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Age, Growth, and Reproduction of Vermilion Snapper (*Rhomboplites aurorubens*) in the North-Central Gulf of Mexico

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AGE, GROWTH, AND REPRODUCTION OF VERMILION SNAPPER (*RHOMBOPLITES AURORUBENS*) IN THE NORTH-CENTRAL GULF OF MEXICO

by

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ABSTRACT

AGE, GROWTH, AND REPRODUCTION OF VERMILION SNAPPER (*RHOMBOPLITES AURORUBENS*) IN THE NORTH-CENTRAL GULF OF MEXICO

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Vermilion Snapper is a commonly harvested species of reef fish in the northern Gulf of Mexico (GOM). It supports both a large commercial and popular recreational fishery, however, knowledge of this fish's life history is limited spatially. Non-linear curve fitting was used to estimate growth parameters and Akaike information criteria (AIC) was used to determine relative model fit. The 2-parameter von Bertalanffy growth function provided the best model fit and lowest AIC score. Histological examination indicated that Vermilion Snapper are batch spawners with asynchronous oocyte development. Additionally, 17% of Vermilion Snapper in the actively spawning phase containing 24 hour POF's suggesting daily spawning is occurring. No immature fish of either sex were collected during this study (139 mm to 535 mm TL). Both histologically-determined phases and gonadosomatic index (GSI) patterns defined the spawning season ranged was from April to September. The spawning interval for Vermilion Snapper was estimated using the hydrated oocyte and post-ovulatory follicle methods, was 1.8 and 2.2 days respectively. Batch Fecundity (BF) estimates of 5,497 to 284,468 eggs/batch were determined using fish macroscopically classified as actively spawning (n = 22). Total fecundity (BF by spawning frequency) was estimated to range from 544,203 eggs/spawning season up to 28,162,332 eggs/spawning season. Mean relative batch

fecundity was 70.7 eggs/g of gonad-free body weight. Estimates from this study can be directly incorporated into population assessments and provide a region-specific overview of life-history for the Vermilion Snapper from the north-central Gulf of Mexico.

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CHAPTER I - INTRODUCTION

Reef fish fisheries in the northern Gulf of Mexico (GOM) consist of a multispecies complex. This complex includes Red Snapper *(Lutjanus campechanus)*, Vermilion Snapper *(Rhomboplites aurorubens)*, Gag *(Mycoptera microlepis)* and other groupers, triggerfishes, amberjacks, and porgies (Weninger and Waters 2003). These fishes often inhabit artificial or natural structure in depths > 10 m to 300 m. Reef fishes exhibit numerous reproductive life-history strategies, from protogyny in groupers (Coleman et al. 1996, Koenig et al. 1996 McGovern et al. 1998), to nest-building in Grey Triggerfish (*Balistes capriscus*; MacKichan and Szedlmayer 2007), and broadcast spawning in lutjanids (Brown-Peterson et al. 2009, Wilson and Nieland 2001, Lowerre-Barbieri et al. 2015). Reef fishes are often long-lived, though differences in maximum sizes exist even on the family level. For example, some snappers, such as the Vermilion Snapper and Yellowtail Snapper *(Ocyurus chrysurus)* only grow to around three kilograms while others, such as the Red Snapper and Mutton Snapper *(Lutjanus cyanopterus)*, can grow upwards of 10 kg or more (Burton 2002, Horst 2004). While many of these fishes exhibit different life-history strategies, all are harvested by both commercial and recreational fisherman.

Declines in reef fish stocks (Red Snapper, Grey Triggerfish, Greater Amberjack (*Seriola dumerili*), etc.) have led to regulatory actions (Polunin and Roberts 1996, Coleman et al. 2004, Doerpinghaus et al. 2014). For example, bag and minimum length limits for Red Snapper have undergone numerous changes starting in the 1990's (SEDAR 31 2013). Red Snapper is also the most well-studied reef fish in the GOM (Gillig et al. 2000, Patterson et al. 2001, Wilson et al. 2001, Wells et al. 2008, Lowerre-Barbieri et al.

2015), based on the magnitude of harvest (> 6000 metric tons/year) in both the recreational and commercial fisheries. However, despite regulatory changes, many stocks such as Red Snapper, Gag, and Gray Triggerfish are classified as "overfished" in the last ten years (SEDAR 9 2006a,b). Because of these observed population declines, one of the outstanding research needs to increase the accuracy and precision of stock assessment output (fishery and stock status) is life-history data of a species throughout its range, as these data are fundamental for stock assessments. For example, an examination of most stock assessment models for reef fish (http://sedarweb.org/) in the GOM are age-structured and require length-at-age estimates. Furthermore, spawning stock biomass, the biomass attributed to the females of the population that are capable of spawning, is estimated by estimating age-at-maturity. Age-at-maturity is defined as the onset of gonadal maturation and also indicates when the fish will start to contribute recruits to the population. For many stocks, life-history characteristics are not well known or are documented for only one region within the stock's distribution. This is especially true for the Vermilion Snapper in the north-central GOM: A single published study (Johnson et al. 2010) has investigated the age and growth of the species, and no information is available for reproductive characteristics such as age- and length-at-maturity, fecundity, and spawning season. The Vermilion Snapper stock is a recreational and commercially exploited stock with a mean total harvest of 1,300 tons caught per year in the GOM (NMFS 2016, Figure 1). In the commercial sector alone, Vermilion Snapper account for $8,000,000 (USD) of total sales per year (NMFS 2016). Despite its value in the commercial market and the popularity in the recreational sector, information on the growth of Vermilion Snapper is scarce for the north-central GOM. Only a small

proportion of fish (< 7%) come from the north-central GOM (Pensacola west to Mississippi River, LA) from fishery-independent surveys, with the majority of samples (85%) coming from fishery-dependent collections in Florida (Allman et al. 2005, Lombard et al. 2015, SEDAR 45 2016). Stock assessments have also collected reproductive data almost exclusively from the eastern GOM (93% of fish collected, Fitzhugh et al. 2015).

Vermilion Snapper (Lutjanidae) is found in temperate and sub-tropical climates from North Carolina to the Caribbean Sea, throughout the GOM, and south to Brazil (Jordan and Evermann 1896, Breder 1929). Despite its cosmopolitan distribution, biological and life-history information is only available for the South Atlantic Bight (SAB) and the eastern GOM (Pensacola to Cedar Key, FL). Vermilion Snapper is typically associated with offshore rock outcroppings and hard bottom reef habitats in the Atlantic Ocean (Grimes 1982) and in the GOM (Collins et al. 2003). The north-central GOM reef habitat is different from the eastern GOM due to the low abundance of hard bottom reef habitats (Rezak and Bright 1985) and the presence of oil platforms, which are high relief artificial structures that serve as habitat for many reef fishes (Gallaway et al. 2009). Differences in habitat type have been hypothesized to lead to changes in growth and other life-history characteristics for fish species (Leggett et al. 1978), including Red Snapper (Woods et al. 2003, Fischer et al. 2004). Differences in habitat prevalence and type may lead to differences in the life-history characteristics of the Vermilion Snapper across the GOM which has not been accounted for in recent stock assessments.

Most studies on the age and growth are based on data from the SAB where Vermilion Snapper is the primary commercial reef fish fishery (Grimes 1978, 1980; Zhao

et al. 1997). Studies in the eastern GOM documented age and growth along with annual mortality rates of fish captured off Panama City and south Florida (Hood and Johnson 1999, Allman et al. 2001, 2005; Collins et al. 2003). Mean reported maximum length of Vermilion Snapper is 600 mm total length (TL) and the mean maximum weight of the species is 3 kg (Bohlke and Chaplin 1968). Annuli formation has been validated by marginal increment analysis (MIA) in multiple studies, which determined that annuli are formed yearly (Campana 2001, Zhao et al. 1997, Hood and Johnson 1999). Age-3 year to age-5 year fish are generally captured in both the commercial and recreational fisheries, although the oldest individual recorded is estimated to be 26 years old (VanderKooy 2009). Zhao et al. (1997) reported a shift in the size-at-age and age-at-maturity to younger and smaller fish in the SAB from 1979 to 1987, which the authors attribute to fishing pressure.

Similar to information on growth, descriptions of reproductive biology of Vermilion Snapper is limited for the north-central GOM. In the eastern GOM, Collins et al. (2003) examined age-at-maturity, spawning season, fecundity, and spawning frequency, primarily examining Vermilion Snapper from spawning locations south of Panama City, FL. Studies from the SAB have been conducted in the past and have examined spawning frequency, age-at-maturity, spawning season, and fecundity (Grimes et al. 1982, Cuellar et al. 1996). In both areas, spawning season was found to be from April to September. Annual fecundity estimates in the eastern GOM range from 1-35 million eggs with a spawning interval of 1.6 days (Collins et al. 2003); however, Fitzhugh et al. (2015) estimated mean batch fecundity (± S.D.) at 76,465 eggs (± 79,093 eggs) and spawning interval to be every 2.6 days in the eastern GOM. In the SAB,

spawning interval was estimated at five days, which leads to much smaller annual fecundity estimates, ranging from 125,000 to 1.7 million eggs (Cuellar et al. 1996). Age-at-maturity has been most recently estimated by Fitzhugh et al. (2015) to be around 0.7 years old (138 mm FL). In the SAB, Zhao et al. (1997) observed a temporal decline in age-at-maturity over an eight-year period from 160 mm TL to 151 mm TL for female Vermilion Snapper.

Vermilion Snapper, like most reef dwelling stocks, support both a commercial and recreational fishery which increases the complexity of management for this species, since needs of both sectors must be considered. For example, the recreational sector consists of three groups: 1) headboats (charter vessels in which rates are charged per "head" or individual, which generally carry above 15 people per trip); 2) recreational fisherman; and 3) for-hire charter vessels. From 2000 to 2011 recreational catch of Vermilion Snapper for the GOM averaged 140 metric tons; however, from 2012 to 2014, the recreational catch increased, averaging 360 metric tons (NMFS 2014a, Figure 1). The increase in harvest is likely in response to the shortened recreational season for Red Snapper (11 total days in year 2014), causing the recreational sector to target other reef species like Vermilion Snapper. Currently, minimum length limits and bag limits for Vermilion Snapper are 25.4 cm TL (10 inches) and 10 fish per person per day in the GOM. The most recent change in management came in 2004 when a bag limit was established after the GOM stock was classified as "overfished" (GMFMC 2004). In 2006, after more biological data were gathered, the classification of "overfished" was overruled, but the bag and size limit did not change from the 2004 regulations (SEDAR 9 2006a). Since 2000, commercial harvest accounted for $7,000,000 (USD) in revenue and

has averaged around 1,100 metric tons (NMFS 2014b). However, total commercial catch increased in inter-annual variation after 2007, likely due to the implementation of individual fishing quotas (IFQs) in the Red Snapper fishery, which lowered quotas for individual fisherman, forcing many to harvest different species. The most recent stock assessment for Vermilion Snapper (SEDAR 45) was conducted in 2015 and showed that the stock appears to be in a healthy state and that currently, no overfishing is occurring. Also, the current spawning potential ratio (SPR), is at 32%, which is above the target value of 0.3. Projected target yields are also within the range of optimal yield, suggesting this fishery is being exploited at a sustainable rate (SEDAR 45). Though the stock seems to be harvested sustainably, the need for information about the stock throughout its range was a recommendation in SEDAR 45, especially information from the recreational sector.

In addition to the increased harvest of Vermilion Snapper by the recreational sector, a new predator, the non-native Red Lionfish (*Pterois volitans*), has invaded reefs throughout the GOM and preys on newly settled juvenile Vermilion Snapper (Dahl and Patterson 2014). Vermilion Snapper are documented as the recreational species found in the highest abundance in Red Lionfish digestive tracts (Dahl and Patterson 2014). Using an ECOSIM model approach, the effects of different Red Lionfish biomass scenarios on reef harvest were simulated and in every harvest and Red Lionfish biomass scenario, Vermilion Snapper abundance declined over a 10 year period (Chagaris et al. 2015). Johnston et al. (2017) also compared lionfish abundance with larval density and using a biophysical computer model, found that with increased abundance of lionfish, Vermilion Snapper abundance would decrease across the GOM. With increased fishing and

predation affecting the stock by a non-native predator, an updated life-history profile will help determine if these added pressures could cause population-level changes in life-history characteristics. To address these critical knowledge gaps in Vermilion Snapper life-history in the north-central GOM, the following objectives were developed:

1. Describe the length-weight relationships and age and growth characteristics of Vermilion Snapper using a suite of non-linear models;
2. Describe the reproductive biology of the Vermilion Snapper using standard histological techniques, and estimate the spawning seasonality, age and length-at-maturity, spawning interval/frequency and fecundity; and
3. Compare the life-history parameters estimated in this study to those reported for the eastern GOM and the SAB regions

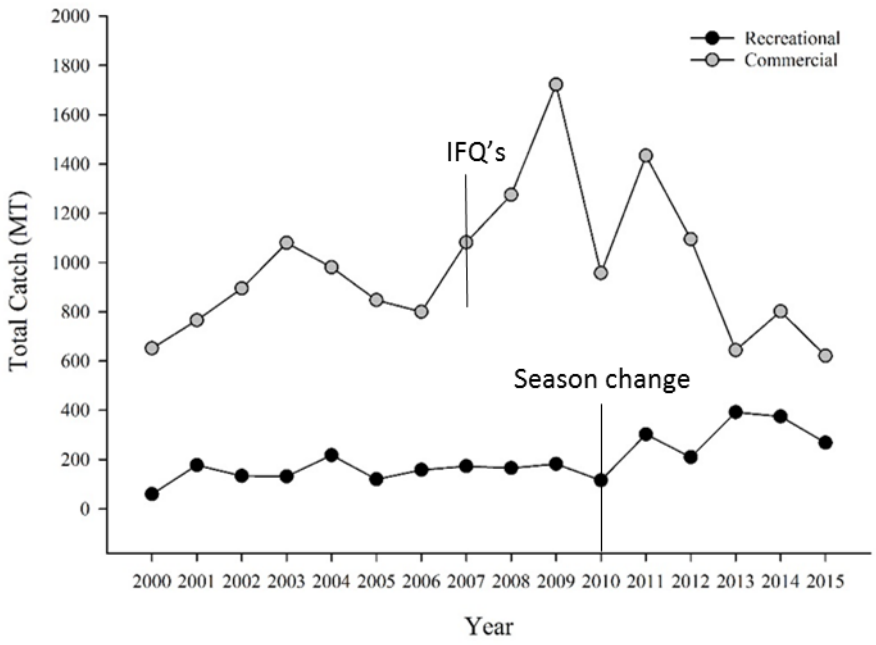

Figure 1. Recreational and commercial catch of Vermilion Snapper

Commercial (gray line) and recreational (black line) total catch for Vermilion Snapper in the Gulf of Mexico from 2000 through 2015. IFQ's = Individual fishing quotas implemented in the Red Snapper commercial fishery, Season change = First year of the shortened Red Snapper seasons in the recreational fishery.

CHAPTER II – METHODS

Fish Collection

Vermilion Snapper in the north-central GOM were collected between May 2015 and October 2016 from Pensacola, FL to the Mississippi River discharge (Figure 2). Fish were collected on petroleum platforms, Rigs-to-Reef sites (rigs cut-off and left as artificial reefs), wrecks, and natural reef habitats which were all located in depths ranging from 35 to 200 m. Fish were collected using multiple sampling methods; for larger fish, hook and line sampling was used onboard recreational and charter vessels, as well as fishery-independent collection using a SEAMAP (Southeast Area Monitoring and Assessment Program), approved vertical line survey. Recreational gear consisted of two and three hook rigs fished during daylight hours from 0900 to 1500 hours. Smaller (< 200 mm TL) fish were collected during fall trawl SEAMAP groundfish surveys conducted aboard the *R/V* Tommy Munro. Additional samples for reproductive analysis were provided by Alabama Marine Resources Division captured during fishery-independent and fishery-dependent sampling events (MRD). Upon collection, fish were immediately placed on ice and brought back to the laboratory for processing. In the laboratory, standard length (SL, mm), fork length (FL, mm) and total length (TL, mm), sex, and weight (TW, kg) were recorded. Linear regressions of SL to FL, SL to TL and FL to TL were used to develop length measurement conversions.

Age and Growth

Otoliths were removed by sawing through the dorsal surface of the head down to the otic capsule (Vanderkooy (2009). A transverse cut was then made from the top of the skull to the point at which the lateral line and operculum meet. This cut exposed the brain

and once the brain was removed, the butterfly-shaped capsule in which the otoliths rested was visible. Otoliths were removed using forceps and rinsed in tap water before being dried on paper towels. Once dry, otoliths were transferred into individually labeled envelopes for storage.

