Characterization of red grouper (Serranidae: Epinephelus morio) reproduction from the eastern Gulf of Mexico

L. A. Collins, G. R. Fitzhugh, L. A. Lombardi-Carlson, H. M. Lyon, W. T. Walling, and D. W. Oliver

National Marine Fisheries Service
Southeast Fisheries Science Center
3500 Delwood Beach Road
Panama City, FL 32408

Panama City Laboratory
Contribution Series 2002-07.
July 26, 2002

Abstract

Red grouper gonads from commercial, recreational and fisheries-independent sources were sampled from the eastern Gulf of Mexico from Panama City to Key West between 1992 through 2001. Sampling of this protogynous hermaphroditic species was evenly distributed across the decade with approximately 50% of the collections made during years 1992 to 1996 and 50% from years 1997 to 2001. Most fish (72%) were collected during the months of March through August. Macroscopic/microscopic examinations were used to assign gonad-maturation stage to 2,311 fish. Sex ratio from histological examination of 573 red grouper varied little for 1996 and 2001: 69 to 71% were female, 29 to 28% were male, and 3 to 1% were transitional, respectively. Size at 50% maturity was estimated to be about 400-500 mm TL in agreement with previous studies. Age and length at 50% transition (from female to male) occurred at about 13 years and 800-900 mm TL respectively. Most spawning occurred in March, April and May but the percentage of females that were active during this period varied between 36% and 82% from 1993 to 2001. Batch fecundity estimates for all years together (n=35) ranged from 24,300 (459 mm total length (TL), 5 year-old sampled in 2001) to 2,322,517 (907 mm TL, 21 year old sampled in 1995). Batch fecundity was best predicted by TL (r^2 =0.57, n=35) rather than by age (r^2 =0.39, n=34). Spawning frequency for 2001 was estimated as 26 spawns from both the hydrated oocyte and postovulatory follicle methods. Annual fecundity was estimated as 631,400 to 17,141,170 for 2001 (n=14). If spawning frequency could be assumed constant across years, then maximum estimated annual fecundity for an individual female with the largest observed batch fecundity would be 60,385,430 hydrated ova.

Introduction

The red grouper (Serranidae: Epinephelus morio) is one of several target species which are the subject of a research project that began in 1991 to characterize reef fish age, growth and reproduction in the Gulf of Mexico. Previous publications/reports on age and growth of red grouper from our laboratory are Johnson and Collins 1994, Johnson et al. 1997, and Lombardi-Carlson et al. 2002. Some estimates of reproductive condition of red grouper were included in Johnson et al. 1997, but the present report is our first contribution concentrating solely on reproduction of this species.

Information on red grouper reproduction in the literature has greatly increased in the last eleven years. The first and most detailed study was 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) describes life history traits of red grouper collected in 1977-1980 based on the characterization of Moe (1969). Coleman et al. (1996) compares spawning traits among three shallow-water groupers (red grouper ,E. morio; gag, Mycteroperca microlepis; and scamp, M. phenax) and states 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) concludes that red grouper can spawn at lengths less than the minimum size limit of 508 mm. Brule et al. (1999) provides information on size-based reproductive traits, seasonality, depth factors in reproduction and spawning behaviors. The most recent research on red grouper reproduction from fish caught off North Carolina and South Carolina describes the first spawning frequency estimates for this species, and also presents other basic estimates on age, growth and reproduction for the south Atlantic region (Burgos 2001).

The main purpose of this report is to update the information on reproduction of red grouper from the eastern Gulf of Mexico (west Florida shelf). While we will be comparing many reproductive traits with earlier studies, our update provides a longer term profile (1992 to 2001) for gonadosomatic indices and macroscopic/microscopic based results, along with new histological results for 1996 and 2001. The first estimates of batch fecundity, spawning frequency (for the eastern Gulf of Mexico), and annual fecundity are provided.

