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The Reproductive Biology of Red Snapper in Mississippi Waters

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Summary

Red Snapper (n = 2,084) were sampled in three depth strata (<20 m, 20-49 m, 50-100 m) and at three artificial structure types (artificial reefs, oil platforms, rigs-to-reefs) monthly from March–November 2016–2020 in Mississippi waters. Captured fish ranged from 168 to 795 mm FL and 0.75–22.25 years. Female fish achieved maturity at a small size and young age (50% physiological maturity at 283 mm FL and 1.33 years; 50% functional maturity at 318 mm FL and 1.80 years), and 39% of age-1 females are spawning capable as they approach their second birthday. However, 50% spawning maturity is achieved at a larger size and older age (374 mm FL and 2.93 years, respectively). Spawning capable females were found from April through October, suggesting Red Snapper in Mississippi waters have a 179-day spawning season, although smaller females have a shorter spawning season. Daily spawning was observed in 21% of spawning capable females captured, and the percentage of daily spawning females significantly increased with increasing age. The spawning interval of spawning capable only females is estimated to be every 2.4–3.4 days, and is significantly different across months but not across age classes. The spawning interval of all mature females is estimated to be every 4.3–6.7 days and is significantly different across both months and age classes. Batch fecundity (BF) is significantly, positively related to fish size but highly variable, ranging from 596 to 349,754 eggs, with a mean relative batch fecundity (RBF) of 73.05 ± 13.73 eggs/g ovary-free body weight; RBF was highest in August and lowest in April. Both BF and RBF varied significantly with age class, with highest fecundities at 5 and 8 years. Some Red Snapper reproductive parameters vary with the type of artificial structure, with a higher percentage of spawning capable fish, a higher fecundity and a longer spawning interval seen at rigs-to-reefs. Depth is an important factor in all Red Snapper reproductive parameters examined, with a higher percentage of spawning capable fish, shorter spawning interval, and greater BF in the deepest depth stratum.

Introduction

Red Snapper *Lutjanus campechanus* is one of the most economically important species in the northern Gulf of Mexico (GOM), supporting large commercial and recreational fisheries. Due to overfishing, Red Snapper stocks began declining in the 1960's in the western GOM and were likely overfished in the eastern GOM by the 1950's, reaching an unsustainable population level by 1990 (SEDAR 2018). Despite the implementation of a variety of regulations beginning in the 1990's the GOM Red Snapper stock was still considered overfished and to be undergoing overfishing in 2005 (SEDAR 2005). New, stricter regulations were enacted following SEDAR 7 (SEDAR 2005), resulting in the determination in 2018 that the Red Snapper stock is recovering and, on a GOM-wide basis, is not overfished, not undergoing overfishing, but has not yet recovered to the Gulf-wide rebuilding target (SEDAR 2018). Despite this classification, stock recovery has not been uniform across the GOM; Red Snapper stocks in the eastern GOM are projected to decline due to lower recruitment and greater discard mortality relative to that occurring in the western GOM (SEDAR 2018). Additionally, data from Mississippi has not been included in previous SEDAR assessments; inclusion of these data, particularly in light of the recent Red Snapper Stock ID recommendation of using three stocks in the upcoming SEDAR assessment (SEDAR 2021), will provide additional important input for stock assessors to better understand GOM Red Snapper stocks for the SEDAR 74 assessment.

Red Snapper are a structure-oriented species, particularly during the first eight years of their life (Gallaway et al. 2009). The density of young fish is greater on artificial structures (i.e., artificial reefs and/or oil and gas platforms) than on natural reefs (Karnauskas et al. 2017). Near-shore Mississippi waters contain 233 artificial reefs, 17 rigs-to-reefs and 169 standing oil platforms (Mississippi Department of Marine Resources 2018; Bureau of Ocean Energy Management, 2018) at depths ranging from 6 to 100 m which potentially provide abundant, but previously undocumented, habitat for Red Snapper. For Mississippi female Red Snapper, immature females captured at artificial reefs are older than those captured at oil and gas platforms, but smaller and younger, immature fish are most often found in shallower, reef-based areas where fishing pressure is highest (Leontiou et al. 2021a).

Although the reproductive biology of Red Snapper has been well studied in the northern GOM over the past 20 years (Collins et al. 2001, Jackson et al. 2006, 2007, Fitzhugh et al. 2012a, Lowerre-Barbieri et al. 2012, Glenn et al. 2017, Kulaw et al. 2017, Downey et al. 2018, Froehlich et al. 2021), none of these studies included fish from Mississippi waters, and few address all aspects of Red Snapper reproductive biology, including interrelationships between reproduction and habitat. Recently, however, a meta-analysis of female Red Snapper reproduction throughout the GOM included data from Mississippi fish (Brown-Peterson et al. 2019), and showed some changes in both duration of the reproductive season and spawning interval over 1991-2017. Furthermore, a random forest analysis of Red Snapper captured in offshore Mississippi waters from 2016-2018 found that depth and month are important predictors for most reproductive parameters, but type of artificial structure is not important (Brown-Peterson et al. 2021).

This report provides information on all aspects of Red Snapper reproduction in Mississippi waters, adding to the body of biological knowledge of this important commercial and recreational species. Specifically, we document size and age at maturity, spawning seasonality, spawning interval, and fecundity of Red Snapper captured from 2016–2020 on artificial structures. Additionally, differences in Red Snapper reproduction among depth strata and reef types are documented, as this information is important for effective management of the species.

Materials and Methods

Sampling Sites

A total of 600 randomly allocated stations containing artificial structures were sampled in Mississippi waters between March and November 2016-2020 (Figure 1). Fish were collected from three depth strata (< 20 m "shallow", 20-49 m "mid", 50-100 m "deep") and three artificial structure types using a stratified random sample design. Structure types included artificial reefs (AR; rubble, concrete culverts, concrete pyramids, bay balls, and/or sunken vessels), oil and gas platforms (platforms) and rigs-to-reefs (R2R; decommissioned oil and gas platforms with the upper structure cut off and then toppled). Platforms occurred in all depth strata, AR were located in the shallow and mid depth strata, and R2R were only located in the deep strata.

Fish and Environmental Parameter Sampling

Fish samples were collected monthly from April to November 2016, April to October 2017, March to October 2018, March to November 2019, and April to September 2020 during daylight hours, using vertical long-lines containing 10 hooks baited with Atlantic mackerel (*Scomber scomber*). Sampling gear consisted of three electric bandit reels rigged with an 8 m vertical mainline outfitted with ten 45.7 cm leaders spaced 0.67 m apart and a 4.5 kg weight at the terminal end. Every leader on the line was rigged with one hook size (8/0, 11/0 or 15/0 circle hooks of zero offset). Lines were fished just off the bottom for 5 min sets. During each sampling event, three simultaneous 5 min sets were made at platform and AR sites at each depth strata and two simultaneous 5 min sets were made at R2R sites, for a total of 17 stations per month. Fish were stored on ice immediately upon capture.

The depth at each station (m) was determined with an on-board down scan depth sounder (Garmin GPSmap 7610xsv). Temperature, salinity and dissolved oxygen (DO, mg/L) measurements were collected throughout the water column with a Seabird CTD instrument deployed at each station.

Fish Sample Analysis

Fish were measured (TL, FL, SL mm), weighed (W, 0.01 kg), and gonads and otoliths were removed within 15 h of capture. Hereafter, all lengths are reported as FL. Gonadal tissue was weighed (GW, 0.01 g) and a mid-section from the right ovary of each female was preserved in 10% neutral buffered formalin for histological analysis. In the laboratory, preserved ovarian tissues were rinsed overnight in running tap water, dehydrated, cleared, embedded in paraffin, sectioned at 4 µm and stained with hematoxylin and eosin following standard histological techniques. A 1-4 g portion of the ovary of all females macroscopically identified in the actively spawning sub-phase was weighed (0.01 g) and preserved for a minimum of three months in Gilson's solution for later fecundity analysis.

Sectioned otoliths were used to estimate age following VanderKooy et al. (2020). Each opaque band (annulus) was considered to represent one year of growth, and the area between the last annulus and otolith edge – the margin – was measured. Three independent readers determined age and margin codes for each individual fish, and later did a joint reading to remedy any discrepancies. All annulus counts were then converted to biological age, which was determined based on annulus count, date of collection, mean birthdate, and mean timing of annuli formation. The Red Snapper birthdate is defined as 1 July, the middle month of their spawning season (VanderKooy et al. 2020).

Reproductive Parameters

Reproductive seasonality was assessed with the Gonadosomatic Index (GSI), where GSI = (GW/W-GW) × 100. Only mature fish were included in GSI analyses. A GSI $\geq$ 1 has been used previously to identify spawning capable Red Snapper (Brown-Peterson et al. 2019). Here, we use a threshold GSI value of $\geq$ 0.5 to represent reproductively active females (i.e., those in the developing, spawning capable or actively spawning phases), based on the mean GSI value of females in the developing reproductive phase (0.30 ± 0.01, range 0.05-0.66). Ovarian development and reproductive phases were assessed histologically following Brown-Peterson et al. (2011). Fish were considered reproductively active if they were in the developing, spawning capable and actively spawning phases and reproductively inactive in the immature, early developing, regressing and regenerating phases. Males in the spawning capable phase were further separated into three histological sub-phases that describe the amount of germinal epithelium (GE) present in the lobules of the testes (Brown-Peterson et al. 2011). To further describe testis development, the Spermatogenic Index (SMI) was calculated following Tomkiewicz et al. (2011). Each histological slide of male Red Snapper was photographed (Olympus BX43 compound microscope with a Q-color 3 Olympus camera and Q-capture Pro 6.0 software for image analysis), and all stages of spermatogenesis were quantified in three randomly chosen views from each fish using ImageJ software and an 80-point grid. The SMI describes testis development on a scale from 0 to 1.

