## **Draft Working Document**

# Characterization of red snapper (*Lutjanus campechanus*) reproduction: for the 2004 Gulf of Mexico SEDAR

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#### Abstract

To better understand reproduction of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico and meet the needs of an upcoming stock assessment, an archived collection of gonads was processed and analyzed. Red snapper were sampled year round from 1991 -2002, with an increase in sampling effort during the spring-summer spawning season. Samples were obtained throughout the northern Gulf of Mexico, from Florida, Alabama, Mississippi, Louisiana, and Texas, although most samples (61%) came from the west coast of Florida. A total of 5,995 red snapper gonads was collected throughout the twelve year period, and 1,956 (females) were used for histological observations. Our findings on seasonality, depth of spawning, maturity, spawning duration and frequency are in broad concordance with- and expand upon results of prior studies. This work was accomplished coincident in time, but independent to an academic MARFIN study of stock differentiation. A major recent finding of the MARFIN supported study was differential reproductive traits between Alabama and Louisiana. Therefore, an additional objective of our study was to further examine evidence for regional differences. By adding data from more locations, particularly from Florida waters, we found more supporting evidence for higher reproductive output at age among young (to age-8) red snapper in the eastern-versus the western Gulf of Mexico. To maximize the ability to fit functional relationships we combined fecundity data from our work and the academic MARFIN project. Even with the increased sample sizes, predictive relationships for fecundity remain a challenge to estimate. Power or exponential functions provide best fits to fecundity-at-length, however a dome-shaped or asymptotic function may provide a better fit for fecundity-at-age.

#### Introduction

Although red snapper in the Gulf of Mexico have been studied intensely, information on spawning conditions and habitats is limited (Collins et al. 2001). In addition, several questions related to estimating reproductive output by age were raised during the last red snapper stock assessment (Schirripa and Legault 1999). In particular, increased sample sizes and better resolution of fecundity was deemed important. The objective of this study was to continue to build data sets on spawning, maturity and fecundity of red snapper in the Gulf of Mexico. Other objectives included: defining the reproductive season using histology and gonadosomatic indices and characterizing red snapper spawning areas. An additional objective, based on recent results of an academic Marine Fisheries Initiative (MARFIN) study (Cowan et al. 2002, Woods 2003), was to examine possible broad regional differences (eastern vs. western Gulf) in spawning traits by size and age.

#### Methods

Red snapper were sampled year round from 1991–2002, with an increase in sampling effort during the spring-summer spawning season. Red snapper were obtained throughout the northern Gulf of Mexico, from Florida (FL), Alabama (AL), Mississippi (MS), Louisiana (LA), and Texas (TX). Fish were collected by commercial boats, recreational headboats, charterboats, tournaments, and fishery-independent surveys. Fish collected were weighed and measured, and the gonads were removed and placed in plastic bags and stored on ice. Gonads were shipped overnight and processed at the Panama City Laboratory. Otoliths were extracted and ages were determined according to Allman et al. (2004).

During processing, excess tissue was removed and each gonad examined microscopically. A small sample was removed and viewed at 250x and the most advanced maturation stage was assigned aided by measurement of oocyte diameter. Most gonads sampled were weighed to the nearest 0.1 g and placed in plastic bags with 10% buffered formalin and later sectioned for histological observation. During the years prior

to 1998, ovaries were selected for histological preparation to minimize processing time and costs. During this period, if there were a number of ovaries from a particular collection, at least n=5 were selected for sectioning as representative of the entire collection based on the microscopic examination. From 1998 on, all collections of ovaries were processed for histological observation. During all years, any obviously hydrated ovaries were identified and set aside for fecundity determinations.

During sectioning, all gonads were removed from formalin, blotted dry, and weighed to the nearest 0.1 g. Using the methods described by Erickson et al. (1985), a randomly selected region (anterior, medial, or posterior) on one or both lobes of the gonad was cross sectioned. In a previous study, ovaries of red snapper have been found to be homogeneous with respect to oocyte size by location (Collins et al. 1996). The samples were placed in individual tissue cassettes along with formalin for histological slide preparation at Louisiana State University School of Veterinary Medicine, Department of Pathology.

