such observations justify re-evaluation of the reproductive biology of this species, especially considering that the only published study on the reproductive biology of Atlantic *C. isodon* populations is over two decades old. Therefore, the goal of this study was to re-examine reproduction in Atlantic Ocean populations of *C. isodon* by analyzing the gross morphology and histological structure of various reproductive organs throughout the reproductive cycle.

MATERIALS AND METHODS

Sampling Methods- Sharks were collected using bottom longlines and gillnets in fishery dependent and independent sampling surveys in the Atlantic waters of Florida, Georgia and South Carolina from 2012-2014. Sampling occurred monthly in estuaries, nearshore coastal waters and offshore coastal locations. Following capture, precaudal length (PCL; tip of rostrum to precaudal pit), fork length (FL; tip of the rostrum to fork in caudal fin) and stretched total length (STL; tip of the rostrum to the posterior end of the extended caudal fin) were measured in cm. Weight was measured in kilograms. Blood was collected via caudal venipuncture for companion studies on reproductive endocrinology. The reproductive organs were removed via dissection and used for morphological and histological assessments of reproductive stage.

Morphological assessments- Morphological measurements of reproductive organs were collected from males and females to assess reproductive maturity and stage. Maturity in males was determined by the presence of calcified claspers that rotated 180° with a freely opening rhipidion. Females were deemed mature based on development of the reproductive tracts as described in Manire et al. (1995). For males, clasper length, testis length, width, and weight (in grams), and head epididymis width was measured in mm using dial calipers. The presence of semen in the seminal vesicle was recorded. For females, uterine width, maximum follicle diameter and oviducal gland width were measured with digital calipers. The ovary was examined by determining the presence and number of vitellogenic and atretic ova. If the female

was gravid, ova or embryos from each uteri were enumerated, measured and sexed. Subsamples of each reproductive organ were collected for histological analysis.

Histology- Subsamples of testis, head epididymis, ovarian follicles, uteri and oviducal glands were taken from mature animals via dissection and stored in 10% buffered saline formalin for 48 h. Samples were trimmed and rinsed in tap water for 24 h, then stored in 70% reagent alcohol. After, trimmed samples were dehydrated in an ascending graded series of reagent alcohol (80-100%), cleared in a limonene-based solvent (CitriSolv, Fisher Scientific, Fair Lawn, NJ) and processed using routine paraffin histology. Samples were cut into 5- μ m transverse sections using a rotary microtome and adhered to slides coated with 0.01% poly-L-lysine. Slides were then stained with Harris Hemotoxylin and Eosin and analyzed using a compound microscope to observe general patterns of and seasonal changes in cellular architecture.

Statistical analysis- Quantitative measurements of reproductive structures were grouped by month of collection and, for females, pregnancy status. Width of the testis and head epididymis in males were analyzed using one-way ANOVA followed by a Tukey's *post hoc* test to determine if significant differences occurred by month. Female maximum follicle diameter and oviducal gland width were initially analyzed via two-way ANOVA to determine if significant differences occurred by month of collection and pregnancy status. Afterwards, monthly differences in maximum follicle diameter and oviducal gland width were analyzed for gravid and non-gravid females separately using one-way ANOVA and *post-hoc* tests. Differences between mean embryo size by month were examined using one-way ANOVA and post-hoc tests.

Correlation between gravid female size in FL and litter size was examined using Pearson's product-moment correlation coefficient. Histology of the reproductive structures was analyzed qualitatively.

RESULTS

Morphology- A total of 63 male *C. isodon* were examined in this study. Mature males ranged from 128 cm – 146 cm STL. Testis width (TW) followed a seasonal pattern in which it increased beginning in September, and continued to increase until it peaked in late April-early May at approximately 39 mm (Figure 1.1a). Testis then quickly declined in size around mid- to late May and were found to be fully regressed (4-7 mm width) in all males from June to August. Significant temporal differences were found in TW based on month (one-way ANOVA, $p < 0.001$). Head epididymis width (HEW) followed a similar pattern of steady increase in size beginning in September with a peak in April-May at 27.5 mm, followed by a marked decline in late May-early June following copulation (Figure 1.1b). There were also significant differences based on month in HEW (one-way ANOVA, $p < 0.001$) Semen was found in the seminal vesicle beginning in early April and remained present until July (Figure 1.2).

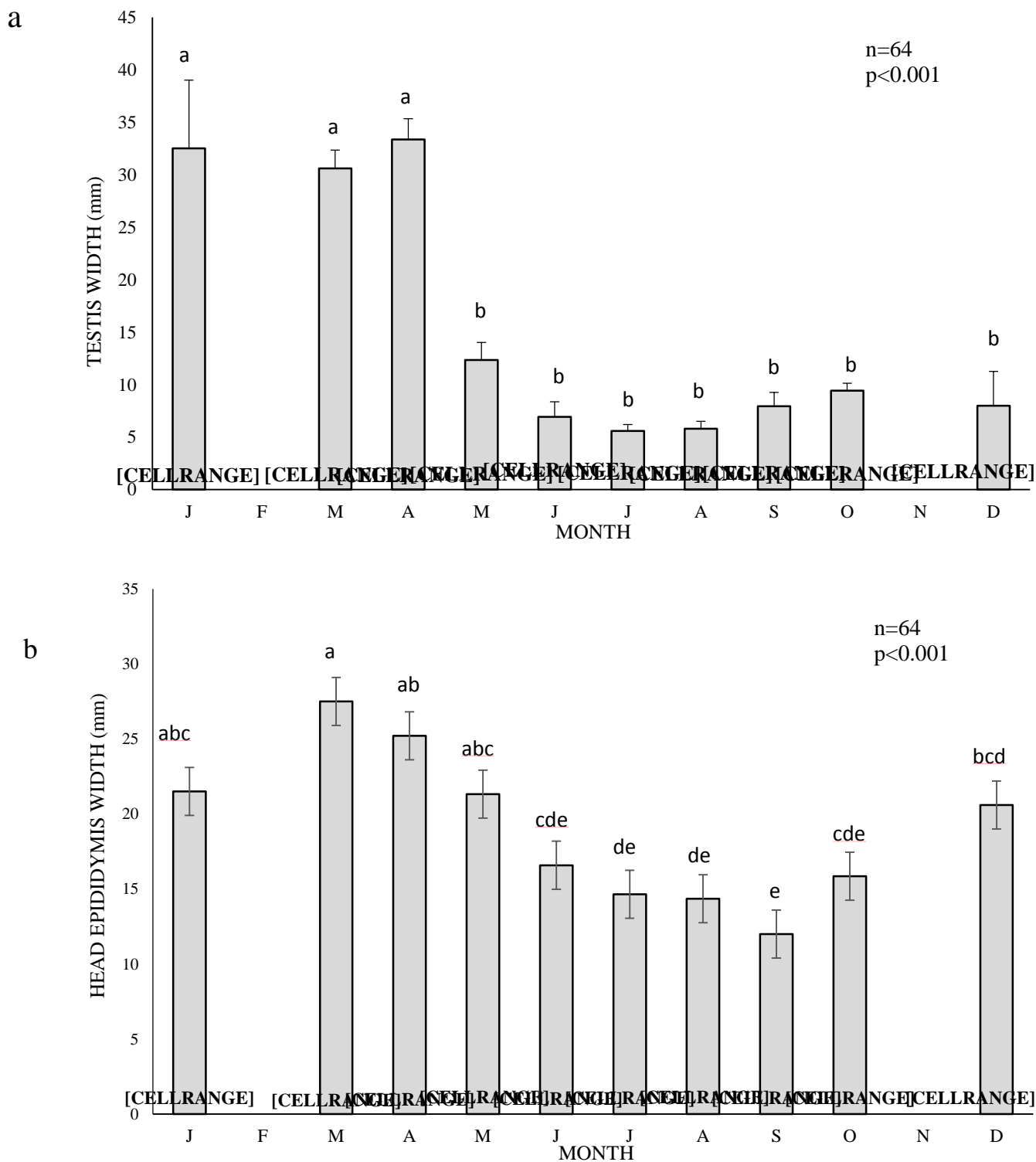


Figure 1.1 Mature male *C.isodon* (a) testis width and (b) head epididymis width by month. Values represent means \pm SEM. Monthly sample size is indicated inside bars. Significance was determined using one-way ANOVA. Letters above bars represent homogenous subsets at $P<0.05$.



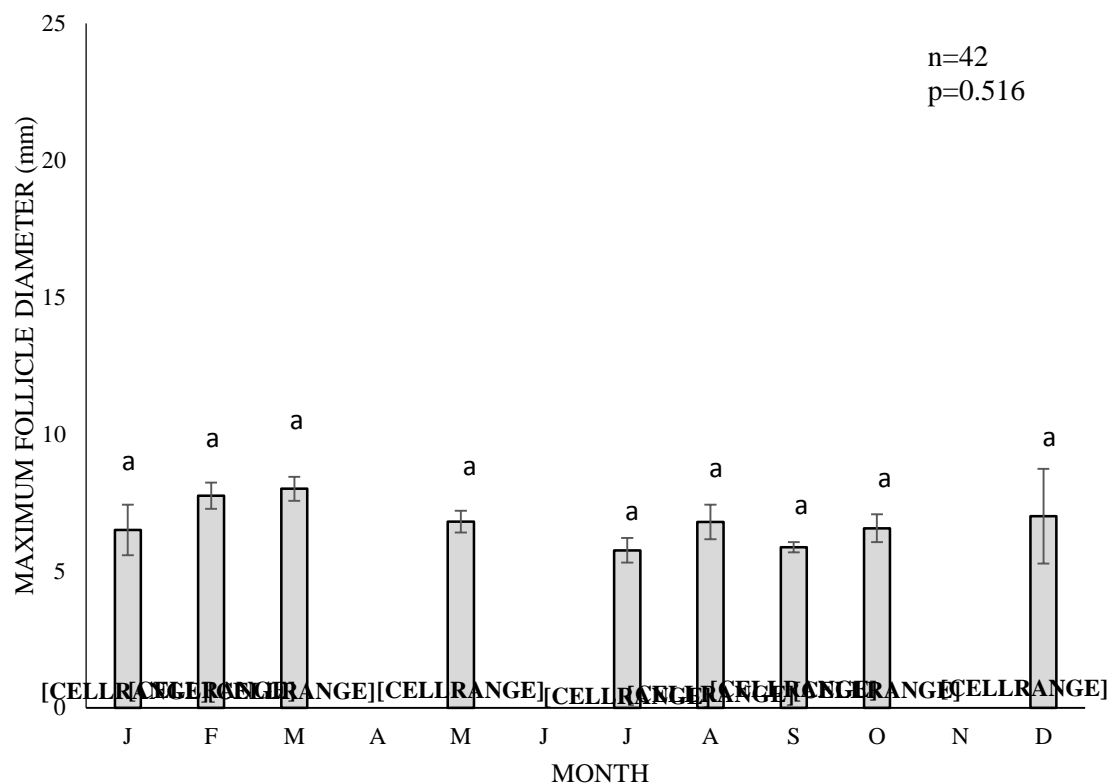
Figure 1.2. a) Reproductive tract of mature male *C. isodon*. At the anterior portion of the tract is the epididymis (EP) leading into the ductus deferens (DD) and terminating in the seminal vesicle (SV). b) SV filled with semen in a mature male caught one month prior to the mating season.

A total of 81 mature female *C. isodon* were examined in this study. Mature female *C. isodon* ranged in size from 132 cm - 162 cm STL. Female morphological data displayed the same temporal patterns in follicle growth as that observed for male testes. Follicle diameter began to increase in non-gravid females in September and peaked in late winter-early spring, but did not vary in gravid females during the same period (Figure 1.3). Significant differences in follicle diameter based on month were found in only non-gravid females (one-way ANOVA, $p=0.032$). Follicular diameter was markedly lower in both gravid and non-gravid females after May, suggesting that ovulation had recently occurred. With the exception of one female, vitellogenic follicles were present in only non-gravid females whereas embryos were present in only non-vitellogenic females, suggesting a biennial reproductive cycle with non-concurrent periods of follicular growth and gestation. Only one of the 81 females examined displayed annual reproductive periodicity (Figure 1.7). This female had large, vitellogenic ova (32 mm) and embryos in both uteri, although the embryos in one uterus were discolored and smaller in size than other embryos caught in the same month. The other uterus contained a pup that had undergone EED.

