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An update of Gulf of Mexico red grouper reproductive data and parameters for SEDAR 12

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Introduction

The first detailed study of red grouper (Serranidae: Epinephelus morio) reproduction was conducted by Moe (1969), who describes spawning season, sex ratio, male/female reproductive stages, fecundity (ovarian egg-number), and gives some information on maturation for this protogynous hermaphrodite from the eastern Gulf of Mexico. Bullock and Smith (1991) describe life history traits of red grouper collected in 1977-1980 based on the characterization of Moe (1969). Coleman et al. (1996) compare spawning traits among three shallow-water groupers (red grouper , E. morio; gag, Mycteroperca microlepis; and scamp, M. phenax) and state that red grouper do not aggregate to spawn unlike the latter two species. Brule and Deniel (1996) document the first results on sexual maturation, spawning period, and sex change of red grouper from the southern Gulf of Mexico (Campeche Bank). Johnson et al. (1997) conclude that red grouper can spawn at lengths less than the minimum size limit (at that time, 508 mm). Brule et al. (1999) provide information on size-based reproductive traits, seasonality, depth factors in reproduction and spawning behaviors. Burgos (2001) describes the first spawning frequency estimates for this species, and also presents other basic estimates on age, growth and reproduction for the southeastern US region.

At the NMFS Panama City laboratory, gonad samples from red grouper with associated catch and demographic data were collected and archived beginning in 1991 and continuing annually, to characterize reproduction in the eastern Gulf of Mexico. Based on these data, a synopsis of reproductive information pertinent to a U.S. Gulf of Mexico red grouper stock assessment was presented as a report in 2002 (Collins et al. 2002). The 2002 report provided the first estimates of batch fecundity, spawning frequency and annual fecundity for the eastern Gulf of Mexico.

Our objective in this report is to provide an update based upon new samples from 2002-2005 and based upon numerous histological sections that were not previously completed. This involved processing of older archived gonad samples from 1991-2002; most of which were not completed by 2002. Additional fecundity information is also presented

and is based upon an improved method of counting hydrated ova. Thus, more information about fecundity, annual spawning activity, maturity and sex transition is available since the last stock assessment.

Methods

Efforts were made to obtain lengths (mm), weights (kg), gonads and otoliths from commercial and recreational fisheries, and fisheries-independent (scientific) surveys from the Gulf of Mexico (Lombardi-Carlson et al. 2006). Commercial boats targeted included both bandit-boats and long-liners. Recreational boats sought were charterboats and headboats. In 2001, a cooperative fishing program was begun to provide reproductive samples at regular intervals throughout the spawning season. To delineate the spawning season, we used a gonadosomatic index that expressed gonad weight as a percentage of total body weight less gonad weight (Burgos 2001). Spawning duration was also examined by looking at the distribution of hydrated and vitellogenic females over time. Differences in duration were examined between the northern and southern areas of the west Florida shelf (N and S of 28˚ N latitude) and between selected years based upon the number of available samples.

Maturity

As red grouper have been found to be protogynous hermaphrodites (female first, then male), all transitional and male fish were considered to be mature. We used two methods to define maturity for female red grouper: 1) we estimated the percentage of females sampled throughout the year that histologically exhibited evidence of spawning activity by size and age and 2) we estimated the percentage of females that exhibited developing or sexually active ovaries (vitellogenic or hydrated ova, postovulatory follicles, or spent) during the peak spawning months (March, April, and May) by size and age.

In the first method, immature females could be distinguished from mature but inactive females by their lack of any evidence of previous spawning including presence of melanomacrophages (brown bodies), remnant hydrated oocytes external to the lamellae, muscle bundles and showing a relatively small ovary size; following criteria in Pears et al. 2006 (and citations within). Females displaying vitellogenic or hydrated oocytes (yolked oocytes) and spent stages were defined as “definitely mature” (consistent with terminology used in the MARMAP program; D. Wyanski personal communication, and in Fitzhugh et al. 2005).

The second method has been defined as a measure of “effective maturity” as it recognizes that despite evidence of prior spawning, not all mature females may be sexually active in a given year (Pears et al. 2006). “Inactive” females included those defined earlier as immature but also included females with primary growth and cortical alveolar-stage oocytes as leading stage and displaying evidence of a prior year’s development to spawning stage. “Active” females exhibited vitellogenic, hydrated, and spent stages. Recently-spent females exhibited a notable proportion of atretic-stage yolked oocytes distributed throughout ovarian lamellae. Inactive mature females that were thought to have developed/spawned in years past exhibited melanomacrophages and muscle bundles as previously mentioned and only sparse remnant-yolked oocytes that persisted external to the lamellae, usually encapsulated in “plugs”.

Specimens were assigned to 5 cm total length classes or age classes and the proportion mature was related to length or age classes using logistic regression weighted by the numbers in each length or age class. The logistic model, based upon the Gompertz function, where P_x = proportion mature in each length or age class, was fitted to the data using maximum likelihood (logistic regression, XLSTAT version 7.5 analytical software).

Spawning frequency

Spawning frequency was estimated based on the average spawning proportion of mature females showing hydrated ova or postovulatory follicles (Day-0 proportion, Fitzhugh et al. 1993, Nieland et al. 2002) out of the total mature (active) females (determined histologically). The inverse of the spawning proportion yields the average expected interval in days between spawning events. The overall spawning season duration in days divided by the average interval yields the expected number of spawns per female per annual reproductive season.