Histological slides were examined microscopically at 32x - 800x magnification to determine oocyte maturation. Using the oocyte maturation characteristics described by Wallace and Selman (1981), oocytes were staged accordingly to determine the leading oocyte stage (see Appendix Figure 1). Females displaying vitellogenic or more advanced oocytes (yolked oocytes) were defined as mature. Females with cortical alveoli or primary growth oocytes as the leading stage, but displaying atretic-yolked oocytes and loosely packed lamellar folds were also classified as mature females (Brown-Peterson et al. 1988, and see Appendix Figure 2). Maturity ogives by size and age were based on females sampled only during the peak spawning months of June-August to further minimize error in assigning maturity status (following Woods 2003). Females were classified as "spawning" depending upon the presence of hydrated oocytes, indicative of imminent spawning, or postovulatory follicles (POF), indicative of recent spawning (Hunter and Macewicz 1985). In addition to histological stages, a gonadosomatic index (GSI = (gonad weight x 100/ (total weight x 1000) – gonad weight) was used to determine the start, peak, and end of the spawning season.

Batch fecundity was determined using the hydrated oocyte method described by Hunter et al. (1985). Samples, including the periphery and center of each ovary (0.02–0.66 g), were cross sectioned and weighed to the nearest 0.001 g (Hunter et al. 1985). Samples were placed in a vial along with 33% glycerol to separate oocytes for the purpose of counting (Collins et al. 1996). Batch fecundity was calculated by multiplying the final hydrated ooctye estimate by the whole ovary weight, and the product was divided by the weight of the sample (Hunter et al. 1985; Collins et al. 1996). Batch fecundity was regressed on FL, TW, and age for all hydrated females (Collins et al. 1996). Any sections showing recent post-ovulatory follicles, suggesting the female had partially spent her current batch, were eliminated from the fecundity estimates.

Spawning frequency was estimated using the time-calibrated method (Fitzhugh et al. 1993, Nieland et al. 2002) which is based on the average spawning proportion (approximately per 24 hour interval) of mature females showing hydrated ova or post-ovlatory follicles. The inverse of the spawning proportion yields the frequency: the average expected interval in days between spawning events.

#### Results

#### Sample sources

A total of 5,995 red snapper gonads were collected between 1991 and 2002 from the northern Gulf of Mexico (Figure 1). A total of 1,956 ovaries was selected for histological observations. Fishery-dependent sampling of gonads deviated from the age structure sampling (Allman et al. 2004) in that reef fish are not commonly landed with gonads intact, and we were dependent upon cooperative fishermen and extra efforts by several port agents and observers to make the collections. In addition, a high proportion of red snapper gonad samples were available to us from scientific surveys and tournaments. Due to these circumstances, charterboats were a source for 37% of all samples, followed by headboats (25%), commercial boats (15%), tournament sources (9%), and scientific survey sources (14%) (Figure 2). Although red snapper were collected in a variety of

gears, the majority (92%) were collected by hook and line gear. Sixty-one percent of the samples were collected from FL, followed by TX (20%), LA (14%), AL (4%), and MS (1%) (Figure 2).

#### Reproductive seasonality

Spawning seasonality ranged from April through October based upon observations of females with hydrated ova (Figure 3). This observation is also supported by the gonadosomatic index, wherein values above 0.5 corresponded approximately to the onset of vitellogenesis (Figure 4). We also found that proportions of spawning females and relative fecundity (hydrated ova per g of ovary) also varied by month (Figures 5 & 6). In general, all seasonal signals indicated that June, July and August are the peak reproductive months.

#### Sexual Maturity

Based upon the peak reproductive months of June-August, a total of 1,137 females were used to examine length-at-maturity. The smallest female observed with hydrated oocytes, indicative of imminent spawning, was 296 mm fork length (FL) and was 2 years old. The smallest female observed with postovulatory follicles, indicative of recent spawning, was 285 mm FL and was 2 years old.

Using logistic regression, maturity ogives revealed that a higher proportion of females were mature-at-size in eastern samples (n=644 from FL, AL, and MS) in comparison to western samples (n=493 from LA and TX) (Figure 7A). Over 75% of females were mature by 300 mm FL for eastern samples, whereas the proportion in the west was slightly below 75% even at 350 mm FL. In both regions, all females were mature upon reaching the 650 mm FL size class. A similar trend was also noted for maturity-at-age, wherein eastern samples showed higher proportions of mature red snapper at ages 2-7 than western samples (Figure 7B).

Some location, temperature, and depth information was available for females in active spawning condition (hydrated, POF) and which were principally sampled via scientific

surveys. Hydrated females were observed across a temperature range of 16-26 C and at depths ranging from 30-126 m (mean=67 m) (Table 1).