Oviducal gland width increased in non-gravid females in concert with follicle growth, peaking significantly in spring (Figure 1.5) (one-way ANOVA, $p=0.005$). Oviducal gland width did not vary in pregnant females by month (one-way ANOVA, $p=0.154$) during the same period, but was slightly greater in newly pregnant females collected during May, perhaps reflecting increases that occurred prior to ovulation (Figure 1.4). Fresh mating wounds were present on females in May. 58.0% of females examined (49 out of 83) were pregnant, providing additional support for a biennial reproductive periodicity. The earliest and latest stages of pregnancy were

found in May, suggesting a 12-month gestation period. Litter size ranged from 2-6 pups with a median of 4 pups/litter (Figure 1.5a). Embryos ranged in size from 65-530 mm STL. Embryo growth was greatest during the first 3 months of gestation starting in July at an average STL \pm SEM of 94.45mm \pm 4.5 and ending in September with an average of 260.2 mm \pm 16.2. Embryos continued to grow rapidly until December when an attenuated period of growth began, during which embryos grew only an average of 81.4 mm \pm 30.4 from December to May (Figure 1.5). Significant differences were found in embryo size based on month (one way ANOVA; $p < 0.001$). A logarithmic growth function best modeled embryonic growth ($R^2 = 0.902$). Maximum observed pup STL was 530 mm with an average maximum size of 499.4 mm \pm 7.7. Early embryonic death (EED), where one or more embryos were partially or fully degraded *in utero*, was found in 14.9% of *C. isodon* litters (Figure 1.6) and occurred in early, mid and late stages of pregnancy.

a



b

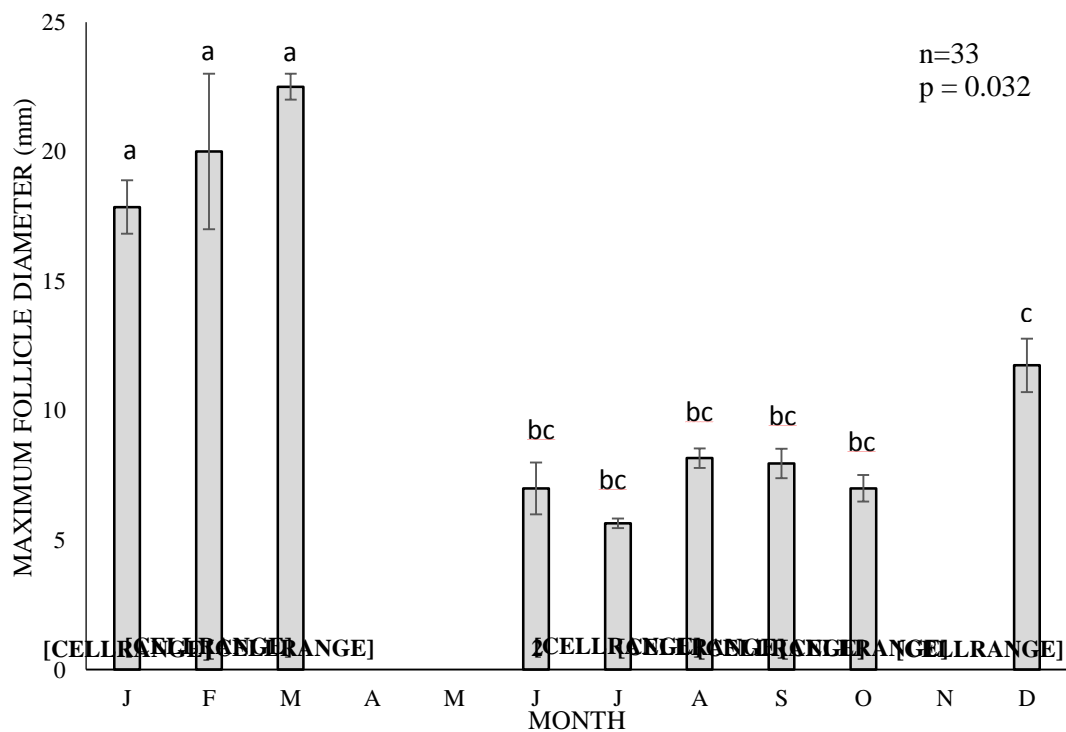


Figure 1.3. Maximum follicle diameter (mm) in a) non-gravid and b) gravid mature female *C. isodon* by month. Values represent means \pm SEM. Sample size is indicated inside bars. Significance was determined using a one-way ANOVA. Letters above bars represent homogenous subsets at $P < 0.05$.

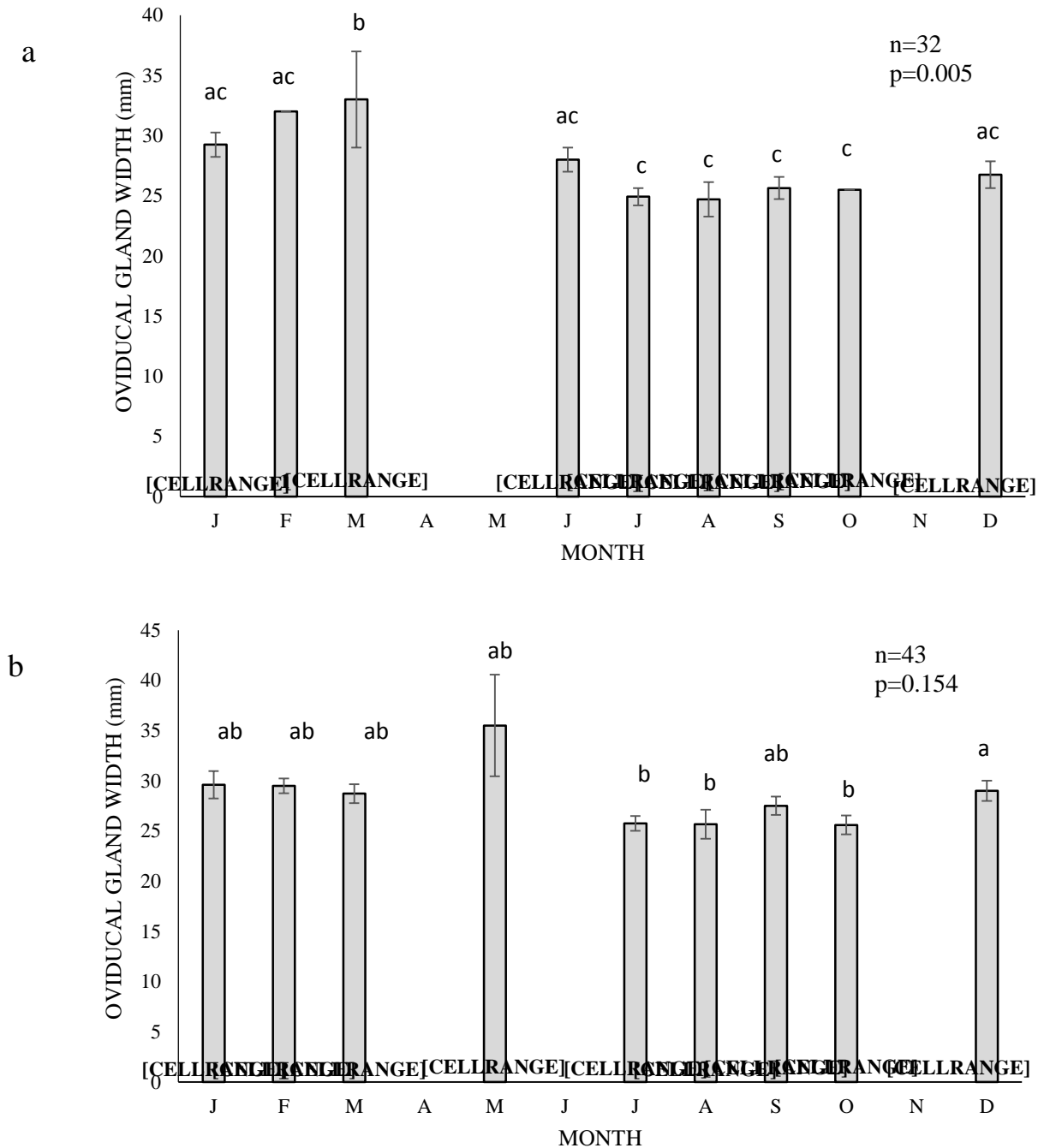


Figure 1.4. Seasonal changes in oviducal gland width of a) non-gravid vs b) gravid mature female *C. isodon*. Values represent means (\pm SEM). Sample size is indicated inside bars. Significance was determined using a one way ANOVA. Letters above bars represent homogenous subsets based on month at $P < 0.05$.

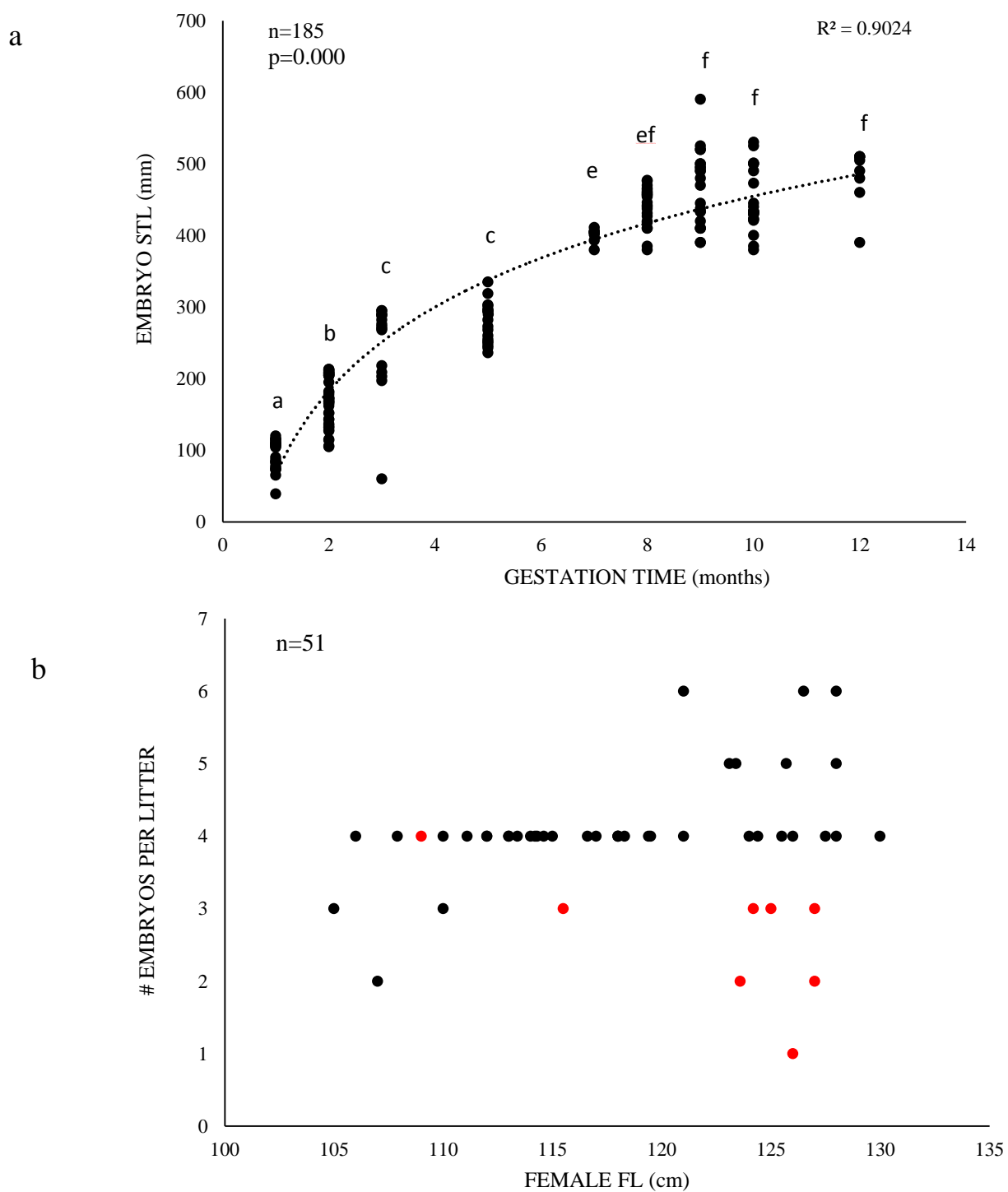


Figure 1.5. a) Stretched total length of *C. isodon* embryos from time of implantation until birth. A logarithmic growth curve was fit to the data ($R^2=0.9024$). b) Number of embryos per litter and fork length (FL) of gravid females. Females with litters that included EED pups are shown in red. Litter size and female size were not significantly correlated (Pearson r , $P = 0.1705$)

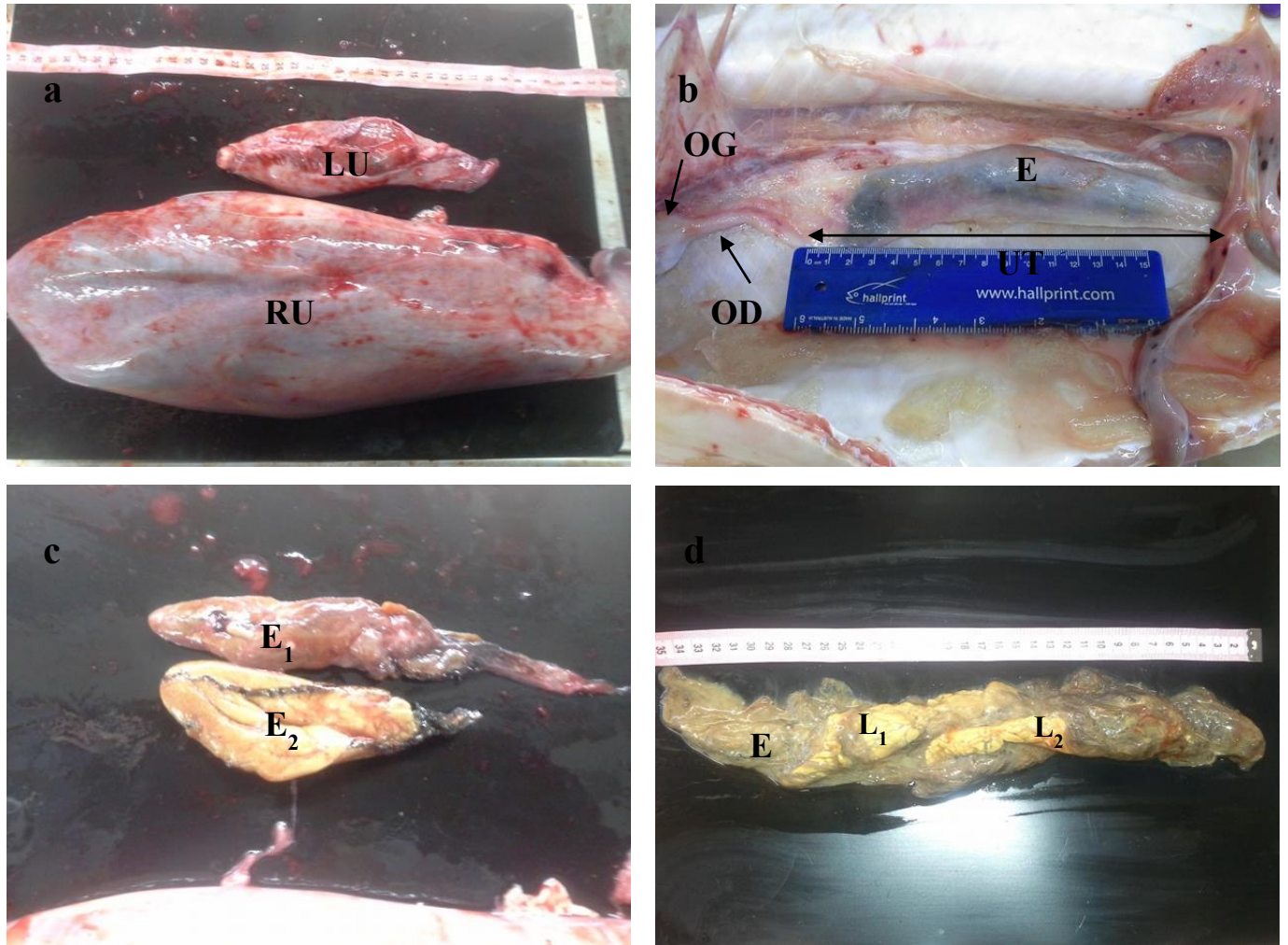


Figure 1.6. Examples of early embryonic death (EED) found in gravid female *C. isodon* uteri: a) Right and left uteri from a pregnant female; the right uterus (RU) contained normally developing embryos whereas the left uterus (LU) contained embryos that had undergone EED. b) Reproductive tract of a gravid female. Oviducal gland (OG) and oviduct (OD) leading to uterus (UT) that contained an embryo (E) undergoing EED. c) The remains of two embryos (E₁ and E₂) that underwent EED. D) Embryo (E) that underwent EED. The two large lobes of the embryonic liver (L₁ and L₂) are visible.