Fecundity

Batch fecundity was determined using the hydrated oocyte method. Ovarian tissue samples were cross sectioned, weighed to the nearest 0.001 g and placed in a vial along with 33% glycerol to separate oocytes for the purpose of counting (Collins et al. 1998). 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 (Collins et al. 1998). Batch fecundity was regressed on total length (TL), whole weight (Wt), and age for all hydrated females (Collins et al. 1998, 2002). Any sections showing recent post-ovulatory follicles, suggesting the female had partially spent her current batch, were eliminated from the fecundity estimates. Since reported in Collins et al. (1998, 2002), our procedure for batch fecundity determination has been improved by taking two fixed ~ 0.075 g samples per ovary and then determining the average fecundity value. The locations of the two samples in each ovary were from the periphery and center portion of the cross-section respectively. The two samples were taken from the same region of each ovary, randomly selected from 6 possible regions (the anterior, middle or posterior region of the left or right ovarian lobe). This method was adopted as an improved technique for dealing with fishes having large gonads.

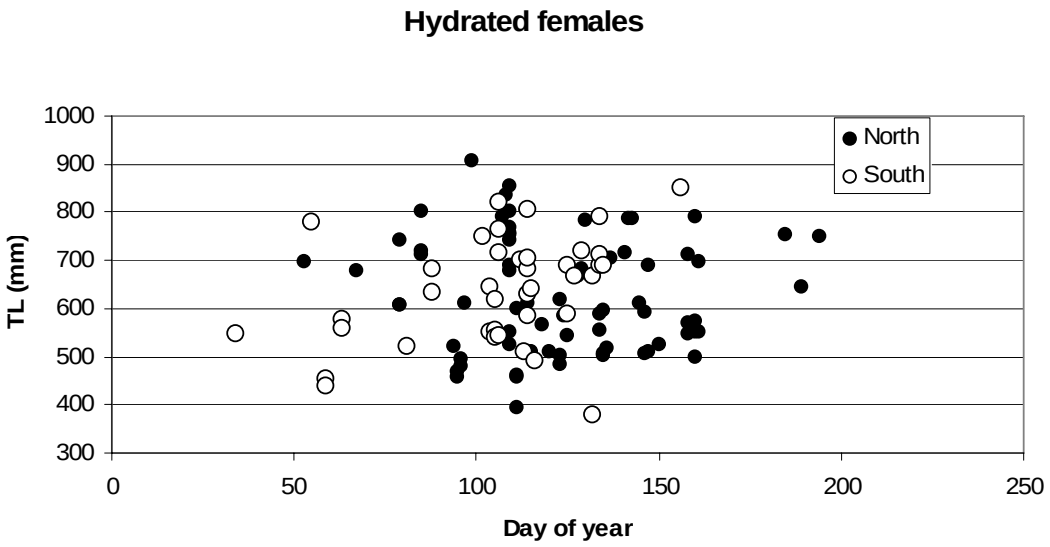
Based on selected females, gonad weight was also examined as a proxy for fecundity. Gonad weight is often available from more females than are fecundity samples. Selection criteria for gonad weight included vitellogenic and hydrated females during the peak spawning months of March, April, and May.

Results

Spawning Seasonality

Based upon presence of hydrated females, spawning duration in north ranged from d 53 to 194 (range 142 d) and in the south from d 34 to 156 (range 123 d) when data were aggregated over all years (1991-2005, Figure 1).

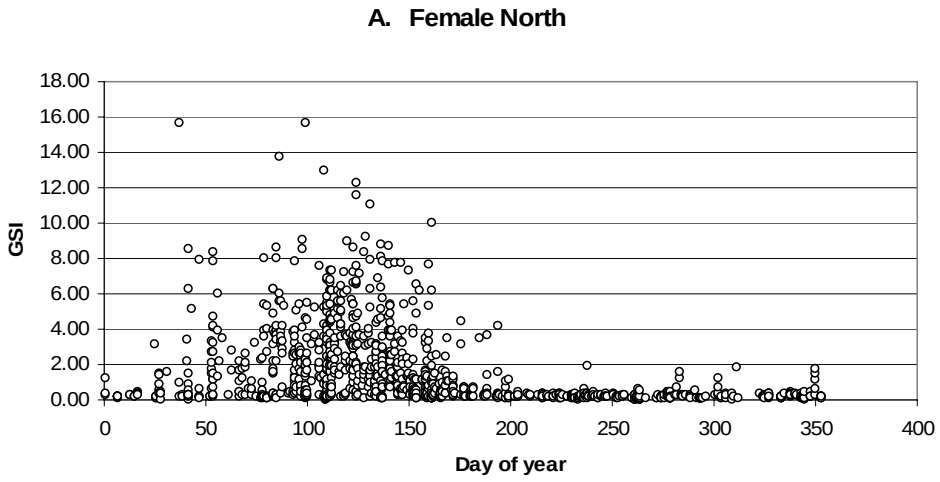
Figure 1. Hydrated females from north and south of 28˚ N latitude (approximately Tampa).