Fish were considered sexually mature if ovarian tissue contained cortical alveolar (CA) oocytes and/or markers of previous spawning (i.e., oocyte atresia, thick ovarian wall, blood vessels, muscle bundles, high percentage of perinucleolar oocytes) or if testicular tissue contained primary spermatocytes (Brown-Peterson et al. 2011), corresponding to physiological maturity. Functional maturity of both females (presence of any stage of vitellogenic oocytes in the ovary) and males (presence of secondary spermatocytes, spermatids or spermatozoa in the testis) was also assessed. Finally, spawning maturity of females (females in the spawning capable phase with the presence of tertiary vitellogenic oocytes, Vtg3) was also determined for fish captured during the prime reproductive season (April – September). The various maturity classifications were examined to determine if there was a difference between size or age at maturity depending on the definition of maturity used. Fish that did not meet the maturity threshold (i.e., containing CA or vitellogenic oocytes) were considered immature for the calculations.

The spawning interval (estimated days between spawns) for female Red Snapper was determined for both spawning capable only females (i.e., an estimation of the number of times females in spawning condition are capable of spawning) as well as for all mature females (i.e., the number of days between spawns for the entire population of mature females, including fish in the early developing reproductive phase). The spawning interval was calculated using two types of spawning markers. For the first method, the reciprocal of the total number of actively spawning females (i.e., those undergoing oocyte maturation (OM) including hydrated oocytes) was divided by the number of spawning capable or mature females. The second method used the reciprocal of the total number of females with postovulatory follicles (POF) $\leq$24 h in the ovary post-spawning divided by the total number of spawning capable or mature females (Brown-Peterson et al. 2019).

Females were classified as daily spawners if their ovaries contained histological evidence of oocytes undergoing OM as well as POF $\leq$ 24 h. The percentage of daily spawners was expressed as both the percentage of actively spawning fish that were daily spawners as well as the percentage of all mature fish that were daily spawners.

For fish histologically verified to be in the actively spawning sub-phase, batch fecundity (BF) was determined volumetrically for six subsamples per individual (Bagenal and Braum 1971). Relative batch fecundity (RBF) was calculated as RBF = BF/(W-GW).

Data analysis

In all cases, data are presented as mean ± standard error. Size and age at maturity for Red Snapper was estimated using a logistic regression, with 0 indicating immature and 1 indicating sexually mature. Differences in size and age of immature fish assessed using the physiological or functional metric were tested with Student's T-test; Equality of Variance was tested using the Levene's test and appropriate adjustments made if there was unequal variance. The size of physiologically mature females in each reproductive phase for each month was tested with ANOVA. The equation FL = (TL - 0.586)/1.064 (Diaz 2004) was used to compare our data to other GOM Red Snapper studies.

Chi-Square tests were used to assess differences in spawning interval of female Red Snapper by month, season, age, structure, and depth using both the OM and POF methods. Due to small sample sizes, fish ages 6 and 7 were combined for the spawning interval analyses, as well as fish ages 8-22. Chi-Square was also used to test differences in reproductively active females by depth or structure type. The relationship between batch fecundity and fish size (TL) for Red Snapper was investigated using multiple models, and the model with the best fit to the data was determined with the Akaike Information Criteria (AIC; Akaike, 1973). Differences in Red Snapper batch fecundity by month, age, depth of capture, and reef structure type, and differences in female TL reproductive phase by month were tested using ANOVA and a Bonferroni post-hoc test. Normality was tested with a Kolmogorov-Smirnov 1-sample test and homogeneity of variance was tested using the Levene Statistic; a Kruskal-Wallis test was used if assumptions were violated with Bonferroni adjustments for pairwise comparisons. All regression analyses were performed using R (R core Team 2017); all other analyses were run using SPSS version 26 (IBM SPSS Statistics). Analyses were considered significant if $p < 0.05$.

Results and Discussion

Fish Collections

Red Snapper analyzed in this study were collected from 327 of the 600 randomly allocated stations containing artificial structures. A total of 2,084 Red Snapper were captured on artificial structures during the course of this project, ranging in size from 168 to 795 mm FL and 0.75-22.25 years. Histological analyses were completed for 2,033 specimens (1,028 females, 1,005 males), and 2,041 fish were aged.

Size and Age at Maturity

Both ageing and histological data were available for 1005 female and 979 male Red Snapper for maturity determinations. Red Snapper reach sexual maturity at a relatively small size and young age. The smallest and youngest sexually mature female was 168 mm FL and age 1, in the developing phase, and captured in July 2017. Females reached 50% physiological maturity (presence of CA oocytes) at 283 mm FL (Figure 2, top left) and 1.33 years (Figure 2, top right) and 50% functional maturity (presence of vitellogenic oocytes) at 318 mm FL (Figure 2, middle left) and 1.80 years (Figure 2, middle right). There is a significant difference in the size of immature females between physiological maturity (307 ± 4 mm FL) and functional maturity (349 ± 5 mm FL; $t_{317} = -6.769$, $p < 0.001$). Physiologically immature females are also significantly younger (2.12 ± 0.06 years) than functionally immature females (2.52 ± 0.07 years; $t_{314} = -4.327$, $p < 0.001$). Size and age at 50% spawning maturity (i.e., fish in the spawning capable or actively spawning phases with the presence of Vtg3 oocytes) is 374 mm FL (Figure 2, bottom left) and 2.93 years (Figure 2, bottom right). The larger size and older age at 50% spawning maturity indicates that female Red Snapper are not contributing significantly to the spawning population until they are ~70 mm FL and one year older than the size and age at first maturity. Size and age at 95% physiological maturity for female Red Snapper is 404 mm FL and 3.84 years, 95% functional maturity is 477 mm FL and 5.17 years, and 95% spawning maturity is 637 mm FL and 7.84 years. All of these values of 95% maturity are greater than the current minimum length limit for Red Snapper in Mississippi (16" TL [equivalent to 381 mm FL]), suggesting larger immature fish are likely landed by anglers, as well as the capture of fish well before their full spawning potential is reached.

Few immature males were captured during the five-year study (n = 14), and thus 50% maturity could not be calculated. The youngest sexually mature male was an early developing fish captured in March 2018 and was 0.75 years old. The smallest sexually mature male was a 190 mm FL fish in the developing phases captured in May 2018; this fish was 0.83 years old.

Previous estimates of Red Snapper maturity have been based on either functional or spawning maturity. Our estimates of 50% functional maturity are larger than previous estimates from Alabama (225-274 mm FL from 1999-2001, Kulaw et al. 2017), Louisiana (290 mm FL, Render 1995) and the southern GOM (295 mm FL, Brulé et al. 2010). Our 50% spawning maturity estimates are larger than those from 1999-2001 in Louisiana (276-324 mm FL, Kulaw et al. 2017), similar to estimates for fish on Louisiana artificial reefs in 2011-2013 (328-374 mm FL, Glenn et al. 2017) but smaller than more recent estimates from Louisiana reefs (425-474 mm FL for fish on natural banks and toppled oil platforms in 2009-2010, Kulaw et al. 2017). It is possible that size-at-maturity has decreased in the northern GOM in the past 5-10 years, or that Mississippi fish have a smaller size-at-maturity than those off Louisiana, but a more robust analysis, including population size estimates, is necessary for accurate determinations of changes in size-at-maturity over time. In contrast, our estimates of age-at-50% maturity for both physiological and functional maturity are lower than previous age estimates for the northern GOM (i.e., $\geq$ 2 years; Render 1995, Lowerre-Barbieri et al. 2012, Kulaw et al. 2017), although Fitzhugh et al. (2012a) did report 50% maturity at < 2 years for a GOM-wide collection of Red Snapper in 2011.

As age-1 female Red Snapper begin to approach their second birthday (July 1 herein), a relatively high percentage were spawning capable or actively spawning in May (age 1.83, 39.6%) and June (age 1.92, 38.5%; Figure 3). Overall, fewer age-1 fish (n = 28) were captured in July (age 1.0), August (age 1.08) and September (age 1.17), and while 46% of these very young fish were physiologically mature, only two spawning capable age-1 fish were captured during those months.

Spawning Seasonality

Red Snapper in Mississippi waters have a long (5-6 month) spawning season, with GSI values of both males and females generally elevated from April through September (Figure 4). Peak female GSI values varied during the 5-year project, with peaks in May and July in 2016, May and August in 2017, August in 2018, June and September in 2019, and April and July in 2020 (Figure 4). The high female April GSI values in 2020 are a likely a result of those fish being collected on 1 May, although still considered an "April" sample by collection protocol. Male GSI peaked in May in 2016, May and June in 2017, June in 2018, May in 2019 and August in 2020 (Figure 4). However, despite variations in male GSI, the SMI, a measure of the amount of spermatozoa in the testis, was relatively stable from April through September in all years (Figure 4), with mean values generally ranging between 0.60 and 0.73 during this six month period.

Previous studies have reported a varying but similar duration in GOM female Red Snapper spawning seasons based on GSI values (April-August, Render 1995; June-September, Collins et al. 1996; April-October, Fitzhugh et al. 2004, 2012a; April-September, Glenn et al. 2017). Meta-analysis of female Red Snapper spawning between 1991 and 2017 in the northern GOM showed peak GSI values occurred from June through August, with a high probability of spawning in May from 1995-2017 (Brown-Peterson et al. 2019). Mississippi Red Snapper females appear to have had a slightly longer spawning season in 2016-2020 compared to results from the meta-analysis, as April GSI values were generally higher than the 0.5 cut-off value, although mean GSI values of Mississippi females during the spawning season were generally low (0.5 – 2; Figure 3).