Figure 1.7 Female *C. isodon* displaying annual reproductive periodicity. a) Arrow is pointing at large, vitellogenic ova (VO) and uteri (UT) containing 2 embryos in the left uterus and 1 embryo undergoing EED in the right uterus (b). c) Viable embryos from the right uterus of annual female (P1, P2) compared to normal embryo from a different female caught during the same month (P3). Embryos 1 & 2 appear smaller in size and slightly discolored compared to the normal healthy embryo.

Histology- The germinal zone and spermatocysts containing primary spermatogonia (Figure 1.8a), secondary spermatogonia (Figure 1.8b), and primary spermatocytes (Figure 1.8c), the latter of which mark the end of mitotic division and the transition into meiosis I, were the only stages of spermatogenesis found in animals collected from July through September (Figure 1.9a), suggesting limited sperm production during this period. Spermatocysts containing germ cells undergoing Meiosis II (Figure 1.8c), spermatids (Figure 1.8d), and mature spermatozoa (Figure 1.8e), were more prevalent in animals collected from December through May, suggesting active spermatogenesis. The delineation between resting and active spermatogenesis is illustrated prevalence of meiotic stages in the cross section (Figure 1.9b). Males that had completed spermatogenesis showed predominantly late stage (Stage V-VI) spermatocysts throughout the entirety of the cross section (Figure 1.9c). Evacuated spermatocysts (Figure 1.8f) were first observed in males collected in March and most prominent in males collected in June and July (Figure 1.9d), reflecting spermiation. All stages of spermatogenesis were observed in testis of samples collected between June to October (Figure 1.9e), after which signs of testicular re-modelling are more prevalent.

Stages of ovarian development observed in females are shown in Fig 1.10. Primordial, developing and primary follicles were found in both gravid and non-gravid females (Figures 1.10a, b, and c, respectively). They were most prevalent in gravid females throughout the duration of pregnancy. The most advanced stage of follicle development found in gravid females were pre-vitellogenic follicles (Figure 1.10d, e) found towards the end of gestation, indicating possible preparation for oogenesis following parturition. Non-gravid females had an abundance of primordial and primary follicles from July-September after which secondary and

pre-vitellogenic follicles were predominant until December suggesting this is the period leading up to vitellogenesis. From December-March, vitellogenic follicles (Figure 1.10f) were the most prevalent with earlier stages present in less abundance. Atretic follicles were found in both gravid and non-gravid females throughout the entirety of the reproductive cycle. The largest atretic follicles were found in late May suggesting ovulation had taken place, follicular development had ceased and degeneration of non-ovulated vitellogenic or post-ovulated follicles had begun.

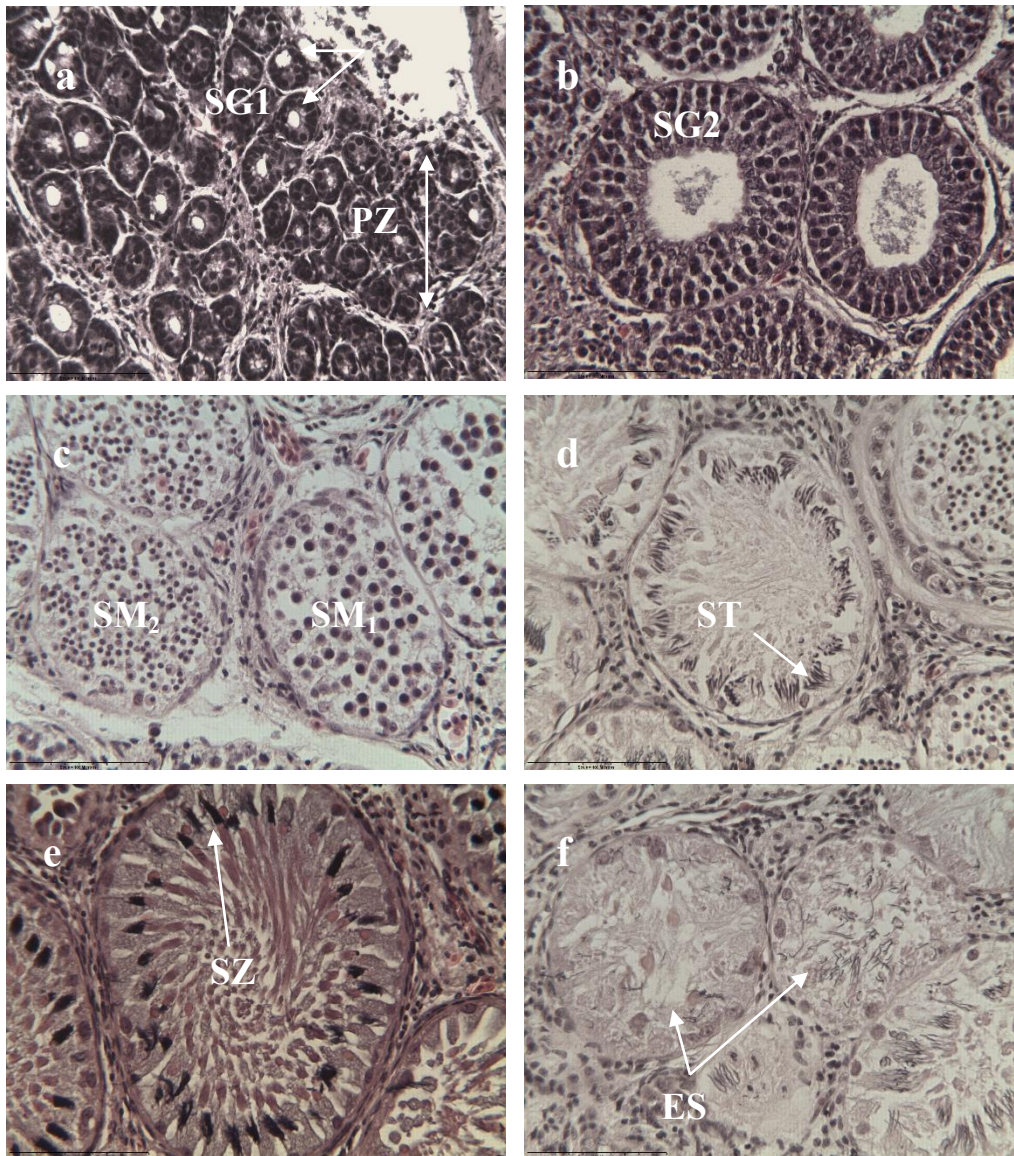


Figure 1.8. Histological transverse sections of male *C. isodon* testes taken at different stages of spermatogenesis. All images are taken at 40x magnification and include: a) Stage I containing the proliferative zone (PZ) and primary spermatogonia (SG1). b) Stage II showing early stage spermatocysts with secondary spermatogonia (SG2) and associated Sertoli cells enclosed in a basement membrane. c) Stage III and IV; Stage III spermatocysts contain primary spermatocytes undergoing Meiosis I (SM₁), Stage IV contain secondary spermatocytes undergoing Meiosis II (SM₂) d) Stage V includes spermatids (ST) undergoing spermiogenesis. e) Stage VI are fully mature spermatozoa (SZ) forming clusters associated with one Sertoli cell/cluster. f) Stage VII shows evacuated spermatocysts (ES).

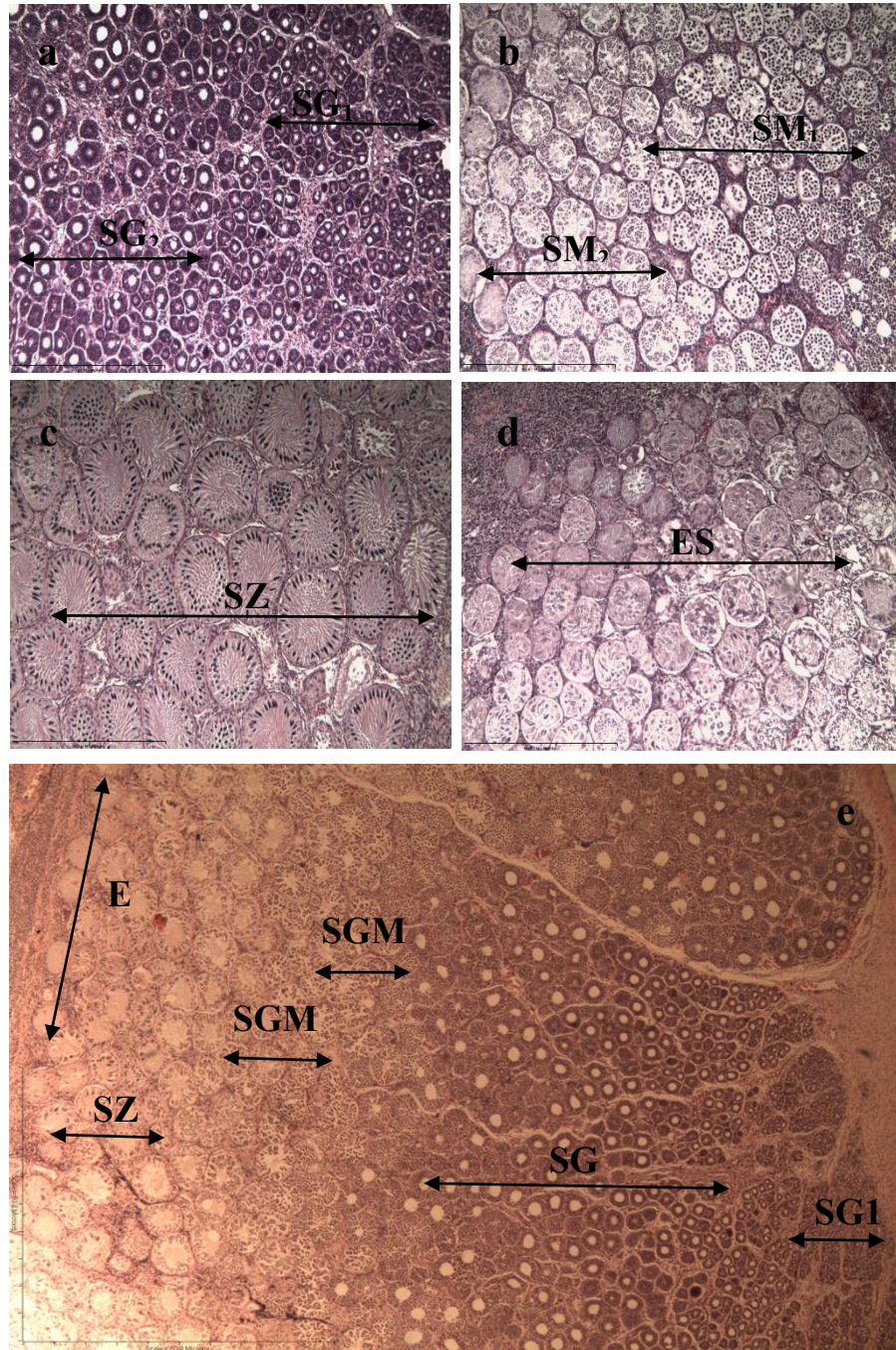


Figure 1.9 Transverse sections of male *C. isodon* testes in different stages of reproductive cycle. Images include: a) 10x section from a male caught in October; section was composed almost entirely of primary and secondary spermatogonia (SG₁ and SG₂, respectively) indicating it is in an inactive stage prior to spermatogenesis; b) 10x section of a male caught in December containing large amounts of spermatocysts undergoing meiosis I (SM₁) and meiosis II (SM₂) indicating the beginning of spermatogenesis; c) 10x section of male caught in April showing mature spermatozoa (SZ); d) 10x section from male caught in August after the mating season was concluded showing large amounts of evacuated spermatocysts (ES); e) 4x entire cross section of testis from animal caught in December showing all stages of spermatogenesis.