The left sagittal otolith was used to estimate the age of Vermilion Snapper whenever available. Poly Sciences embedding molds (22 x 22 x 20 mm) were used to mount the otoliths in resin. First, a small layer of resin (West Systems 105 epoxy resin and West Systems 206 slow hardener) was added into the molds to form a base for the otolith to sit upon. Once this mixture had set for 24 hours, sagittal otoliths were placed in the molds and oriented centrally. Otoliths were then covered in resin and cured for 24 hours.

After the resin had cured, the resin block was removed from the mold and smoothed using coarse sandpaper to allow for a proper fit in the saw chuck. A line was drawn vertically on the resin block to indicate where the best cut for aging was located. The ideal section is near the junction of the ostium and sulcus, and if sectioned properly, will produce a V-shaped groove with annuli radiating out from the core (Vanderkooy 2009). The block was securely placed on the saw chuck and aligned with the vertical line on the block. Sections were cut with an Isomet low-speed wafering saw and Norton diamond wheel blade into a recommended 0.5 mm thickness to allow for the best reading (Vanderkooy 2009). Ideal sections with clearly defined annuli were chosen that best estimate the true age of the fish and were placed onto a slide for mounting. The slide was placed on a flat surface and each section was covered with Cytoseal, a thermoplastic

adhesive that clears and seals the section to the slide. Slides were left overnight for 24 hours or until dry before aging the sectioned otoliths.

Two independent readers examined mounted otoliths to estimate age using a Nikon SMZ1000 microscope with a digital sight for computer screening. Readers determined age based on the formation of bands on the otolith section. Bands consist of both opaque and translucent coloration patterns and indicate periods of slow and fast growth, respectively (Secor et al. 1991). The slow growth opaque rings (annuli) were used to determine age of each specimen (Figure 3). Once readers determined the age of specimens, results from each reader were compared. If any discrepancies arose, the otolith in question was examined for a third time and if no agreement was reached, the otolith was removed from analysis. Biological age was calculated based on a July 1st birthdate and a fractional year estimate (Vanderkooy 2009). Percent of fish ages for binned length classes (20 mm) were used to construct an age-length key.

Using a multi-model approach, length-at-age was described using the two-parameter Von Bertalanffy growth function, three-parameter Von Bertalanffy growth functions (von Bertalanffy 1938), and the logistic growth function (Ricker 1975). The two-parameter Von Bertalanffy growth function is:

$$L_t = L_\infty(1 - e^{-kt});$$

where L_t represents the TL (mm) at (t) in years, L_∞ is the hypothetical mean maximum TL (mm), and k is the growth coefficient. The three-parameter Von Bertalanffy growth function equation is:

$$L_t = L_\infty\,[1 - e^{-k(t-t_0)}];$$

this function includes a third parameter, t_0, which is the theoretical age of a fish at length zero. The logistic growth equation (Ricker 1975) is:

$$L_t = L_\infty / (1 + e^{-k(t-t_i)}),$$

where the growth parameter *k* and t_i are incorporated to limit growth to a maximum size.

Mean parameter estimates were compared to the 95% confidence intervals of the opposite sex to determine if growth was significantly different between sexes. If the mean parameter estimate fell between the 95% confidence intervals of the opposite sex, then growth between sexes was determined not to be different. An analysis of the residual sum of squares (ARSS) was also used for the most-supported model to compare growth between sexes. Model support across all three equations was compared for combined sexes using Akaike information criterion (AIC; Burnham and Anderson 2004). The model with the lowest ΔAIC value was the candidate model with the best support. All models were fit to both sexes and TL so that comparisons could be made to past studies of the species.

Weight-at-length was described using the power function:

$$W = aTL^b;$$

where W is total weight (kg), TL is total length (mm), *a* is a scaling coefficient, and *b* is an exponent describing the change in TL relative to weight. Similar to length-at-age models, differences in weight-at-length between sexes was determined by comparing the mean parameter estimate to the 95% confidence interval of the opposite sex.

Reproduction

All gonads were removed and weighed (GW, 0.01g) at the laboratory within 24 hours of capture. Gonads were assigned a macroscopic phase and sex based on physical

appearance and size (Tables 1 and 2) following Brown-Peterson et al. (2011). A cross section (< 1 cm^3) was removed from the middle of the right gonad and placed into a labeled cassette for histological analysis. These cassettes were preserved in 10% neutral buffered formalin (20:1 ratio liquid to tissue) for at least one week to ensure proper preservation and penetration of the tissues. In cases where an actively spawning fish was sampled, a subsample of the gonad (~5g) was removed, weighed (0.01 g) and put into Gilson's fluid (Bagenal and Braum 1978) for three months for fecundity determination.

Spawning seasonality, the portion of the year in which the population is reproductively active, was determined using two methods. The first is the gonadosomatic index (GSI), which is used to measure spawning preparedness throughout the year. GSI is calculated as:

$$\mathrm{GSI} = \frac{\mathrm{GW}}{\mathrm{GW-TW}} \times 100.$$

Prior to statistical analysis, GSI values were tested for normality and homogeneity of variance using a Levene's and Shapiro-Wilke's test. If the assumptions were met, mean GSI values were compared by month for each sex using a one-way analysis of variance (ANOVA). If significant *F*-values were found, monthly values were separated using a Sidak pairwise test ($\alpha < 0.05$). If the assumptions were not met, a Welch's ANOVA along with a Games-Howell posthoc test were used ($\alpha < 0.05$). A linear regression of gonad-free body weight (GFBW) and GSI was calculated for both sexes separately to ensure fish weight was independent of GSI values (Jons and Miranda 1997). If the two were correlated, I conducted a one-way ANOVA of GSI and GFBW by month with sexes separated, and then plotted each by month for visual and statistical comparison; these were used to ensure GSI was not being influenced by GFBW.

The second method used to estimate spawning seasonality was histological examination of gonadal tissue. For histological analysis, formalin-preserved tissues were first rinsed overnight in running tap water for 24 hours, then dehydrated by placing cassettes in 60% ethanol for two hours followed by placement in 70% ethanol for two hours then placed again in 70% ethanol before being processed. Next, the tissues were put into a Shandon Excelsior ES Tissue processor (Thermo-Fischer Scientific), where they were further dehydrated in a series of graded ethanols (Appendix 1). Once fully dehydrated, tissues were cleared in Xylene Sub (Thermo-Fischer Scientific) and impregnated with Histoplast LP (Thermo-Fischer Scientific). Tissues were removed from the processor and transferred to a Shandon Tissue Embedding Center (Thermo-Fischer Scientific), where they were embedded in steel molds filled with Paraplast X-tra paraffin wax (McCormick), within an hour of being processed. Embedded tissues were sectioned at 4µm on a rotary microtome (American Optical) and mounted onto slides for staining using Stay-ON slide adhesive (Thermo-Fischer Scientific). Slides were placed on a LAB-LINE Instruments slide warmer for two hours and then stained using Hemotoxylin 2 and Eosin Y (Richard-Allan Scientific, Appendix 2). Coverslips were placed on top of stained tissue using Richard-Allen Scientific mounting media and slides were allowed to dry overnight before analysis under a compound microscope. Microscopic classification of each fish followed histological descriptions and terminology from Brown-Peterson et al. (2011, Tables 1 and 2).

Quantification of oocyte and spermatogenetic stages was conducted using ImageJ software (Schneider et al. 2012). Three areas were randomly selected from the histological slides of the tissue and photos were taken at 4× for females and 40× for

males using a Nikon compound microscope with DCIM imaging software. An ImageJ software 80 point grid was overlaid on the photo and for each grid point oocyte stages were counted (Figure 4). After all grid points were examined and empty grids or grids containing non-oocyte tissues were excluded, the percent coverage of each oocyte stage (primary growth (PG), cortical alveolar (CA), primary vitellogenic (Vtg1), secondary vitellogenic (Vtg2), tertiary vitellogenic (Vtg3), oocyte maturation (OM), post-ovulatory follicle complex (POF), or atresia (A)) was calculated for all three photos, and a grand mean was calculated. For males, the spermatogenetic maturity index (SMI) was used to quantify gonadal development (Tomkiewicz et al. 2011). This method allows the experimenter to estimate percent coverage of each testis tissue type (somatic cells (Ts), spermatogonia (Sg), spermatocytes (Sc), spermatids (St), spermatozoa (Sz)). Methods for estimation matched techniques used for female analysis with three areas randomly selected and photographed for incorporation of an ImageJ software grid. The SMI equation used was:

$$\mathrm{SMI} = 0.0\mathrm{F_{Ts}} + 0.4\mathrm{F_{Sg}} + 0.6\mathrm{F_{Sc}} + 0.08\mathrm{F_{St}} + 1.0\mathrm{F_{Sz}};$$

where F is the frequency of each indicated cell type. The index describes testis development on a scale from 0 to 1.

Age-and length-at-maturity were defined using histological criteria so that estimates would be as accurate as possible. Females were classified as sexually mature when cortical alveolar oocytes were present in the ovaries whereas for males the presence of primary spermatocytes indicated sexual maturity (Brown-Peterson et al. 2011).

Batch fecundity (BF) was estimated for all fish macroscopically classified and histologically-confirmed in the actively spawning sub-phase. Actively spawning fish are

those fish whose oocytes were hydrated or were undergoing oocyte maturation (OM). Oocyte maturation represents the final stages of growth before an oocyte is ovulated. Histological evidence of oocyte maturation includes lipid and yolk coalescence (LC, YC), germinal vesicle migration (GVM), germinal vesicle breakdown (GVBD), and hydration. Once oocytes were histologically confirmed to be undergoing OM, the Gilson's Fluid sample was washed with flowing tap water for 12 hours to ensure removal of the fixative. Batch fecundity was estimated using the volumetric method presented in Bagenal and Braum (1978). This method involves eggs placed in a volume of water (50 to 250 ml) and gently stirred until eggs are distributed homogenously throughout the solution. Once mixed, six one mL sub-samples were taken with replacement. An oocyte size frequency distribution of all oocytes over > 80 µm was used to determine which oocytes to count for BF calculations. This was conducted using a spawning capable and an actively spawning female (Figure 5). The largest size bin of oocytes (> 450 µm) were considered hydrated or undergoing OM and were used for BF estimates. All hydrated eggs were counted in each subsample and BF was estimated using the formula:

$$\mathrm{BF}=\mathrm{N}\left(\frac{\mathrm{DL}}{\mathrm{DLS}}\right)\left(\frac{\mathrm{GW}}{\mathrm{PGW}}\right);$$

where N is the number of oocytes in the largest size bin, DL is the volume of water used to dilute the sample (ml), DLS is the volume of water in the subsample, GW is gonad weight (g) and PGW is the portion of the gonad used for the analysis (g). Relative Batch Fecundity (RBF) was estimated using the equation:

$$\mathrm{RBF}=\frac{\mathrm{BF}}{\mathrm{OFBW}};$$

where OFBW is the ovary-free body weight (g). Linear regressions of both raw and log-transformed estimates were used to determine the relationship between BF and age, as well as BF and total length.

Spawning interval was estimated two ways using both the presence of oocytes undergoing OM and the presence of 24hr POFs (Hunter and Macewitz 1985). Calculation of the spawning interval (SI) was estimated with the following equation:

$$SI = \frac{N(SC)}{N(POF \text{ or } OM)};$$

where N(SC) is the total number of fish defined as spawning capable (including actively spawning sub-phase) and N(POF or OM) is the total number of fish that are undergoing OM or that contain POFs. Bi-monthly estimates of the spawning interval were averaged to yield annual spawning interval. To calculate the spawning frequency, I divided the total number of days within the spawning season by the annual spawning interval. This number was then multiplied by BF to estimate total annual fecundity for an individual at age.

Table 1

Female phase descriptions

Phase	Macroscopic Description	Histological Description
Developing	Enlarged ovaries with a translucent-orange coloration, blood vessels present but not distinct	Contains primary growth, cortical alveolar, primary vitellogenic and secondary vitellogenic oocytes. Little or no tertiary vitellogenic oocytes present.
Early-developing subphase	Enlarged ovaries with translucent-orange coloration, blood vessels present but not distinct	Contains both primary growth and cortical alveolar cells. Little or no vitellogenesis present.
Spawning capable	Large ovaries with opaque-orange coloration, blood vessels prominent and throughout ovary	All stage of oocyte development occurring with the exception of oocyte maturation. Tertiary vitellogenic oocytes abundant with small lipid particles surrounding the nucleus. Post-ovulatory follicle complex may be present.
Actively spawning subphase	Large inflated ovaries with a reddish-orange mottled coloration, blood vessels present along with clear spacing in between oocytes	Abundance of oocytes undergoing oocyte maturation, with lipid coalescence, germinal vesicle migration, germinal vesicle breakdown or hydration occurring. Post-ovulatory follicles may be present.
Regressing	Flaccid ovaries with a dark orange-red coloration, blood vessels prominent	Primary growth and cortical aveolar oocytes most abundant with all stages of vitellogenic oocytes undergoing multiple stages of atresia.
Regenerating	Small ovaries, blood vessels present but not distinct	Contains only primary growth oocytes, with most oocytes in the peri-nucleolar stage. Interstitial tissue and blood vessels present throughout.

Description of macroscopic and microscopic features in female Vermilion Snapper found in each reproductive phase (following Brown-Peterson et al. 2011).

Table 2

Male phase descriptions

Phase	Macroscopic Description	Histological Description
Developing	Enlarged testes with a translucent yellow-white coloration.	Contains all stages of spermatogenesis within the spermatocysts of the lobule. Lumens may be present but do not contain any spermatozoa.
Early-developing subphase	Enlarged testes with a translucent yellow-white coloration	Contains only primary and secondary spermatogonia, along with primary spermatocytes. Lumens may or may not be present.
Spawning capable	Large opaque testes, white in coloration.	All stages of spermatogenesis occurring, spermatozoa present in the lumen. Spermatozoa may be present in the duct. Germinal epithelium (GE) can be continuous or discontinuous.
Early GE subphase	Histological only	Continuous GE throughout testes
Mid GE subphase	Histological only	Continuous GE in the periphery, discontinuous GE near duct
Late GE subphase	Histological only	Discontinuous GE throughout the testes
Actively spawning subphase	Large opaque testes, white in coloration. Milt is released with gentle abdominal pressure applied.	Macroscopic only
Regressing	Testes reduced in size and often firm or hard to the touch	Spermatogonial proliferation reduced to primary and secondary spermatognia in the periphery, residual spermatozoa left in the lumens. No active spermatogenesis with few spermatocysts present.

Description of the macroscopic and microscopic features for male Vermilion Snapper in each reproductive phase (following Brown-Peterson et al. 2011).

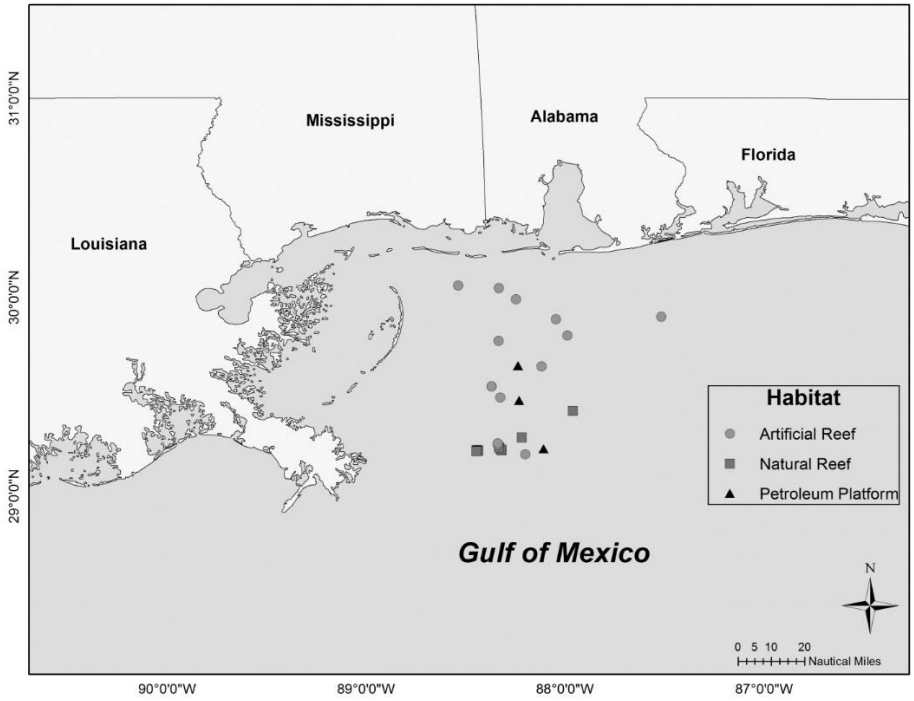

Figure 2. Sampling Map

Map of sampling area in the north-central Gulf of Mexico with each point marking a reef location where fish were collected by habitat type.