Histological inspection of Red Snapper gonads allows further refinement of the reproductive seasonality. Females in the spawning capable and actively spawning phases were found from April through October, suggesting that at least some females were spawning for 7 months (Table 1), similar to histological findings in Fitzhugh et al. (2004, 2012a). However, actively spawning females were most common from May through September (Table 1), indicating this is the peak reproductive season for female Red Snapper in Mississippi waters, which corresponds to the GSI data. The generally lower GSI values in April correspond to the highest percentage of females in the early developing and developing phases during that month. Some females in the regressing phase were seen from June through August, suggesting those fish may have had a shorter reproductive season compared to the population as a whole. The high percentage of females in the regressing phase in September and October (Table 1) signals the end of the spawning season. By November, all females but one were reproductively inactive, with the majority in the regenerating phase. The reproductive season appears to begin for some females in March with the appearance of fish in the early developing sub-phase, but most March females were still in the regenerating phase. Immature females were found during all months of sampling, but were most common in the early (March-April) and late (October-November) collection seasons. Based on the histological presence of spawning capable and actively spawning females over the five-year period, female Red Snapper are estimated to have a potential 179-day spawning season, with the first spawning capable female captured on 19 April and the last on 14 October.

The mean monthly size of females in each reproductive phase during the spawning season gives insight into population reproductive dynamics. During the peak spawning season (April through August), females in the early developing phase were always significantly smaller than spawning capable fish (Table 2), indicating that smaller, younger fish continue to enter the reproductive cycle throughout the reproductive season. Additionally, actively spawning females were also larger than early developing females from April through August, although this difference was only significant in July and August (Table 2). In addition, females in the regressing phase in August and September were smaller than spawning capable females (significant difference in September, Table 2), indicating smaller females cease spawning sooner than larger females. By October, when the majority of the mature females are regressing or regenerating (Table 1), there is no difference in FL among the phases. Taken together, these data suggesting that smaller and younger Red Snapper have a shorter spawning season than larger and older fish, as they enter the reproductive cycle later and leave it sooner. Therefore, smaller fish likely have a spawning season shorter than the potential 179 days estimated for the population as a whole.

The great majority of males were spawning capable (i.e., spermatozoa in the sperm ducts) from April through October (Table 3). However, despite some males being spawning capable for nine months, examination of the spawning capable sub-phases shows that spermatogenesis decreases as the spawning season progresses; in March, all spawning capable males were in the early GE sub-phase, while all spawning capable males in November were in the late GE sub-phase (Table 3). The decline in male GSI values after August in most years (Figure 4), despite the majority of males being spawning capable through September (Table 3), is likely due to reduced spermatogenesis (the majority of males in the late GE sub-phase) despite the presence of lobules full of spermatozoa; the presence of amble spermatozoa is reflected in the relatively stable SMI values during the entire spawning season (Figure 4). Males have a longer reproductive season than females, with the majority of males captured in March reproductively active (i.e., developing or spawning capable phases). Furthermore, while the majority of males captured in November were at the end of the reproductive season (i.e., regressing or regenerating phases), 33% were still in the late GE sub-phase of the spawning capable phase in November (Table 3).

Spawning Frequency

Red Snapper females are batch spawners, with the same individual spawning multiple times during the reproductive season; histological evidence of batch spawning can be seen in ovaries of spawning capable females containing POF (Figure 5A). Additionally, 21.8% of the actively spawning females examined during 2016–2020 were daily spawners (Table 4), with ovaries containing non-ovulated oocytes undergoing OM (indicating spawning within the next 12 h) as well as POF $\leq$ 24 h (indicating spawning within the past 24 h; Figure 5B). The percentage of daily spawning fish increased as the reproductive season progressed, with 14.7% of the actively spawning females spawning daily in spring while 42.8% of actively spawning females were daily spawners in the fall (Table 4). The percentage of daily spawning fish was lower when considering all mature fish, but still increased throughout the spawning season, from 0.8% in April to 14.8% in September, with 5.3% of all mature fish exhibiting daily spawning throughout the year (Table 5). While many authors have reported the presence of new (< 2 h) POF in conjunction with ovulated oocytes, this is not an accurate indicator of daily spawning as it represents newly ovulated oocytes during a current spawn; daily spawning percentages of Red Snapper have not been previously discussed in the literature. However, calculations from data presented in Woods (2003) shows daily spawning in 19.3% of actively spawning female Alabama Red Snapper, similar to the overall percentage in Mississippi fish (Table 4), but only in 7.7% of actively spawning Louisiana Red Snapper. Figure 8 in Kulaw et al. (2017) indicated daily spawning, but no estimation of the percentage of daily spawning female Red Snapper was included. Thus, recent publications (Brown-Peterson et al. 2019, 2021) are the first to discuss daily spawning of GOM Red Snapper.

Red Snapper spawning interval varied depending on both the data set and the method used for calculations, with spawning interval estimates using all mature fish longer than those using spawning capable only fish. For spawning capable and actively spawning only fish, spawning interval varied from once every 1.6 days in July to every 9 days at the end of the reproductive period in October (Table 4). Over the April-October spawning season, spawning interval was estimated to be every 2.4 days (OM method) to every 3.4 days (POF method, Table 4). There was a significant difference in spawning interval among months for both the POF (χ^2 = 11.44, p = 0.043) and the OM (χ^2 = 11.62, p = 0.04) methods, with the shortest interval in September for the POF method (2.6 days) and in July for the OM method (1.6 days) and the longest spawning interval in October for both methods (9 days, Table 4). The OM method consistently provided a shorter spawning interval estimate when using only spawning capable and actively spawning fish than the POF method, and this difference was significant in April, July, and in the summer, as well as overall (χ^2 overall = 11.55, p < 0.001, Table 4). There was no significant difference in spawning interval among seasons for either method, although the time between spawning events decreased from spring (3.4 days) to fall (2.9 days) using the POF method, spawning interval was lowest in summer (2.2 days) for the OM method, and methods only differed in the summer (Table 4).

Although estimated spawning intervals are longer when looking at the entire population of mature female Red Snapper (4.3–6.7 days between spawns overall, Table 5), trends are similar to spawning intervals of spawning capable and actively spawning individuals only (Table 4). Spawning interval is longer using the POF method (χ^2 overall = 9.839, p = 0.002), there are significant differences among months for spawning interval for both methods, and there is only a difference between the POF and OM methods in April and July (Table 5). Spawning interval is shortest in September for both methods (3.8 days, POF method and 3.0 days, OM method); spawning interval was shortest in July when only considering spawning capable and actively spawning females, however. There is no difference in spawning interval by season for the POF method when considering all mature females, but spawning interval is significantly shorter in the summer for the OM method (χ^2 = 7.032, p = 0.030, Table 5), differing from spawning capable and actively spawning females only (Table 4).

There are no differences in spawning interval by age for either the POF or the OM method when using spawning capable and actively spawning female only (Table 6), although spawning interval in age-1 fish as estimated by POF was longer than any other age class. There was a significant difference in spawning interval estimate between the POF and OM methods for age-1 fish (χ^2 = 9.624, p = 0.002), but no difference between the two methods for any other age class (Table 5). However, the percentage of actively spawning fish that were daily spawners increased with age, with one-third of fish ages 7-13 spawning every day (Table 6). It has been previously shown that most species show increased spawning frequency with increasing age (Fitzhugh et al. 2012b). While actual spawning interval is not different across age classes of spawning capable and actively spawning Mississippi fish, the percentage of daily spawning is, suggesting there may be an overall increase in the number of spawning events at increasing age for an individual female. However, small sample sizes in the older age classes suggest caution in interpretation of the data presented here.

In contrast to spawning interval data for spawning capable and actively spawning only females, there is a significant difference in spawning interval for all mature fish across age classes using the POF method (χ^2 = 24.541, p < 0.001), with spawning interval of the oldest fish (8-22 years) the shortest (Table 7). There is no difference in spawning interval by age for all mature fish using the OM method, although interval is shortest for the oldest fish. Red Snapper in the South Atlantic with an age distribution similar to that of Mississippi fish showed no differences in spawning interval among age classes for all mature fish using either the POF or OM method (Lowerre-Barbieri et al. 2015). The only significant difference between methods for all mature fish is for age 1 females, with the interval estimated using the POF method much higher than the OM method ($\chi^2 = 7.553$, $p = 0.006$). The percentage of all mature fish that were daily spawners also increased with age, with only 1.7% of age-1 females daily spawners, while 13.3% of females ages 8-12 were daily spawners (Table 7).