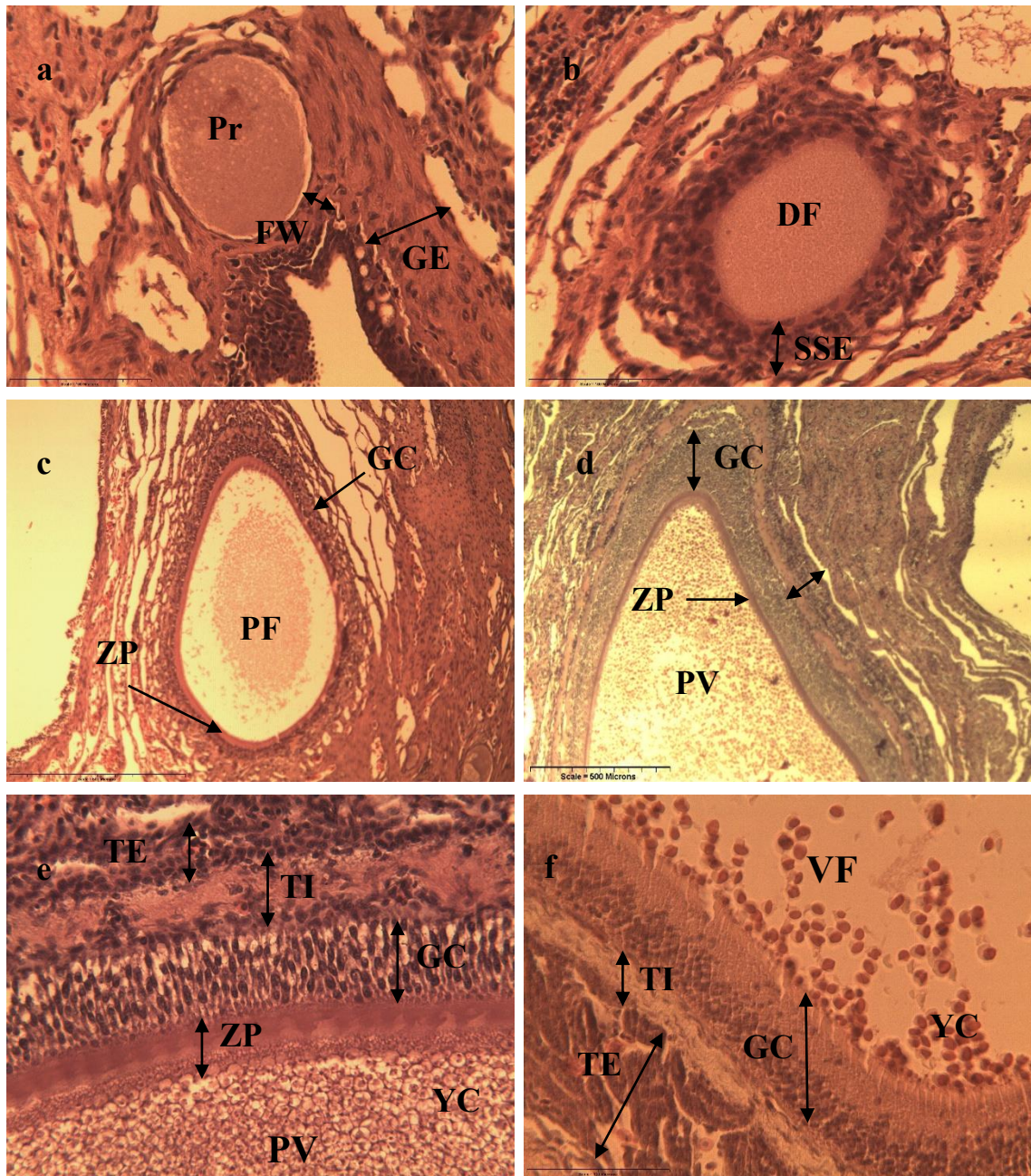


Figure 1.10 Transverse sections of ovaries taken from mature female *C. isodon* in different stages of the reproductive cycle. Images include a) 40x magnification of section taken from a newly gravid female in May; section shows primordial follicle (Pr) surrounded by simple squamous epithelium surrounded by the follicular wall (FW) attached to the germinal epithelium (GE); b) 40x magnification of a developing follicle (DF) taken from a gravid female in July. The epithelial layer has become stratified squamous epithelium (SSE); c) 40x magnification of a primary follicle (PF) taken from a non-gravid female caught in September. The SSE has now become the granulosa cell layer (GC) and surrounds a thick zona pellucida (ZP); d) 10x magnification of a pre-vitellogenic follicle (PV) taken from a non-gravid female caught in October. The GC has continued to thicken and surrounds the zona pellucida (ZP). The theca cell layer (TL) is now visible; e) 40x magnification of a pre-vitellogenic follicle (PV) showing the theca interna (TI) and theca externa (TE) layers. The granulosa cell layer (GC) is now pseudostratified columnar surrounding the ZP. Yolk crystals (YC) have begun to form in the ooplasm indicating this is the stage right before vitellogenesis begins; f) 40x magnification of a vitellogenic follicle (VF) taken from a female caught in February. The GC is now simple columnar surrounded by TI and TE layers. YC are now block like and the ZP is no longer visible.

DISCUSSION

The results of this study confirm that Atlantic populations of *C. isodon* use a seasonal, biennial reproductive strategy. Morphological and histological analysis of the reproductive structures demonstrated temporal changes in reproduction in which gametogenesis occurs in both mature males and females during the months of December through April/early May. All gravid females caught showed an absence of vitellogenic ova. Conversely, all non-gravid mature females had vitellogenic ova present in their ovary, indicating they dedicate an entire year exclusively to gestation and the entire following year exclusively to follicular development. Ovulation most likely took place in late April-early May followed by copulation in late May-June. Gestation lasted for approximately 12 months with pupping taking place in May-June the following year. Litter sizes ranged from 2-6 pups/litter with a median litter size of 4. Embryos were 460-530 mm STL at the end of gestation.

Results from this study are consistent with earlier reports on Atlantic populations of finetooth sharks. Castro (1993) also found evidence for a biennial reproductive strategy with concurrent periods of gestation and folliculogenesis in gravid and non-gravid females, respectively, and a 12 month gestation period. Size at birth was similar to that suggested by Carlson et al (2003) based on back-calculated size-at-birth estimates from finetooth sharks from the Gulf of Mexico.

Notwithstanding similarities between this study and earlier reports, there have been reported differences in other life history parameters between Gulf of Mexico and northwest Atlantic finetooth shark populations. For example, Atlantic populations were found to have larger size-at-age individuals and slower growth coefficients than their Gulf of Mexico counterparts (Carlson et al, 2003; Drymon et al, 2006). To date, there is only one published account of finetooth reproductive biology in the Gulf of Mexico. This study indicated that Gulf of Mexico finetooth sharks displayed both annual and biennial reproductive cycles, a pattern not typically seen in Atlantic specimens (Driggers and Hoffmayer, 2009). A change in reproductive strategies could be energetically based where the sharks are allocating their reserves more towards reproduction than somatic growth allowing for mating and parturition to occur in a one year period of time. This hypothesis would be consistent with the smaller sizes and early sizes at maturity reported for Gulf of Mexico populations (Drymon et al, 2006). Therefore, Gulf of Mexico populations may have a higher density of prey availability and more energy may be available for reproductive efforts. In contrast, with the exception of one individual, all specimens collected in the current study displayed a biennial reproductive strategy (n=81). The variability displayed could indicate reproductive plasticity in response to environmental stimuli such as prey abundance, temperature or fishing pressures. Variability in intraspecific reproductive strategies have been reported in other elasmobranchs such as the blacknose shark, *C. acronotus*, which has been reported as both annual and biennial in the Atlantic (Ford et al., unpublished). It has also been reported in some species of viviparous snakes such as the adder *Vipera berus*, which typically exhibits biennial or triennial periodicity but was found to reproduce annually during a year that had unusually warmer temperatures, allowing for more time to forage for prey (Strugariu et al, 2014). It has also been suggested that migratory patterns

could impose an energy deficit on animals that undergo large seasonal migrations, reducing the amount of energy available for reproductive efforts (Sulikowski et al, 2007). Although there is limited data on migratory patterns of *C. isodon* in the Gulf of Mexico, the distribution in that ocean basin lies along the same latitude, suggesting migration would not be necessary since it is driven primarily by temperature (Castro, 1993). Atlantic populations have a distinct migratory pattern that spans the coasts of three states (Castro, 1993) and may explain in part the requirement for biennial vs annual reproduction in the Atlantic.

The differences that occur in reproduction between Gulf of Mexico and Atlantic populations can have consequences for population growth and management. Using theoretical longevity estimates from Gulf of Mexico and Atlantic finetooth shark females from Carlson et al (2003) and Drymon et al (2006) of 14.4 and 18.2 years, respectively, and an estimated age at maturity of 4.3 and 6.3, respectively, annual Gulf of Mexico finetooth sharks would have approximately 10.1 reproductive events/lifetime and a lifetime fecundity between 20.1-60.1 pups whereas a biennial Atlantic finetooth shark would have approximately 5.95 reproductive events/lifetime and a lifetime fecundity of 11.9-35.7 pups, almost half that of their Gulf counterparts. This would suggest that Atlantic finetooth sharks may be more susceptible to overfishing and may need to be managed as a separate distinct population.

Potentially adding to susceptibility of the Atlantic Ocean finetooth populations is the non-trivial rate of EED (nearly 15%) observed in the current study. While there is little previously published material on EED in elasmobranch fishes, high rates of infertility can clearly have

negative effects on population growth. Cortes and Parsons (1996) conducted a demographic study that demonstrated that unsuccessful fertilization and EED in the bonnethead shark, *Sphyrna tiburo*, could reduce population growth rate by as much as 22%. More data on this phenomenon is necessary to determine whether the rate of EED displayed by *C. isodon* is an anomaly or is prevalent throughout the elasmobranchs.

Results from this study conclude the use of a biennial reproductive strategy with concurrent cycles of folliculogenesis and gestation and a median litter size of 4 pups/litter, all reproductive characteristics that are intermediate to those in the small coastal shark (SCS) and large coastal shark (LCS) complexes. Their biennial reproductive strategy distinguishes them from all other SCS species and is a quality more characteristic of LCS species. Since species-specific life history information on *C. isodon* has been limited, it was placed in the small coastal shark complex. Information from this study indicates that placement of this species in the SCS complex may not be accurate since many of its reproductive and life history characteristics more closely resemble those species belonging to the LCS complex, a notion shared by Carlson et al (2003) and Drymon et al (2006). Placement in the SCS complex may prove detrimental to future stock structures and overall abundance since the SCS complex is not as tightly regulated as the LCS complex. In recent assessments of elasmobranch fisheries, species and region specific management practices been implemented and are more in line with emerging ecosystem based fisheries management, which consider the functional role of a species in a holistic context. Future considerations on managing this stock in this manner may need to be taken in order to properly sustain Atlantic Ocean populations of *C. isodon*.

COMPONENT 2: THE REPRODUCTIVE ENDOCRINOLOGY OF FINETOOTH SHARK, *CARCHARHINUS ISODON*, IN THE NORTHWEST ATLANTIC OCEAN

INTRODUCTION

The elasmobranch fishes are one of the most basal of all living vertebrate groups (Hamlett, 2005; Carrier et al, 2012), having been present throughout evolutionary history for over 400 million years. Although the living members of this group number much less than other living vertebrate groups, they have still managed to evolve a broad number of reproductive strategies that have presumably contributed to their success and survival. These reproductive strategies range from egg-laying or oviparity to live-bearing or viviparity, the latter of which includes aplacental forms of viviparity, in which embryos are nourished by yolk from unfertilized eggs and placental viviparity, in which embryos are nourished directly by umbilical connections to the female (Wourms, 1977). Regardless of the mode of embryonic nourishment, elasmobranch reproduction generally requires a dynamic and complex hormonal system to regulate these reproductive modes. An understanding of these strategies can give insight into the evolution of reproduction in other vertebrate taxa. For example, the hypothalamus-pituitary-gonadal (HPG) axis that controls release of reproductive hormones such as estrogen and testosterone in the elasmobranchs (Awruch, 2012) has been found in the most basal vertebrates, the hagfish and lampreys and has been highly conserved throughout vertebrate evolutionary history (Uchida et al, 2010; Callard et al, 1989b; Kah et al, 2007).

There is also a worldwide fishery for elasmobranchs and they have become increasingly popular fishery targets since the advent of the shark fin trade. As a result, population declines have been observed in many chondrichthyan families (NMFS, 1993; Dulvy et al, 2000; Cortes et al, 2006). Management initiatives have been implemented for sharks and rays beginning in 1993 (NMFS, 1993; NMFS, 1999). A working knowledge of life history characteristics such as age and size at maturity, life span, gestation length and reproductive periodicity are essential components of an effective management plan (Walker, 2005). However, the traditional methods of obtaining these data generally require lethal sampling and subsequent analysis of morphological changes to the gonads throughout the reproductive cycle. Recently, due to ethical concerns and declining populations of sharks, lethal sampling methods have come into question and more interest is being taken into developing and using non-lethal sampling techniques.

Non-lethal methods include the analysis of circulating sex hormones found in blood plasma. A number of steroid hormones, specifically 17- β estradiol (E_2), progesterone (P4), and testosterone (T), are correlated with reproductive events in sharks and their relatives. Increases in circulating E_2 concentrations have been linked with ovarian follicular development in both oviparous and viviparous species of elasmobranchs (Manire et al, 1993; Tsang and Callard, 1987; Hoffmayer et al, 2010; Sulikowski et al, 2004) via stimulation of the hepatic production of the protein vitellogenin, which accumulates in developing follicles as yolk proteins (Dodd and Sumpter, 1984). E_2 is also believed to play a role in regulating various aspects of reproductive tract function development (Callard et al, 2005; Gelsleichter and Evans, 2012), in some cases by influencing the expression or actions of other hormones such as relaxin (Koob and Callard, 1991). P4 has also been linked with a number of reproductive processes such as ovulation, the

cessation of vitellogenesis (Tricas et al, 2000; Prisco et al 2008), embryonic diapause (Waltrick, 2012), and early stages of pregnancy (Sobrera and Callard, 1995). Last, testosterone has been suggested to be the dominant androgen hormone regulating male reproductive events such as spermatogenesis (Gelsleichter and Evans, 2012; Manire and Rasmussen, 1997). It has also been linked to sexual maturation in males and elongation of claspers (Sulikowski et al, 2005). In female vertebrates, testosterone has also been proposed to play various roles in regulating reproduction like sperm storage (Roy and Krishna, 2010) along with its primary role in serving as the precursor for estradiol production via conversion by the enzyme p450 aromatase (Greenspan, 1997).