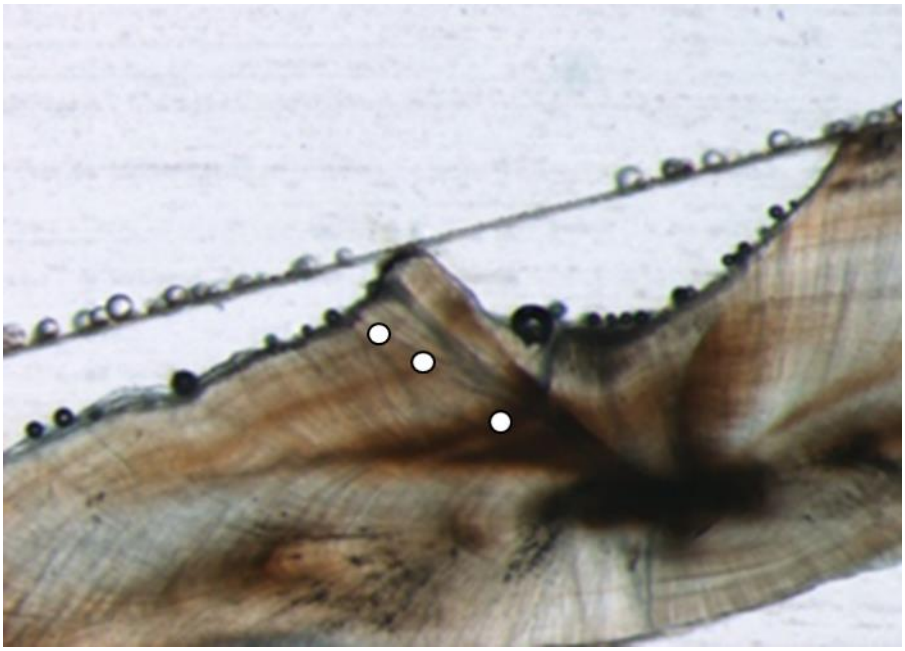

Figure 3. Vermilion Snapper otolith

Photo of a three-year-old Vermilion Snapper with annuli enumerated.

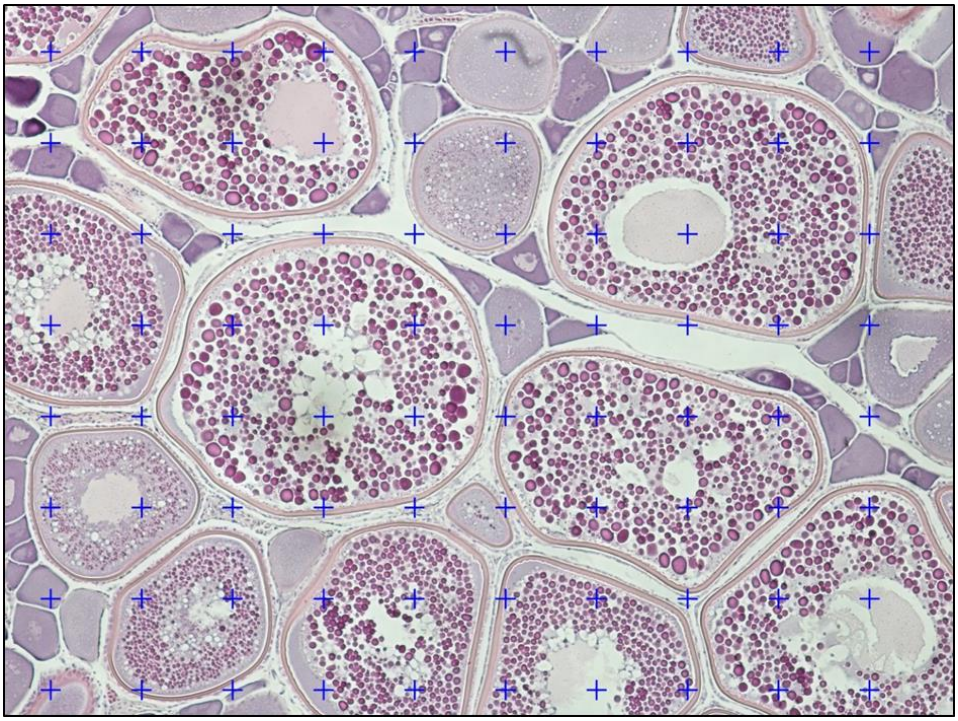

Figure 4. ImageJ analysis grid

Photomicrograph of an actively spawning female Vermilion Snapper with an ImageJ 80-point grid overlaid onto the image. For each cross, oocyte stage is recorded to yield the overall percent coverage of each oocyte stage.

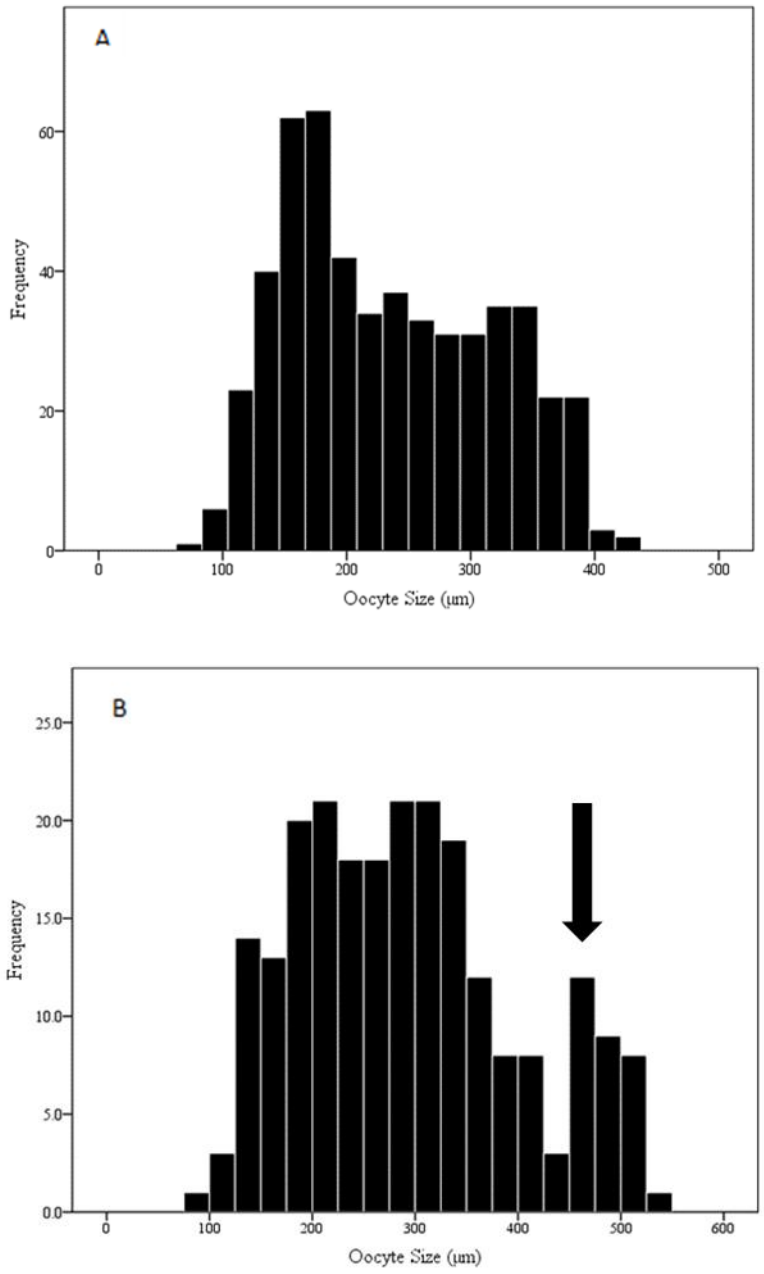

Figure 5. Oocyte size frequency distribution

Oocyte size frequency distribution of a spawning capable (A) and actively spawning (B) female Vermilion Snapper. All oocytes > 450 µm (arrow) were considered undergoing OM and counted for batch fecundity estimation.

CHAPTER III - RESULTS

Fish Collection

A total of 445 Vermilion Snapper were collected from May 2015 to October 2016 including 348 from hook and line, 16 from SEAMAP trawls, and 75 from fishery-independent vertical line sampling. Female Vermilion Snapper were collected during all months with the exception of November and December 2015. Males were collected for all months with the exception of November and December 2015 as well as January 2016. Fish were collected from all habitats; however, most fish came from artificial reefs (Table 3). Lengths ranged from 139 to 510 mm TL and a total of 226 females and 219 males were collected, yielding a sex ratio of 1.03:1 in favor of females.

Age and Growth

Linear regressions were used to compare relationships between TL, FL, and SL all showed high correlation ($r^2 > 0.98$). No differences were found between the slopes of males and females when comparing length measurements. Equations derived from the linear regressions are as follows:

$$\text{TL} = 1.264 \times (\text{SL}) - 0.620;$$

$$\text{TL} = 1.128 \times (\text{FL}) - 2.112;$$

$$\text{FL} = 1.126 \times (\text{SL}) - 0.820; \text{ and}$$

$$\text{FL} = 0.884 \times (\text{TL}) - 2.845.$$

A total of 370 Vermilion Snapper were collected for age estimation with ages ranging from 0.8 up to 13 years old. Reader agreement was 73% for the first separate reading; however, during the second joint reading, agreement increased to 98%. 20 mm

length bins were used to construct an age-length key for Vermilion Snapper and showed wide overlap in length-at-age (Table 4).

Growth models were first separated by sexes and fit to TL. Comparison of the 95% confidence intervals showed no significant differences for growth between sexes, with the exception of the logistic growth function, where the mean L_∞ values did not lie within the confidence interval of the opposite sex (Table 5). To ensure that growth between sexes was not different, an ARSS of the two-parameter VBGF was calculated and found that growth was not significantly different ($F_{2,370}$ = 1.06, P = 0.65), thus combined sex data were used to analyze across growth models. All growth models showed similar mean TL-at-age estimates of Vermilion Snapper (Figure 6). All models were fit to both sexes and TL so that comparisons could be made to past studies of the species (Table 6). For combined sexes, the two-parameter VBGF fit to TL provided the lowest ΔAIC score and was the most supported model (Table 7). For female-specific growth, the logistic growth function provided the best fit, though ΔAIC scores were all similar (ΔAIC < 1.1) whereas, for males, the two-parameter VBGF provided the best fit overall (Table 6). Sex-specific parameter estimates for all models can be found in Table 5.

The weight-at-TL relationship was fit using the power function for both sexes, and showed no significant differences between sexes (Table 8). For combined sexes, *a* was estimated to be 2.74e-08 (95% CI: 1.70×10^{-8}-08 to 4.36×10^{-8}) and *b* was estimated at 2.86 (95% CI: 2.79 to 2.94, Figure 7).

Reproduction

A total of 444 Vermilion Snapper were collected with intact gonads for reproductive analysis and were used for estimating spawning seasonality using GSI. A total of 386 fish were used for histological examination. No immature fish for either sex were collected during this study. All other reproductive phases were present in females and males, with the exception of males in the regenerating phase. The smallest female captured was 155 mm TL and was actively spawning and the smallest male captured was 139 mm TL and was spawning capable.

Histological Descriptions

Each reproductive phase for Vermilion Snapper was described histologically. Immature fish were not found during this study, thus this phase is not described. Females in the regenerating phase contained only primary growth oocytes (PG), mostly in the perinucleolar (PN) stage in the ovary (Table 9), along with blood vessels interspersed in the tissue (Figure 8). Ovaries in the early-developing subphase were also dominated by primary growth oocytes, but the presence of cortical alveolar (CA) oocytes showed that the oocytes were beginning to mature in response to hormonal cues (Figure 9, Table 9). Developing phase females were defined as those beginning the process of vitellogenesis and ovaries contained primary and secondary vitellogenic oocytes (Vtg1, Vtg2) in addition to PG and CA oocytes (Figure 10, Table 9). Ovaries in the spawning capable phase were characterized by the presence of tertiary vitellogenic oocytes (Vtg3, Table 9), although other stages of vitellogenesis were also observed (Figure 11). A low percentage of spawning capable females had atretic oocytes in the ovary (Table 9). Many spawning capable female ovaries also contained POFs which indicate that these fish are batch

spawners. This was supported by the presence of all oocyte stages throughout the spawning season along with the presence of post-ovulatory follicle complexes. Females in the actively-spawning subphase were determined by the presence of oocyte maturation (OM, Table 9), which was characterized by lipid coalescence (LC), germinal vesicle migration (GVM), germinal vesicle breakdown (GVBD) or hydrated oocytes (H, Figures 12a,b). Additionally, 17% of Vermilion Snapper in the actively spawning phase containing 24 hour POF's suggesting daily spawning is occurring (Figure 12a). POF complexes are small in size and quantity and thus were not enumerated in the ImageJ analysis of histological slides (Table 9). Females in the regressing phase were characterized by ovaries with a higher percentage of alpha, beta, and gamma atresia compared to other phases (Table 9). Vitellogenic oocytes were not seen in females in this phase and although some CA oocytes were evident, PG oocytes in the perinucleolar stage dominated (Figure 13).

The immature and regenerating phases were not found in males, thus no histological description will be given. The actively spawning sub-phase could only be determined macroscopically for males and therefore is not considered a histologically identifiable reproductive phase (Table 2). Males in the early-developing sub-phase were those with testes containing primary spermatogonia (Sg1), secondary spermatogonia (Sg2), primary spermatocytes (Sc1) and secondary spermatocytes (Sc2), although spermatogonia were the dominant spermatogenic stage (Figure 14). Early-developing Vermilion Snapper contained no spermatozoa in the spermatocysts or in the lumen, and lumens were often hard to distinguish. Males in the developing phase contained all stages of spermatogenesis; however, spermatozoa were found in spermatocysts but not in the

lumens, and in many cases, no lumens were observed (Figure 15). The majority of males collected were in the spawning capable phase (Figure 16), and had all stages of spermatogenesis as well as spermatozoa in the lumens and sperm ducts. Spawning capable males were differentiated based on the condition of the germinal epithelium (GE). Spermatocysts are formed in the germinal epithelium and as the spawning season progresses and spermiation increases, the epithelium begins to become discontinuous, with whole sections containing no active spermatogenesis (Figure 16b). Early-GE subphase was assigned when all lobules had a continuous GE and were completely surrounded by spermatocysts (Figure 16) and were typically found in the beginning of the spawning season (Table 10). The mid-GE subphase was assigned when discontinuous germinal epithelia were found near the sperm duct and late-GE was assigned when discontinuous germinal epitheliums were observed throughout the gonad (Figure 16b). Male testes in the regressing phase had lobules with spermatozoa but little active spermatogenesis and few spermatocysts occurring in the GE (Figure 17). Spermatogonial proliferation could be observed at the periphery of the testis in regressing males (Figure 17).

Spawning Seasonality

To determine if GSI could be used as a valid metric to describe spawning seasonality, the relationship between fish size and GSI was compared separately for both sexes. There was a significant ($p < 0.001$) relationship for females, and GFBW accounted for 16% of the variation in GSI ($r^2 = 0.16$). There was also a significant ($p < 0.001$) relationship in males with 34% of the variation in GSI accounted for by GFBW ($r^2 = 0.34$). A visual comparison of mean monthly GFBW values plotted with GSI values for

males shows GFBW was relatively constant over the year and does not mirror the GSI pattern (Figure 18a). Thus, despite the moderate but significant r^2 value, GSI can be used as a proxy for male spawning preparedness. However, female values showed similarities to the monthly GSI pattern, particularly at the end of the season (Figure 18b). In January through March, GSI remained level while GFBW declined; however, as GSI values increased in April so did GFBW. April through July had relatively constant GFBW values, however, GSI values peaked in May and showed a sharp decline in June and July which did not coincide with the GFBW pattern. Finally, from August through October both GSI and GFBW declined (Figure 18b). A one-way ANOVA with a Sidak post-hoc test was used to test differences in both mean GFBW and mean GSI by month. Significant differences in mean GSI values were found when comparing May to July, but mean GFBW values showed no significant difference between those months. Thus, it is unlikely that GFBW is responsible for the fluctuation in mean GSI values during the spawning season. However, it should be noted that the significant but moderate r^2 value and similar trends in GFBW and female GSI may suggest that GSI is influenced by fish size, particularly at the end of the season.

Spawning seasonality was determined by plotting mean GSI values (± SE) by month for both sexes (Figure 19). For females, GSI values were lowest during the months of January-March and in October. During the summer months (April-September) elevated values were observed with the peak GSI value (2.7%) in May, suggesting that Vermilion Snapper were spawning during these months. Female mean GSI values were significantly different when analyzed across months using a Welch's ANOVA ($F_{9,210}$ = 6.113, $p < 0.001$), and a posthoc Games-Howell test indicated that April, May, July,

August, and September were significantly higher than values observed in January, February, March, and October. Male GSI values were similar to trends of females, with elevated values found from April through September, but due to large variation and numerous high values, the Games-Howell posthoc did not show clear differences when comparing months. Both male and female GSI values showed a decrease in July during the spawning season, which was attributed to the amount of small individuals captured within the month.