#### Spawning Duration

Spawning duration estimates, based on first and last observation of hydrated females and females exhibiting post ovulatory follicles (POF) ranged from 89 to 193 d. A paired T-test for the means of the hydrated oocyte method compared to POF method revealed no significant difference (p=0.159). In order to make a single best estimate, we examined the years when histological sample sizes were >100 and collection dates clearly spanned the months April-October. These criteria were met five years out of twelve, and the average duration using both approaches was 151 d (sd = 36) (Table 2).

#### Spawning frequency

Based upon the precedent set by the recent academic MARFIN project (Cowan et al. 2002 and Woods 2003), we examined spawning frequency estimates by age and region (age data aggregated across years). We found evidence that expected intervals between spawning by age are of shorter duration in the east in contrast to the west (Figure 8). Based upon the slope of the linear relationship of age on spawning frequency, this difference is most apparent for the younger ages (e.g., age 2-8).

Spawning frequencies are commonly determined annually and then used to extrapolate annual fecundity estimates. Differences in spawning frequency estimates were apparent by year and between regions by year. However, across all years, mean spawning frequency by region was similar (3.0 in east versus 2.9 in west) (Figure 9).

#### Batch Fecundity

Batch fecundity is typically determined from a relatively small subset of reproductive samples as only visually apparent hydrated females (by microscopic observation) are chosen for further processing. It was apparent from the last stock assessment that additional fecundity samples were needed, especially from larger and older females (Schirripa and Legault 1999). Taking into account increased efforts since the last full

assessment and by combining our fecundity data with data from the academic MARFIN project (Woods 2003), we report fecundities from 563 females. By age, the fecundity information still appears quite variable, especially beyond age 10 where sample sizes still drop off despite the increased collection efforts (Figure 10). By size, batch fecundity appears to increase dramatically in range and variance beyond 750 mm FL (Figure 10 B). This result is also matched, and the pattern perhaps better revealed, by the notable increase in range and variance of gonad weight of active females (vitellogenic and more advanced stage) at a size greater than about 700 mm FL (Figure 11). Gonad weight by-age shows a slightly different relationship than by-length with many older fish displaying relatively low ovarian weights (Figure 12).

#### Discussion

#### Seasonality

There was evidence of spawning from April through October, but clearly June, July and August were the peak months. These months were noted for having the greatest synchrony of spawning females, highest percent of females with vitellogenic and more advanced ova, and highest mean GSI values. In addition, relative fecundities were highest during these three months and indicated that within individuals, reproductive output was also at a peak level. These results are in broad agreement with seasonality reported elsewhere for the Gulf of Mexico (Futch and Bruger 1976, Wilson et al. 1994, Collins et al. 1996, Woods 2003).

#### Depth and temperature of spawning

Our results, together with other studies, confirmed that spawning of red snapper is occurring in the offshore waters of all the Gulf states. Further, results show spawning occurs across the entire shelf and upper slope (18-37 m—Moe 1963, 24-29 m—Futch and Bruger 1976, 15-73 m Szedlemayer and Furman 2000, and 30-126 m—our current results). Red snapper also appear to spawn across a relatively broad thermal zone. The temperature range in which we observed hydrated females was 20-25 C. But females with post-ovulatory follicles have been found over a broader temperature range (16-29 C;

Collins et al. 2001, Woods 2003 and this study). A pattern of seasonal depth-related movements of adult red snapper has been generally related as directed toward shallower water (inner-mid shelf) in the spring/summer months and offshore (mid-outer shelf) in the winter months (Moe 1963 Appendix I, Bradley and Bryan 1975). Bradley and Bryan (1975) further speculated that the inshore spring/summer movement may be related to spawning. We note that for spawning-condition females, mean collection depth was similar for the months of June and August (69 m and 68 m respectively). While still speculative since Bradley and Bryan's 1975 paper, possible movements related to spawning bear further investigation.

