Female Red Snapper Reproductive Parameters differ across Depth

Los parámetros reproductivos del pargo rojo hembra difieren según la profundidad

Les paramètres de reproduction du vivaneau rouge femelle diffèrent selon la profondeur

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EXTENDED ABSTRACT

Red Snapper (*Lutjanus campechanus*) is a reef associated species that is documented to be strongly associated with natural and artificial structure. Red Snapper is one of the most targeted species by recreational anglers in the northern Gulf of Mexico (GOM) and were overharvested in the 1990's, although current assessments state the GOM population is no longer overfished nor undergoing overfishing (SEDAR 2018). While there is a large body of literature on the reproductive biology of this species, an aspect that has received little attention is how reproductive parameters interact with changes in depth. Previous reproductive biology studies on Red Snapper have focused on differences between structures with limited inclusion of depth as a variable. A recent study by Brown-Peterson *et al*. (2021) found that depth predicted reproductive performance better than artificial structure type. Our objective is to investigate the differences in female Red Snapper reproductive parameters across three depth strata.

Red Snapper were collected using bandit reels following a random, stratified sampling design of artificial structures off the Mississippi coast from March-November of 2016-2020. Stations consisted of oil platforms, rigs-to-reefs, and artificial reefs and were found in three depth strata (shallow: <20 m, mid: 20-49 m, and deep: 50-100 m). Fish collected were processed within 24 hours of capture where fork length (FL, mm), body mass (g), and gonad mass (g) were measured. A thin mid-section of the gonad was fixed in 10% neutral buffered formalin for histological analysis. If the fish was macroscopically identified as actively spawning, an additional portion of the gonad was removed, weighed (0.01 g), and placed in Gilson's fluid for later fecundity analysis. Fixed gonadal tissue was rinsed overnight in running tap water, dehydrated, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin following standard histological procedures. Stained tissue was histologically classified into one of the six reproductive phases outlined by Brown Peterson *et al.* (2011). Percentages of females identified in the spawning capable phase and actively spawning sub-phase were calculated by depth and tested using a chi square analysis. Mean FL of fish captured in each depth strata were calculated and significant differences were tested using a Welch's test and Games-Howell post-hoc test. Each histological slide produced was photographed and three random views were chosen to calculate percentages of two oocyte stages, tertiary vitellogenic oocytes and oocyte maturation. We used Image J to identify and count oocytes present in each of the three views, and a percentage of each oocyte stage present was calculated. A Kruskal-Wallis test was used to investigate significant differences across depth. Fecundity samples were rinsed overnight in running tap water and suspended in 50-200 ml of water for calculation of batch fecundity using the volumetric method. Relative batch fecundity was calculated by dividing the batch fecundity by the body mass to eliminate the effect of fish size on fecundity. The mean batch fecundity and relative batch fecundity for each depth strata were calculated and tested using a Welch's test. The Gonadosomatic Index (GSI) was calculated using a standard GSI equation. Mean GSI was calculated for each depth strata across months and an ANOVA

Table 1. Female Red Snapper reproductive parameters across three depth strata in the northern Gulf of Mexico (Shallow: >20 m, Mid: 20-49 m, Deep: 50-100 m). The asterisk (*) indicates parameters that were significantly different.

Reproductive Parameter	Shallow	Mid	Deep	p
Fork Length (mean ± SE)	351 ± 4.7	377 ± 3.5	463 ± 7.2*	**p < 0.0001**
Percentage of Spawning Capable Females	29	39	56*	**p < 0.0001**
Percentage of Tertiary Vitellogenic oocytes	34	32	33	p = 0.7674
Percentage of oocytes undergoing Oocyte Maturation	16	15	16	p = 0.1981
Batch Fecundity (mean ± SE)	27,461.79 ± 7,053.882	36,774.42 ± 7,955.967	86,083.32 ± 31,340.44	p = 0.3162
Relative batch fecundity (mean ± SE)	115.37 ± 35.78	46.55 ± 13.12	191.05 ± 109.06	p = 0.2365
Spawning Interval (days)	4.06	4.53	2.12*	**p = 0.0001**