Histological analysis was used to further elucidate the spawning season. All females captured in January, March, and October were in the regenerating phase (Table 10, Figure 6). Gonadal recrudescence was first observed in February with the appearance of the early-developing phase (Table 10, Figure 7). Actively spawning and spawning capable fish were found from April through September, supporting the GSI trend of an April through September spawning season. Additionally, the first actively spawning individual was captured on 4 April and the last was captured on 26 September, leading to an estimated 172 day spawning season. Some females were in the regenerating phase throughout the spawning season, with the highest percentage during April and July, months that also had the lowest GSI values (Table 10, Figure 19). Females were also observed in the regressing phase as early as May, indicating that some individuals may not spawn throughout the season.

Males captured in February were undergoing gonadal recrudescence and by March, spawning capable fish were observed (Table 11). Spawning capable males were found in high percentages from April through September in all sub-phases, consistent with elevated GSI values during these months. Increased presence of the LGE sub-phase

near the end of the spawning season was observed. Regressing males were first observed in September and all males captured in October were in the regressing phase.

The Spermatogenic Index (SMI) was used to further describe the relative spawning preparedness of males throughout the spawning season. The SMI values increased gradually up to April, and then sustained values of around 0.79 until October, when SMI values increased to 0.92 (Figure 20). This index shows the increasing presence of spermatozoa in the testis relative to other spermatogenic stages, thus as the season progressed and spermatogenesis decreased, the percentage of spermatozoa in the testes increased and the SMI reached maximum values.

One keynote on spawning strategy is that actively spawning females were found on all structure types sampled, including petroleum platforms and Rigs-to-Reef sites (Table 3). Further analysis showed that 26% of female Vermilion Snapper caught on natural reefs were actively spawning, 14% of fish caught on artificial reefs, and 16% of the fish caught on petroleum platforms were actively spawning.

Spawning Frequency

The spawning interval calculations showed that Vermilion Snapper spawn frequently from April through September. The spawning interval using the HO method for the months of April and May was estimated to be 1.3 days between spawning events in the beginning of the season. In June and July, the spawning interval increased to 2.2 days between spawning events and in August and September, it decreased back to every 1.9 days at the end of the season. Combining all months together, a spawning interval of every 1.8 days (Table 12) was obtained, and when incorporated into a 172 day spawning season yielded a potential spawning frequency of 95 spawn events/season using the HO

method. Using the POF method, results varied slightly from the HO method (Table 12) and showed a potential annual spawning interval of 2.2 days between spawning events. Incorporating this spawning interval into a 172 day spawning season yielded a potential spawning frequency of 78 spawn events/season. Histological evidence shows that some females are capable of daily spawning (Figure 16A), supporting the calculated spawning interval of < 2 days.

Fecundity

Batch fecundity was estimated from 22 fish ranging from 394 to 513 mm TL. Estimates ranged from 5,497 to 284,468 eggs/batch. While BF did not show a significant relationship when compared to fish size ($p = 0.19$) or age ($p = 0.23$) for both raw and log-transformed data, a general trend of increasing BF with increasing fish size can be seen visually (Figure 21 a, b). Relative batch fecundity yielded estimates of 8.1 eggs/g of GFBW up to 276.9 eggs/g of GFBW with a mean RBF value of 70.7 eggs/g of GFBW. A linear regression of RBF and fish size ($r^2 = 0.02$, $p = 0.548$) showed no relationship. Annual fecundity was estimated by multiplying BF and the spawning frequency of the HO method and ranged from 544,203 eggs/spawning season up to 28,162,332 eggs/spawning season.

Table 3

Vermilion Snapper Collection

		Females only	
Structure Type	**n**	**% Actively Spawning**	**% Spawning Capable**
Artificial Reef	234	14.5	11.1
Natural Reef	64	26.5	6.2
Petroleum Platform	61	16.4	11.5

Number of Vermilion Snapper caught on each structure type in the north-central Gulf of Mexico along with the percentage of females found actively spawning or spawning capable on the structure.

Table 4

Age-length key

TL (mm)	N	age-1	age-2	age-3	age-4	age-5	age-6	age-7	age-8	age-9	age-10	age-11	age-12	age-13
130-149	2	100.0	0	0	0	0	0	0	0	0	0	0	0	0
150-169	7	85.7	14.3	0	0	0	0	0	0	0	0	0	0	0
170-189	2	100.0	0	0	0	0	0	0	0	0	0	0	0	0
190-209	2	50.0	50.0	0	0	0	0	0	0	0	0	0	0	0
210-229	2	100.0	0	0	0	0	0	0	0	0	0	0	0	0
230-249	5	20.0	60.0	20.0	0	0	0	0	0	0	0	0	0	0
250-269	15	0	46.7	46.7	6.7	0	0	0	0	0	0	0	0	0
270-289	27	0	33.3	59.3	7.4	0	0	0	0	0	0	0	0	0
290-309	39	0	38.5	43.6	10.3	0	0	2.6	0	0	0	0	0	0
310-329	39	0	20.5	61.5	15.4	0	0	0	0	0	0	0	0	0
330-349	29	0	17.2	55.2	20.7	0	0	0	0	0	0	0	0	0
350-369	26	0	11.5	69.2	15.4	0	0	0	0	0	0	0	0	0
370-389	25	0	8.0	72.0	20.0	0	0	0	0	0	0	0	0	0
390-409	23	0	0	52.2	34.8	0	0	0	0	0	0	0	0	0
410-429	29	0	3.4	51.7	17.2	13.8	10.3	3.4	0	0	0	0	0	0
430-449	34	0	0	32.4	23.5	14.7	2.9	0	5.9	2.9	8.8	0	2.9	2.9
450-469	34	0	0	20.6	17.6	14.7	8.8	5.9	8.8	14.7	2.9	0	2.9	0
470-489	22	0	0	4.5	13.6	13.6	9.1	0	31.8	18.2	4.5	4.5	0	0
490-509	6	0	0	0	0	16.7	0	33.3	16.7	16.7	0.0	16.7	0	0
510-529	1	0	0	0	0	0	100.0	0	0	0	0	0	0	0
530-549	1	0	0	0	0	0	0	100.0	0	0	0	0	0	0
550-569	1	0	0	0	0	0	0	0	0	0	100.0	0	0	0

Age-length key with lengths separated into 20 mm bins for Vermilion Snapper from the north-central Gulf of Mexico for combined sexes. TL = total length, N = number of fish

Table 5

Length-at-age parameter estimates

Model	Parameter	Parameter Estimate	95% CI
Two-Parameter VBGF			
Male	L_∞	452.75	428.65 to 479.24
	k	0.48	0.42 to 0.55
Female	L_∞	470.39	450.77 to 491.60
	k	0.54	0.48 to 0.62
Three-Parameter VBGF			
Male	L_∞	489.29	454.35 to 536.82
	k	0.31	0.22 to 0.41
	t_0	-0.8	-1.52 to -0.32
Female	L_∞	479.67	455.54 to 510.55
	k	0.45	0.32 to 0.60
	t_0	-0.38	-1.20 to 0.12
Logistic Growth Function			
Male	L_∞	421.49	396.37 to 451.42
	k	0.52	0.40 to 0.65
	t_i	1.23	0.89 to 1.72
Female	L_∞	472.1	450.61 to 497.38
	k	0.66	0.48 to 0.87
	t_i	0.98	0.53 to 1.30

Length-at-age parameter estimates for all models by sex of Vermilion Snapper in the north-central Gulf of Mexico. VBGF = Von Bertalanffy Growth Function,

L_∞ = hypothetical mean maximum total length (mm), k = growth coefficient (y^{-1}),

t_0 = theoretical length at age 0, t_i = age at maximum growth rate.

Table 6

Published length-at-age parameter estimates

Citation	Location	Sex	*n*	L_∞ (mm)	*k*	t_0
Johnson et al. 2010	north-central GOM	Male	242	862 (± 35.3)	0.05 (±0.04)	-5.67 (± 1.56)
		Female	317	655 (± 4.7)	0.13 (±0.03)	-2.78 (± 0.56)
		Combined	621	707 (± 6.4)	0.09 (± 0.01	-3.97 (± 0.59)
Zhao et al. 1997	SAB	Combined	192	562	0.202	-0.117
Grimes 1978	SAB	Combined	815	626.6	0.198	-0.128
Schirripa 1992	SAB	Combined	886	535	0.203	-0.940
Potts et al. 1998	NC to FL Keys	Combined	1,465	650.24	0.144	-0.238
Hood and Johnson 1999	eastern GOM	Combined	858	297.18	0.25	-3.9
This Study 2016	north-central GOM	Male	187	489.29 (± 34.95)	0.31(± 0.09)	-0.80 (± 0.72)
		Female	183	479.67 (± 24.13)	0.45(± 0.13)	-0.38 (± 0.82)
		Combined	370	483.28 (± 21.48)	0.38(± 0.08)	-0.55 (± 0.49)

Length-at-age parameter estimates from previous studies of Vermilion Snapper. All comparisons are for the three-parameter Von Bertalanffy Growth Function; 95% confidence intervals are displayed in parentheses if given. GOM = Gulf of Mexico, SAB = South Atlantic Bight, L_∞ = hypothetical mean maximum total length (mm), *k* = growth coefficient (y^{-1}), t_0 = theoretical length at age 0.

Table 7

AIC comparison of growth models

Model	Parameter	Parameter Estimate	95% CI	ΔAIC
Two-Parameter VBGF	L_∞	464.08	446.48 to 482.94	0
	k	0.50	0.45 to 0.59	
Logistic Growth Function	L_∞	472.46	454.27 to 492.87	2.76
	k	0.59	0.48 to 0.71	
	t_i	1.12	0.87 to 1.34	
Three-Parameter VBGF	L_∞	483.28	461.80 to 509.20	9.22
	k	0.38	0.30 to 0.46	
	t_0	-0.55	-1.04 to -0.20	

Length-at-age parameter estimates for Vermilion Snapper in the north-central Gulf of Mexico from the three growth functions for combined sexes. Mean parameters are displayed along with the 95% confidence intervals and ΔAIC values. VBGF = Von Bertalanffy Growth Function, L_∞ = hypothetical mean maximum total length (mm), k = growth coefficient (y^{-1}), t_0 = theoretical length at age 0, t_i = age at maximum growth rate.

Table 8

Weight-at-length parameter estimates

Weight-at-Length	Parameter	Parameter estimate	95% Confidence interval
Combined	*a*	2.74E-08	1.70E-08 to 4.36E-08
	b	2.86	2.79 to 2.94
Male	*a*	5.05E-08	2.23E-08 to 1.13E-07
	b	2.76	2.63 to 2.90
Female	*a*	1.97E-08	1.28E-08 to 3.02E-08
	b	2.913	2.84 to 2.98

Weight-at-Length parameter estimates for combined sexes, and males, and females separately for Vermilion Snapper in the north-central Gulf of Mexico.

Table 9

Percent coverage of oocyte stage

Phase	PG (%)	CA (%)	Vtg 1 (%)	Vtg 2 (%)	Vtg 3 (%)	OM (%)	POF (%)	Alpha (%)	Beta/Gamma (%)
Regenerating	100.0	0	0	0	0	0	0	0	0
Early Developing	83.1	16.9	0	0	0	0	0	0	0
Developing	37.3	10.0	20.4	32.3	0.0	0	0	0	0
Spawning Capable	27.2	8.7	9.2	11.4	41.2	0	1.7	0.2	0.1
Actively Spawning	17.0	7.2	9.4	11.5	7.9	46.0	0	0.7	0.2
Regressing	54.0	21.8	0	0	0.0	0	0	14.0	10.2

Mean percent coverage of each oocyte stage as determined by ImageJ analysis for each reproductive phase found for Vermilion Snapper during in the north-central Gulf of Mexico. Sub-phases listed in italics. PG = primary growth, CA = cortical alveolar, Vtg1 = primary vitellogenic, Vtg2 = secondary vitellogenic, Vtg3 = tertiary vitellogenic, OM = oocyte maturation, POF = post ovulatory follicle, Alpha = alpha atresia, Beta/Gamma = Beta and gamma atresia.

Table 10

Percent occurrence of female reproductive phase

Phase	N	RGN	EDEV	DEV	SC	AS	RGR
January	4	100.0	0	0	0	0	0
February	3	66.7	33.3	0	0	0	0
March	10	100.0	0	0	0	0	0
April	34	44.1	8.8	2.9	14.7	29.4	0
May	55	20.0	5.5	0	18.2	54.5	1.8
June	9	22.2	0	0	22.2	44.4	11.1
July	15	40.0	6.7	0	40.0	13.3	0
August	24	12.5	0	0	29.2	50.0	4.2
September	30	16.7	0	0	33.3	46.7	3.3
October	7	100.0	0	0	0	0	0

Percent occurrence of each reproductive phase by month for female Vermilion Snapper in the north-central Gulf of Mexico. RGN = regenerating, EDEV = early developing, DEV = developing, SC = spawning capable, AS = actively spawning, and RGR = regressing.

Table 11

Percent occurrence of male reproductive phase

					SC			
Phase	**N**	**EDEV**	**DEV**	**SC**	**EGE**	**LGE**	**MGE**	**RGS**	**RGN**
February	3	33.3	66.7	0	0	0	0	0	0
March	12	0	33.3	66.7	83.3	0	16.7	0	0
April	33	0	0	100	61.8	5.9	32.4	0	0
May	38	0	0	100	40.5	2.7	56.8	0	0
June	15	0	0	100	28.6	28.6	42.9	0	0
July	25	0	0	100	54.5	22.7	22.7	0	0
August	40	0	2.9	97.1	39.4	9.1	51.5	0	0
September	48	0	0	91.3	38.1	19	42.9	8.7	0
October	3	0	0	0	0	0	0	100	0

Mean percent occurrence of each reproductive phase by month for male Vermilion Snapper in the north-central Gulf of Mexico. Note: EGE, LGE, and MGE are sub-phases of spawning capable and percentages represent spawning capable males only. EDEV = early developing, DEV = developing, SC = spawning capable, EGE = early germinal epithelium, LGE = late germinal epithelium, MGE = mid-germinal epithelium, RGS = regressing, and RGN = regenerating.

Table 12

Bi-monthly spawning interval estimates

Months	N (SC,AS)	HO Method	N (SC,POF)	POF Method
April - May	55,40	1.3	55,18	3.1
June - July	13,6	2.2	13,6	1.3
August - September	26,14	1.9	26,14	2.2
Mean	31,20	1.8	31,14	2.2

Bi-monthly spawning interval estimates (days between spawns) for Vermilion Snapper in the north-central Gulf of Mexico using both the post-ovulatory follicle (POF) and hydrated oocyte (HO) methods following Hunter and Macewitz (1985). SC = spawning capable, AS = actively spawning.

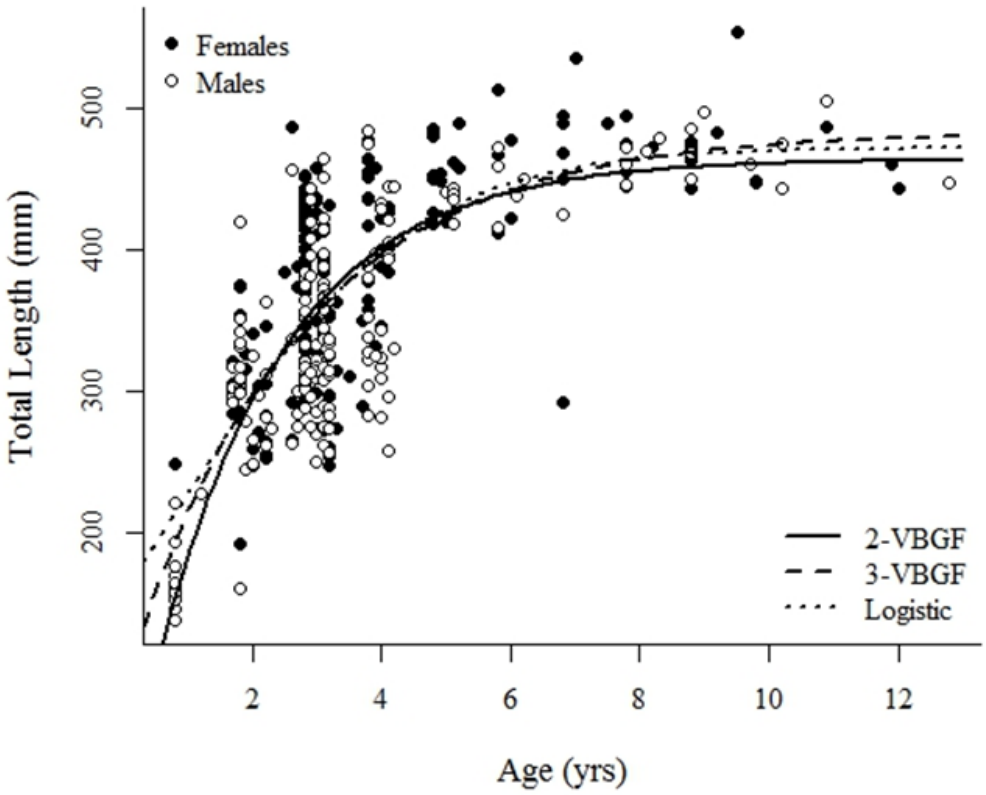

Figure 6. Multi-model growth curve comparison

Plot of multiple models describing the length-at-age relationship of male and female Vermilion Snapper from the north-central Gulf of Mexico (N = 370). Models include the two-parameter Von Bertalanffy Growth Function (2-VBGF), three-Parameter Von Bertalanffy Growth Function (3-VBGF), and the Logistic Growth Function (Logistic).