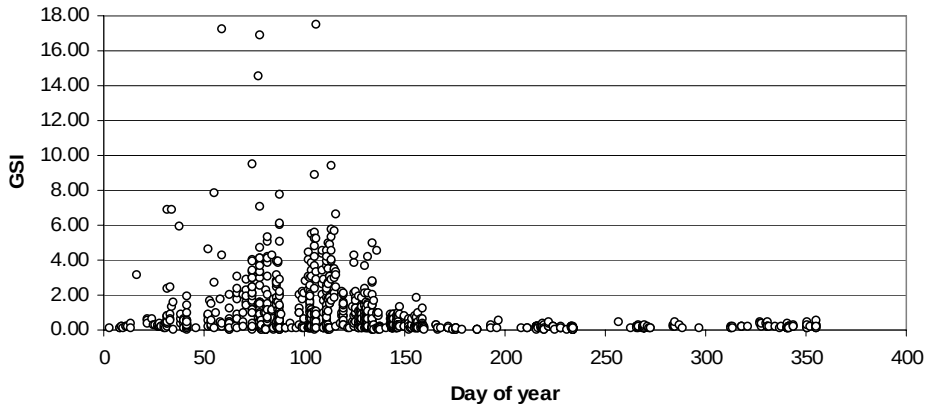


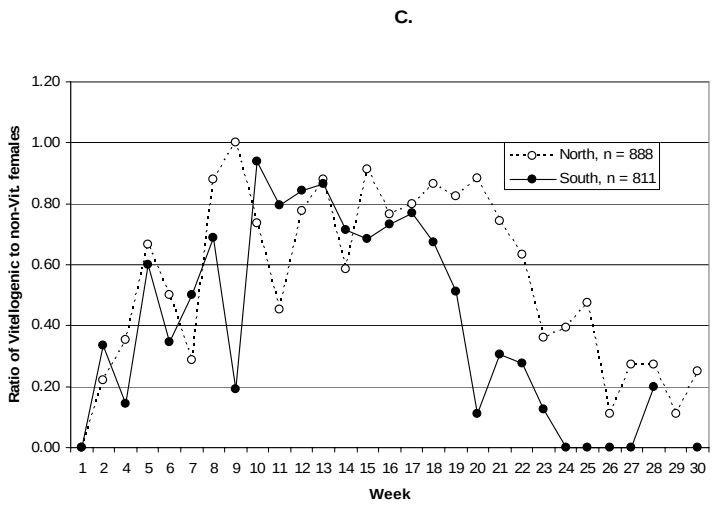
Other results, including the gonadosomatic index (GSI) and the ratio of vitellogenic to non-vitellogentic females, also suggest a slight offset in reproductive season between northern and southern areas of the west Florida shelf (Figure 2). With data aggregated across years, southern red grouper appeared to begin development about the same time as northern fish, but tended to cease reproductive activity a few weeks before northern fish, exhibiting a slightly shorter period (Figure 2).

In any given year, the duration of spawning is likely to be shorter than indicated in Figure 1, as was found to be the case in four select years in which we had a relatively large number of samples (n> 250 each year) distributed throughout the spawning season (Table 1). Spawning durations ranged from 87 to 113 days with an average of 98 days. Despite a N-S offset, the overall spawning peak in the eastern Gulf occurs during March - May (about 90 days) as has been shown in earlier reports (Collins et al. 2002).

Figure 2. Gonadosomatic (GSI) indices for red grouper north (A) and south (B) of 28˚ N latitude, and (C) the ratio of vitellogenic to non-vitellogenic females for the north and the south.







Sexual Maturity

Red grouper are known to be protogynous hermaphrodites (female first, changing to male later in life). Consequently, sexual maturity is reported for females only. Male sexual maturity is addressed under "Sexual Transition" below.

As indicated in Collins et al (2002) and earlier by Moe (1969), Brule et al. (1999), and Burgos (2001), clear determination of size and age of maturity for red grouper has been difficult. Collins et al. (2002) suggested that some mature females may skip spawning in some years, or show little reproductive synchrony among females (e.g., some spawn early and stop, while some may delay and spawn late in the reproductive season), which may explain the difficulty. Recent results from related Epinephelid groupers also show that skipped spawning or highly asynchronous spawning may be occurring (Fennessy and Sadovy 2002, Pears et al. 2006). Discerning skipped from highly asynchronous spawning is also challenging, but we note there is a growing amount of evidence and theory that skipped spawning may be common in relatively long-lived marine fishes and that skipped spawning is more likely to occur in the few years following first spawning than later in life (Rideout et al. 2005, Jorgensen et al. 2006). So we followed a strategy outlined in Pears et al. (2006) of applying a traditional approach (maturity) and comparing it to active fish (effective maturity) during the peak spawning months.

Figure 3. Length at maturity based on definitely mature and immature female red grouper. Logistic regression function (Gompertz): Proportion = EXP(-EXP(-(-2.79 + 1.13E-02*TL))), n=1149, L50 maturity = 280 mm TL.

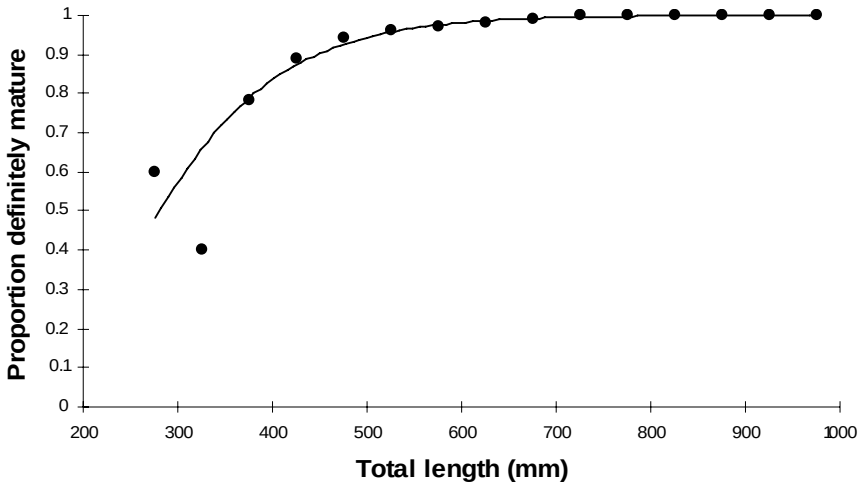


Figure 4. Age at maturity based on definitely mature and immature female red grouper. Logistic regression function (Gompertz): Proportion = EXP(-EXP(-(-1.15 + 0.74*Age))), n=1067, A50 maturity = 2.