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. 2002). 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, based largely on the efforts of a single bandit fisherman, to provide reproductive samples at regular intervals throughout the spawning season.

To delineate the spawning season and estimate reproductive potential, we used a gonadosomatic index that expressed gonad weight as a percentage of total body weight less gonad weight (Burgos 2001), as well as macroscopic, microscopic and histological gonad staging. Macroscopic (staged by eye) and microscopic (staged microscopically from a raw tissue smear) staging of fresh ovaries and testes followed Collins et al. 1998: 1=resting/immature; 2 =early developing; 3=late developing; 4=spawning; and 5=spent. Histological staging by sex is defined in Table 1. We used histological stages as the best indicator of the spawning season for 1996 and 2001, with the beginning and end of spawning shown by the first and last occurrence of active (Stage 3-6) ovaries.

Sex ratio by length and age was determined to identify the transition from female to male. Length and age at maturity were estimated to see if changes occurred over time (c.f. Moe 1969) and compared with those estimates from other geographic areas (Brule et al. 1999, Burgos 2001). Maturity was defined for females sampled during peak spawning months (March-May) based on active ovaries (exhibiting vitellogenic and more advanced ova) and based on ovaries exhibiting cortical alveolar-stage ova and notable atresia (evidence of prior spawning). We used the hydrated oocyte method for batch fecundity estimates (Hunter et al. 1985) and the hydrated oocyte and postovulatory follicle methods for estimates of spawning frequency and annual fecundity (Hunter and Macewicz 1985).

Results and Discussion

Sample number and origin

A total of 2,311 red grouper with gonads were sampled from the U. S Gulf of Mexico from Key West to the Florida panhandle region. Sampling was evenly distributed across the decade with approximately 50% of the collections made during years 1992 to 1996 and 50% from years 1997 to 2001. A majority of samples (72%) were sampled during the months of March through August encompassing the peak spawning season.

Most samples came from landings in northwest Florida (65%) and west-central Florida (19%). Most (49%) samples came from commercial sources (primarily long-line and hook-and-line gears), followed by recreational (35%) and scientific survey (12%). Fishery-dependent sampling of gonads deviated from the age structure sampling (Lombardi-Carlson et al. 2002) 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 to make the collections. Due to this circumstance, our commercial samples were more representative of bandit catches (76%) than long-line catches (24%), the reverse of the actual fishery. A total of 571 gonads, collected during 1996 and 2001 (n= 213 and 358, respectively), were analyzed histologically. All 6 stages of ovaries and 4 stages of testes were found, along with transitional gonads.

Sex ratio and size/age at transition

Based on 1996 and 2001 histology samples, 69% and 71% of the red grouper were females, respectively; the remaining fish were males (28.6% and 27.9%, respectively) and transitionals (2.8% and 1.1%, respectively), 1 male/transitional :2.3 females. Compared to other studies, these west Florida shelf numbers reflect a relatively high ratio of males to females. A study from the Campeche Banks showed a male to female ratio of 1:3.4 (Brule et al. 1999) and a recent survey from the U.S. south Atlantic showed a male to female ratio of 1:6.6 (Burgos 2001). Moe's (1969) earlier findings on the west Florida shelf also indicated that males were relatively more rare than our current findings (1 male/transitional: 5 females). These results may be related to the observation that we generally found larger red grouper in our samples than has been reported in other studies even taking into account the possible size differences between hook and line and long-line sources of samples (Lombardi-Carlson et al. 2002).

We determined that the macroscopic-examination of fresh gonads was usually an accurate method of determining sex when compared to histological examination. The macroscopic visual method correctly identified 83% of females (n=365) and 94% of males (n=47) (Table 2). However, transitional fish could not be macroscopically determined with any certainty. Transitional fish, later confirmed histologically, were most often staged as males during initial examination (Table 2). Therefore we aggregated males and transitionals, versus females, for our macroscopic/microscopic statistics.