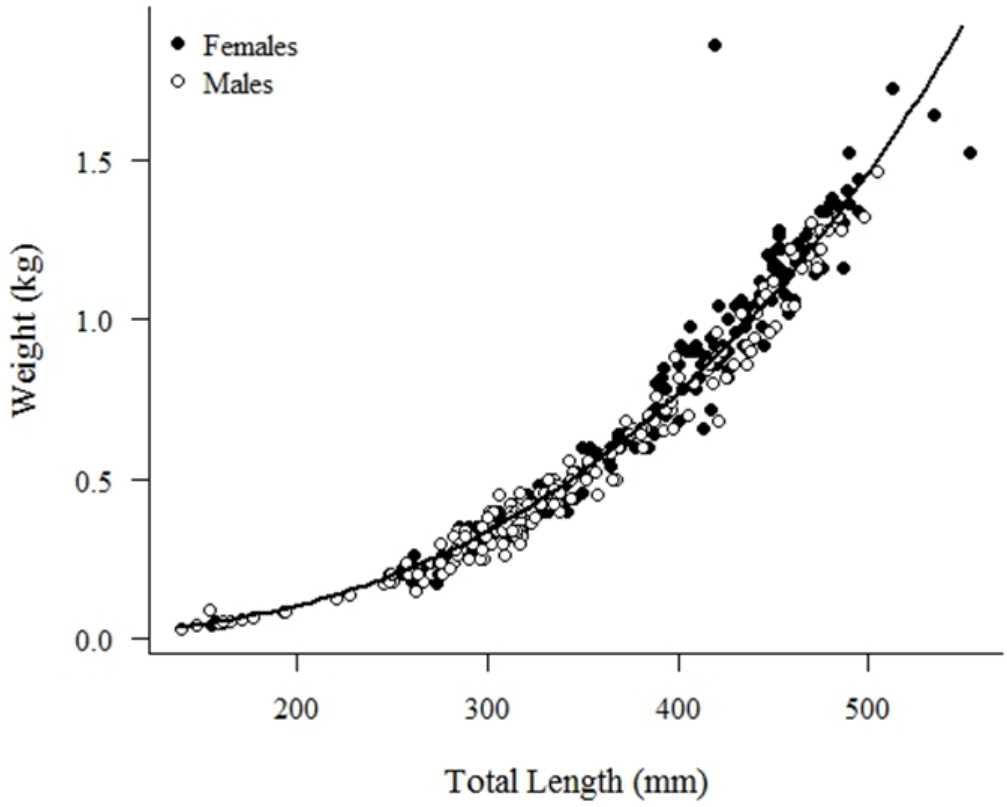

Figure 7. Weight-at-length model

Plot of the weight-at-length relationship of male and female Vermilion Snapper from the north-central Gulf of Mexico. The power function was used to fit the data.

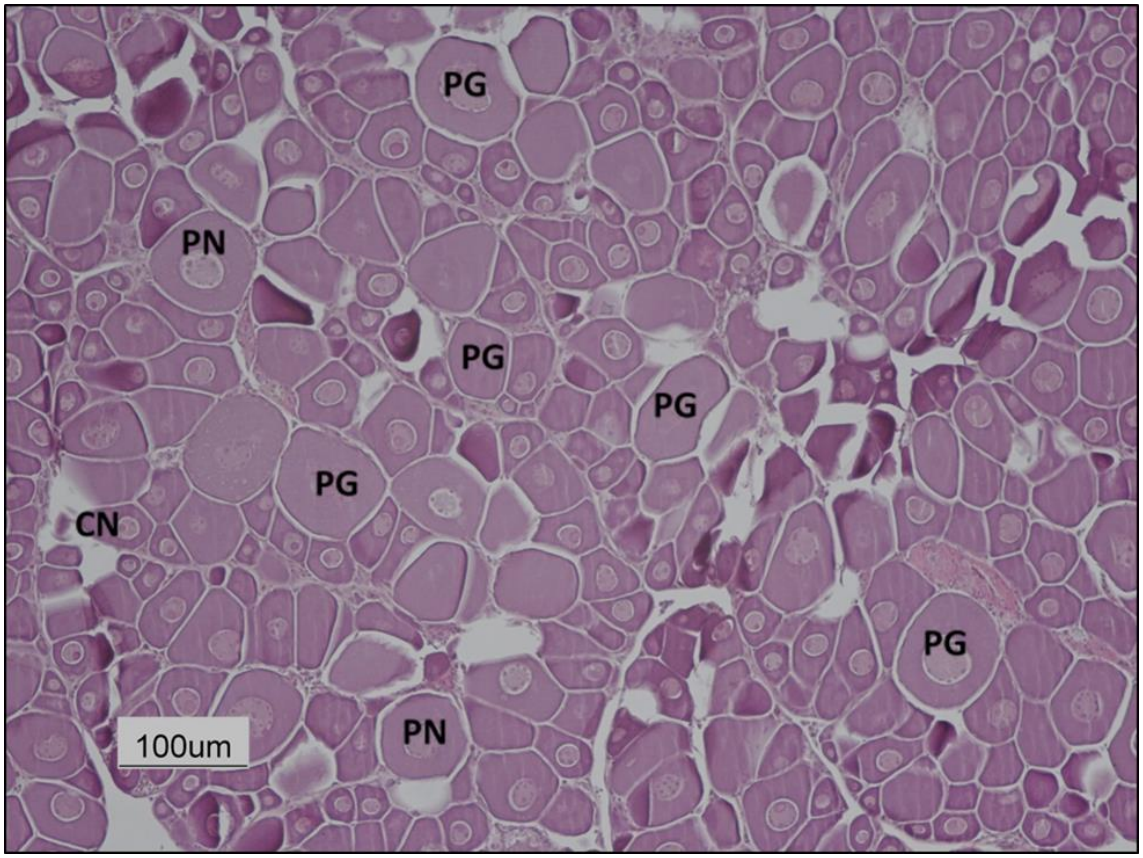

Figure 8. Regenerating female

Photomicrograph of a 346 mm TL female Vermilion Snapper captured in July from the north-central Gulf of Mexico in the regenerating phase with primary growth oocytes (PG). Most PG oocytes were in the perinucleolar stage (PN) and also in the chromatin nucleolar stage (CN) labeled.

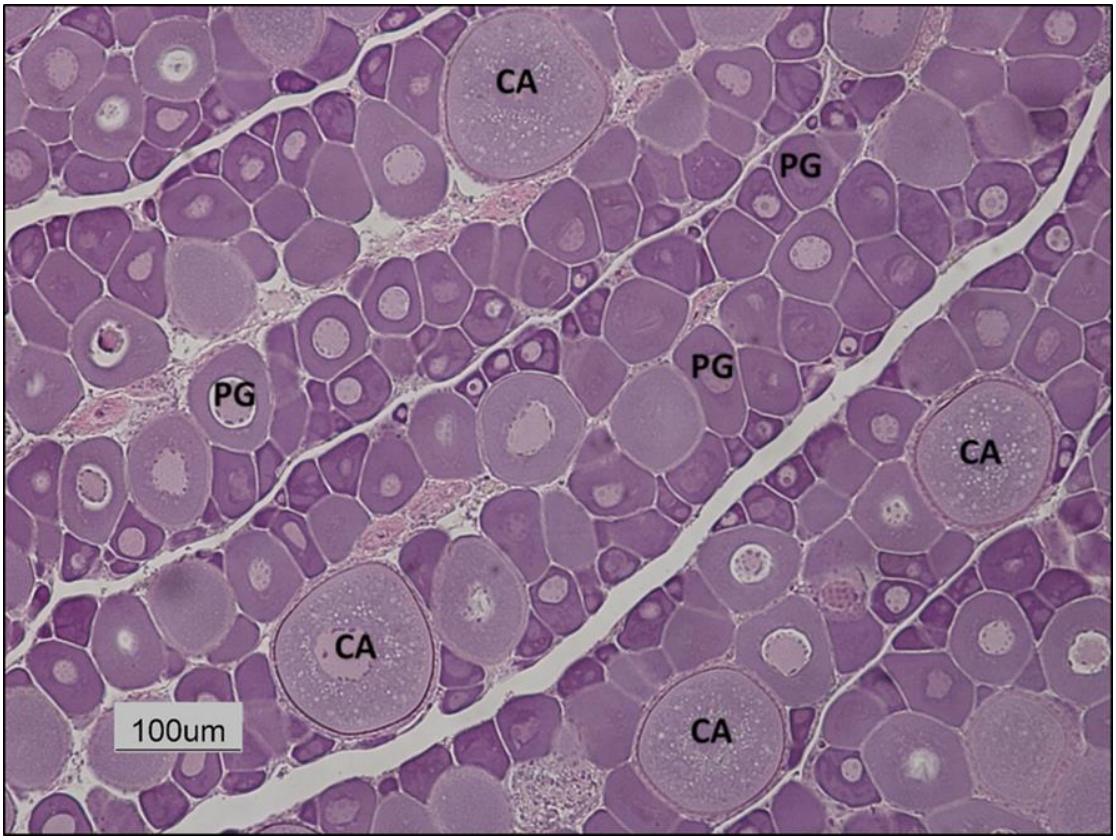

Figure 9. Early-developing female

Photomicrograph of a 485 mm TL female Vermilion Snapper captured in April from the north-central Gulf of Mexico in the early-developing sub-phase with both primary growth (PG) and cortical alveolar (CA) oocytes.

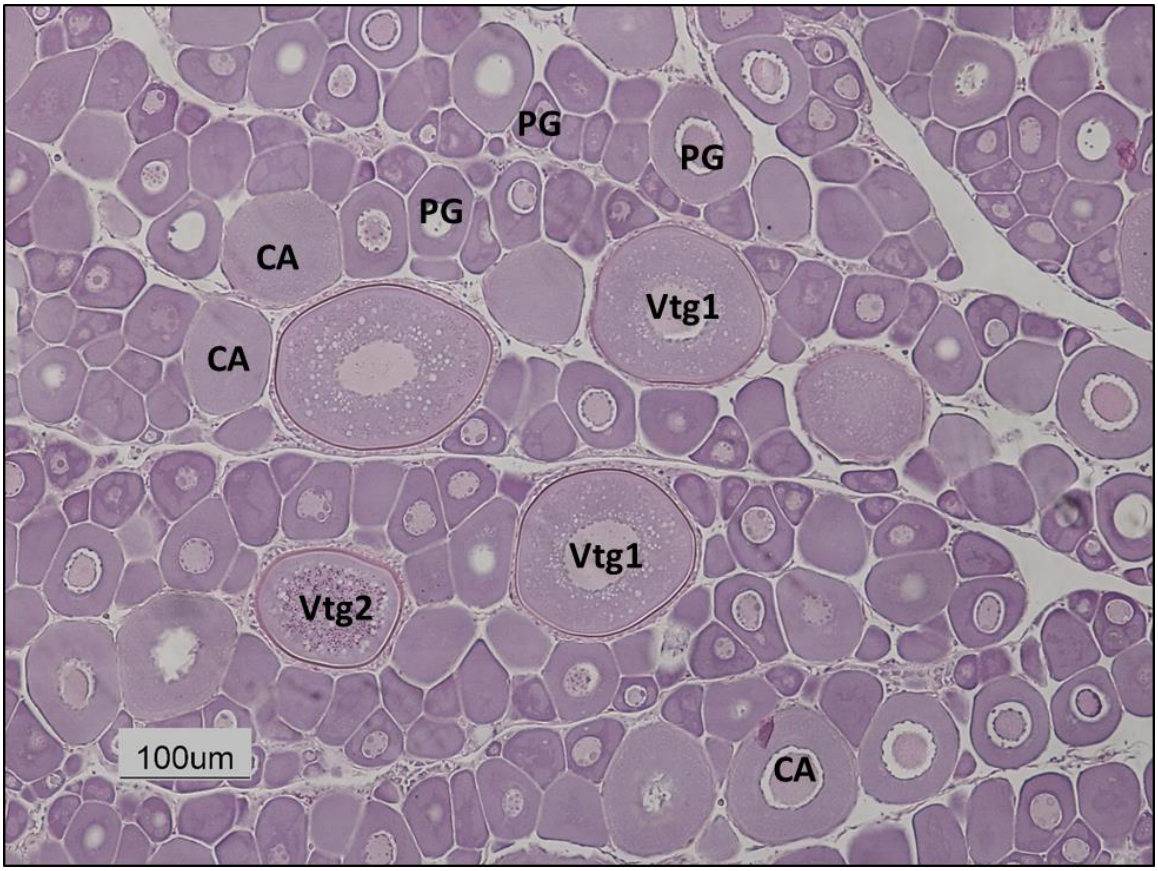

Figure 10. Developing female

Photomicrograph of a 490 mm TL female Vermilion Snapper captured in April from the north-central Gulf of Mexico in the developing phase with primary growth (PG), cortical alveolar (CA), primary vitellogenic (Vtg1) and secondary vitellogenic (Vtg2) oocytes.

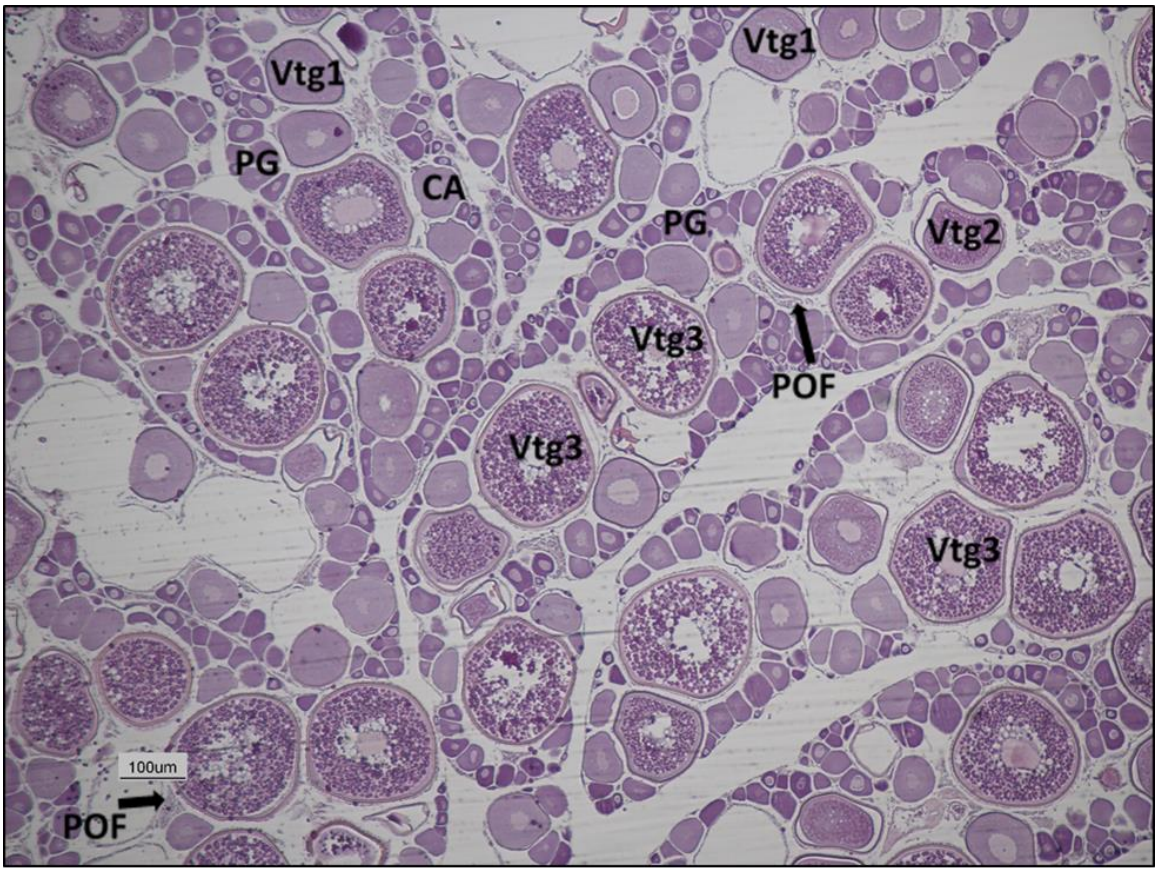

Figure 11. Spawning capable female

Photomicrograph of a 385 mm TL female Vermilion Snapper captured in August from the north-central Gulf of Mexico in the spawning capable phase showing asynchronous oocyte development. Primary growth (PG), cortical alveolar (CA), primary vitellogenic (Vtg1), secondary vitellogenic (Vtg2), tertiary vitellogenic (Vtg3) oocytes and post-ovulatory follicle complex (POF) are present.