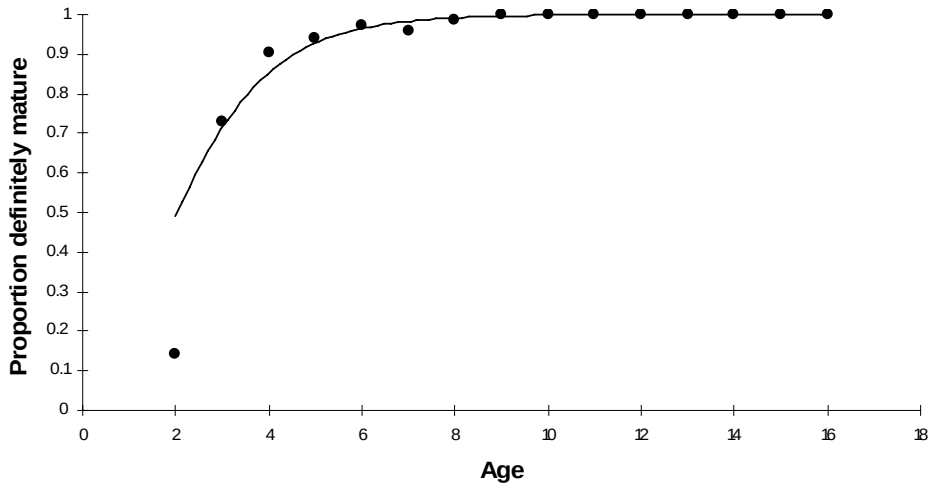


Figure 5. Length at maturity based on effectively mature (active) and inactive female red grouper during the peak spawning period. Logistic regression function (Gompertz): Proportion = EXP(-EXP(-(-2.0 + 6.22E-03*TL))), n=1279, L50 maturity = 380 mm TL.

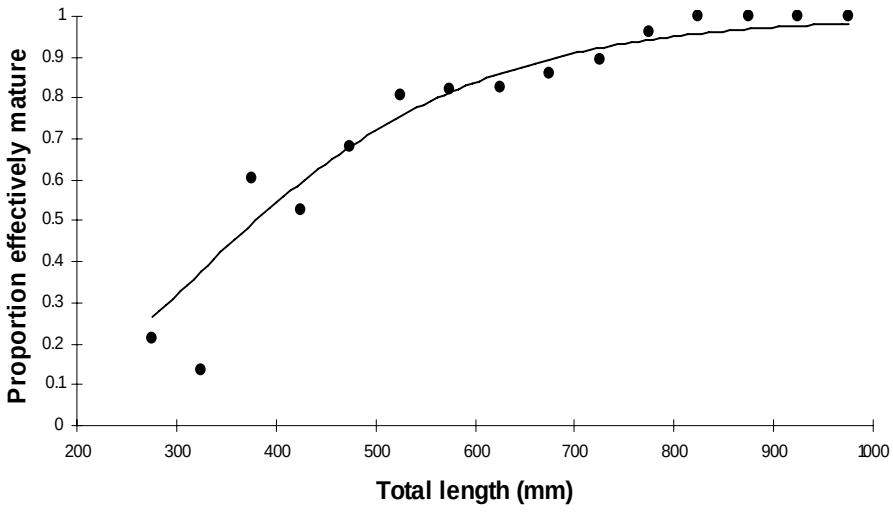
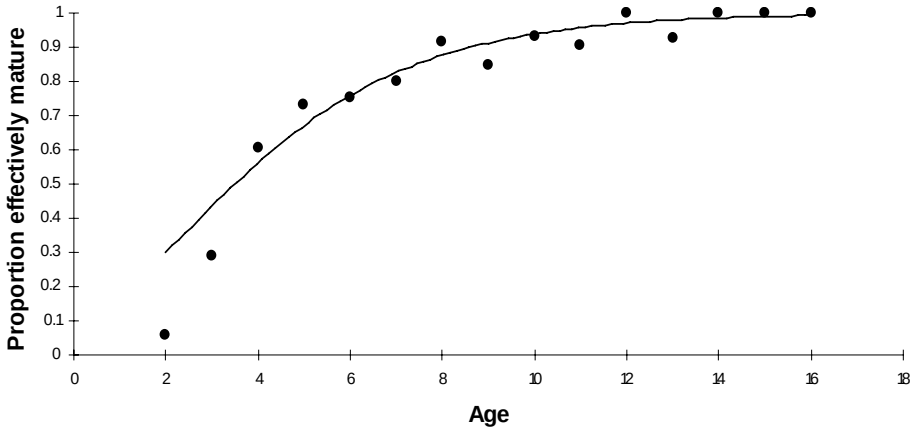


Figure 6. Age at maturity based on effectively mature (active) and inactive female red grouper during the peak spawning period. Logistic regression function (Gompertz): Proportion = EXP(-EXP(-(-0.92 + 0.369*Age))), n=1237, A50 maturity = 3.5 yrs.



Based on our histological interpretations of 'definitely' mature and immature females (showing no evidence of prior spawning), the size and age at 50% maturity were approximately 280 mm TL at age 2 (Figures 3 and 4). As expected, based on observations in similar species (Pears et al. 2006, Jorgensen et al. 2006), effective maturity determined from active females during the spawning season reflected an increased size and age at 50% maturity, to 380 mm TL at 3.5 years (Figures 5 and 6). By comparison, previous workers note higher estimates for length at 50% maturity (509 mm FL, Brule et al. 1999; 487 mm TL, Burgos 2001) or approximate length at maturity (485 mm FL, Moe 1969). Burgos (2001) also estimated maturity by age, as 50% mature at 2.4 years in the southeastern US. In the 2002 assessment, all fish older than 2 years were assumed to be mature (NMFS 2002, pg. 4).

Sexual Transition

As noted in Collins et al. (2002) the proportion of males increased- and females decreased- with increasing length and age. With more histological samples completed (> 3000) we detected a smaller size and younger age at 50% transition than in the 2002 report: 50 % transition between 800-900 mm TL by age 13 (Collins et al. 2002), versus 50% transition at 765 mm TL by age 10.5 years (Figures 7 and 8). Moe (1969), Brule et al. (1999) and Burgos (2001) noted fairly rapid transition between 600-700 mm TL. Burgos (2001) estimated 50% of females changed sex at 690 mm TL and age 7.2 years. Brule et al. (1999) estimated the median size at sexual transition at 597 mm FL. In the 2002 assessment, various data sets were combined to fit the proportion female; 50% at approximately 16 years based on visual interpolation of Figure 4 in the assessment (NMFS 2002, pg. 35).