Although research into hormone cycles has increased, little published work has focused on regulatory mechanisms for these hormones. Identifying sites of the various hormone receptors could offer insight into how steroid hormones operate within the body. Since the cartilaginous fishes are the first taxa to possess these receptors, (Ogino et al, 2009), they could serve as a model organism for understanding the ancient roles of these hormones in other vertebrate taxa.

The steroid hormone receptors are members of the nuclear receptor (NR) superfamily, which also include the glucocorticoid and mineralocorticoid receptors, among others. NRs exert their biological effects by binding to specific intranuclear sequences, acting as transcription factors that regulate gene expression (Molina, 2006). Androgen receptor (AR) and progesterone receptor B (PRB) under basal conditions exist in the cytoplasm whereas estrogen receptor- α (ER α) is generally found in the nucleus.

The goal of the present study was to investigate the reproductive endocrinology of the finetooth shark, *Carcharhinus isodon*. To achieve this, concentrations of circulating steroid hormones and locations of hormone receptors during different stages of the reproductive cycle were determined. This species has come under increasing fishing pressure in the last 20 years and was previously found to be overfished in past stock assessments (NMFS, 2002; NMFS, 2007). In order to properly manage a stock, information on life history and reproductive biology is essential. However, the last published study of this species in the Atlantic was conducted over 20 years ago and was limited to only gross morphology. As a result, updated information on the life history and reproductive biology of this species is urgently needed for improved management (NMFS, 2007). In this study, plasma concentrations of E₂, P4 and T in mature male and female *C. isodon* were measured to characterize the reproductive endocrinology of this species. These methods were validated by comparison to changes in the morphology and histology of reproductive organs. Immunocytochemistry was conducted to identify target organs for the function of gonadal steroid hormones.

MATERIALS AND METHODS

Sampling methods- Mature male and female *C. isodon* were collected from July 2012-October 2014 using gillnet and bottom longline fishing in coastal waters of the Atlantic Ocean from South Carolina to central Florida. Sample collection methods are described in detail in Chapter 1. Sampling was conducted on a monthly basis to obtain samples from all reproductive stages. Males were deemed mature when they possessed calcified claspers that rotated 180° with a freely opening rhipidion. Female maturity was determined by examination of the reproductive tract following criteria described in Manire et al. (1995). Blood was obtained from live animals via caudal venipuncture with a sterile syringe with an 18-gauge needle and transferred into a vacuum tube containing acid-citrate dextrose (ACD) anticoagulant modified for elasmobranchs. Plasma was clarified via centrifugation at 1300g for 5 minutes and frozen at -20°C until used for hormone analysis. Samples of mature male and female reproductive structures were removed following blood sampling and stored in 10% saline buffered formalin. For males, sections of the testis and head epididymis were taken. For females, sections of ovarian follicles, uterus and oviducal glands were taken. Upon removal, samples were fixed in 10% buffered formalin in elasmobranch-modified phosphate buffered saline for a minimum of 48 h. They were then trimmed and rinsed in tap water for 24 hrs and stored long-term in 70% EtOH until used for examining hormone receptor distribution using histology and immunocytochemistry.

Hormone concentrations- Circulating concentrations of the sex steroid hormones 17- β estradiol (E_2), progesterone (P4) and testosterone (T) were measured using the commercially available AccuLite chemiluminescent immunoassay (Monobind, Lake Forest, CA) following the manufacturer's instructions. Chemiluminescence was measured using the Synergy HT Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT) in luminescence mode. Validation of each kit for use with *C. isodon* was determined using parallelism to determine whether serial dilutions of samples (1/1 to 1/50) behaved according to a standard curve generated using known amounts of hormone standards as provided in each kit. A 'cold spike' was also used to determine percent recovery by adding a known amount of hormone standard to each sample and calculating the recovery. Recovery results were used to determine the ideal dilution of plasma to avoid matrix effects. Hormone concentrations for each sample were calculated by multiplying by the dilution factor used.

Immunocytochemistry- Sub-samples of organ samples were dehydrated in a graded series of EtOH (80-100%), cleared in CitriSolv (Fisher Scientific, Fair Lawn, NJ) and processed for routine paraffin histology. Transverse 5- μ m sections were prepared using a rotary microtome and adhered to microscope slides pre-treated with 0.01% poly-L-lysine. Immunocytochemistry was performed to identify the presence of androgen and progesterone receptors (AR and PR, respectively) in males, and estrogen receptor- α (ER) and PR in females. Sections were deparaffinized in CitriSolv followed by rehydration in a descending graded series of histological grade EtOH (100-95%) and rinsed in tap water. Antigen retrieval was performed by incubating sections in sodium citrate (10mM, pH 6) at 95°C for 20 min to expose antibody binding sites. Slides were then cooled to room temperature, rinsed in phosphate buffered saline (PBS) and

blocked for non-specific antibody binding with 2% goat serum overnight at 4°C. Slides were rinsed twice with PBS the following day followed by incubation in the appropriate primary antibody for 24 h at 4°C (Table 1.1). Endogenous enzyme activity was quenched by incubating sections in a 1:1 mixture of 3% hydrogen peroxide and 100% methanol prior to addition of primary antibody. Control sections were incubated with diluent in place of primary antibody. On the final day of the protocol, slides were rinsed once in PBS containing 0.05% Tween-20 (PBS-T) and then twice with PBS. Sections were then incubated in secondary antibody (anti-rabbit Ig for AR and ER- α or anti-mouse Ig for PR, ImmPRESS Polymer Detection Kits, Vector) for 30 min at room temperature, after which they were rinsed three times in PBS for 5 min each. Antibody binding sites were then revealed by addition of the peroxidase substrate 3,3'-diaminobenzidine (ImmPACT DAB, Vector). Once substrate-enzyme binding induced a noticeable color change (~ 5 min), slides were transferred to a running tap water bath for 10 minutes. They were then counterstained with 2% methyl green (Vector) for 30-45min at 37°C and rinsed in tap water for 2 min followed by dehydration in an ascending, graded series of alcohols (95-100%) and clearing with CitriSolv. Slides were coverslipped and mounted using Cytoseal-60 (Richard-Allan Scientific, Kalamazoo, MI) and allowed to dry.

Data Analysis- Hormone concentrations in males were grouped by month and analyzed using one-way ANOVA followed by a Tukey's *post hoc* test to determine if significant differences were present. For females, hormone concentrations were analyzed via two-way ANOVA followed by multiple comparison tests to determine if significant differences occurred by month and/or reproductive stage. Non-parametric alternatives were used when data sets did not meet the assumptions of parametric tests. Spearman's ρ was used to determine whether gonads were

significantly correlated to hormone concentrations. Hormone receptor localization was analyzed qualitatively via light microscopy to determine temporal and/or stage related changes.

Table 2.1 Primary antibodies and their corresponding secondary antibodies used to isolate hormone receptors in the reproductive tract of mature male and female *C. isodon*.

Receptor	Receptor Type	Manufacturer
Androgen receptor	AR-21	Millipore, Temecula, CA
Estrogen receptor	ER α C1355	Millipore, Temecula, CA
Progesterone receptor	PR-22	ThermoScientific, Rockford, IL

RESULTS

Hormone concentrations

Females- A total of 41 and 67 females were analyzed for E₂ and P₄, respectively. Plasma E₂ concentrations varied significantly based on pregnancy status, with non-gravid females having significantly higher concentrations than gravid individuals (two-way ANOVA, $p < 0.001$). Temporal variations in plasma E₂ were also observed in non-gravid females in relation to reproductive stage. Plasma E₂ concentrations were very low in recently pupped females following parturition then increased in females collected in winter and spring (Jan-March), the period of maximum follicular growth (Figure 2.1). E₂ levels then declined markedly in post-ovulatory and newly gravid females collected in May, females that would have been categorized as non-gravid prior to this stage. E₂ was significantly correlated with follicle diameter (Figure 2.2; Spearman's ρ , $P < 0.001$). In contrast, there were no clear patterns in plasma P₄ concentrations throughout the reproductive cycle (Figure 2.3). Concentrations were relatively low in both gravid and non-gravid females in all months measured, with spikes occurring in only a few non-gravid individuals collected during spring and fall.

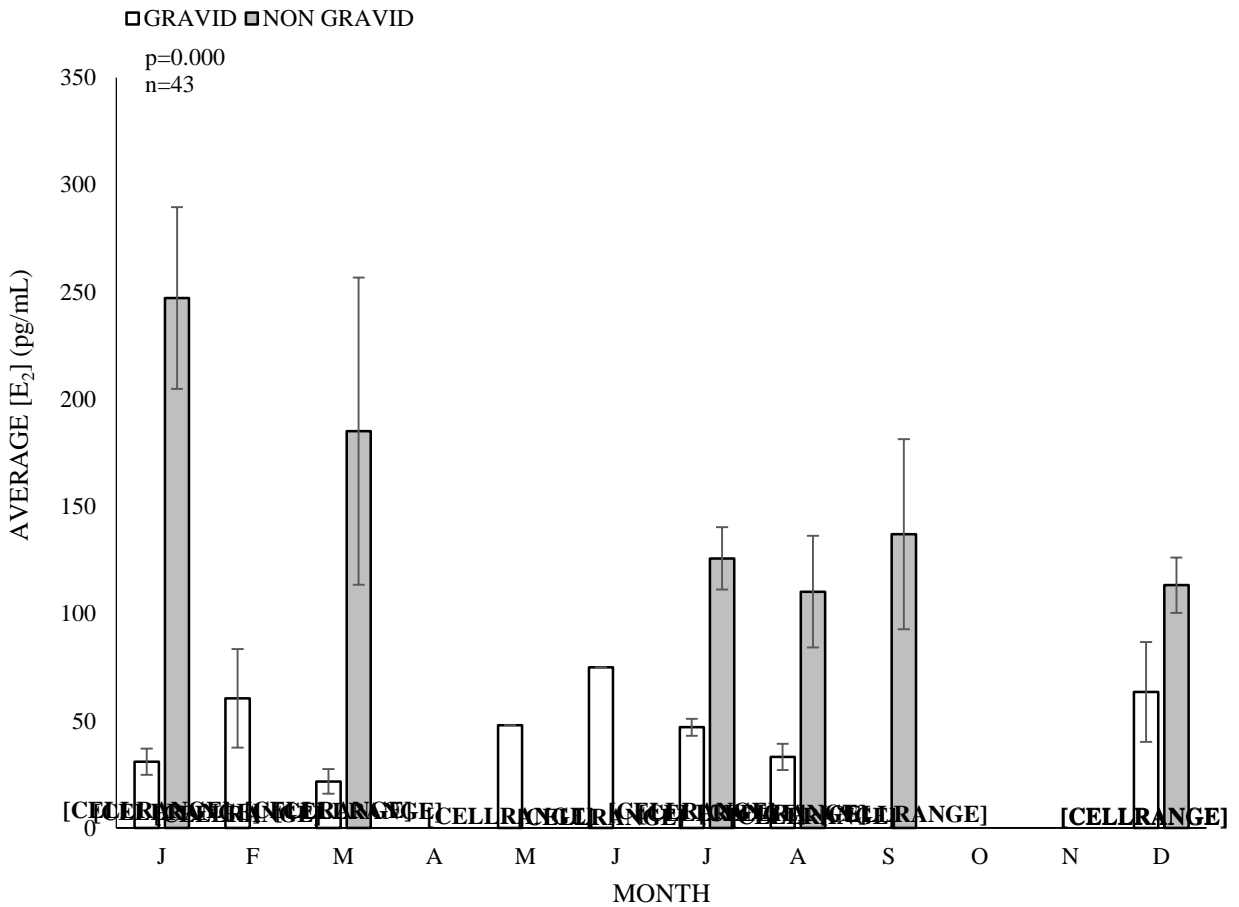


Figure 2.1. E₂ concentrations of gravid and non-gravid mature female *C. isodon* in the northwest Atlantic Ocean. Bars represent means \pm SEM. Sample size is represented inside bars. Significance was determined using a two-way ANOVA (p=0.276 for month; p<0.001 for reproductive condition).