Most previous estimates of spawning interval for GOM Red Snapper have been based on all mature females. Both the overall OM and POF spawning interval values estimated for all mature Mississippi fish are longer than spawning intervals previously reported for GOM Red Snapper by Kulaw et al. (2017) of 2.5 and 5.2 days for the OM and the POF methods, respectively. Glenn et al. (2017) estimated 4.5 days between spawns for Red Snapper on artificial reefs off Louisiana using a time-calibrated method that considers both OM and POF combined, while Fitzhugh et al. (2012a) estimated a spawning interval of 3.6 days for Red Snapper captured throughout the northern GOM in 2011 using the POF method for all mature fish. Meta-analysis of spawning interval for spawning capable and actively spawning only females across 26 years in the northeastern GOM (an area that includes Mississippi) showed spawning intervals slightly longer than results from our five years of Mississippi data, with mean spawning interval of 3.18 ± 0.26 days using the OM method and 6.51 ± 2.45 days with the POF method (Brown-Peterson et al. 2019). Assuming a 179-day annual spawning period when combining data for the five years, our spawning interval estimates suggest an individual spawning capable female could spawn between 53 and 75 times during the reproductive season, while spawning frequency for the population of mature females is 27 and 42 times. This annual spawning frequency in Mississippi is similar to a previous estimate by Porch et al. (2015) of 29–71 times for Red Snapper GOM-wide with an April–October spawning season; these authors also found that the number of spawns increases annually with the size of the fish. Other researchers have assumed a 150-day spawning season, and have calculated spawning frequency estimates to be 15–19 times (Texas, Downey et al. 2018), 33–39 times (Louisiana, Glenn et al. 2017), 44 times (Louisiana, Kulaw et al. 2017), and 25–71 times (GOM-wide, Fitzhugh et al. 2004). If the spawning season for Mississippi fish was reduced to

150 days, our spawning frequency estimates for all mature females would be 22–35 times, within the ranges previously reported for the GOM.

Batch Fecundity

Batch fecundity (BF) was estimated from 93 Mississippi Red Snapper and is quite variable, with a low of 596 eggs in a 376 mm FL fish and a high of 349,754 eggs in a 568 mm FL fish. We found that the linear equation best predicted the relationship between BF and FL based on AIC comparisons of four models although there was little difference in the AIC values among models (Table 8). Batch fecundity is significantly, positively correlated with fish length ($p < 0.001$, Figure 6), although the linear predictive equation (BF = 398.06(FL) – 122,839.14) only explains 21.7% of the variation in fecundity by FL. Batch fecundity also varied by age, although few of the 90 fish with both fecundity and age values were >4 years (n = 8). There was a significant difference in BF by age ($KW_{8,90} = 19.37$, $p = 0.013$, Figure 7), with BF generally higher in fish > 4 years although post-hoc tests found no significant differences among ages. Batch fecundity was not significantly different among months ($KW_{5,92} = 9.7$, $p = 0.084$), although May and July had the highest mean BF values, while values were lowest in April and September (Table 9).

Due to the significant relationship between BF and FL, relative batch fecundity (RBF), which adjusts for fish size, should be used when comparing fecundity across fish of different sizes. There is not a significant relationship between RBF and FL ($p = 0.339$), and therefore it is appropriate to use RBF to compare among fish of different sizes. The RBF ranged from 0.29–1,752 eggs/g ovary-free body weight; mean RBF was 73.05 ± 13.73 eggs/g ovary-free body weight (Table 9). There was a significant difference in RBF among months ($KW_{5,92} = 12.36$, $p = 0.03$), with highest values in August and lowest in April (Table 9), although the Bonferroni-adjusted post-hoc test was unable to differentiate among months. There was also a significant difference in RBF by age ($KW_{8,90} = 17.22$, $p = 0.028$, Figure 7), with RBF highest at ages 5 and 8, although there were low sample sizes and high variation for all ages >4.

The batch fecundity values presented here are lower than those reported in previous studies of Red Snapper in the northern GOM. However, the majority of fecundity values (82%) are from fish ≤ 3 years, and small Red Snapper are known to have low fecundity (Lowerre-Barbieri et al. 2015). Additionally, ovaries were preserved in Gilson's solution for fecundity counts, which can result in deformation and potential destruction of hydrated oocytes although fecundity samples were not left in Gilson's solution longer than 4 months and hydrated oocytes were always easily identified. Low fecundity counts corresponded with histological observations of few hydrated oocytes in the ovary; small batch sizes in Red Snapper have been previously reported (Lowerre-Barbieri et al. 2015), suggesting fish are capable of advancing very small batches of oocytes for spawning. Batch fecundity of Red Snapper captured at artificial and natural reefs in Louisiana from 2011-2013 ranged from 6,991 to 1,194,993 eggs per batch, and fish captured on natural reefs had a significantly higher batch fecundity than those captured on artificial reefs (Glenn et al. 2017). Collins et al. (2001) reported batch fecundity of Red Snapper from all Gulf states except Mississippi to range from 200 to >3,000,000 eggs, with younger ($\leq$ 4 years) fish having very low batch fecundities. Fitzhugh et al. (2012a) found similar results for the same region, with maximum batch fecundities of ~1,500,000 eggs. Batch fecundity increases exponentially in larger and older Red Snapper, particularly in fish > 700 mm FL and >10 years (Fitzhugh et al. 2012a, Porch et al. 2015, Lang and Falterman 2017, Fitzhugh et al. 2017). Unfortunately, no batch fecundity data from fish of this size or age were obtained in the current project. There is little published information regarding RBF for GOM Red Snapper. However, the mean RBF for 2016-2020 in Mississippi is higher than 2017 northeastern GOM (west of the Mississippi River) estimates of 51.4 eggs/g ovary-free body weight (25% and 75% credible intervals, 1.1 – 62.9; Brown–Peterson et al. 2019).

Red Snapper Reproductive Parameters Related to Artificial Structure and Depth

Although artificial structure is important to Red Snapper (only 0.4% of all Red Snapper were captured at control sites with no structure during the five years of sampling), the type of artificial structure does not generally impact Red Snapper reproductive parameters. Artificial structure type does not accurately classify maturity, spawning seasonality (GSI), reproductively active females, spawning indicators or fecundity (Brown-Peterson et al. 2021), and there is no difference in the percentage of reproductively active females among structure types (p = 0.171, Table 10). However, there is a difference in the percentage of spawning capable females by structure type (χ^2_{1029} = 7.52, p = 0.023, Table 10), with the highest percentage of spawning capable females at R2R structures. Artificial structure height, measured as height above the sea bottom, is an important predictor of female Red Snapper maturity (Brown-Peterson et al. 2021), and immature fish are older at artificial reefs than at platforms, with no differences at R2R (Leontiou et al. 2021b). Bayesian analysis of the presence/absence of POF, indicating recent spawning, suggests there is a 95% probability that more recent spawners will be associated with R2R than platforms (Brown-Peterson et al., in review) although there is no significant difference in spawning interval of spawning capable and actively spawning females using the POF method across the three structure types (χ^2 = 0.086, p = 0.958, Table 11. However, spawning interval of spawning capable and actively spawning females is significantly lower at artificial reef structures using the OM method (χ^2 = 11.08, p = 0.004), and this is the only structure type with a difference between the OM and POF method (χ^2 = 14.48, p < 0.001; Table 11). There is a difference in mean batch fecundity by structure types ($KW_{2,92}$ = 17.25, p = <0.001), with BF significantly different among all structures, with highest values at R2R and lowest values at platforms (Table 12); RBF showed the same trend of highest fecundity at R2R, but the difference was not significant (p = 0.060).

Depth is an important factor in all Red Snapper reproductive parameters examined. Depth was important for classifying maturity (Brown-Peterson et al. 2021), and FL increased at a greater rate per meter of depth for immature than mature females (Leontiou et al. 2021b), suggesting immature females in the deep strata grow faster than mature females. Depth is also important for correctly classifying spawning seasonality, reproductively active females, and spawning indicators (Brown-Peterson et al. 2021). There is a significant difference in the percentage of fish in reproductively active phases across depth strata (χ^2_{1031} = 53.65, p < 0.001), as well as a significant increase in the percentage of spawning capable females with increasing depth (χ^2_{1031} = 33.77, p < 0.001, Table 10). Additionally, there is a 95% probability that the percentage of tertiary vitellogenic (Vtg3) oocytes in spawning capable females increases with increasing depth (Brown-Peterson et al. in review). Spawning interval of spawning capable and actively spawning females estimated by POF is significantly shorter (χ^2 = 35.82, p < 0.001) in the deep stratum compared to the mid and the shallow strata (Table 11), and the POF method provided a shorter estimate of spawning interval in the deep stratum than the OM method (χ^2 = 6.44, p = 0.011). There are also differences among depth strata for spawning interval of spawning capable and actively spawninf females estimated by OM (χ^2 = 11.66, p = 0.003), with the shortest spawning interval seen in shallow water, where the percentage of daily spawning females was also highest (31%, Table 11). Although batch fecundity was not significantly different by depth strata (p = 0.677; Table 12), RBF did show a significant difference by depth ($KW_{2,92}$ = 6.94, p = 0.031). Interestingly, BF was highest in the deep stratum (860,83 ± 31,340 eggs), while RBF was significantly lowest in the mid stratum (56.55 ± 13.12 eggs/g ovary-free body weight).

Although Red Snapper are known to be structure-oriented, particularly when <7 years of age (Karnauskas et al. 2017), there is little information on differences in reproductive variables among artificial reef structures in the northern GOM. Female red snapper captured on nearshore artificial reefs in Texas exhibited more active spawning and higher fecundity than those taken from offshore artificial reefs (Froehlich et al. 2021), despite similarities in depth between nearshore and offshore reefs. There was no reported difference in female Red Snapper reproduction between artificial and natural reefs in the same depth zones in Texas (Downey et al. 2018). In contrast, Red Snapper from artificial reefs off Louisiana are reported to have a lower reproductive potential than those from natural reefs (Glenn et al. 2017), although the artificial reefs occurred in shallower depths than the natural reefs. Thus, the differences described by Glenn et al. (2017) may be more related to depth than to reef type. Similarly, it should be noted that the R2R structures in our study are only found in the deepest depth stratum. Thus, differences in reproductive parameters noted at R2R structures may be confounded by their deeper depth; while platforms are also found at deep depths, they are not exclusive to that depth stratum.