Figure 7. Proportion female by size, assessed histologically. Logistic regression function (Gompertz): Proportion = EXP(-EXP(-(3.57 -4.33E-03*TL))), n = 3479, L_{50} transition = 740 mm TL.

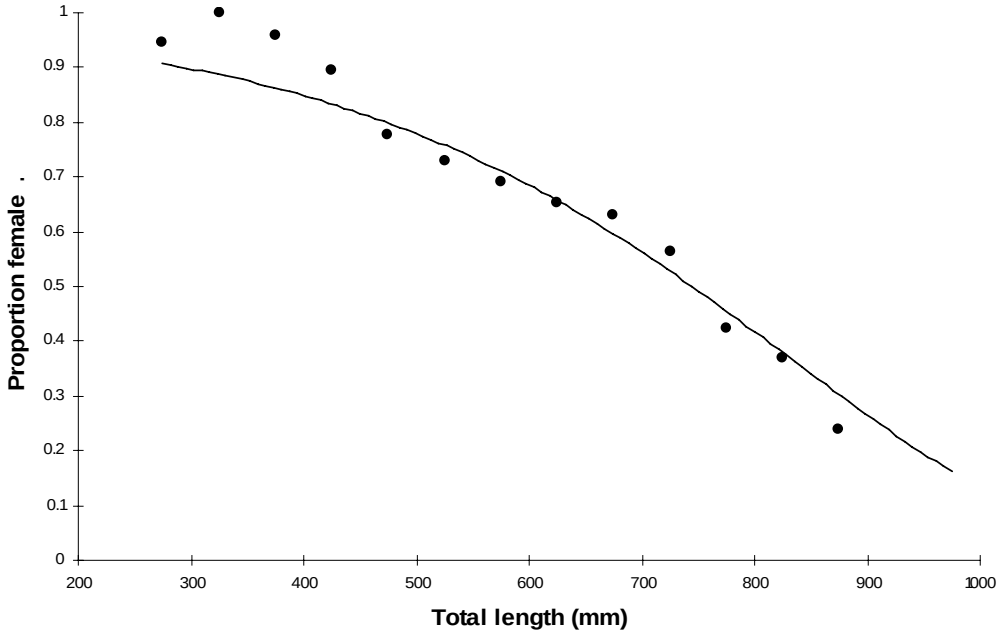
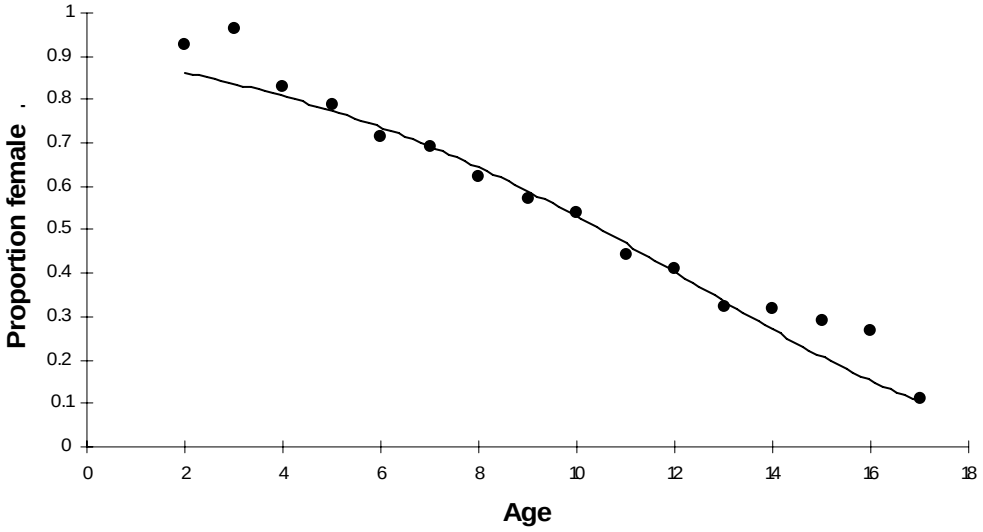


Figure 8. Proportion female by age, assessed histologically. Logistic regression function (Gompertz): Proportion = EXP(-EXP(-(2.27 -0.18*Age))), n = 3277, A_{50} transition = 10.5 years.



Batch Fecundity

Batch fecundity increased with age, length and weight of females and linear regressions are shown in Figures 9-11. The number of hydrated samples were doubled (to n=73) compared to Collins et al. (2002) but the summary statistics remained similar: range 1.5 thousand to 2.8 million ova, mean 284 thousand ova, standard deviation 37 thousand ova.

Figure 9. Batch fecundity by total length. Regression equation: Batch fecundity (number of hydrated ova) =1984.6*TL - 902126, r^2 = 0.38, n=77.

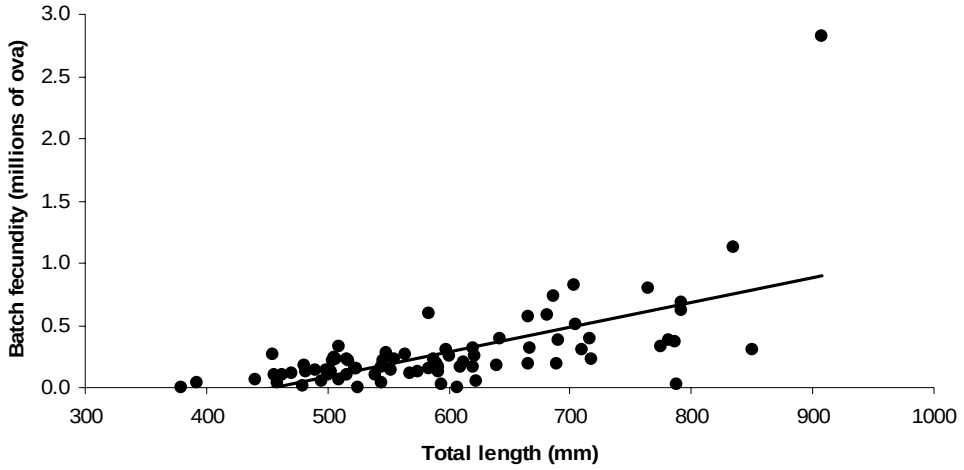


Figure 10. Batch fecundity by age. Regression equation: Batch fecundity (number of hydrated ova) = 97986*Age - 409775, r^2 = 0.49, n=73.