#### Regional differences, sexual maturity, spawning frequency

Because of the recent findings of differences in reproductive output at size and age between Alabama and Louisiana locations (academic MARFIN project: Cowan et al. 2002, and Woods 2003), we made independent regional contrasts with samples from the east (largely Florida waters) and samples from the west (Texas and Louisiana). In doing so, we also found evidence for regional differences in maturity and spawning frequency which supports findings by these investigators. Our samples from the east were characterized by higher proportions of mature fish among smaller size classes (to 650 mm FL) and younger ages (to 8 years) than fish from the west. Fish were mature at age-2 and reaching 75% maturity proportions by 300 mm FL (east) and 350 mm FL (west). These results are similar to findings in the MARFIN project and also similar to an earlier study from Florida waters (age-2 and 300-320 mm fork length-at-50% maturity; Futch and Bruger 1976).

Our eastern samples showed higher spawning frequencies (averaged fewer days between spawns) in the east versus the west to about age-8. Again, this is similar to the academic MARFIN results for the time-calibrated method. Our spawning frequency range across age (2.1-5.9) was close to the results of Woods 2003 (2.7-5.1). Our overall estimates of spawning frequency (averaged across years and ages) were slightly lower than the MARFIN results (3.0 east, 2.9 west—ours; and 3.4 east, 4.2 west—Woods 2003). However, all of these findings, are in general agreement with previous annual spawning

frequency estimates aggregated across ages (4.0-6.0—Collins et al. 1996, 4.0— Szedlmayer and Furman 2000).

We have not fully examined fecundity relationships by region due to high variances (addressed below), but our results together with the academic MARFIN results, certainly supports the inference that reproductive output among the younger age classes is higher in the east than the west.

#### Spawning duration—number of spawns

Our overall best estimate of a spawning duration of about 151 d corresponds well with other investigations (150 d—Woods 2003, 142 d—Szedlemayer and Furman 2000). For example, a duration of 150 d and a range of spawning frequencies (2.1-5.9), results in the average number of annual spawning events ranging by age from 25 to 71. Our results suggest that there may be year-to-year differences, but annual distinctions are difficult to make given the often haphazard and opportunistic schedule for obtaining reproductive samples.

#### Batch fecundity

The amount of fecundity information notably increased in the last assessment (Schirripa and Legault 1999) and even more information exists for this assessment (the results herein plus Woods 2003 and Szedlemayer and Furman 2000). During the last assessment, fecundity results were available by length and were converted to age (based on Wilson et al. 1994, Collins et al. 1996). To date, much more red snapper fecundity information is available by age directly, and age-fecundity data may be most preferred for equation fits in order to minimize errors in extrapolation. For example, use of direct fecundity-age data was thought to be beneficial during the last red grouper assessment (Gulf of Mexico Fisheries Management Council 2002). However, when viewing the distribution of the length-based versus age-based fecundity information for red snapper, the choice of function to best fit the data remains an open question.

Female gonad weight is often used as a proxy for fecundity. While a less direct measure, gonad weights are often available when fecundity data are sparse and gonad weight data may reveal patterns due to the much greater sample sizes typically available. When we plotted the raw gonad weight data by length, we saw that gonad weight of larger active females increased rapidly; a pattern that is usually fit with an exponential or power function. We also noticed that there was high variance in gonad weight as size increased. However, when we plotted gonad weight by age directly, an exponential or power function seemed inappropriate. Instead, the variance in the data and relatively low gonad weights of a number of active older females (>age 16) suggested an asymptotic or domed relationship; initial best fits were achieved with a  $2^{nd}$  order polynomial equation.

Because batch fecundity is based on gonad weight, similar patterns were also seen when batch fecundity by weight and age were plotted (our data and Woods 2003 combined). An initial response might be to throw out the seemingly high outliers (a single individual of nearly 8 million ova—Woods 2003, and an individual of over 3 million ova—our results). However, viewing the gonad weight data suggests this would be inappropriate and these results seem to be biologically valid.

At the other extreme, very low batch fecundity values (100s of ova) have been noted from young red snapper just maturing (our results, Chesney and Filippo 1994, Szedlemayer and Furman 2000, Woods 2003). Schirripa and Legault (1999) discussed this problem, but treated low values as outliers and relied on the maturity functions to minimize any error in doing so. None-the-less, they were concerned that the stock assessment model may have overestimated the reproductive value of females in the 10-20 inch range (254-508 mm).

In summary, simulating the reproductive output of red snapper remains a challenge. The variability in size-at-age precludes simple extrapolation of fecundity-at-length to age. The variation observed in gonad weight and relative fecundity (ova per g) combine to yield batch fecundities that range in the extreme from 100s to millions and would be further exaggerated when extrapolated to annual fecundities. The best fit fecundity-age

relationships may be dome-shaped given the relatively low values for several older fish, but this seems an area for continued examination.