Overall, there is little published information regarding Red Snapper reproductive output in relation to depth other than data generated by the present study (i.e., Brown-Peterson et al. 2021, Brown-Peterson et al. in review). Karnauskas et al. (2017) found both higher abundances and higher fecundity estimates of Red Snapper at 50-90 m along the coasts of Texas and Louisiana compared to other areas of the GOM. Similarly, greater numbers of eggs and larvae have been reported from the northwestern Gulf (Lyczkowski-Shultz and Hanisko 2007, Hanisko et al. 2017), suggesting there is potentially a higher reproductive output in this region. Modeling by Porch et al. (2015) suggested evidence that depth may have a strong effect on Red Snapper spawning indicators even though it did not explain a substantial amount of the variation in the parameter. The occurrence of larger Red Snapper at deeper depths (Millender and Brown-Peterson 2022) suggests the potential for greater reproductive output at depth, as large Red Snapper are known to have disproportionally higher fecundity (Lang and Falterman 2017). The strong evidence that depth is an important factor in multiple Red Snapper reproductive parameters in Mississippi waters suggests this would be a fruitful area for continued research.

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Table 1. Monthly percentages of female Red Snapper (*Lutjanus campechanus*) in various reproductive phases collected in Mississippi waters from 2016-2020. All depths and habitat types combined. Phase assignment based on histological evaluation of the ovaries following Brown-Peterson et al. (2011). N—number of fish; Imm—immature; EDev—early developing; Dev—developing; SC—spawning capable; AS—actively spawning; Rgs—regressing; Rgn—regenerating.

Month	N	Imm	EDev	Dev	SC	AS	Rgs	Rgn
March	53	38	17	0	0	0	0	45
April	189	25	30	12	14	11	0	8
May	225	13	12	12	37	21	0	4
June	123	11	14	11	35	23	4	3
July	118	8	14	10	25	27	12	4
August	89	18	16	8	35	18	4	1
September	76	7	5	9	28	26	21	4
October	102	25	6	11	8	1	19	31
November	53	36	0	2	0	0	11	51

Table 2. Monthly mean (± se) size (FL, mm) of physiologically mature female Red Snapper (*Lutjanus campechanus*) in various reproductive phases during the spawning season. n—number of fish each month. EDev—early developing; Dev—developing; SC—spawning capable; AS—actively spawning; Rgs—regresssing; Rgn—regenerating. *P*—significant difference in monthly phases (ANOVA; Kruskall-Wallace for July and August); superscript letters indicate significant differences among phases within a month.

Phase	April n = 142	May n = 195	June n = 110	July n = 109	August n = 72	September n = 71	October n = 78
EDev	373 ± 10^{a}	371 ± 12^{a}	363 ± 16^{a}	313 ± 14^{a}	329 ± 13^{a}	$300 \pm 24^{a,c}$	358 ± 27
Dev	$399 \pm 2^{a,c}$	366 ± 12^{a}	357 ± 16^{a}	$354 \pm 26^{a,c}$	$316 \pm 13^{a,c}$	$358 \pm 21^{a,c}$	414 ± 32
SC	$447 \pm 21^{b,c}$	$439 \pm 11^{b,c}$	$429 \pm 11^{b,c}$	$420 \pm 22^{b,c}$	405 ± 18^{b}	428 ± 24^{a}	421 ± 32
AS	$426 \pm 25^{a,c}$	$420 \pm 12^{a,c}$	$423 \pm 15^{a,c}$	$421 \pm 18^{b,c}$	$411 \pm 30^{b,c}$	$394 \pm 18^{a,c}$	427 ± 0
Rgs	---------	--------	$362 \pm 18^{a,c}$	$426 \pm 18^{b,c}$	$313 \pm 5^{a,b}$	$341 \pm 17^{b,c}$	419 ± 21
Rgn	$402 \pm 8^{a,c}$	$444 \pm 19^{a,c}$	$467 \pm 34^{a,c}$	$372 \pm 39^{a,c}$	$369 \pm 0^{a,b}$	$383 \pm 63^{a,c}$	408 ± 19
P	**0.009**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**0.024**	0.847

Table 3. Monthly percentages of male Red Snapper (*Lutjanus campechanus*) in various reproductive phases collected in Mississippi waters from 2016-2020. All depth and habitat types combined. Phase assignment based on histological evaluation of the testes following Brown-Peterson et al. (2011). N—number of fish; Imm—immature; EDev—early developing; Dev—developing; SC—spawning capable; Rgs—regressing; Rgn—regenerating. Spawning capable sub-phase percentages based on number of spawning capable fish only; EGE—early germinal epithelium; MGE—mid germinal epithelium; LGE—late germinal epithelium.

Month	**N**	**Imm**	**EDev**	**Dev**	**SC**	**SC sub-phases**			**Rgs**	**Rgn**
						EGE	**MGE**	**LGE**		
March	60	13	20	43	18	100	0	0	0	5
April	175	1	4	29	65	89	9	1	1	1
May	178	1	1	15	83	77	16	17	0	1
June	89	0	0	3	96	27	32	41	1	0
July	133	0	0	4	91	21	18	61	5	0
August	95	0	0	2	97	23	27	50	0	1
September	113	0	0	1	94	10	7	83	4	1
October	105	2	1	7	47	4	4	91	25	19
November	54	4	4	0	33	0	0	100	31	28

Table 4. Monthly, seasonal, and overall spawning interval (SI) of spawning capable and actively spawning female Red Snapper (*Lutjanus campechanus*) in Mississippi waters from 2016-2020. N—number of spawning capable fish; POF—postovulatory follicle method; OM—oocyte maturation method. P—difference between methods or months determined by Chi-square test; bold indicates significant difference ($p < 0.05$). Daily spawner percentage calculated as the percentage of actively spawning fish containing oocytes undergoing oocyte maturation as well as POF ≤ 24 h in the same ovary. Spring—April and May; Summer—June, July and August; Fall—September and October.

Month	**N**	**SI from POF** (days)	**SI from OM** (days)	***P*** (method)	**Daily spawners** (%)
April	48	8.0	2.3	**0.001**	4.8
May	131	2.8	2.8	0.0897	19.1
June	71	2.9	2.5	0.486	17.5
July	53	3.8	1.6	**<0.001**	18.7
August	47	3.9	2.9	0.367	37.5
September	41	2.6	2.0	0.373	45.0
October	9	9.0	9.0	1.000	0
P (month)*		**0.043**	**0.040**		
Spring	179	3.4	2.6	0.073	14.7
Summer	165	3.3	2.2	**0.004**	22.4
Fall	50	2.9	2.4	0.510	42.8
P (season)		0.781	0.468		
OVERALL	400	3.4	2.4	**0.001**	21.8

*October excluded from analysis due to low sample size

Table 5. Monthly, seasonal and overall spawning interval, in days, of mature female Red Snapper (*Lutjanus campechanus*) in Mississippi waters from 2016-2020. N—number of mature fish (early developing, developing, spawning capable, actively spawning, regressing and regenerating phases); POF—postovulatory follicle method; OM—oocyte maturation method. P—difference between methods or months determined by Chi-square test; bold indicates significant difference ($p < 0.05$). Daily spawner percentage calculated as the percentage of actively spawning fish containing oocytes undergoing oocyte maturation as well as POF $\leq$ 24 h in the same ovary relative to all mature fish captured. Spring—April and May; Summer—June, July and August; Fall—September and October.

Month	**N**	**SI from POF (days)**	**SI from OM (days)**	***P*** (method)	**Daily Spawners (%)**
April	128	21.3	6.4	**0.002**	0.8
May	166	3.9	3.9	0.903	5.4
June	85	6	3.3	0.506	5.9
July	95	10.6	3.1	**0.002**	6.3
August	62	5.2	4.1	0.390	9.7
September	61	3.8	3.0	0.373	14.8
October	77	77	77	---	0
P (month)*		**<0.001**	**0.002**		
Spring	294	6.1	4.7	0.097	0.3
Summer	242	6.9	3.4	**0.006**	4.5
Fall	138	8.1	5.1	0.475	10.9
P (season)		0.227	**0.030**		
OVERALL	674	6.7	4.3	**0.002**	5.3

*October excluded from analyses due to low sample size

Table 6. Spawning interval (SI) of spawning capable and actively spawning female Red Snapper (*Lutjanus campechanus*) in Mississippi waters from 2016-2020 by age class. N—number of spawning capable fish; POF—postovulatory follicle method; OM—oocyte maturation method. P—difference between methods or months determined by Chi-square test; bold indicates significant difference ($p < 0.05$). Daily spawner percentage calculated as the percentage of actively spawning fish containing oocytes undergoing oocyte maturation as well as POF $\leq$ 24 h in the same ovary.

Age (year)	N	SI from POF (days)	SI from OM (days)	*P* (method)	Daily Spawners (%)
1	42	7	2.2	**0.002**	10
2	165	3.2	2.4	0.192	19
3	110	3.2	2.6	0.260	21
4	29	2.4	2.6	1.000	27
5-6	31	4.4	4.4	1.00	28
7-13	18	2.0	2.0	1.00	33
P (age)		0.074	0.441		

Table 7. Spawning interval (SI), in days, of mature female Red Snapper (*Lutjanus campechanus*) in Mississippi waters from 2016-2020 by age class. N—number of mature fish (early developing, developing, spawning capable, actively spawning, regressing and regenerating phases); POF—postovulatory follicle method; OM—oocyte maturation method. P—difference between methods or months determined by Chi-square test; bold indicates significant difference ($p < 0.05$). Daily spawner percentage calculated as the proportion of actively spawning fish containing oocytes undergoing oocyte maturation as well as POF $\leq$ 24 h in the same ovary to all mature fish.