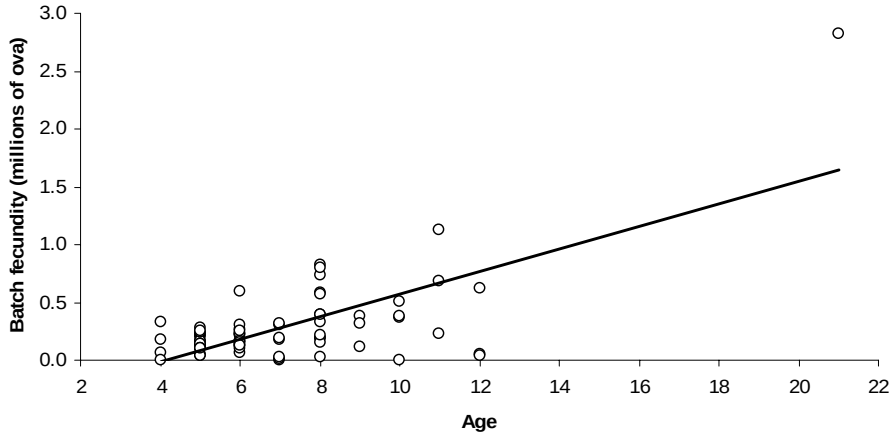
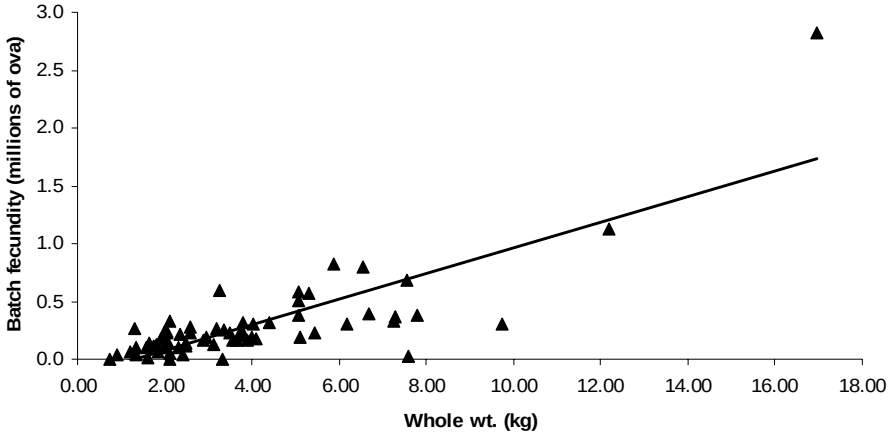


Figure 11. Batch fecundity by whole weight (kg). Regression equation: Batch fecundity (number of hydrated ova) = 10035*whole wt. - 137820, r^2 = 0.65, n=66



Spawning Frequency

We examined spawning frequency for four years in which we had relatively numerous samples (Table 1). For the year 2001, we obtained similar estimates to Collins et al. (2002); about a 5.4 day interval between spawning. Based upon estimates of the 2001 spawning season duration, this extrapolates to estimates of 26 spawns per year (Collins et al. 2002) compared to our 21 spawns per year (Table 1). Burgos (2001) cited within Collins et al. (2002) found a spawning interval based upon HO and POF methods of about 8.8 d in the late 1990s. In our more recent years, it seems the average interval between spawns can be even longer—to 35 days for the HO method in 2005 (Table 1). While there were year differences, the frequency of hydrated females within a year was very similar to the frequency of females detected with recent postovulatory follicles. Thus, the large differences between some years are observed whether the HO or POF method is used (Table 1).

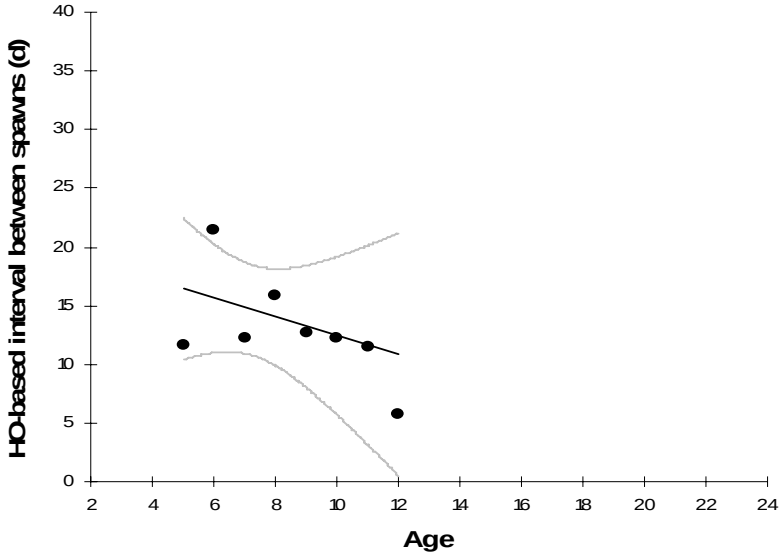
To determine whether there may be an age effect to spawning frequency, we calculated the average interval between spawns based upon the samples summarized in Table 1. We found that there was a possible age relationship whether the HO or the POF method was used; older fish had shorter intervals and thus would be expected to spawn more times per year. However, the regressions, weighted by the number of active females in each age class, were highly variable and non significant (p= 0.38 and 0.31 for HO and POF methods respectively, Figure 12).