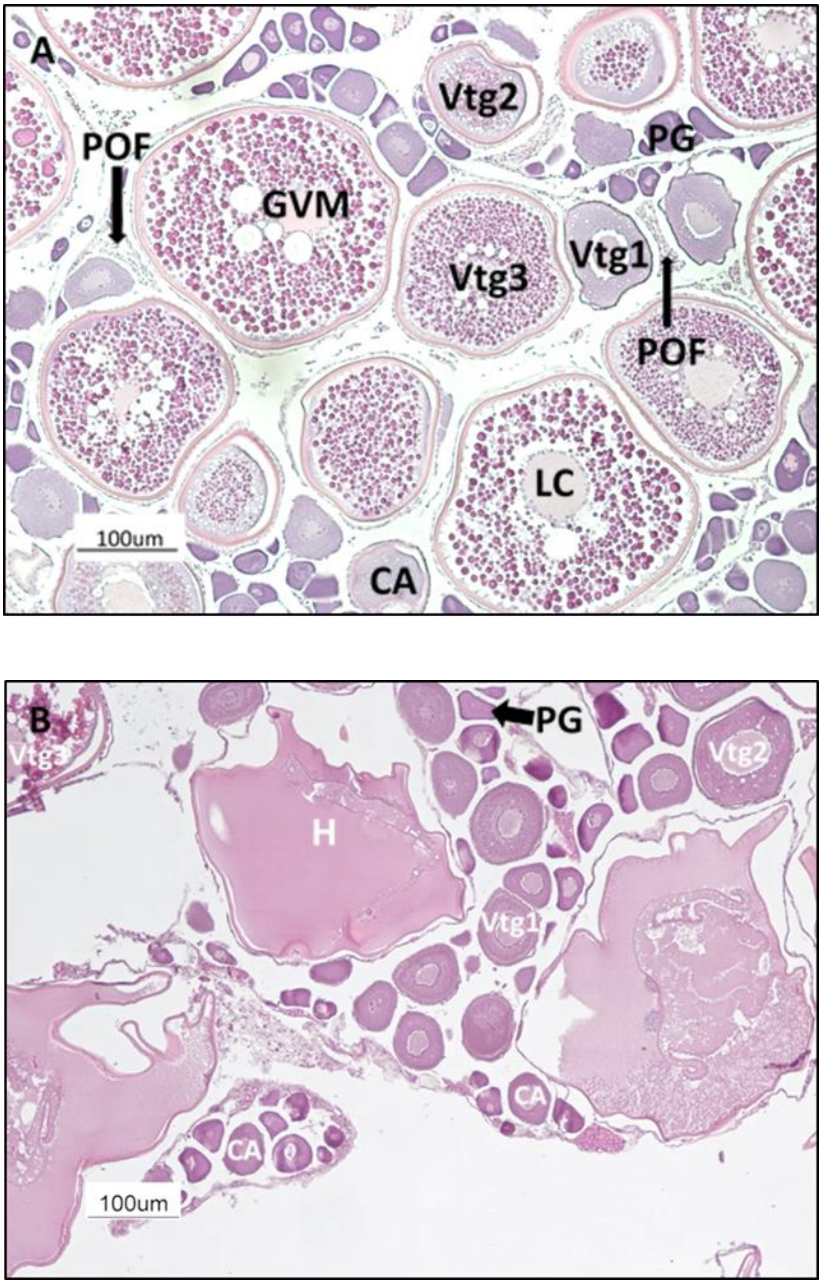

Figure 12. Actively spawning female

Photomicrographs of female Vermilion Snapper from the north-central Gulf of Mexico in the actively spawning sub-phase. A). A 462 mm TL female captured in August undergoing oocyte maturation. Primary growth (PG), cortical alveolar (CA), primary vitellogenic (Vtg1), secondary vitellogenic (Vtg2), tertiary vitellogenic (Vtg3), lipid coalescence (LC), germinal vesicle migration (GVM) and post-ovulatory follicles (POF) labeled. B). A 433 mm TL female captured in May undergoing the late stages of oocyte maturation. Primary growth (PG), cortical alveolar (CA), primary vitellogenic (Vtg1), secondary vitellogenic (Vtg2), tertiary vitellogenic (Vtg3), hydration (H) and germinal vesicle breakdown (GVBD) oocytes are present.

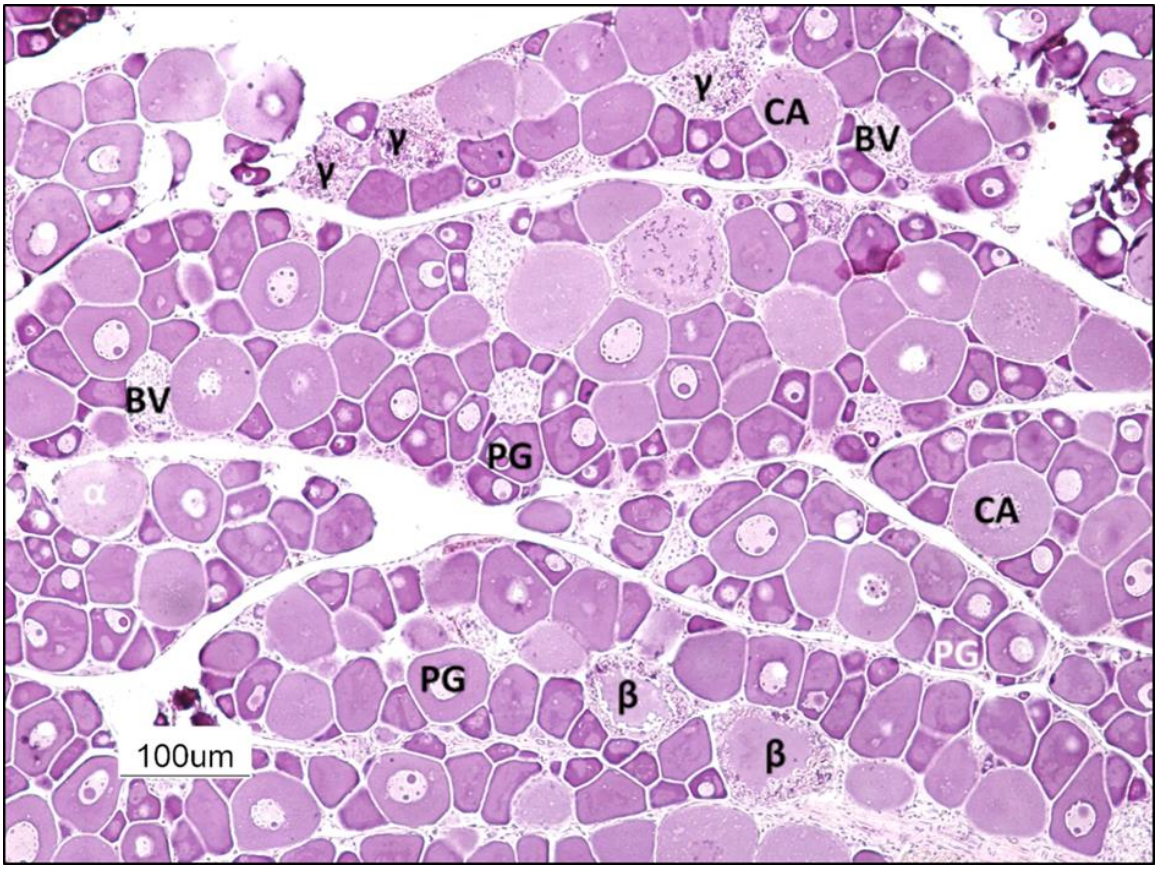

Figure 13. Regressing female

Photomicrograph of a 337 mm TL female Vermilion Snapper captured in September from the north-central Gulf of Mexico in the regressing phase with primary growth (PG), cortical alveolar oocytes (CA) blood vessels (BV) and atretic oocytes β = beta atresia, γ = gamma atresia present.

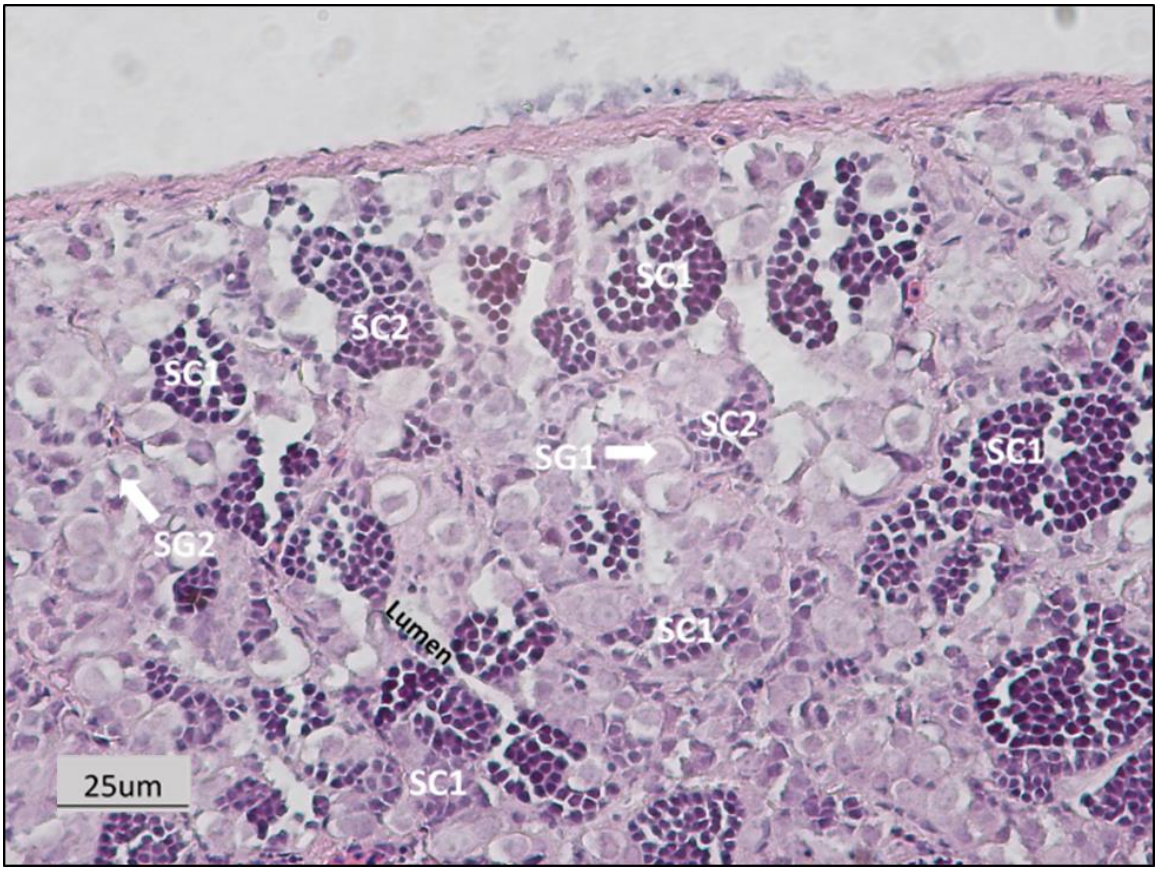

Figure 14. Early-developing male

Photomicrograph of a 337 mm TL male Vermilion Snapper captured in February from the north-central Gulf of Mexico in the early-developing subphase with primary spermatogonia (SG1), secondary spermatogonia (SG2), primary spermatocytes (SC1), and secondary spermatocytes (SC2).

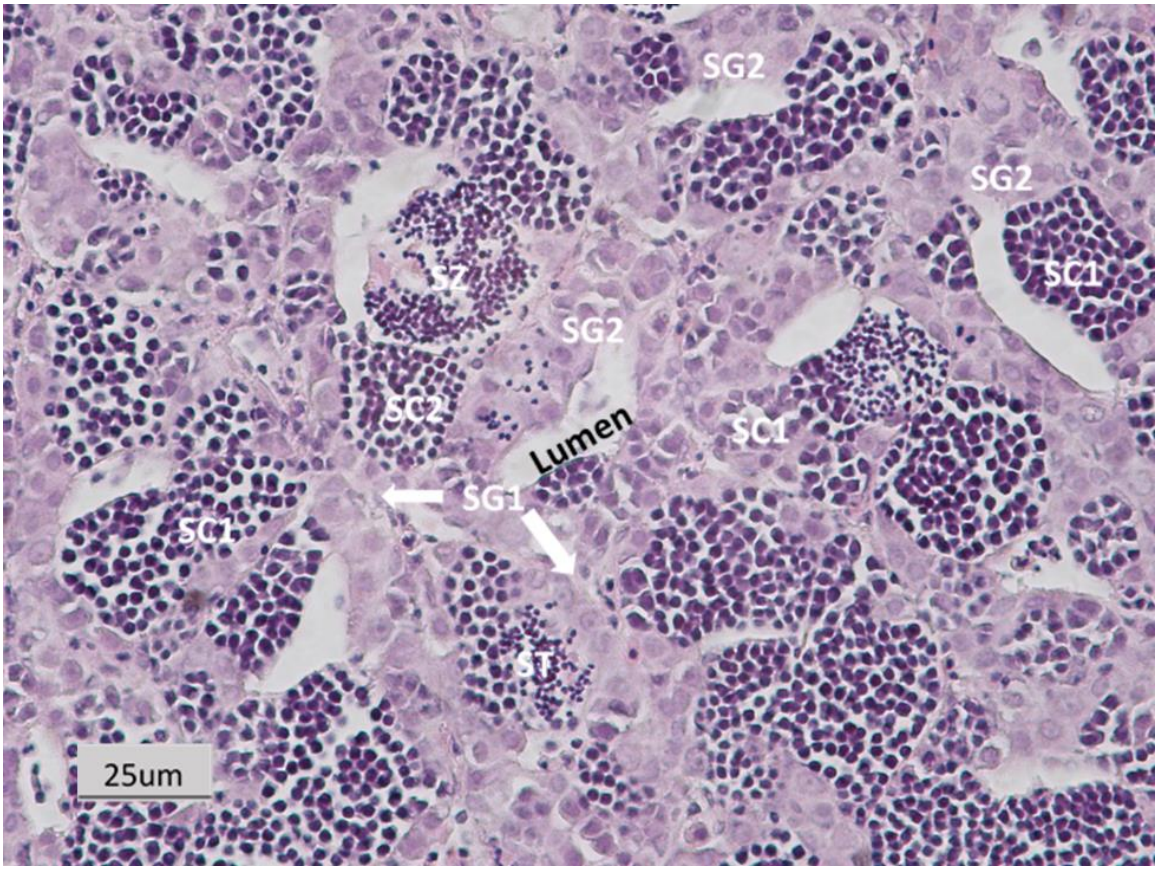

Figure 15. Developing male

Photomicrograph of a 301 mm TL male Vermilion Snapper captured in February from the north-central Gulf of Mexico in the developing phase with primary spermatogonia (SG1), secondary spermatogonia (SG2), primary spermatocytes (SC1), secondary spermatocytes (SC2), spermatids (ST) and spermatozoa (SZ) present in spermatocysts.