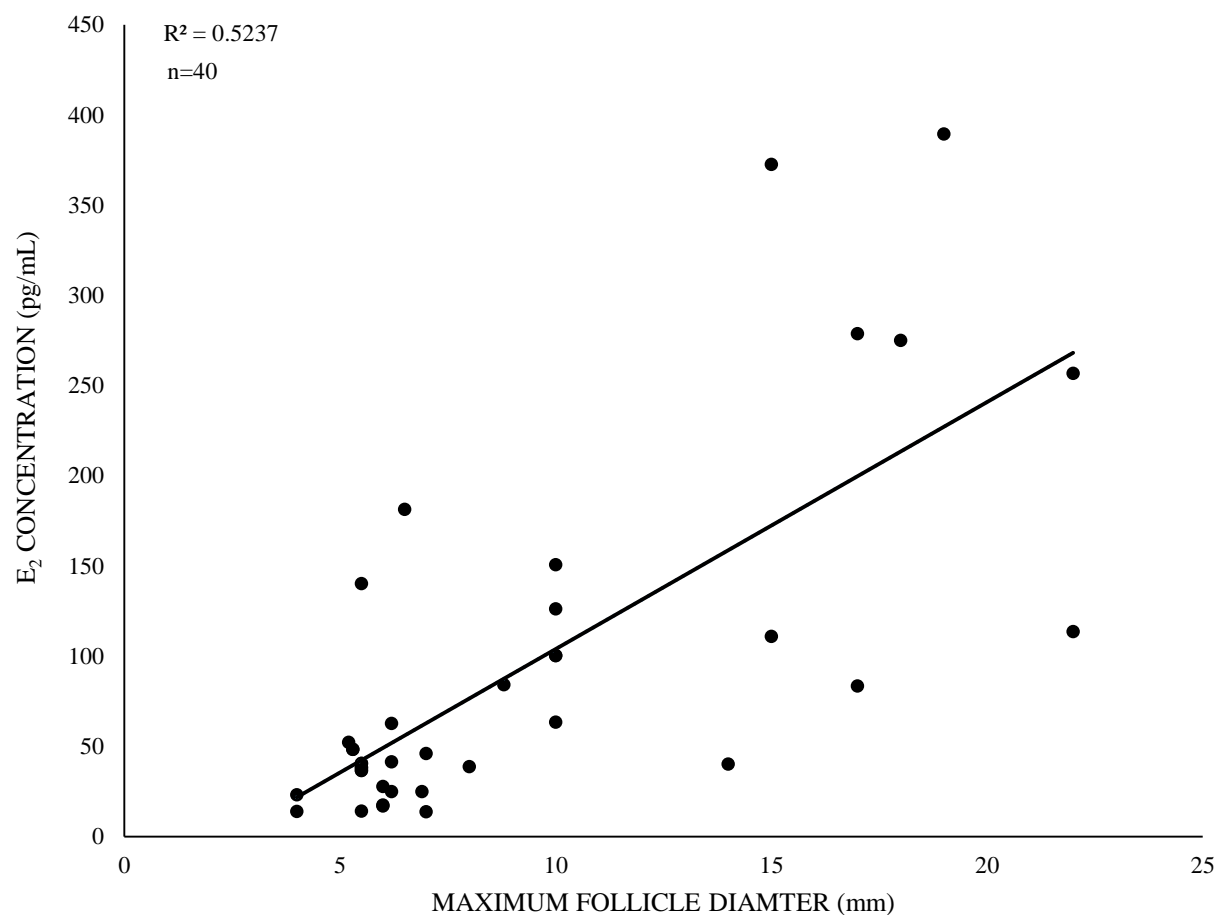


Figure 2.2. Plasma E₂ concentration and maximum follicle diameter in mature female *C. isodon* from the northwest Atlantic Ocean. E₂ concentrations were significantly correlated with follicle diameter (Spearman's ρ , $P < 0.001$).

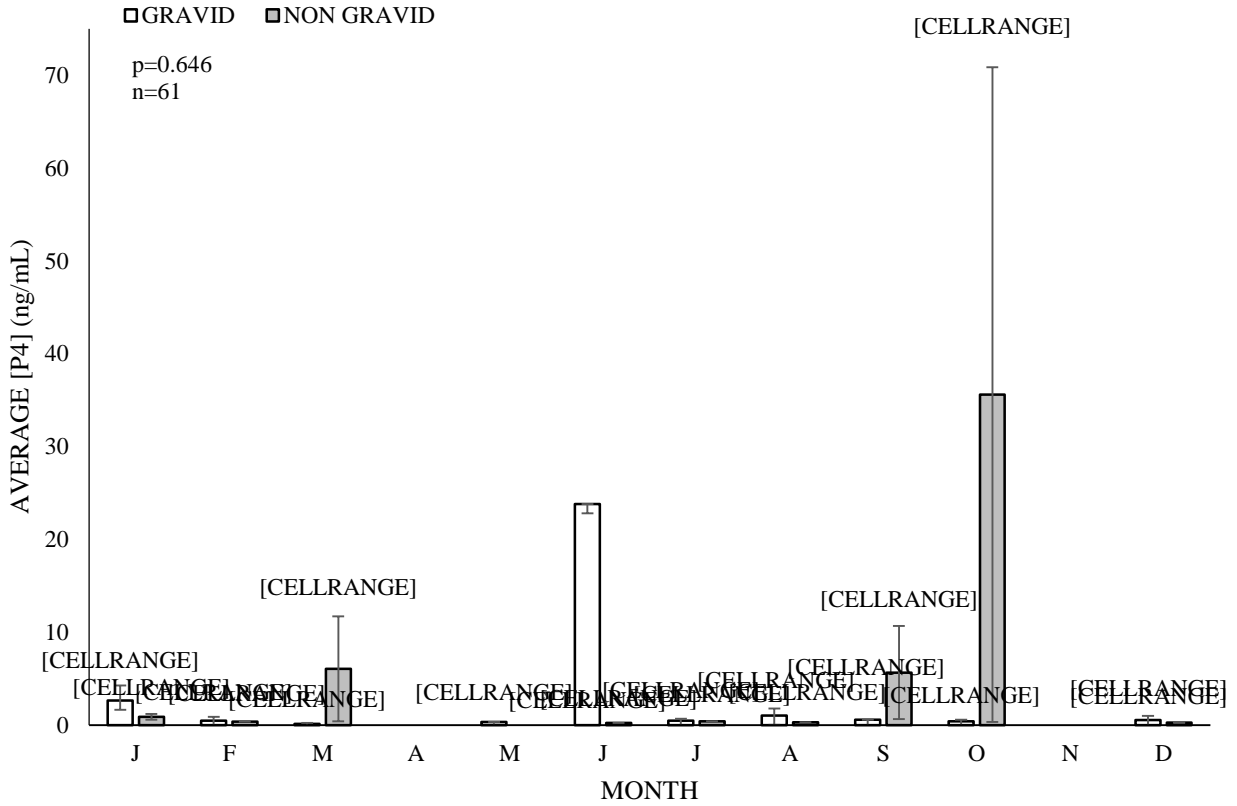


Figure 2.3. Plasma P4 concentrations of gravid and non-gravid mature female *C. isodon* from the northwest Atlantic Ocean. Bars represent means \pm SEM. Sample sizes are shown inside or above bars. Significance was determined via a one way ANOVA ($p=0.646$).

Males- Plasma T concentrations were measured in a total of 48 mature male *C. isodon* and displayed significant temporal fluctuations (one-way ANOVA, $p < 0.001$). T levels were elevated winter through spring, reaching their maximum concentrations in April (Figure 2.4). This peak coincided with the period of greatest testicular growth and a predominance of late stage mature spermatocysts, indicating this is the period of spermatogenesis. Plasma T concentrations were drastically lower in May, coinciding with the beginning of the mating season. Low T levels were observed from May to October corresponding to a period of minimum testicular growth and high quantities of early stage spermatocysts indicating little sperm production occurred during this time period. T concentrations were significantly correlated with testis width (Figure 2.5, Spearman ρ , $P < 0.001$).

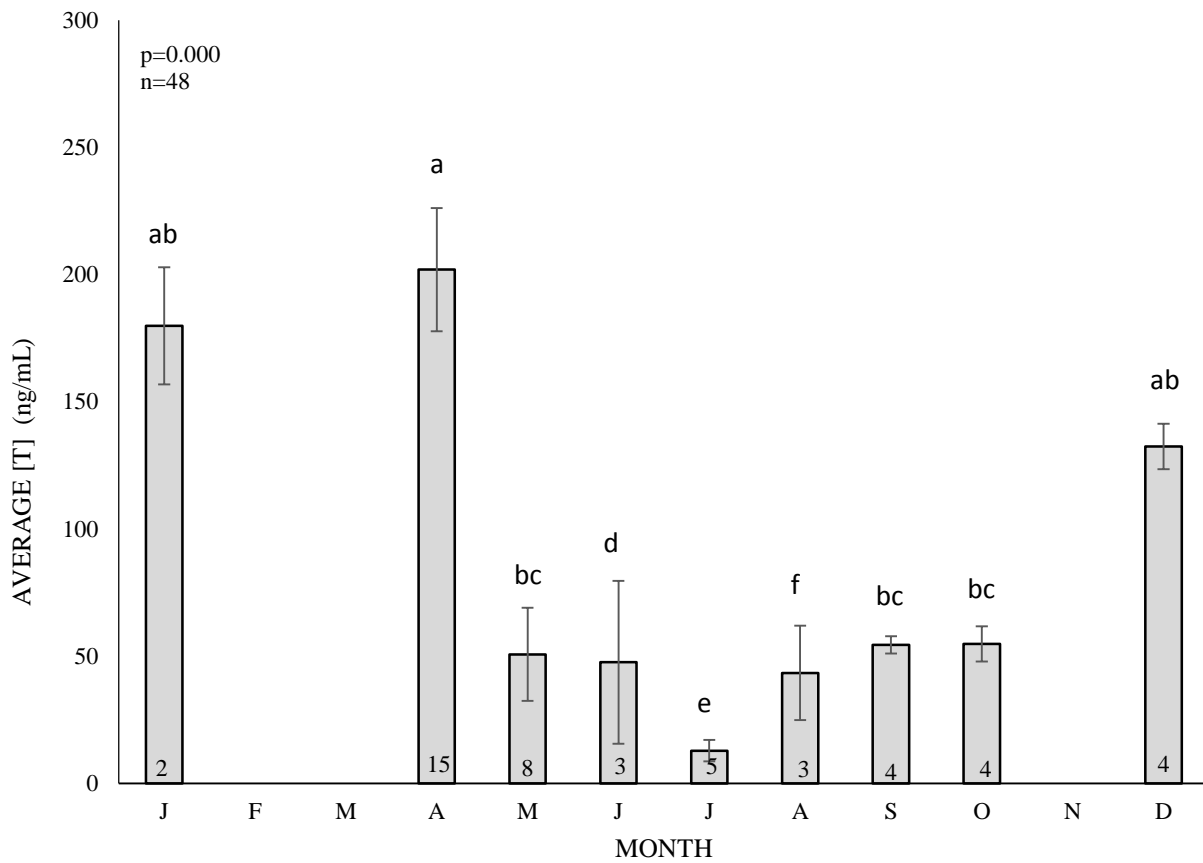


Figure 2.4. Plasma T concentrations in mature male *C. isodon* in the northwest Atlantic Ocean. Bars represent means \pm SEM. Sample sizes are shown inside bars. Significance was determined using a one-way ANOVA ($p < 0.001$). Letters above bars represent homogenous subsets at $p < 0.05$.

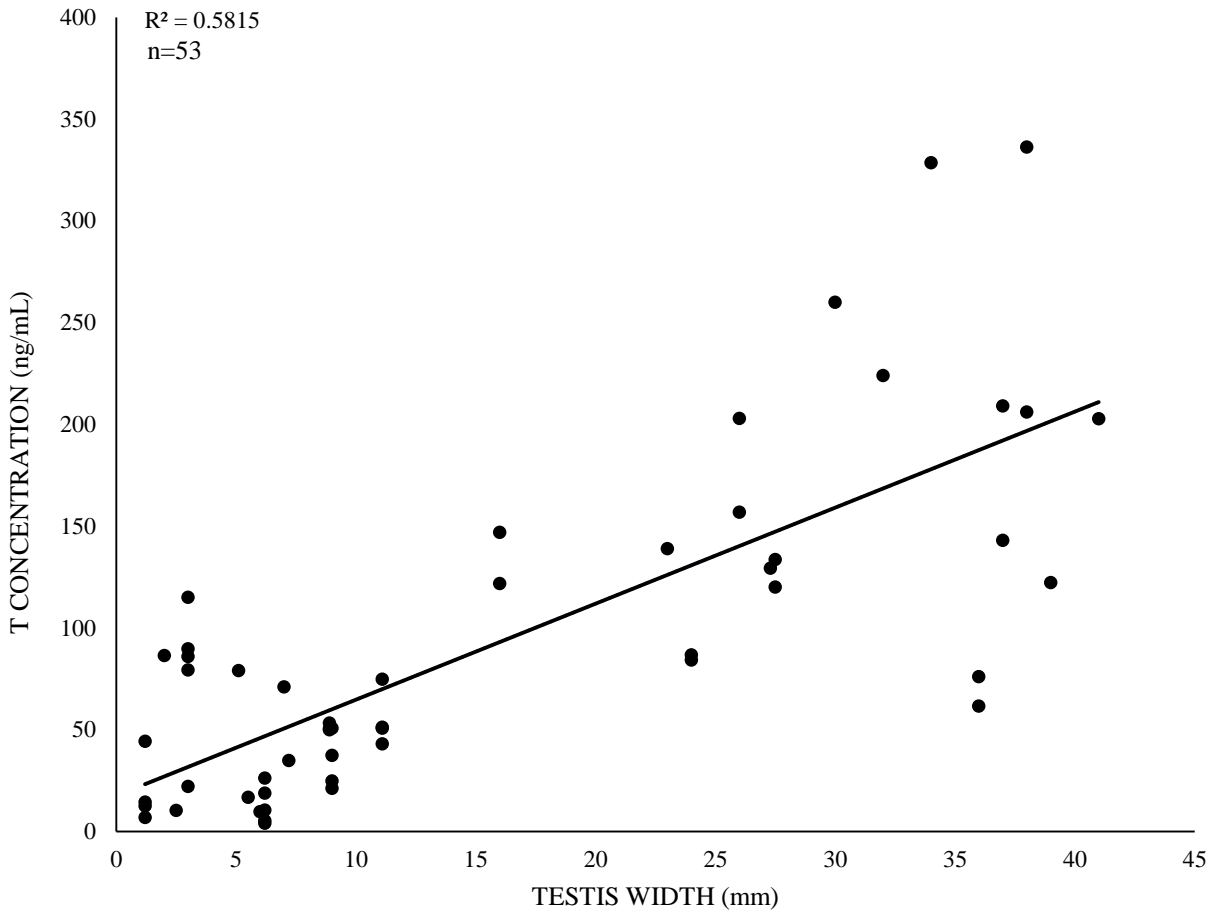


Figure 2.5. Plasma T concentration and testis width in male *C. isodon* from the northwest Atlantic Ocean. Plasma T concentrations were significantly correlated with testis width (Spearman ρ , $P < 0.001$).