Table 1. Annual spawning frequency estimates for years in which more than 250 females ovaries were histologically examined and in which >100 active mature females were sampled during the spawning season (active includes vitellogenic, hydrated and spent stages). Season duration is based on the period in which spawning females (possessing hydrated oocytes-HO, or postovulatory follicles-POF) were observed.

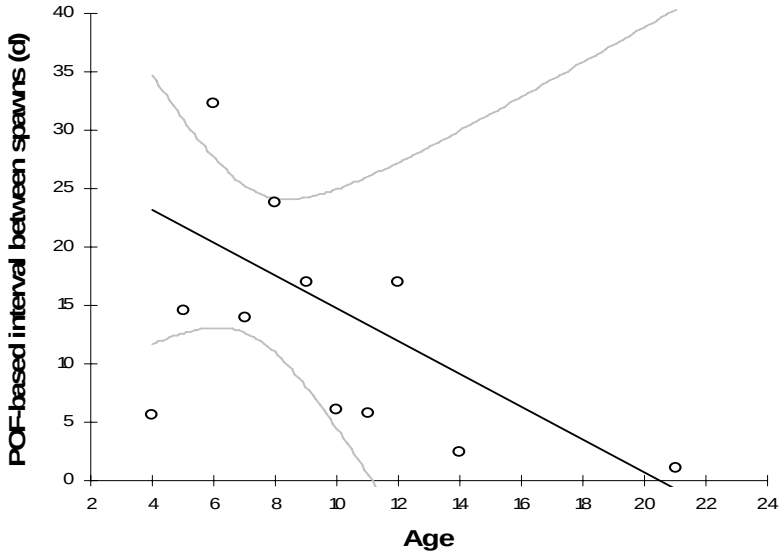
Year	# Active	HO	New POF	HO Spawning interval (d)	POF Spawning interval (d)	Estimated season duration (d)	HO-based estimate of annual spawns	POF-based estimate of annual spawns
2001	102	19	15	5.37	6.80	111	21	16
2002	179	6	11	29.83	16.27	80	3	5
2004	203	15	15	13.53	13.53	113	8	8
2005	139	4	5	34.75	27.80	87	3	3
Averages				20.87	16.10	98	5	6

Figure 12. Estimates of spawning frequency (average interval in days between spawning events per active female) by age based upon females (A.) exhibiting hydrated oocytes (HO) and (B.) exhibiting recent postovulatory follicles (POF) during years 2001, 2002, 2004 and 2005. Grey lines indicate the 95% confidence intervals for mean predicted values. Weighted regressions: HO-interval between spawns = 20.4 – 0.79*Age, r^2 = 0.13, mean interval = 14.6 days, mean age in regression = 7.3 years, n= 44 hydrated females/595 active females. POF-interval between spawns = 28.8 –1.41*Age, r^2 = 0.115, mean interval = 18.6 days, mean age in regression = 7.3 years, n=46 POF females/607 active females.

A.



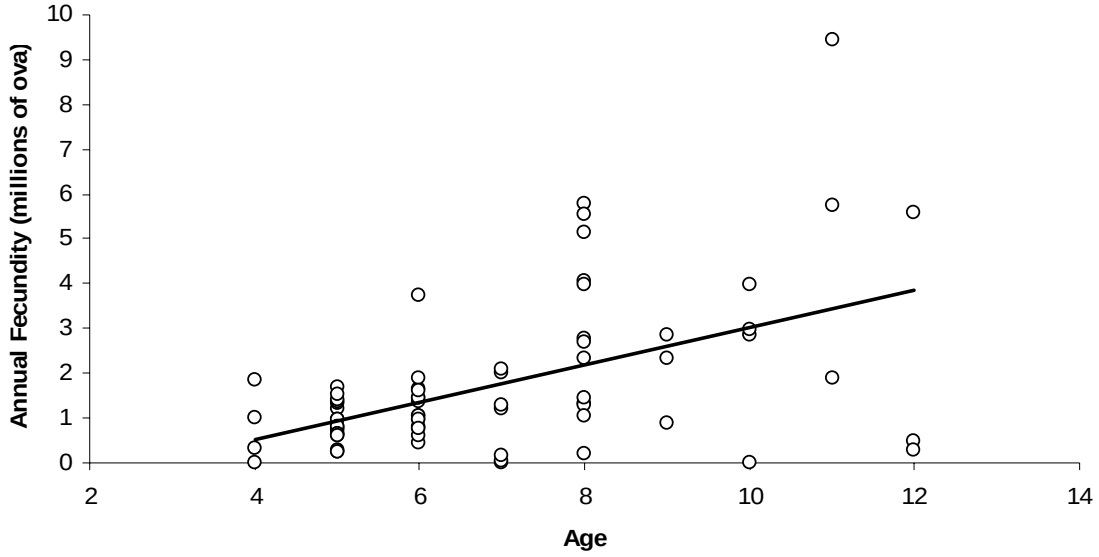
B.



Annual Fecundity

Annual fecundities are the product of batch fecundities and the expected number of spawns per year. If there is no age effect effect upon spawning frequency, batch fecundity by age can serve to calculate age-specific relative fecundities. Assuming there is an age effect as shown in Figure 12A, mean annual fecundity increases to 1.73 million ova and standard deviation increases to 1.72 million ova (Figure 13).

Figure 13. Annual fecundity by age based upon a 98 d average season (Table 1). Regression equation: Annual fecundity (number of hydrated ova) = 416068*Age - 1E+06, $r^2 = 0.26$, n=72. In this regression, a single value for an age 21 female was not included (estimated fecundities: batch- 2.8 million ova, annual- 72.6 million ova).



Gonad Weight Proxy For Fecundity

The gonad weight of developed females (with vitellogenic and more advanced ova) has been used as a proxy for fecundity and relationships of gonad weight to length, whole weight and age are shown in Figures 14, 15 and 16 respectively. During the 2002 assessment, a similar power function of gonad weight and age (but with fewer observations) was used to calculate per capita fecundity at age (NMFS 2002).