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Table 1.--- Gulf of Mexico spawning location characteristics based upon red snapper females observed with early and late hydrated oocytes (H) or postovulatory follicles (POF). Time caught is the end of a one-hour longline set for most observations. An asterisk denotes a hook-and-line capture where time was known.

State	Date	Degrees Latitude (N)	Degrees Longitude (W)	Depth (m)	Bottom Temperature (°C)	Time Caught	Spawning State
FL	6/01/1995	2858.58	8456.49	41.15		2030	POF
FL	6/02/1995	2905.07	8449.25	36.58		2100	POF
FL	6/02/1995	2859.00	8449.00	42.67		1130	Н
FL	6/03/1995	2900.00	8400.00				POF
FL	6/09/1995	2900.00	8600.00	88.39		1100	Н
FL	6/09/1995	2929.00	8558.00	67.36		1445	POF
FL	6/21/2000	3004.46	8602.37	30.00	25	0818	Н
FL	6/27/2000	3004.07	8602.98	30.00	26		POF
FL	7/25/2000	2936.73	8604.87	58.00	22		POF
FL	7/26/2000	3004.07	8602.98	30.00			POF
FL	7/27/2000	3004.07	8602.98	30.00		0315	POF, H
FL	6/08/2001	2545.00	8349.00	106.68			POF
FL	6/15/2001	2949.51	8559.90	41.00	23	0948	POF, H
FL	6/26/2001	3004.46	8602.37	30.17	24	0855	POF, H
FL	7/10/2001	2900.00	8500.00	121.92			POF
FL	7/18/2001	2936.06	8558.51	41.45	24	0948	Н
FL	8/25/2001	2936.06	8558.51	40.20		1410*	Н
FL	6/03/2002	2400.00	8200.00	60.96			POF, H
LA	8/07/2000	2828.94	9228.12	51.20			POF
LA	8/07/2000	2827.40	9204.50	53.00			Н
LA	8/02/2001	2822.40	9034.30	45.00	22	0605	POF
LA	8/02/2001	2813.78	9023.26	76.00	21	0221	POF
LA	8/08/2001	2811.93	9132.28	85.00	21	1155	POF
LA	8/09/2001	2815.34	9211.81	68.00	21	2207	POF
LA	8/10/2001	2824.56	9227.64	56.00	22	0446	POF
LA	8/16/2001	2814.62	9322.13	56.00	21	2155	POF
LA	8/16/2001	2814.84	9319.96	60.00	22	0022	POF
LA	8/18/2001	2818.18	9354.99	62.00	21	1230	POF, H
LA	8/02/2002	2819.68	9016.09	65.84		0033	POF
LA	8/02/2002	2819.86	9011.49	108.81		0030	POF
LA	8/05/2002	2824.68	9145.95	57.42		1331	POF, H
LA	8/06/2002	2810.78	9230.52	73.33		0542	POF
LA	8/06/2002	2758.41	9243.82	126.19		0931	POF