Immunocytochemistry- Immunocytochemistry was performed on a total of 38 female and 39 male *C. isodon* during different stages of the reproductive cycle. In the ovaries, ER α was absent in primordial and primary follicles (Figure 2.6a). Pre-vitellogenic follicles displayed scattered positive ER α staining in the granulosa and theca cell layers (Figure 2.6b). ER α staining was most prominent in vitellogenic follicles where deep staining occurred in the granulosa cell layer and scattered staining was found in the theca cell layers (Figure 2.6c). Positive ER α staining was also observed in yolk crystals of pre-vitellogenic and vitellogenic follicles (Figures 2.6b, 2.6c). Atretic follicles displayed positive staining in nuclear regions of the infolding granulosa cells (Figure 2.6d). In the oviducal gland, ER α staining was found in pre-ovulatory non-gravid and early pregnant females in epithelial cells lining secretory tubules that are associated with egg encapsulation (Figure 2.7a, 2.7b). In the uterus, ER α staining was found in luminal epithelial cells and stromal cells of non-pregnant females (Figures 2.8a, 2.8b).

There was strong PR immunoreactivity in the oviducal gland of early, mid- and late-stage pregnant females, specifically in the ciliated epithelial cells lining luminal projections (Figure 2.8c, 2.8d, 2.8e). This staining was absent in oviducal glands of pre-ovulatory vitellogenic females. Stromal, luminal epithelial and myometrial muscle cell PR staining was also observed in the uterine folds of non-gravid, pre-ovulatory females (Figure 2.9c, 2.9d).

In males, AR immunoreactivity was present in late stage spermatocysts (Stage V-Stage VI) that were actively undergoing spermatogenesis (Figure 2.10a). Immunoreactivity occurred in Sertoli and ductal cells (Figures 2.10b, 2.10c, respectively) as well as on individual spermatozoa,

specifically in the head and mid-piece regions (Figure 2.10d). ARs were absent in early stage spermatocysts (Stage I-Stage IV). In the epididymis, ARs were found in basolateral epithelial cells (Figure 2.10e).

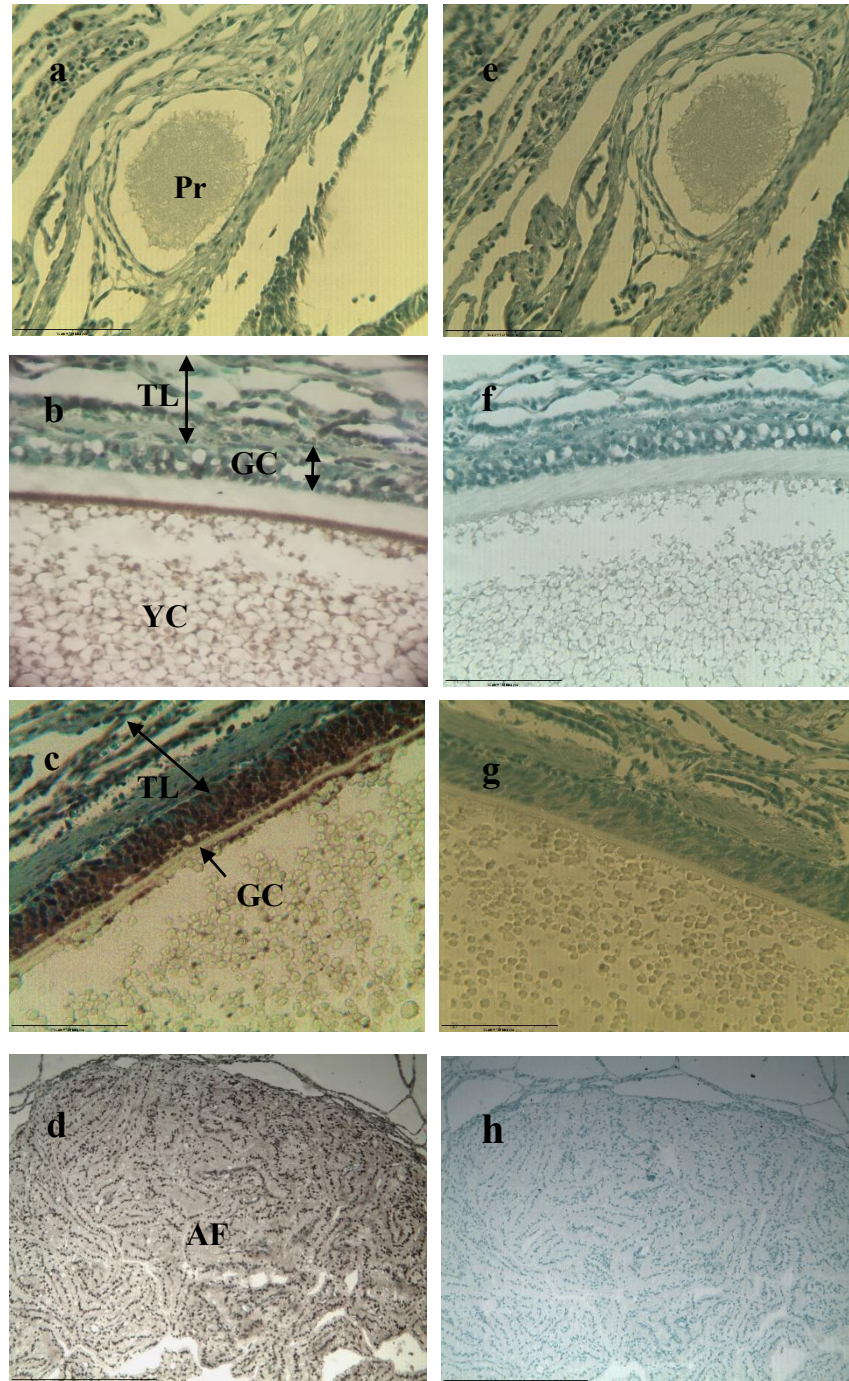


Figure 2.6. Immunocytochemical analysis of the presence of ER α in female *C. isodon* ovaries through different stages of the ovarian cycle. a-d represent samples that were stained for ER α ; e-h are negative controls. a) 400x magnification of a primordial follicle (Pr) with no positive staining. b) 400x magnification of a pre-vitellogenic follicle with scattered positive ER α staining in the granulosa (GC) and theca cell (TL) layers. c) 400x magnification of a vitellogenic follicle displaying deep positive ER staining in the GC layer and scattered positive staining in the TL. d) 10x magnification of an atretic follicle with deep nuclear staining of infolding GC layer.

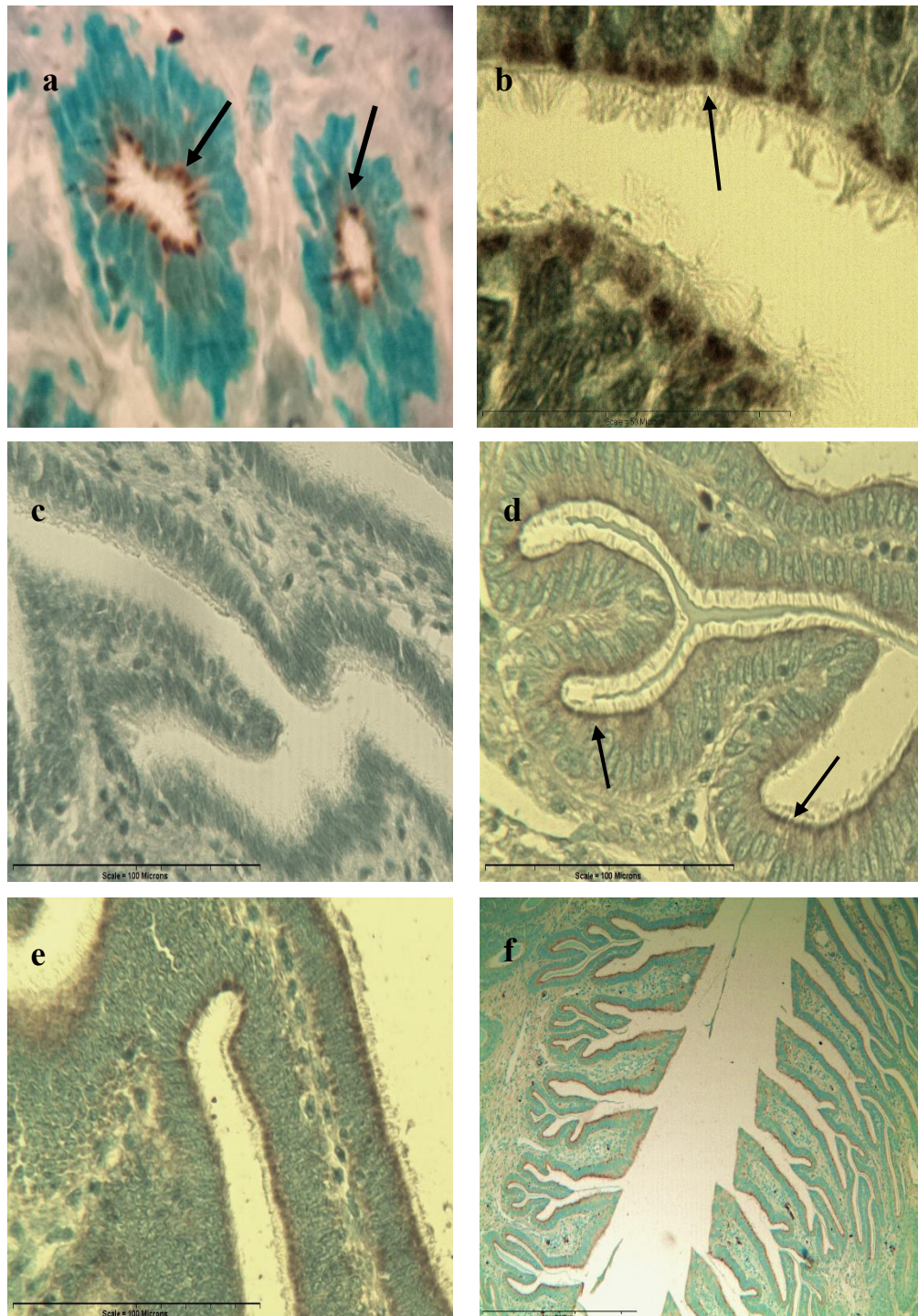


Figure 2.7. Immunocytochemical analysis of female *C. isodon* oviducal glands. a-b were stained with ER α ; c-f are stained with PR. a) 1000x magnification of secretory tubule of oviducal gland displaying deep positive ER α staining in luminal epithelial cells in an early pregnant female. b) 1000x magnification of positive ER α staining in epithelial cells of luminal projections in an early pregnant female. c) 400x magnification of luminal projection showing no positive PR staining in an early vitellogenic female. d) 400x magnification showing positive PR staining in ciliated epithelial cells of luminal folds in an early pregnant female. e) 400x magnification showing positive PR staining in luminal epithelial cells of a mid-stage, pregnant female. f) 100x magnification showing positive staining in luminal folds of a late-stage, pregnant female.

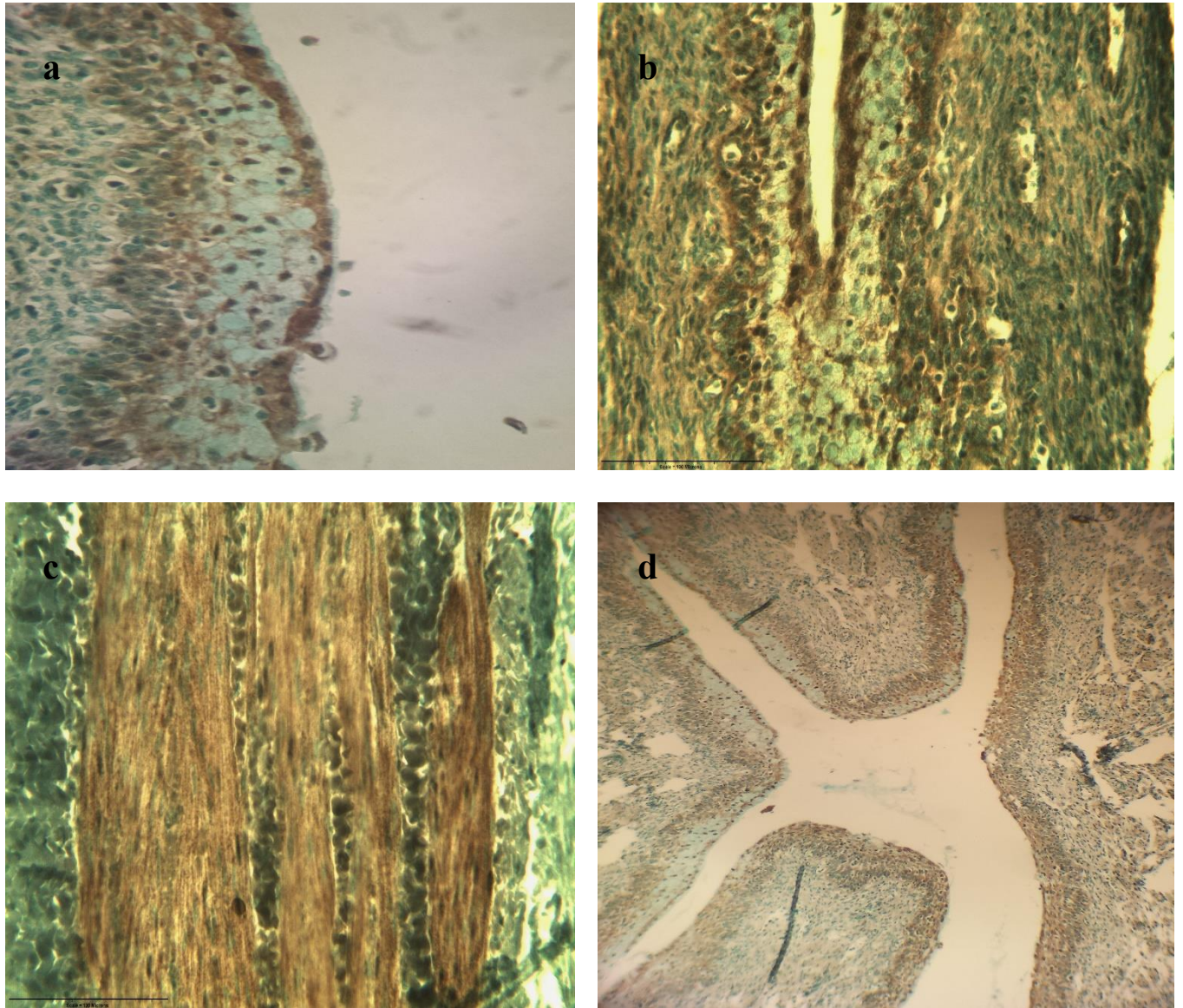


Figure 2.8. Immunocytochemistry analysis of female *C. isodon* uterus. a-b were stained with ER α ; c-d were stained with PR. a) 400x magnification showing positive ER staining in the epithelial and stromal cells of a pre-ovulatory uterus. b) 400x magnification showing positive staining of stromal and epithelial cells in uterine folds of a pre-ovulatory female. c) 400x magnification of positive PR staining in myometrial muscle cells of pre-ovulatory uterus. d) 100x magnification showing positive PR staining in uterine folds of pre-ovulatory uterus.