Age	N	SI from POF (days)	SI from OM (days)	P (method)	Daily Spawners (%)
1	118	19.6	6.2	**0.006**	1.7
2	371	7.1	5.4	0.243	3.5
3	305	9.0	7.3	0.332	3.0
4	57	4.8	5.2	1.000	5.3
5	24	8.0	8.0	1.000	4.2
6-7	30	5.0	5.0	1.000	6.7
8-22	15	2.1	3.0	0.465	13.3
P (age)		**<0.001**	0.587		

Table 8. Models used to determine the best prediction of fecundity by fork length for female Red Snapper, *Lutjanus campechanus*, captured from Mississippi waters 2016-2020. The lowest AIC value indicates the best fit (in bold). BF—batch fecundity. RBF—relative batch fecundity.

Model	BF AIC	RBF AIC
Linear	**2324.61**	**1270.11**
Quadratic	2325.53	1271.99
Cubic	2326.01	1271.55
Quartic	2328.01	1270.58

Table 9. Mean (± SE) fecundity of Red Snapper (*Lutjanus campechanus*) captured during April through September 2016-2020 from Mississippi waters. N = number of fish; BF = batch fecundity (# eggs); RBF = relative batch fecundity # eggs/g ovary-free body weight); *P* = significance of difference among months, Kruskal-Wallis test. No difference among months by Bonferroni-adjusted pairwise comparisons.

Month	N	BF ± SE	RBF ± SE
April	10	12,666 ± 3,072	19.17 ± 4.02
May	35	52,418 ± 11,818	60.03 ± 6.82
June	11	40,047 ± 3,072	66.30 ± 46.67
July	16	56,457 ± 21,309	45.08 ± 21.17
August	11	19,283 ± 4,892	206.04 ± 76.70
September	9	11,091 ± 3,305	74.31 ± 46.00
P		0.084	**0.030**
All Combined	93	39,316 ± 6,667	73.05 ± 13.73

Table 10. Percentages of reproductively active female Red Snapper (*Lutjanus campechanus*) captured from Mississippi waters from March through November 2016-2020 by depth and structure. N—total number of females captured; DEV – developing, SC—spawning capable; AS—actively spawning; *P*—Significance of difference among structures and depths determined by Chi-Square analysis.

	N	% DEV	% SC	%AS	*P* (Active)
Structure					
Platform	560	9	26	16	0.171
Artificial Reef	425	12	20	16	
Rigs-to-Reefs	44	9	34	14	
P (structure)		0.279	**0.023**	0.913	
Depth					
Shallow (< 20 m)	249	7	14	16	**>0.001**
Mid (20-49 m)	591	12	23	16	
Deep (50-100 m)	191	9	38	17	
P (depth)		0.062	**<0.001**	0.285	

Table 11. Spawning interval (days between spawns) of spawning capable and actively spawning female Red Snapper (*Lutjanus campechanus*) captured from April through October 2016- 2020 in Mississippi waters by depth and structure. OM—oocyte maturation method; POF—postovulatory follicle method; Daily—percentage of daily spawners based on actively spawning fish with 24 h POF; *P*—Significance of difference determined by Chi-Square test.

	N	OM	POF	P (method)	Daily
Structure					
Platform	235	2.58	2.97	0.251	26
Artificial Reefs	152	2.27	4.47	**<0.001**	38
Rigs-to-reefs	21	3.5	3.5	0.741	17
P		**0.004**	0.958		
Depth					
Shallow (< 20 m)	73	1.87	3.84	**0.001**	31
Mid (20-49 m)	231	2.46	4.44	**<0.001**	17
Deep (20-49 m)	105	3.28	2.10	**0.011**	25
P		**0.003**	**<0.001**		

Table 12. Mean (± SE) fecundity of Red Snapper (*Lutjanus campechanus*) captured during April through September 2016-2020 by depth and structure. N = number of fish; BF = batch fecundity (# eggs); RBF = relative batch fecundity (# eggs/g ovary-free body weight). *P* = significance of difference, Kruskal-Wallis Test. Superscript letters indicate homogeneous subgroups, based on Bonferroni-adjusted pairwise comparisons.

	N	BF ± SE	RBF ± SE
Structure			
Platform	47	17,660 ± 2,819[a]	88.57 ± 22.93
Artificial Reef	41	46,071 ± 10,073[b]	45.22 ± 13.21
Rigs-to-Reef	4	224,531 ± 47,754[c]	165.47 ± 96.61
P		**<0.001**	0.060
Depth			
Shallow	26	27,462 ± 7,054	115.37 ± 35.78[a]
Mid	51	36,774 ± 7,956	56.55 ± 13.12[b]
Deep	16	86,083 ± 31,340	88.74 ± 32.69[a,b]
P		0.677	**0.031**

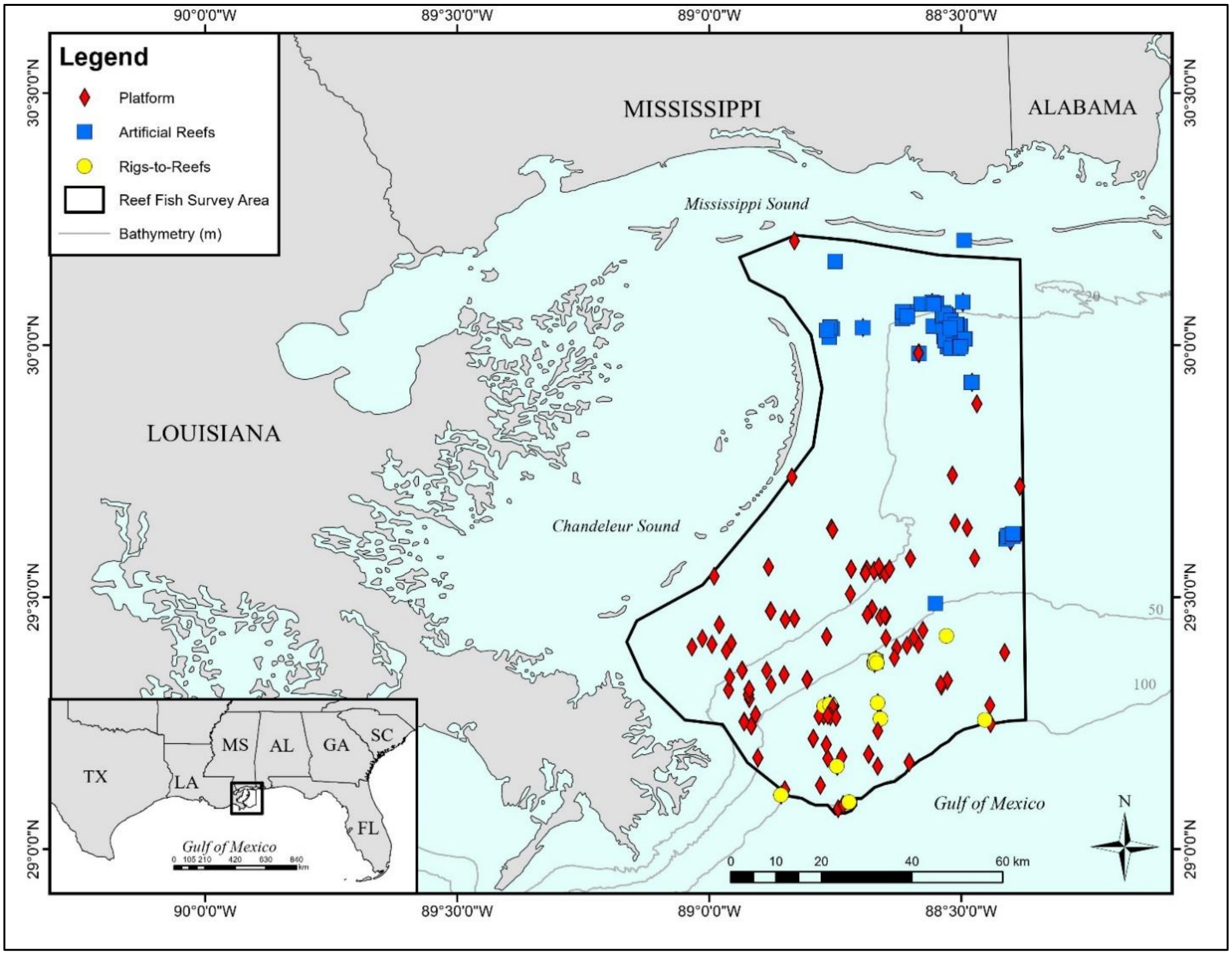

Figure 1. Locations of artificial structure stations sampled for Red Snapper (*Lutjanus campechanus*) in Mississippi waters 2016-2020. Monthly sampling occurred in three depth strata for a total of 17 stations/month. Some stations were sampled multiple time during the five years of the project.