LA	8/07/2002	2802.25	9316.17	95.83		2133	POF
LA	8/08/2002	2830.46	9233.30	49.01		0934	Н
LA	8/11/2002	2837.94	9309.10	36.03		1208	POF, H
LA	8/11/2002	2817.35	9330.08	58.70		1958	POF
LA	8/12/2002	2830.48	9345.42	41.33		0203	POF
LA	8/12/2002	2817.57	9356.23	59.62		0839	POF
LA	8/27/2002	2819.42	9105.43	60.53		1837	POF
LA	8/28/2002	2828.14	9006.27	65.29		0816	POF
MS	7/03/2001	2851.90	8934.00	70.00	20	1518	Н
ΤX	6/08/2000	2750.86	9410.08	121.00	16		POF
ΤX	6/09/2000	2759.23	9433.76	69.00	21		Н
ΤX	6/10/2000	2803.21	9456.03	68.00	21		POF
ΤX	6/10/2000	2753.20	9508.04	111.00	17		POF
ΤX	6/12/2000	2752.00	9524.22	85.00	20		POF
ΤX	6/13/2000	2746.51	9605.61	72.00	22		POF
ΤX	6/13/2000	2736.55	9616.90	87.00	21		POF
ΤХ	6/13/2000	2734.81	9613.56	99.00	19		POF
ΤX	6/14/2000	2738.19	9623.96	68.00	22		POF
ΤХ	6/14/2000	2726.51	9625.82	89.00	20		Н
ΤХ	6/16/2000	2633.15	9633.35	81.00	20		POF
ΤХ	8/19/2001	2801.39	9439.98	66.00	23	0530	POF, H
ΤХ	8/23/2001	2737.15	9625.34	74.00	22	1205	POF, H
ΤХ	8/23/2001	2738.29	9600.43	126.00	19	0110	POF
ΤX	8/24/2001	2702.00	9635.95	102.00	21	1012	Η
ΤX	8/24/2001	2702.42	9651.52	62.00	23	0639	POF, H
ΤX	8/14/2002	2834.17	9411.06	36.76		0303	POF
ТΧ	8/19/2002	2740.10	9556.09	113.57		1407	POF
ТΧ	8/20/2002	2742.47	9632.92	48.65		0706	POF
ТΧ	8/20/2002	2726.11	9640.51	59.98		2033	POF
ТΧ	8/21/2002	2701.81	9651.27	58.52		2323	POF
ТΧ	8/22/2002	2614.19	9632.51	57.61		2024	POF
ТΧ	8/22/2002	2630.30	9633.02	74.62		1354	POF
ТΧ	8/22/2002	2626.55	9629.87	72.79		1635	POF, H
ТΧ	8/22/2002	2630.30	9633.02	74.62		1354	Н
ΤХ	8/23/2002	2622.93	9446.07	43.34		1601	POF, H
ΤХ	8/23/2002	2650.79	9637.60	95.28		2357	POF
ΤХ	8/24/2002	2715.63	9641.62	70.59		0437	POF
ΤX	8/25/2002	2753.74	9543.61	57.61		0511	POF

Table 2. Estimated duration of spawning season in days based upon the  $1^{st}$  and last observation of hydrated oocytes (H) and post-ovulatory follicles (POF). Estimates in bold represent years in which histological sample size was >100 and samples clearly spanned the months from April to October.

Year	Н	POF
1991	102	99
1992	123	98
1993	158	153
1994	142	193
1995	140	140
1996	114	106
1998	91	129
1999	159	183
2000	188	172
2000	89	118
2001	105	160



Figure 1. Number of red snapper gonads sampled by year and provided to the NMFS, Panama City Laboratory.



Figure 2. Sources of red snapper gonad samples provided to the Panama City lab by fishing mode, gear and state.



Figure 3. Frequency of the leading oocyte stage histologically observed from individual ovaries by month (all years combined). For abbreviations, see legend for Appendix Figure 1.





January - December

Figure 4. Mean (± sd) gonadosomatic index (GSI) (A.) by leading oocyte stage and (B.) by month (all years combined). For oocyte stage abbreviations, see Appendix Figure 1.



Figure 5. Mean ( $\pm$  sd) number of hydrated ova per gram of ovarian tissue by month (all hydrated females, all years).



Figure 6. Spawning proportions for females sampled from eastern and western regions by month.



B.

A.



Figure 7. Proportions of mature females and fitted logistic equations based upon (A.) fork length and (B.) age, for the eastern and western regions.



Figure 8. Spawning frequency by age for females sampled from the western and eastern regions (all years).



Figure 9. Spawning frequency estimates by region and year.

10 A.



10 B.



10 C.



Figure 10. Batch fecundity relationships by (A.) age (B.) fork length and (C.) total weight. These plots represent combined data from the NMFS Panama City Laboratory (n= 265 observations) and The University of South Alabama (n=298 observations; Woods, 2003).



Figure 11. Gonad weight by fork length determined from active females (vitellogenic and more advanced stages determined histologically and microscopically). Further, the data are restricted to females sampled from the peak reproductive months of June-August (n=1069, all years).



Figure 12. Gonad weight by age determined from active females (see legend Figure 11) from the peak reproductive months of June-August (n=859, all years).



### Appendix: Histological Images from Red Snapper Gonads

Appendix Figure 1. Red snapper oocyte stages. A. Primary Growth (PG). B. Cortical alveoli (CA). C. Vitellogenic (V). D. Hydrated (H).



Appendix Figure 2. Red snapper ovaries showing signs of alpha atresia of yolked oocytes (A and B), alpha atresia of unyolked oocytes (D) and beta atresia of yolk oocytes (C). Images A, B and C indicate mature females while image D would not be considered mature.