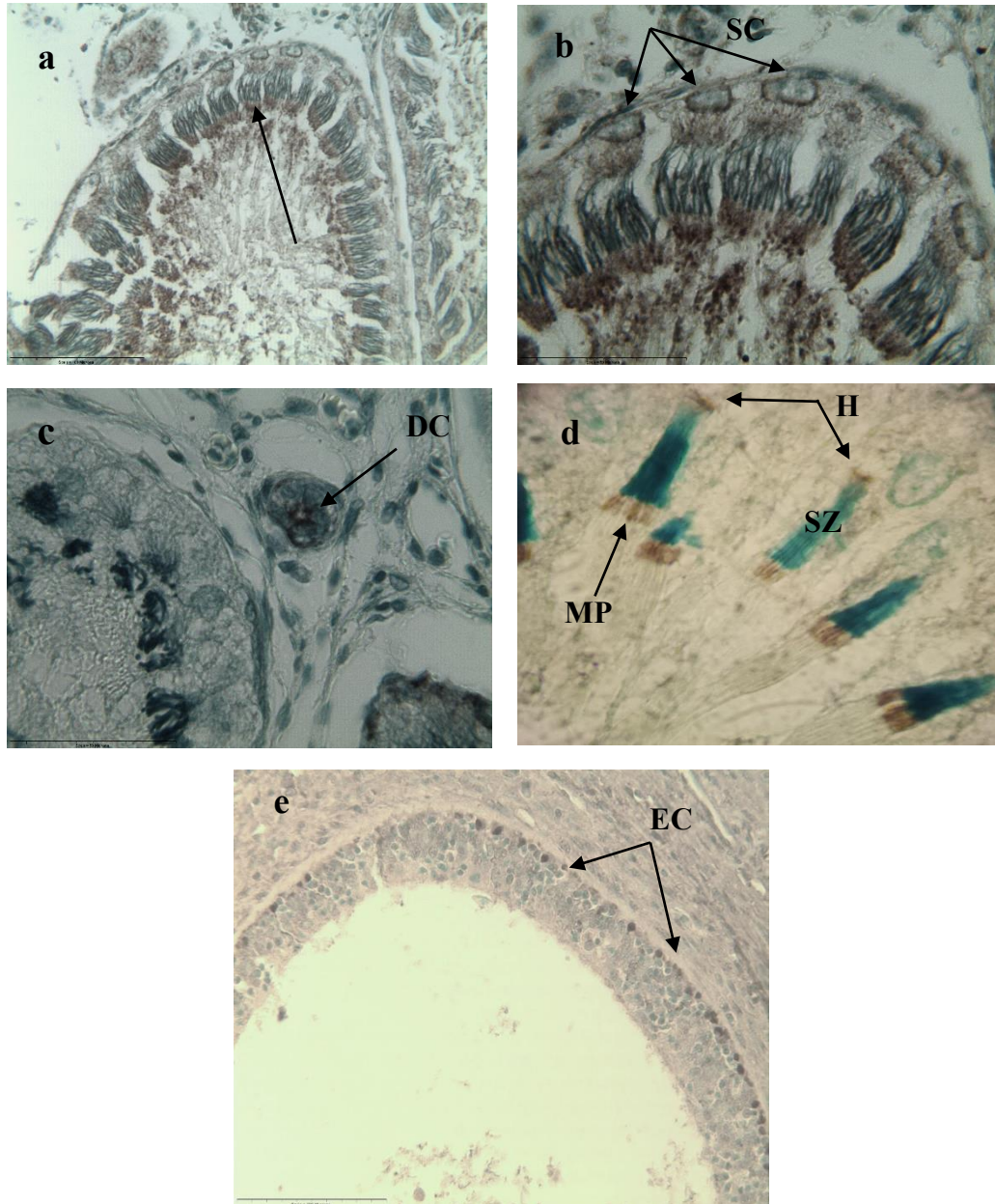


Figure 2.9. Immunocytochemical analysis of male *C. isodon* gonads. (a-d) represent AR staining in the testis, (e) shows AR staining in the epididymis. a) 400x magnification showing positive AR staining in Stage VI spermatocyst. Arrows indicate positive staining on mid-piece region of spermatozoa. b) 1000x magnification of a) showing positive AR staining surrounding Sertoli cells (SC). c) 1000x magnification showing positive AR staining in ductal cell (DC) on the outside of a spermatocyst. d) 1000x magnification showing arrows pointing to positive AR staining on head-piece (H) and mid-piece (MP) regions of spermatozoa in the testis. e) 400x magnification with arrows pointing to positive staining in basolateral epithelial cells (EC) of epididymis.

DISCUSSION

The results of this study show an association between reproductive events and plasma hormone profiles in both male and female *C. isodon* in the northwest Atlantic Ocean. In females, plasma E₂ concentrations displayed a temporal trend with peaks occurring during follicular development, suggesting a role in vitellogenesis. This was supported by evidence for the presence of ER α in granulosa and theca cells of vitellogenic follicles. In contrast, plasma P4 concentrations did not display a clear temporal trend in female *C. isodon*, remaining relatively constant in both gravid and non-gravid females throughout the duration of the reproductive cycle. However, positive detection of PR suggested possible roles for this hormone in regulating various aspects of reproductive tract function including roles in the oviducal gland and uterus. Male *C. isodon* also displayed plasma T concentrations indicative of a seasonally reproducing shark, increasing specifically during the period of spermatogenesis. ARs were prominently detected in both supporting cells (i.e., Sertoli cells) and germ cells in the male testis as well as the epithelial cells of the epididymis, suggesting both direct and indirect effects on male gamete function.

The patterns observed in E₂ concentrations in female *C. isodon* were comparable with those observed in other seasonally reproducing sharks such as *Sphyrna tiburo*, *Rhizopriondon terraenovae* and *Hemiscyllium ocellatum* (Manire et al, 1995; Hoffmayer et al, 2010; Heupel et al, 1999). Elevated E₂ concentrations have been previously correlated with vitellogenesis in female elasmobranchs, in which this hormone is believed to stimulate hepatic production of the protein vitellogenin as well as its accumulation in growing follicles (Dodd and Sumpter, 1984).

This trend has been observed in both placental viviparous sharks (Manire et al, 1995) as well as oviparous sharks and rays (Tricas et al, 2000; Heupel et al, 1999; Koob et al 1986). This study also showed that these changes reflect specific roles for these hormones in the reproductive tracts of females. With the increase in E_2 during vitellogenesis, the presence of $ER\alpha$ was also detected in the follicles, specifically in the granulosa cell layer. The granulosa cell layer is a well-established target of this hormone where aromatization of androgens diffused from the theca cells into estrogens occurs (Molina, 2006). Immunoreactivity of $ER\alpha$ increased as the follicles matured and accumulated yolk and was absent in primordial and primary follicles of non-vitellogenic females. These findings are similar to previous studies that also found positive staining in the granulosa cell layer (Wang et al 2000) and an increase in $ER\alpha$ mRNA in the ovary towards the end of the ovarian cycle (Nagler et al, 2012). The increase in the presence of $ER\alpha$ was also consistent with elevated levels of plasma E_2 . $ER\alpha$ were also detected in the oviducal gland of pre-ovulatory and early pregnant females, specifically in the secretory tubules. Since these tubules are responsible for egg encapsulation (Hamlett, 2005), E_2 may be present in anticipation of ovulated follicles in pre-ovulatory females. Similar staining patterns have also been found in multiple vertebrate species throughout the reproductive cycle (di Cosmo et al, 2002; Wang et al, 2000; Socorro et al, 2000).

Variations in plasma P4 concentrations in mature female *C. isodon* were not consistent with those observed in other elasmobranchs for which this hormone has been examined, most of which exhibit increased levels of P4 during ovulation (Manire et al., 1995). However, the role that P4 plays in reproduction has not been consistently connected with any reproductive event in female elasmobranchs to date. P4 has mainly been connected with ovulation and the suppression

of vitellogenesis in elasmobranchs (Perez and Callard, 1992), but these stages were missing in this study. Nonetheless, PR were detected in the oviducal gland throughout the entirety of pregnancy in gravid females, suggesting possible roles in ovulation and/or egg encapsulation. In the uterus, P4 was found in luminal epithelial, stromal and myometrial muscle cells. These areas have shown positive PR staining in multiple taxa and have been suggested to play roles in the preparation of the uterus for pregnancy and intrauterine muscle contractions (Vermiersch et al, 2000; Snijers et al, 1992).

Temporal patterns in plasma T concentrations in male *C. isodon* were consistent with those observed in males from other seasonally reproducing elasmobranchs and corroborates a well-established role this hormone has been suggested to play in spermatogenesis (Manire et al, 1995; Gelsleichter and Evans, 2012; Hoffmayer et al, 2010). Immunolocalization of AR during different stages of the reproductive cycle also supported these roles. AR immunoreactivity was found in the Sertoli and germ cells of late stage spermatocysts in the testes of males undergoing spermatogenesis, corresponding with high levels of plasma T and an increase in testicular growth. ARs were mostly absent in early-stage spermatocysts throughout the entirety of the reproductive cycle and from specimens with low T levels and minimal testicular growth. The presence of AR during spermatogenesis is consistent with the well-established role that T has in that process (Walters et al, 2010; Manire et al, 1995; Gelsleichter and Evans, 2012; Hamlet 2006). ARs have been previously reported to be found in Sertoli and Leydig cells of animals actively undergoing spermatogenesis, but absent or weakly expressed in aspermatogenic animals (Okuyama et al, 2014; Pearl et al, 2011). Sertoli cells are thought to have critical central roles in spermatogenesis such that interruption of that process would occur if either are missing or

nonfunctional. For example, recent studies have demonstrated that deletion of the AR gene in mice Sertoli cells can result in complete arrest of spermatogenesis at the spermatid stage (Wang et al, 2009). Other studies have also correlated elevated T concentrations with an increase in AR expression in Sertoli cells (Verhoeven and Cailleau, 1988). Interestingly, immunoreactive AR was also found in the head and mid-piece regions of individual spermatozoa, which has previously been reported in human sperm (Solakidi et al, 2005, Aquila et al, 2007) although the role of androgens in maturing sperm is still unknown and the presence of AR on individual spermatozoa is controversial. In the epididymis ARs were found in basolateral epithelial cells lining the epididymis. AR receptors have also been shown in the epididymis and efferent ducts in mature rats (Zaya et al, 2013) deer (Kaziol and Kaziorowski, 2013) and humans (Ungefroren et al, 1997). ARs in the epididymis bind androgens that further aid the maturation of sperm once it leaves the testes (Ungefroren et al, 1997) and is also required for normal post-natal epididymal development (You, 1998).

In conclusion, seasonal changes were observed in plasma concentrations of T and E₂ in male and female *C. isodon* in the northwest Atlantic. Although P4 displayed relatively low, constant concentrations, spikes were observed in individuals suggesting this hormone may have a secondary role in maintaining various reproductive processes, although additional samples are needed to further investigate this notion. Strong evidence suggests that T contributes to the regulation of spermatogenesis and its effects are mediated by ARs in the testis and epididymis. E₂ regulates vitellogenesis in non-gravid females and has its effects through ER α in the ovaries, oviducal gland and uterus. PRs were found in the cytosol of developing spermatocysts and may contribute to sperm maturation and spermeiogenesis. This study sheds additional light on the role

the steroid hormones play in the reproductive cycle of a relatively understudied species of commercially viable shark. It is also one of the few studies to qualitatively investigate the location of hormone receptors in an elasmobranch in an effort to understand their cellular functions. Future directions should focus on quantitatively measuring cellular receptors in relation to different periods of the reproductive cycle in an effort to shed further light on the functions of the steroid hormones in reproduction.

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VITAE

NAME OF AUTHOR: Amanda Nevada Brown

DEGREE AWARDED:

May 2009: B.S. Biology, Florida Atlantic University, Boca Raton, FL

PROFESSIONAL EXPERIENCE:

2013-2014: Graduate Teaching Assistant, University of North Florida
Anatomy and Physiology I, Anatomy and Physiology II Laboratory
2012-2013: Graduate Teaching Assistant, University of North Florida
General Biology I, General Biology III Laboratory
2012-2013: Graduate Research Assistant, UNF Shark Biology Research Program
Investigating reproduction in *C. isodon*
2010: Fisheries Ecology Intern, Dauphin Island Sea Lab
Investigating effects of Deepwater Horizon oil spill on fish populations
2010: Research Assistant, Bimini Biological Field Station
Investigating effects of habitat loss on lemon shark nursery
2009: Research Assistant, Virginia Institute of Marine Science
Assisting in shark longline survey

AWARDS:

2006: Dean's list, Santa Fe Community College
2008: Dean's list, Florida Atlantic University