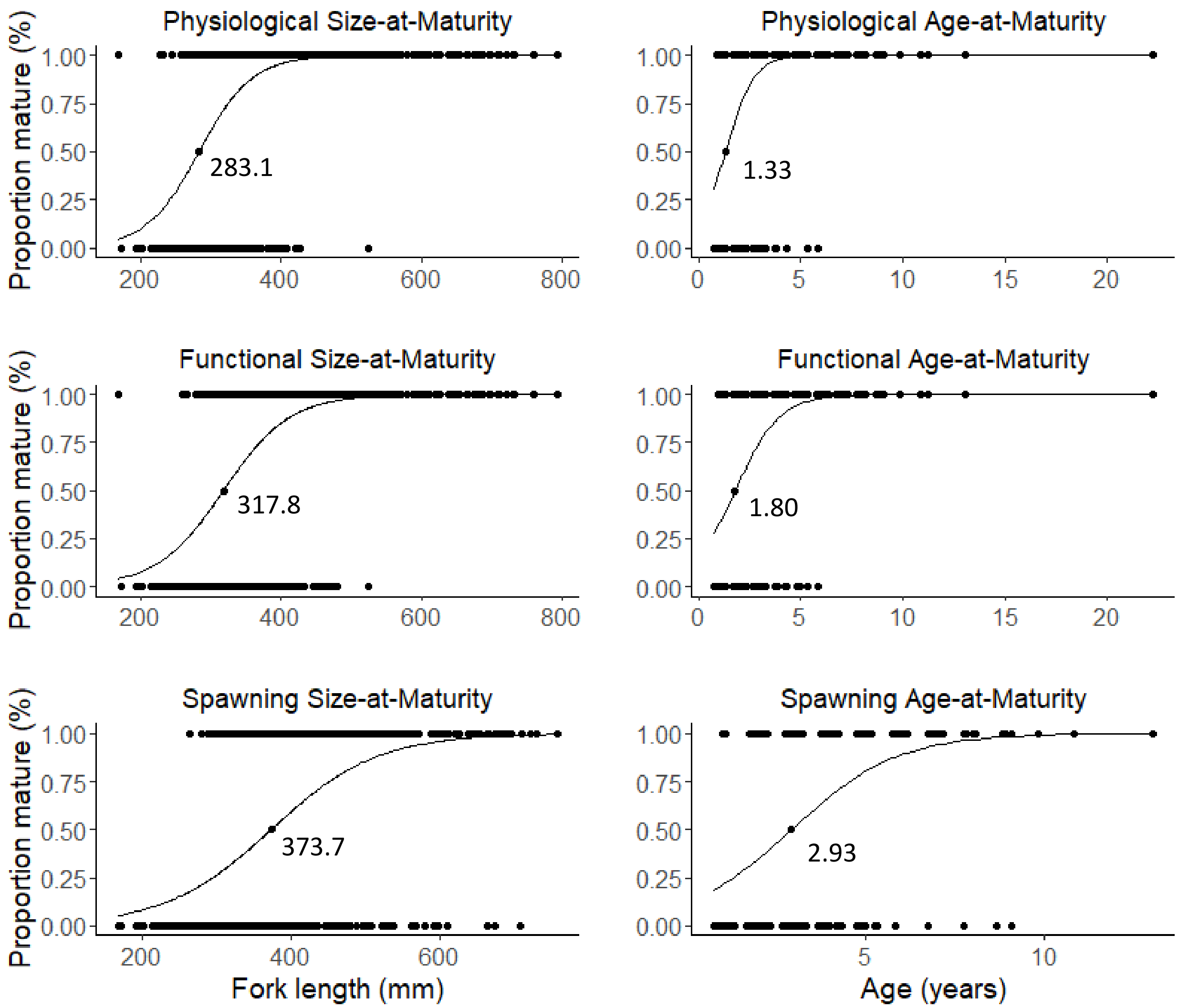

Figure 2. Maturity ogives for female Red Snapper (*Lutjanus campechanus*) captured in Mississippi waters 2016-2020. The 50% maturity value is indicated in each graph.

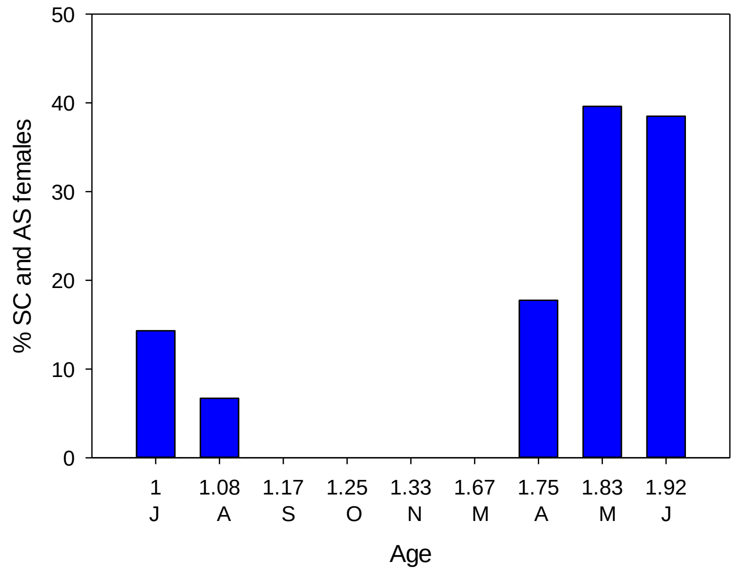

Figure 3. Percentage of age-1 spawning capable (SC) and actively spawning (AS) female Red Snapper (*Lutjanus campechanus*) captured each month from Mississippi waters in March through November 2016-2020. Assigned birthdate is 1 July; ages represent fractional age each month, beginning in July.

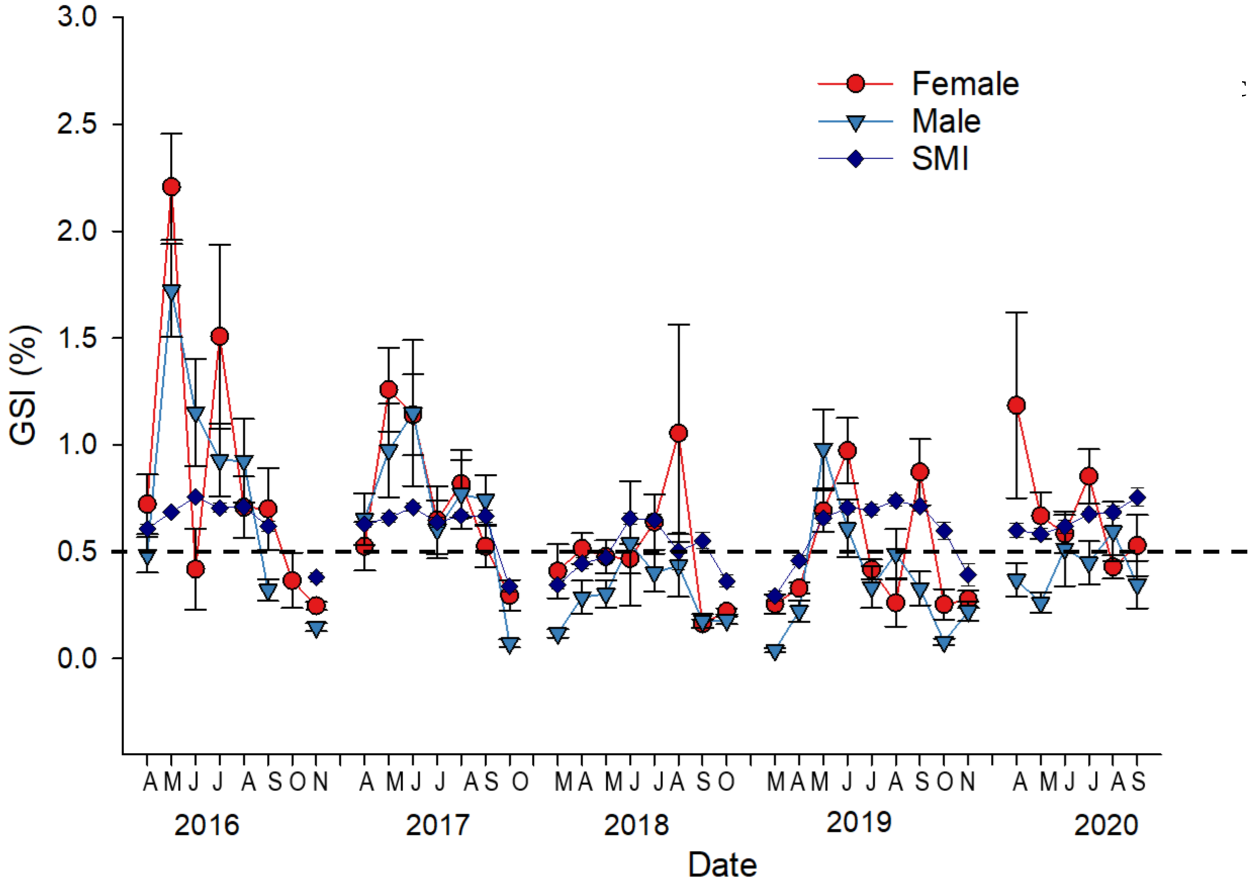

Figure 4. Spawning seasonality of Red Snapper (*Lutjanus campechanus*) in Mississippi waters 2016-2020. Data represents mean (± se) of Gonadosomatic Index (GSI) for males and females and Spermatogenic Index (SMI) of males. The dashed line represents the threshold GSI value for reproductively active females.