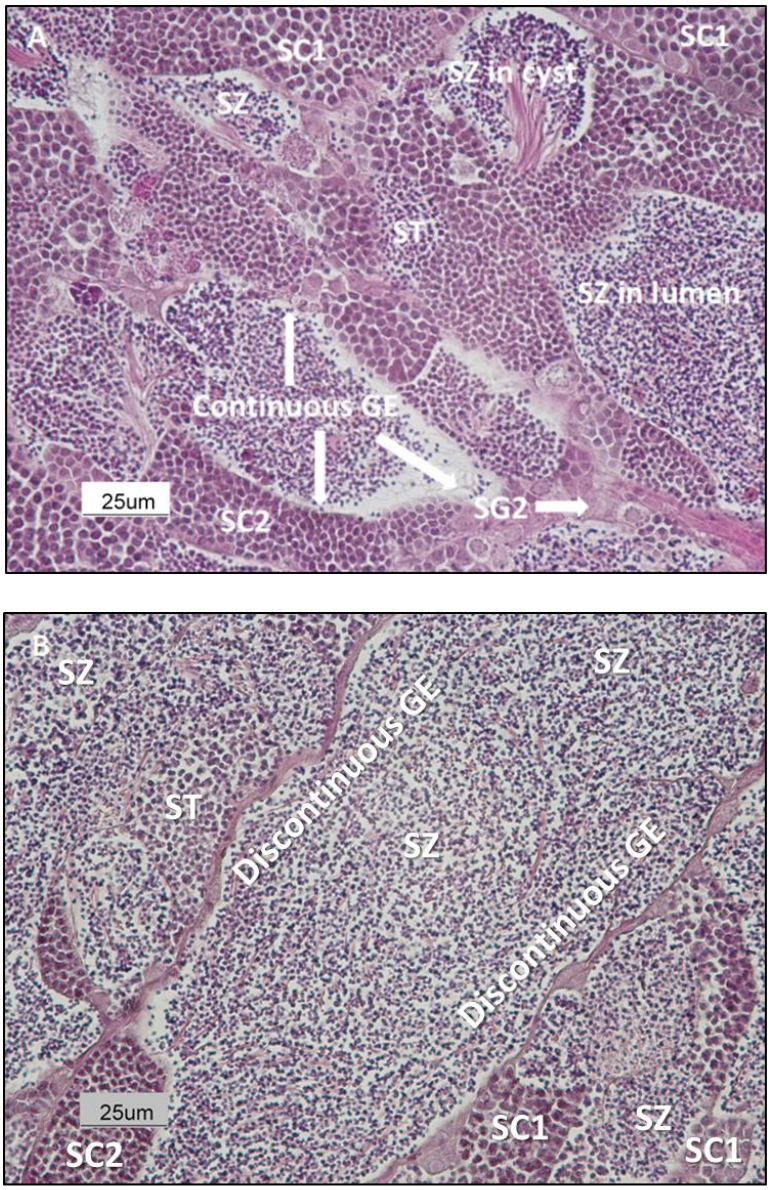

Figure 16. Spawning capable male

Male Vermilion Snapper from the north-central Gulf of Mexico in the spawning capable phase. A). A 371 mm TL male captured in May in the early GE subphase with continuous germinal epithelium, spermatozoa (SZ) in both the lumen and spermatocyst, spermatids (ST), primary spermatocytes (SC1), secondary spermatocytes (SC2) primary spermatogonia (SG1) and secondary spermatogonia (SG2). B). A 348 mm TL male captured in August in the late GE subphase with discontinuous germinal epithelium, spermatozoa (SZ) in both the lumen and spermatocysts, spermatids (ST), primary spermatocytes (SC1), secondary spermatocytes (SC2) and secondary spermatogonia (SG2).

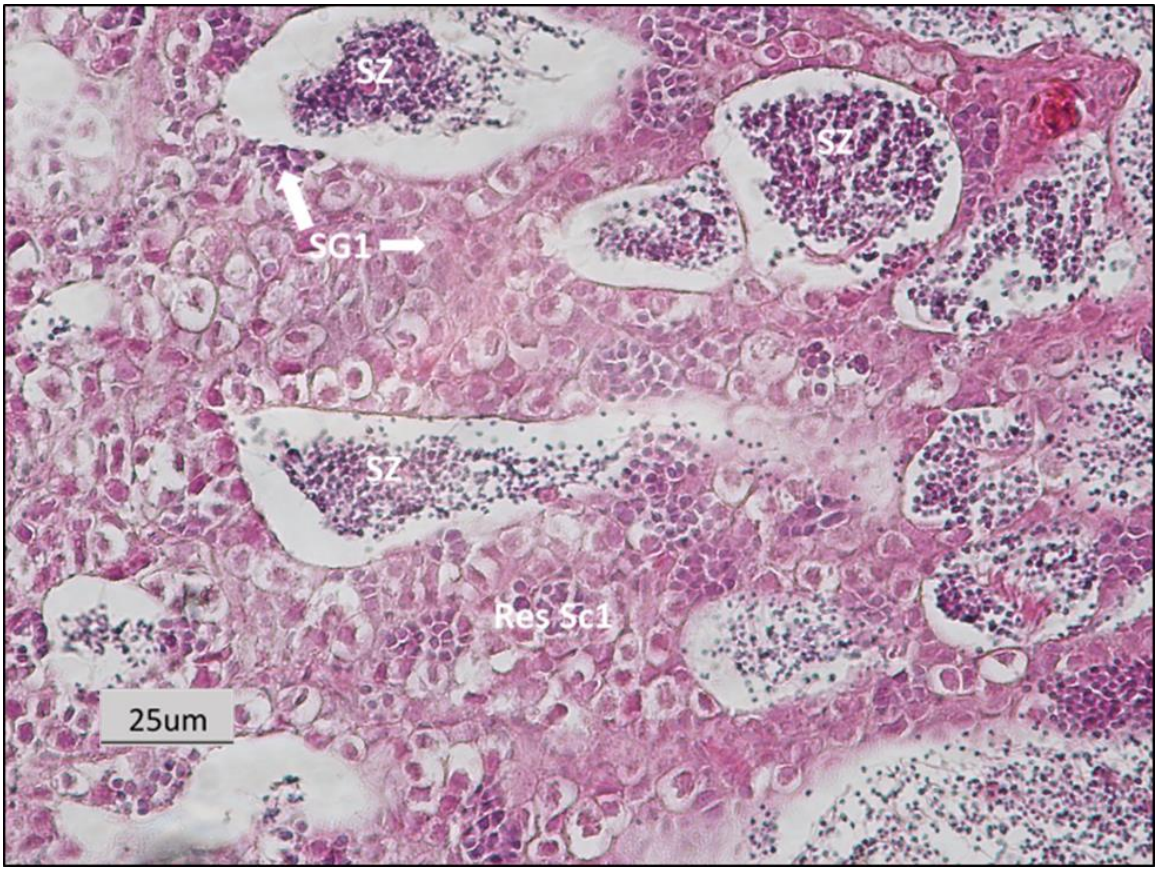

Figure 17. Regressing male

Photomicrograph of a 330 mm TL male Vermilion Snapper captured in October in the regressing phase with spermatozoa (SZ) in the lobules, primary spermatogonia (SG1), and residual spermatocytes (Res Sc) annotated. Spermatogonial proliferation is occurring in the periphery and in the GE but no active spermatogenesis is occurring.

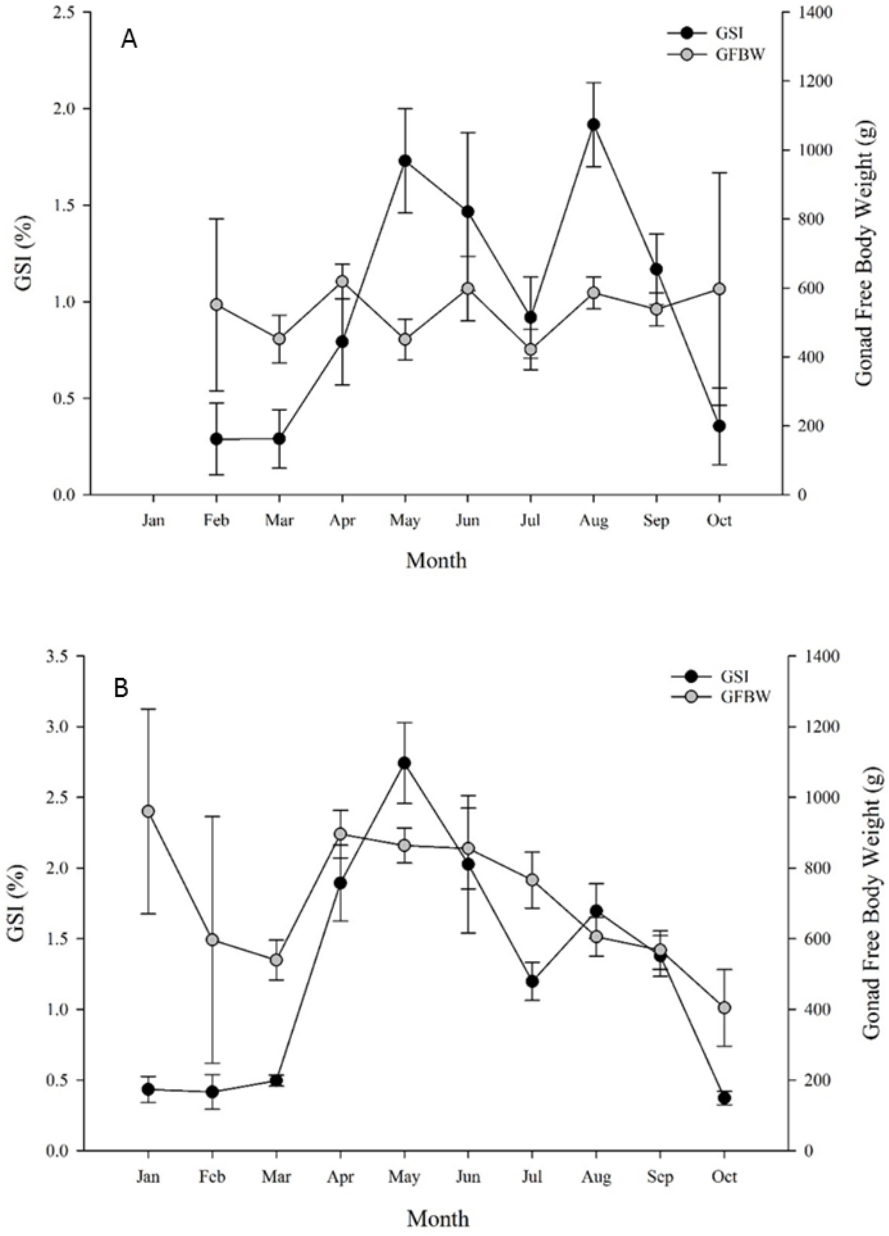

Figure 18. GSI vs GFBW

Comparison of the mean (±SE) monthly gonad free body weight (GFBW) relative to the gonadosomatic index (GSI) for Vermilion Snapper from the north-central Gulf of Mexico. A). Male. B). Female.

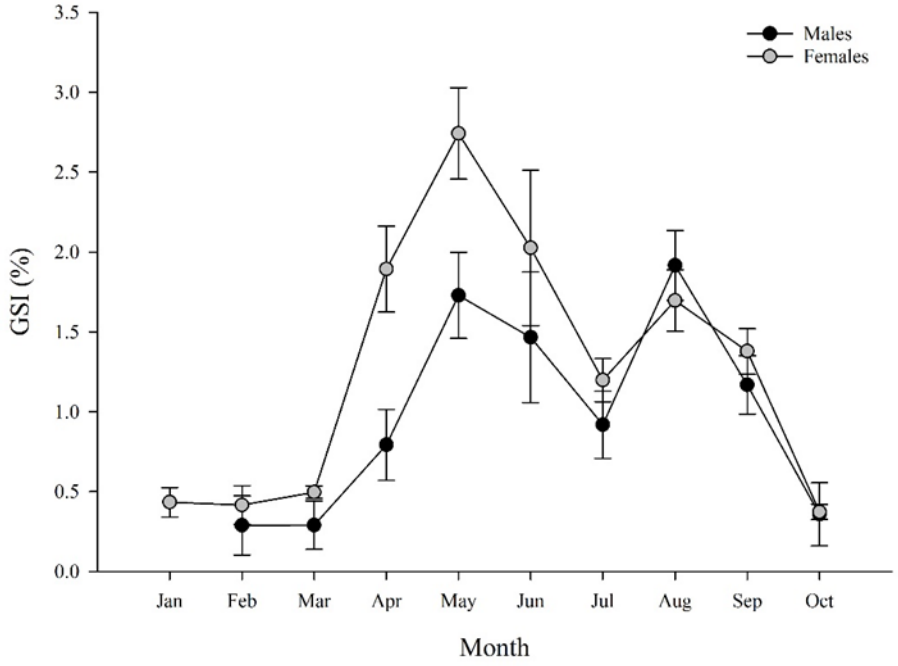

Figure 19. Monthly GSI for both sexes

Mean monthly gonadosomatic index (GSI ± SE) for both male and female Vermilion Snapper in the north-central Gulf of Mexico.

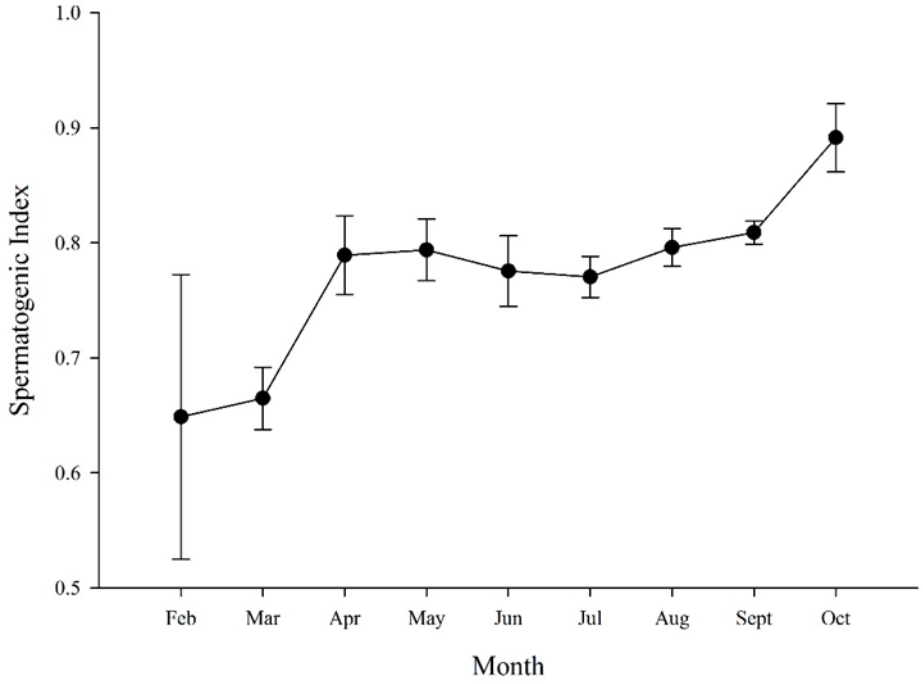

Figure 20. Monthly SMI values

Mean (± SE) monthly Spermatogenic Index (SMI) score for male Vermilion Snapper from the north-central Gulf of Mexico throughout the sampling period.

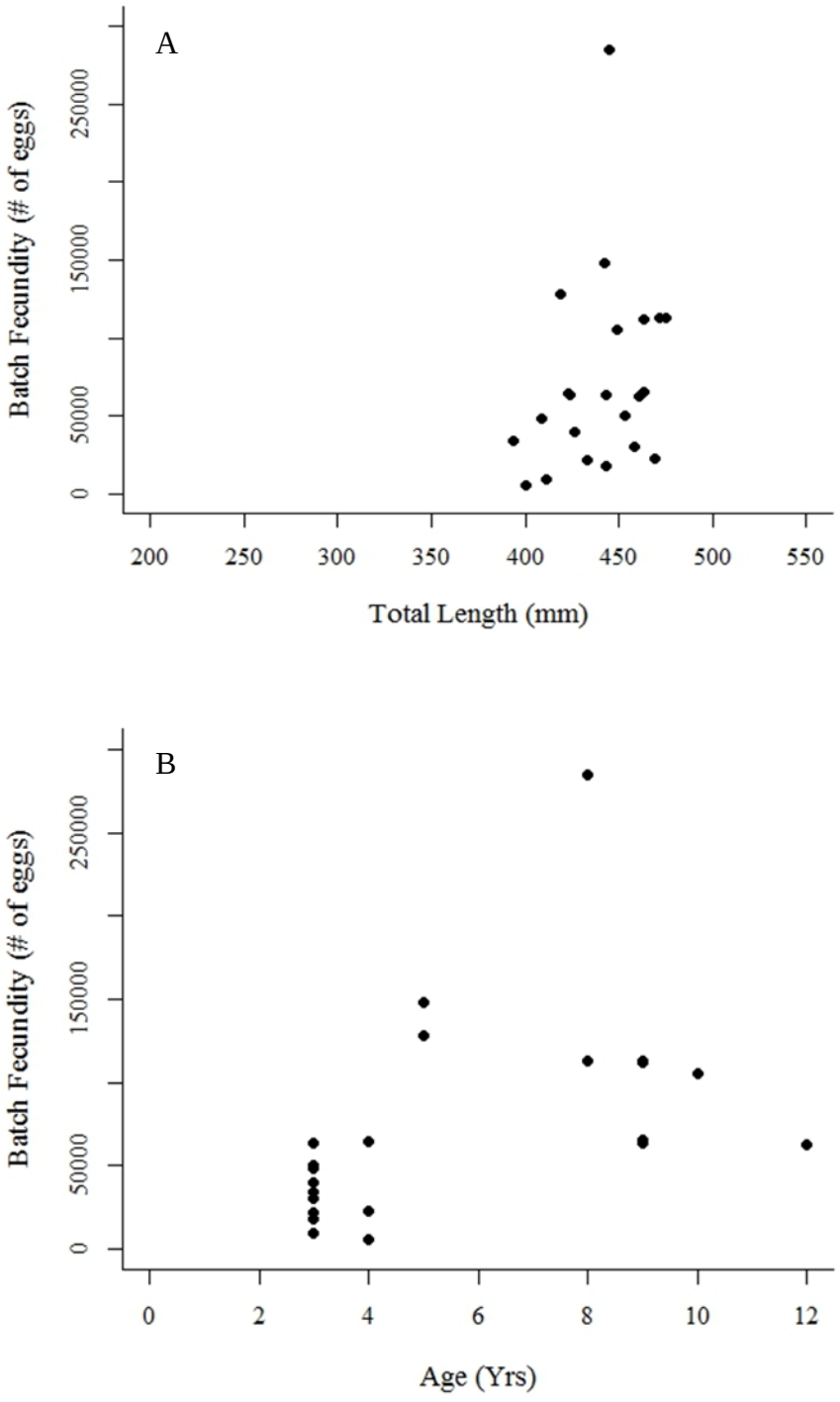

Figure 21. Batch fecundity estimates

The relationship of batch fecundity of Vermilion Snapper from the north-central Gulf of Mexico to A). total length and B). age.