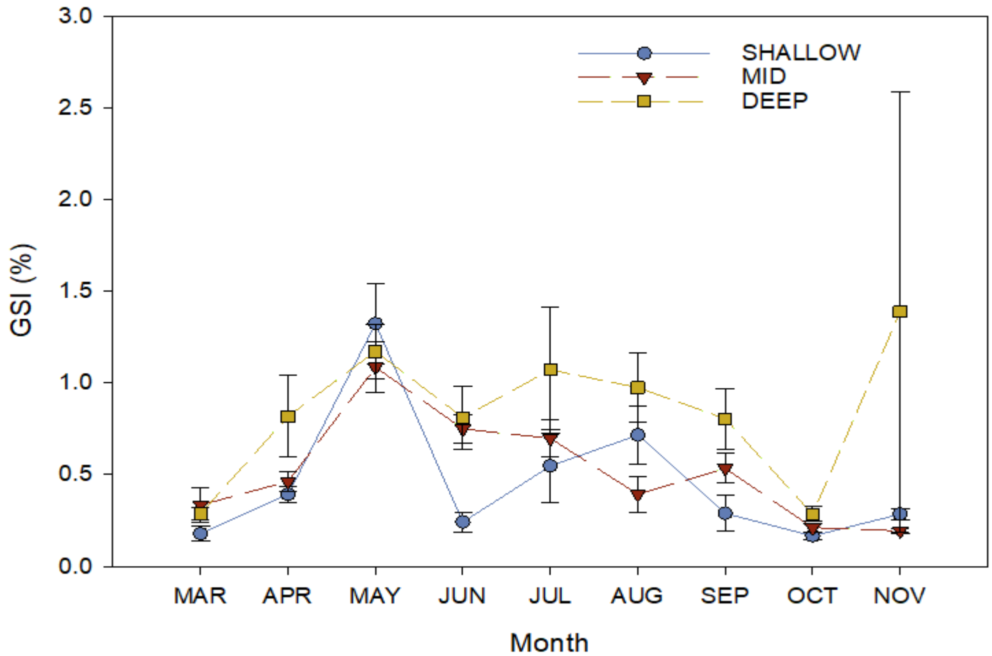

Figure 1. Mean (± SE) Gonadosomatic Index of female Red Snapper across three different depth strata in the northern Gulf of Mexico (Shallow: >20 m, Mid: 20-49 m, Deep: 50-100 m). No significant differences present.

was used to test for significant differences. Spawning interval, or the number of days between spawns, was calculated using the post ovulatory follicle method. The relationship between depth and spawning interval was tested using a chi square analysis. All results were considered significant if $p < 0.05$.

A total of 1,028 females were captured over the 5-year period and ranged from 168-795 mm FL. Mean fork length increased as depth increased and the largest females were found in the deep stratum ($F_{2,\ 427.675}$=84.920, p<0.0001, n=1,027; Table 1). In most species, larger fish are more common in deep waters, however this may be a result of fishing pressure rather than an ontogenetic movement (Frank *et al.* 2018). The percentage of spawning capable females was found to be highest in the deep stratum, similar to results found by Glenn *et al*. (2017), and increased from 29% to 59% from shallow to deep stratum (Table 1). There was a significant relationship between depth and percentage of spawning capable fish, and the deep stratum contributed the most to that significance (X^2_2=31.108, p<0.001, n=1,028). The percentage of tertiary vitellogenic oocytes and oocytes in oocyte maturation in ovaries of females were homogenous across depth and no significant differences were present (Table 1). Batch fecundity was calculated for 93 females and increased with depth. Relative batch fecundity was highest in deep depths, but there were no significant difference in fecundities among depth strata (Table 1). Gonadosomatic Index (GSI) was generally highest in the deep stratum, although there were no significant differences in GSI among the depth strata (Figure 1). Spawning interval was significantly shorter at deep depths (2.12 days) compared to mid (4.23 days) and shallow (4.06 days) depths (X^2_2=22.619, p=0.001, n=408; Table 1).

The results from this study show that females in the deep stratum are larger and more reproductively active. Deep water females spawn a greater number of eggs at a faster rate, and therefore have a greater reproductive potential than those in mid or shallow stratum. Our results support previous work by Brown-Peterson et al. (2021) who found that depth was an important predictor of maturity, spawning seasonality, reproductive phase, and recent or imminent spawning. This information on the relationship between depth and reproductive potential will be important for future stock assessments and useful in management decisions. For instance, creation of new artificial reefs in deep waters may help increase the Red Snapper GOM population through enhanced reproduction.

KEYWORDS: Reproductive potential, fecundity, spawning, histology, Gulf of Mexico .

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