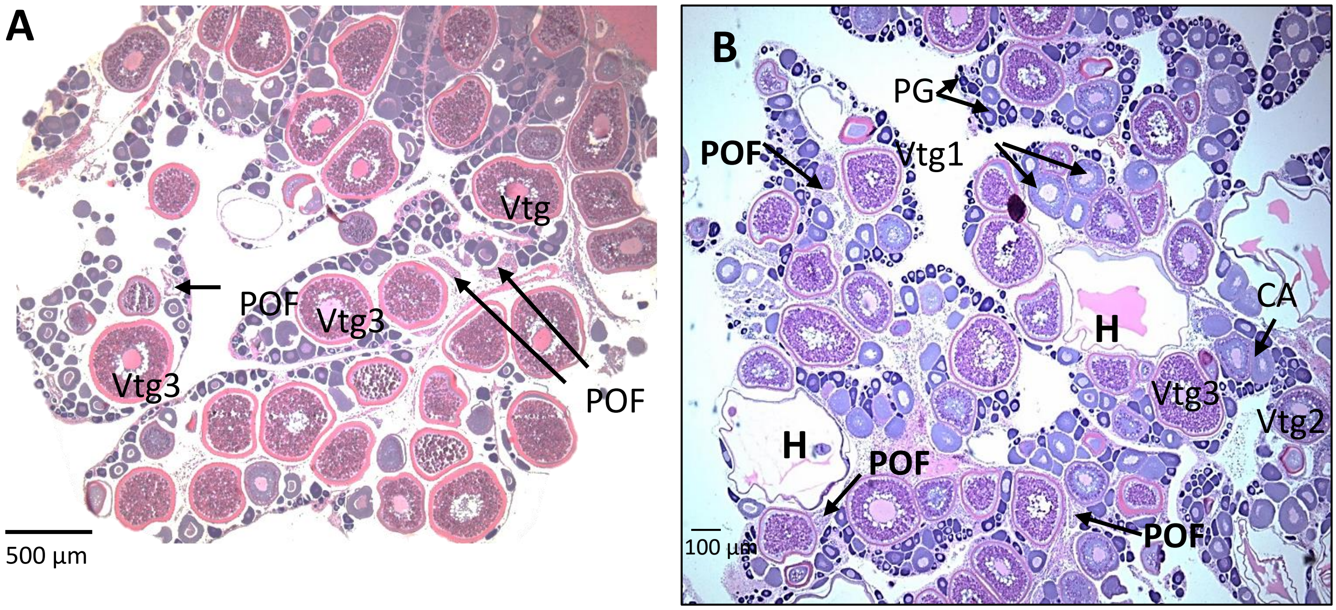

Figure 5. Histological photos of ovary of female Red Snapper (*Lutjanus campechanus*) from Mississippi waters. A. Spawning capable phase showing evidence of batch spawning (Vtg3 and POF). Spawning capable fish such as this were found from April through October. B. Actively spawning reproductive sub-phase. This fish was a daily spawner, as indicated by the presence of both 24 h POF and hydrated oocytes (H). Daily spawning fish such as those pictured here were found from April through September. CA—cortical alveolar oocyte; H—hydrated oocyte; PG—primary growth oocytes; POF—24 h postovulatory follicle; Vtg1—primary vitellogenic oocyte; Vtg2—secondary vitellogenic oocyte; Vtg3—tertiary vitellogenic oocyte.

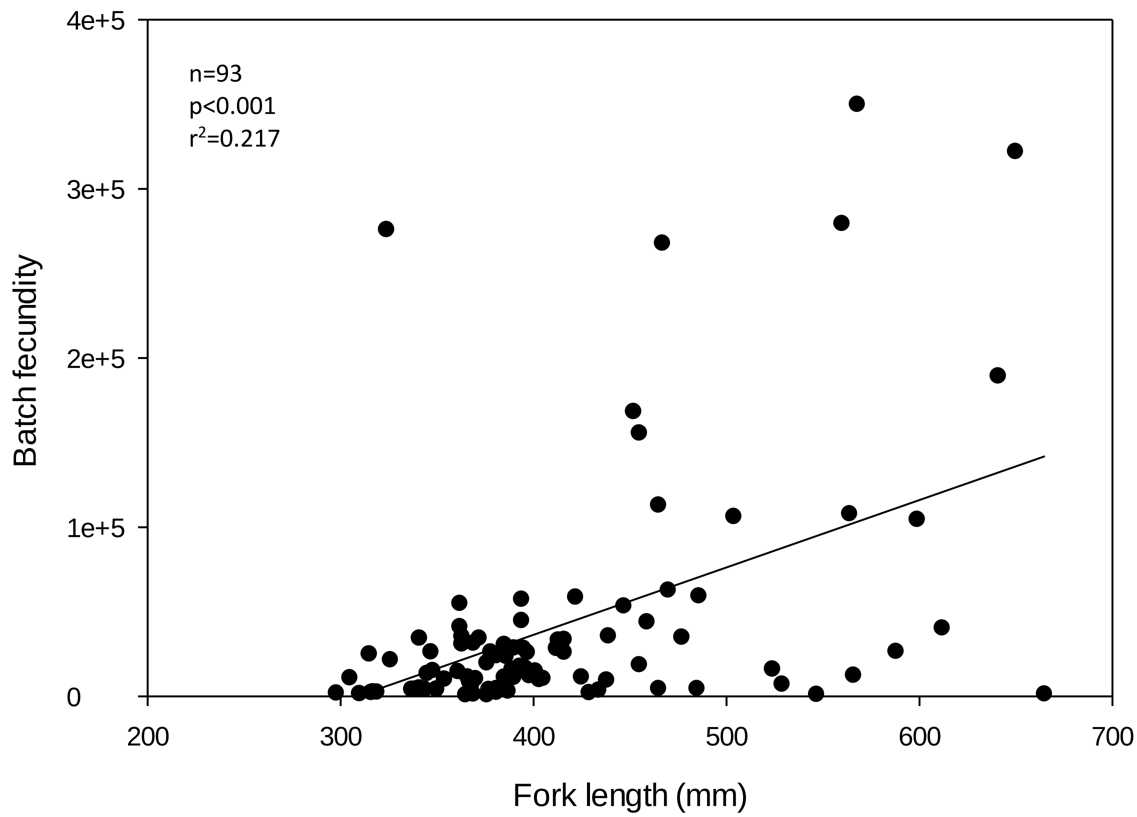

Figure 6. Batch fecundity of 93 actively spawning Red Snapper (*Lutjanus campechanus*) by fork length (FL) in Mississippi waters 2016-2020. The fecundity-TF relationship is best explained by the linear equation BF = 398.06(FL) – 122,839.14.

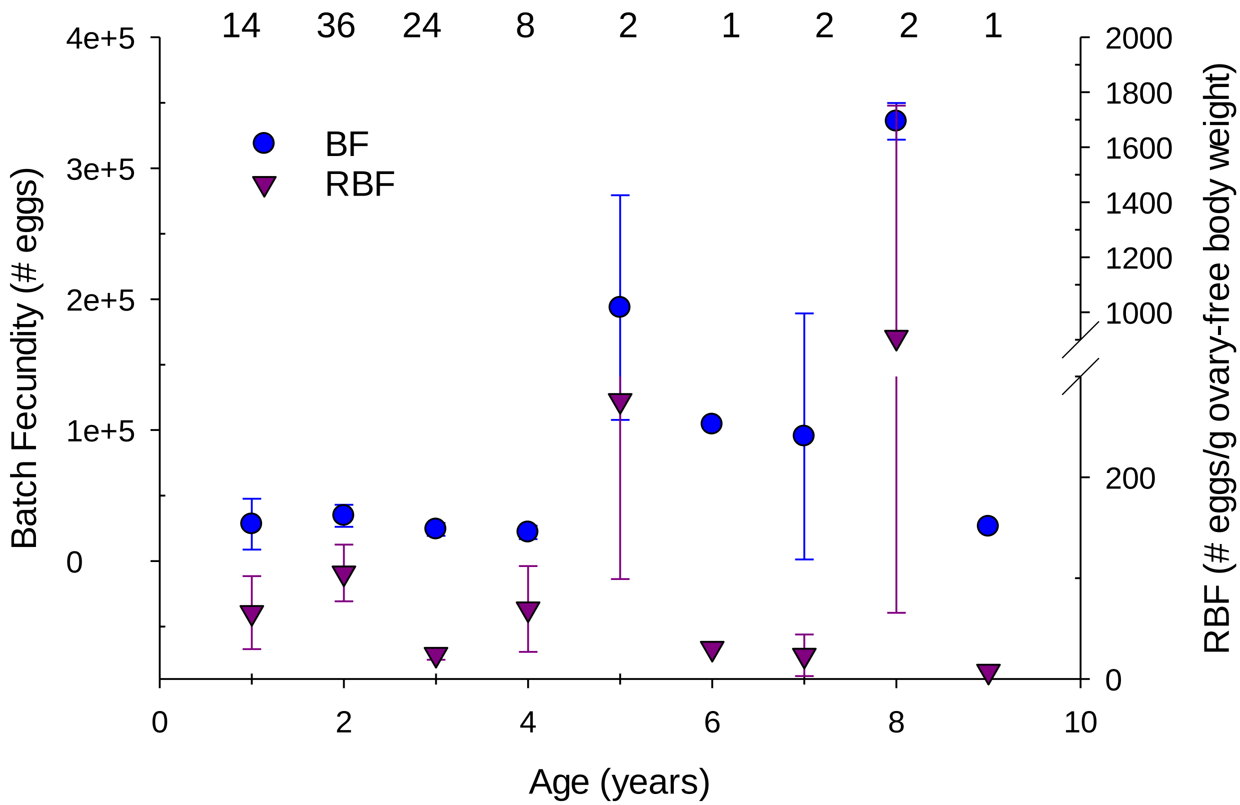

Figure 7. Mean (± se) batch fecundity (BF) and relative batch fecundity (RBF) by age class for 90 Red Snapper (*Latjunus campechanus*) captured in Mississippi waters 2016-2020. Number of fish in each age class shown at top of graph. Significant difference across age classes for both BF ($p = 0.013$) and RBF ($p = 0.028$) with Kruskall-Wallace one-way Analysis of Variance, although there were no significant differences in pair-wise comparisons among ages using Bonferroni adjustment.