Figure 14. Gonad weight (g) by total length (mm). Least squares fit of the power function: GWT =9E-09TL$^{3.5528}$, r^2 = 0.35, n=505.

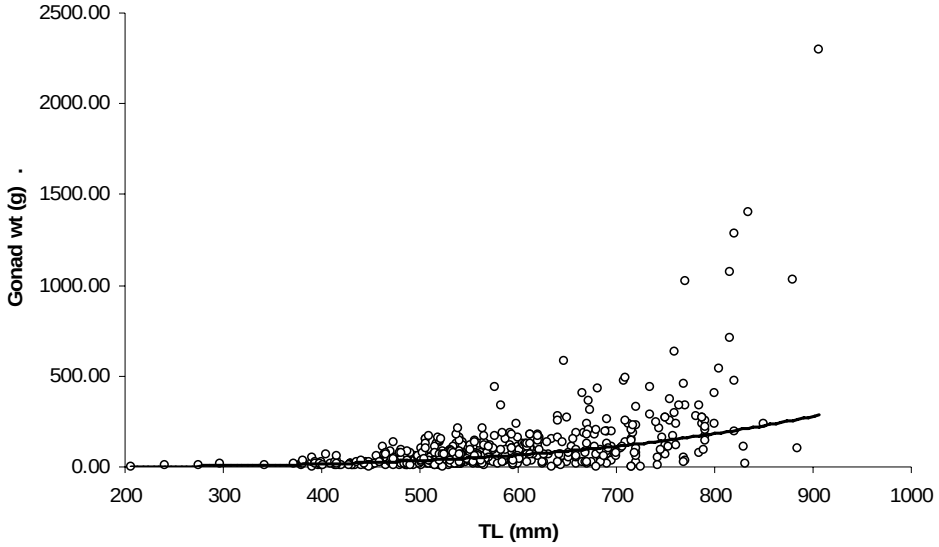


Figure 15. Gonad weight (g) by whole weight (kg). Regression equation: GWT= 55.539WT - 79.622, r^2 = 0.45, n=505.

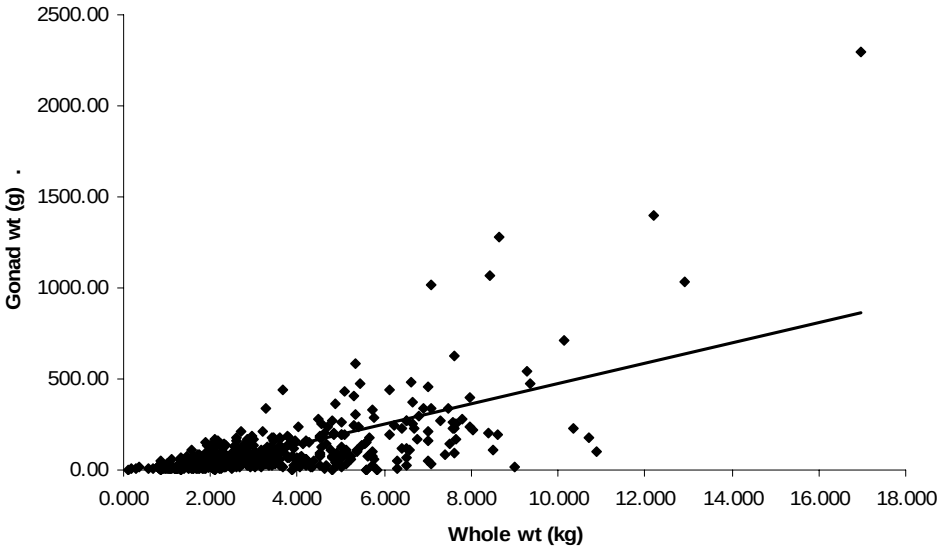
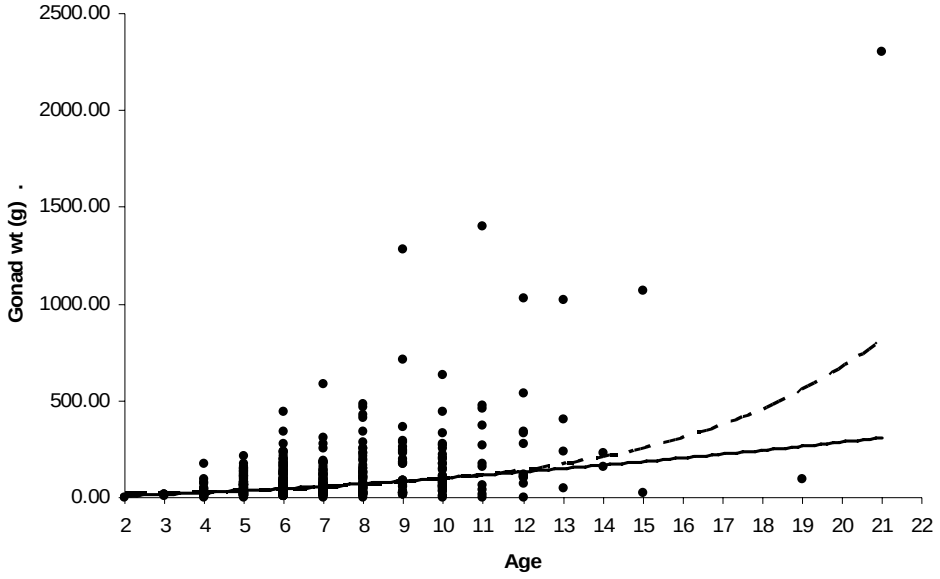


Figure 16. Gonad weight (g) by annual age. Least squares fit of the power function (solid line): GWT = $3.2107Age^{1.4998}$, r^2= 0.15. Fit of the exponential function (dashed line): GWT= $4.365e^{0.1924Age}$, r^2 = 0.14, n=505.



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