CHAPTER IV – DISCUSSION

This study investigated the life history characteristics for the Vermilion Snapper and provides pertinent parameter estimates from the north-central GOM, a region whose reef structure and substrate are distinctly different from the eastern GOM (Rezak and Bright 1985). Vermilion Snapper growth has been described using the three-parameter VBGF throughout their range (Zhao et al. 1997, Potts et al. 1998, Hood and Johnson 1999, Allman et al. 2007, Johnson and Powers 2010), but when compared to the only previous study in the north-central GOM (Johnson et al. 2010), the calculated $L\infty$ and k estimates in this study were significantly lower when compared with 95% CI's (Table 6). Differences may be due to the lack of large fish and collection of fish smaller than 200 mm TL in the current study which has been shown to increase the accuracy of growth curves (Wilson et al. 2015). Although Johnson et al. (2010) collected Vermilion Snapper over a slightly different size range (200 to 594 mm TL) compared to fish collected for this study (139 to 535 mm TL), they estimated an L_∞ of 707 mm TL for combined sexes compared to 483 mm TL for fish collected for this study. The lack of small fish likely influenced their k values which weren't representative of the rapid growth shown in younger ages, and since k and L_∞ are negatively correlated (Pilling et al. 2011). Conversely, Hood and Johnson (1999) estimated a L_∞ of 298 mm TL, with fish being collected from 192-585 mm TL in the eastern GOM. In this case, they had a large range in sizes, but most of the fish collected were small (87% between 201 and 325 mm TL), which will dictate the shape of the growth curve. Thus, for all of these studies, considerable variation in growth along with sampling bias was hypothesized to have led to differences in parameter estimates and thus other important vital metrics. Parameter

estimates in the current study likely provide the most precise description of length-at-age as a larger size range of fish were captured with an equal distribution of sizes.

Determining the age and size at sexual maturity is a critical component of the population dynamics of a species (Stearns 1992, Trippel 1995). Though no immature fish of either sex were found during this study, reproductively active males were collected as small as 139 mm TL (0.8 years old). Increased fishing pressure has been shown to affect age/length-at-maturity (Beverton and Holt 1957), often resulting in fish achieving sexual maturity at a smaller size due to compensatory responses after population declines (Colby and Nepsky 1981, Trippel 1995). Since Vermilion Snapper have not been well studied throughout their historic exploitation in the GOM, a change in age and TL-at-maturity could have gone undetected. In the SAB, a temporal shift in TL/age-at-maturity was observed over a 9-year period and was hypothesized to be linked to fishing pressure (Zhao et al. 1997). The most recent stock assessment for Vermilion Snapper in the GOM estimated 50% length/age-at-maturity to be around 138 mm FL (0.7 years old; Fitzhugh et al. 2015), similar to our findings in actively spawning fish. Considering this species can live upwards of 26 years (Barber 1989), this early age-at-maturity is surprising. Vermilion Snapper do not grow to a large size as adults as in other species in the family Lutjanidae. The only disparity is that they are relatively long-lived, however, when examining the age distribution from fish captured in this study and others, the majority of the fish were between ages 3 and 5, with a small percentage > age 6.

Male and female GSI values peaked in May and again in August, with a decline in GSI in the months of June and July in the north-central GOM. Female mean GSI values within the spawning season were significantly different than values outside of the

spawning season ($P < 0.001$), with the exception of June, which had a low sample size and large amount of variance. This supports histological evidence of a spawning season of April to September in the north-central GOM. However, Hood and Johnson (1999) found elevated values from May to September while Collins et al. (2002) estimated a single peak in June in the eastern GOM. The June-July decline documented in this study is not seen in Red Snapper in the same general area (Glenn and Cowan 2014), however. This decline could be due to multiple reasons, including gear bias, regional temperature differences, energetics or forage availability; however, the most likely cause was that all fish captured under 350 mm TL during these months were in the regenerating phase suggesting these females had already ceased spawning for the season. Smaller fish are known to have much shorter spawning seasons than their larger counterparts (Lowerre-Barbieri et al. 2011, Fitzugh et al. 2012). Male GSI values were also equal to and sometimes higher than female values during the spawning season, results that are not common in many teleost fishes. One reason may be that males are undergoing sperm competition, a biological mechanism that is common to fishes that spawn in large groups or in aggregations such as Lutjanids and Serranids (Grimes 1987, Peterson and Warner 1998, Heppell 2007). This strategy allows males to increase the total number of possible fertilizations in a given spawning event (Peterson and Warner 1998).

An assumption of using GSI as an index of reproductive preparedness is that GFBW has no influence on GSI values (Jons and Miranda 1997), which must be tested to support the use of GSI. In this study, GSI and GFBW for females showed similar declines at the end of the spawning season, suggesting that GFBW may be influencing GSI values. Past studies of Vermilion Snapper have not investigated the relationship

between GSI and GFBW to determine its validity as a reproductive metric. Though this effect is documented here, it is important to note that histology supported the trends in GSI. This proves that while GSI may not be the most precise method of estimating spawning seasonality, it can still be used as an approximation of spawning preparedness throughout the year.

The SMI has been used in recent literature to accurately quantify spawning preparedness and the level of spermatogenesis for a given male individual (e.g., Tomkiewicz et al. 2011, Corey et al. 2017). The SMI values for Vermilion Snapper increased in February and March to 0.79 and maintained similar values through September, indicating that fish were spawning capable and spermatogenesis was still actively occurring. However, in October, the SMI value increased to 0.92. This is counterintuitive since all fish were in the regressing phase in October. The reason the SMI increased at the end of the season is likely due to the weighting scheme used to calculate in SMI. The SMI was developed with the European Eel (*Anguilla anguilla*) (Tomkiewicz et al. 2011), which is a total spawner that migrates long distances to spawn and generally releases all sperm in one large, relatively short, spawning event. Since Vermilion Snapper have an extended spawning season, they are undergoing spermatogenesis throughout the spawning season. However, at the end of the spawning season, spermatogenesis ceases and spermatozoa begins to be the dominant stage of spermatogenesis in the lobules. This proportional increase in spermatozoa is what appears to drive the increased values of SMI at the end of the spawning season. Similar results were found when using SMI to describe male development of Southern Flounder (*Paralichthys lethostigma*), where elevated SMI values were found from December

through March, and contradicting GSI patterns during that time (Corey et al. 2017). In future studies of male fish with extended spawning seasons, a correction factor could be used to down-weight fish that are no longer undergoing active spermatogenesis.

The batch spawning strategy of Vermilion Snapper is quite common in many species of lutjanids, including Red Snapper (Brown-Peterson et al. 2009, Gallaway et al. 2009, Brule et al. 2010). This strategy allows for a large number of eggs to be released over a protracted spawning season, increasing the chances of larval survival. While this strategy is advantageous, it also requires considerable and consistent energy, which may cause fish to not spawn all season long. This was evident for Vermilion Snapper of smaller sizes, as multiple fish < 350 mm TL were found in the regenerating and regressing phase during the spawning season.

This study was the first to report Vermilion Snapper actively spawning on artificial structures in the GOM. The north-central GOM has considerable amount of petroleum platforms, Rigs-to-Reef sites, and other high profile artificial reefs that provide habitat for numerous reef fish species. Hydrated female Vermilion Snapper were found on most high-relief artificial structures; however, small, low-relief artificial structures such as chicken coops that were sampled did not yield any actively spawning females, though these areas were not sampled as frequently. Since many state agencies dedicate effort to putting out artificial structures, perhaps effort could be made to provide more large, high-profile structures for reef-fishes like the Vermilion Snapper. Petroleum platforms in the GOM that are reaching the end of their expected lifetime are being removed which decreases the amount of available habitat for reef fishes such as the Vermilion Snapper. Spawning on artificial reefs highlights the importance of the high-

relief structures to the north-central GOM, as these structures may also be a source of fisheries production as well (Carr and Hixon 1997, Powers et al. 2003, Gallaway et al. 2009).

Mean BF (± S.D.) was 73,004 (± 60,925) eggs for Vermilion Snapper in the north-central GOM but showed no relationship with TL or age. With only a narrow range of fish sizes (394-513 mm TL) examined in this study coupled with considerable variation in estimates, it is not unexpected that no relationship exists between BF and fish size. Wide ranges in BF have also been documented in past studies of Vermilion Snapper (Collins et al. 2003, Fitzhugh et al. 2015, Table 13). For example, the recent Vermilion Snapper stock assessment showed that BF ranges from 6,106 - 407,570 eggs/batch, with a mean (± S.D.) of 76,465 (± 79,093) eggs/batch (Fitzhugh et al. 2015), similar to the mean BF value found in the present study (Table 13). However, large variation in BF estimates are typical of batch spawning species since large amounts of energy are required to produce a single batch of eggs and thus the size of the batch can have large variability throughout the spawning season (Hunter et al. 1985).

Relative batch fecundity in the north-central GOM was estimated at 70.7 (± 57.9 [S.D.] eggs/gram of GFBW), lower than the previous estimate by Fitzhugh et al. (2015; 224 ± 112 [S.D.] eggs/gram of GFBW) based on eastern GOM data, but within the estimated 95% confidence intervals in this study (Table 13). The two values showed no statistical significant difference when comparing the 95% confidence intervals, though increased sample size of the north-central GOM region could further elucidate potential differences in RBF in the future.

Annual fecundity in the north-central GOM ranged widely from 500,000 to 27 million eggs but appears similar to estimates in the eastern GOM from Collins et al. (2003) (700,000 to 35 million eggs, Table 13). In the SAB, the decreased spawning frequency leads to lower estimates of annual fecundity, with estimates nearly one-half as large as those found in the GOM (Cuellar et al. 1997).

The spawning interval of 1.8 to 2.2 days estimated in this study was similar to that for the eastern GOM which averaged 1.6 days between spawns (Hood and Johnson 1999, Collins et al. 2003, Table 13). The spawning frequency in the north-central GOM of 78 to 95 spawns/season is similar to the 83 spawns/season estimated for the eastern GOM (Collins et al. 2003). These estimates vary greatly from the SAB, where Cuellar et al. (1997) found a spawning interval of 5 days between spawns and a spawning frequency of 35 spawns per season, although duration of the spawning season was similar (Table 13). Spawning interval estimates using the HO method are based on the number of actively spawning fish observed during collection. Spawning interval calculations may be skewed based on the time of day the fish were captured since generally Vermilion Snapper spawn around dusk (Collins et al. 2003). Also, fish undergoing OM could be more active and therefore more susceptible to the gear than non-spawning fishes, which would support the high numbers of hydrated fish caught in May in this study. This behavior is well noted in aggregate spawning fishes since most fish are located in a small area or around a single reef structure (van Overzee and Rijnsdorp 2015).

Current management regulations for the Vermilion Snapper are a 25.4 cm (10 inch) minimum length limit in both the commercial and recreational fishery. Based on the growth documented in this study, individuals may be vulnerable to the fishery

between the ages of 2 and 4 years old. Since Vermilion Snapper mature at less than one year of age, this means they can be harvested after they have reproduced for at least 1 to 3 years; thus, allowing for a large number of eggs to be spawned to contribute recruits back to the population.

In conclusion, there are limited and variable growth and reproduction data in the GOM that can be easily and accurately used in stock assessments and management. For example, Vermilion Snapper growth has only been documented in the north-central GOM in one other study (Johnson et al. 2010) in which authors found differing parameter estimates from those found in this study, illustrating the need for regional data sets. The current study is the first to quantitatively document various aspects of reproduction of Vermilion Snapper in the north-central GOM including a detailed histological description for both males and females of all phases captured, as well as to documenting daily spawning of females. Finally, this study represents the first report of Vermilion Snapper spawning on artificial structures; these structures are more prevalent in the north-central GOM than the eastern GOM. Information collected during this study provides a region-specific overview for Vermilion Snapper growth and reproduction which can be incorporated into future stock assessments of this species allowing for increased accuracy and reduced variability of stock assessment output. Region-specific growth and reproduction estimates will increase clarity of population-level characteristics as well as provide a more robust understanding of the life-history of this commercial and recreationally important species, leading to continued sustainable management in the future.

Table 13

Published reproductive estimates

Citation	Location	Length-at-maturity (mm)	Age-at Maturity	Spawning Frequency	Annual Fecundity (# eggs)	Batch Fecundity (# eggs)	Spawning Season
Grimes and Huntsman (1980)	SAB	186-324	3 to 4	N/A	8,168 to 1.79 million	N/A	April to September
Cuellar and Wyanski (1996)	SAB	No Immature	No Immature	35	140,175 to 3.15 million	4,000 to 90,000	April to September
Fitzhugh et al. 2015 (SEDAR)	GOM	100-200	N/A	82	N/A	76,465 (± 2,628)	April to September
Collins et al. (2003)	GOM	No immature	N/A	87	N/A	7,385 to 407,570	April to September
Hood and Johnson (1999)	GOM	<200 mm	N/A	N/A	N/A	5,535 to 86,811	May to September
This study (2016)	NCGOM	< 155 mm	< 0.8	78 to 95	544,203 to 28.2 million	5,497 to 284,468	April to September

Reproductive estimates for Vermilion Snapper from past studies in the South Atlantic Bight (SAB), Gulf of Mexico (GOM), and north-central Gulf of Mexico (NCGOM). N/A = no data available

APPENDIX A – Histological Procedures

Table A1.

Tissue Processing

Step	Solution	Time
1	70% EtOH	1hr
2	80% EtOH	1hr
3	95% EtOH	40min
4	95% EtOH	40min
5	95% EtOH	40min
6	100% EtOH	1hr
7	100% EtOH	1hr
8	100% EtOH	1hr
9	Xylene Substitute	1hr
10	Xylene Substitute	1hr
11	Xylene Substitute	1hr
12	Paraplast Plus	40min
13	Paraplast Plus	40min
14	Paraplast Plus	40min

Processing sequence for dehydration of gonad tissues in the Shandon Tissue Processor

Table A2.

Tissue Staining

Step	Solution	Duration
1	Xylene Sub.	3 min.
2	Xylene Sub.	3 min.
3	Xylene Sub.	3 min.
4	100% EtOH	10 dips
5	100% EtOH	10 dips
6	95% EtOH	10 dips
7	95% EtOH	10 dips
8	80% EtOH	10 dips
9	80% EtOH	10 dips
10	50% EtOH	10 dips
11	Distilled Water	1 min.
12	Hematoxylin 2	3-5 min.
13	Water – rinse well	------
14	Acid water	2 dips
15	Water – rinse well	------
16	Blueing water	30 sec.
17	Water – rinse well	------
18	95% EtOH	10 dips
19	Eosin Y	1-1.5 min.
20	Blot Blot Blot	------
21	95% EtOH	10 dips
22	95% EtOH	10 dips
23	95% EtOH	10 dips
24	100% EtOH	1 min.
25	100% EtOH	1 min.
26	100% EtOH	1 min.
27	Xylene Substitute	1 min.
28	Xylene Substitute	1 min.
29	Xylene Substitute	1 min.
30	Xylene Substitute	1 min.

Outline of the tissue differential staining process.

APPENDIX B – IACUC Approval Letter

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

118 College Drive #5116 | Hattiesburg, MS 39406-0001
Phone: 601.266.6791 | Fax: 601.266.4377 | iacuc@usm.edu | www.usm.edu/iacuc

NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER:	16101302 (Replaces 14081401)
PROJECT TITLE:	Red Snapper and Reef Fish Assessment for Mississippi Coastal and Nearshore Gulf Waters
PROPOSED PROJECT DATES:	10/2016 – 09/2019
PROJECT TYPE:	New
PRINCIPAL INVESTIGATOR(S):	Read Hendon
DEPARTMENT:	Coastal Sciences
FUNDING AGENCY/SPONSOR:	N/A
IACUC COMMITTEE ACTION:	Full Committee Approval
PROTOCOL EXPIRATON DATE:	September 30, 2019

Jake Schaefer, PhD
IACUC Chair

10/19/16
Date

IACUC approval letter

REFERENCES

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