Similar to other studies, we found that males increased in proportion with increasing length and age (Figures 1 and 2). Our results are very similar to Moe (1969), Brule et al. (1999) and Burgos (2001) with proportion male at about 20% or less through about 600 mm TL. But Moe, Brule et al. and Burgos noted fairly rapid transition between about 600-700 mm TL with about 50% male proportion occurring by 700-800 mm TL. We observed a slower male transition (to 50 % by 800-900 mmTL; Figure 1). Age at 50% transition was more inconclusive; by about age 9 and 17 (Burgos 2001 and Moe 1969 respectively) and by age 13 from our data (Figure 2). These findings may be explained by different sources of samples and limited sample numbers from the larger size- and older age classes. But there is also some evidence that asymptotic size is larger now than during earlier studies from the eastern Gulf of Mexico (Lombardi-Carlson 2002) and this may be correlated to our larger size (if not age) at transition.

Spawning season

GSI varied by month (Figure 3) with the greatest values (> 8) occurring from February through June. Histological assessment indicated a similar seasonality; that spawning occurred from March 23 to June 20 in 1996 (a period of 89 days) and from February 6 to June 26 in 2001 (140 days). In general the main spawning season was from March through May, as indicated by GSI values (Figures 3 and 4) and histology (Table 3). Although a few individuals were detected with relatively high values for GSI outside of the March-May period (e.g., June of 1995), annual differences were not apparent given the available sample sizes (Figure 3). One large and old red grouper with a large histological-stage 4 (early hydration) ovary was found on August 25, 1996, suggesting that larger/older fish may spawn longer than smaller/younger fish. Unfortunately, the gonad samples available thus far have not included enough old fish to test this hypothesis.

Batch fecundity

Batch fecundity estimates are typically based on a few sparse observations as hydration is a short term event occurring during final maturation of ova (Hunter et al. 1985). Hydrated females were only detected in years 1994, 1995, 1996 and 2001. Based on 35 females, batch fecundity ranged from 24,300 (for a 459

mm TL, 5 year-old) to 2,322,517 (for a 907 mm TL, 21 year old) (Figure 5). Our batch fecundity regressions indicated that total length was a better predictor of batch fecundity than age (Figure 5).

Spawning frequency and annual fecundity

We estimated spawning frequency for 1996 and 2001, the two years for which histology sections have been completed. But because batch fecundity estimates are so dependent upon frequent sampling and commonly based on haphazard fishery-dependent sampling, results can be variable if the timing and source of the samples are not taken into account (Burgos 2001). We sampled more active females in 2001 (107 versus 53 for 1996). Also more complete sample coverage in 2001 resulted in better temporal characterization, particularly during the earliest part of the sampling season (Table 3) and therefore we chose 2001 as the best year for this estimate. The estimate for 2001 was 26 using both the hydrated oocyte and postovulatory follicle methods (Table 3). By comparison, Burgos (2001) reported a spawning frequency estimate of 42 from red grouper sampled off North and South Carolina (see Table 3).

Based on the 2001 spawning frequency and batch fecundity estimates, annual fecundity was then estimated to range from 631,400 to 17,141,170 ova (n=14). If spawning frequency was the same (26) in 1995 when the largest hydrated female was collected, then our maximum annual fecundity estimate for this female would be 60,385,430 hydrated ova. The only other estimate of fecundity was based upon counts of yolked oocytes (448,400 to 5,735,700 oocytes; n=14 females; Moe 1969). However, a yolked oocyte estimate has been determined to be an inaccurate estimate of spawning potential in fishes that are batch spawners (Hunter et al. 1995).

Maturity and vitellogenesis

Size-at-maturity information is often used in stock assessments to define the size or age at which individuals are reproductively active and producing offspring. It is a fundamental principal of fisheries management to allow individuals to grow to maturity before allowing substantial harvest. It is often found and thus generally assumed that as individuals increase in size and age, they are more likely to be reproductively active. The fractions of mature individuals within size-classes are often plotted as a

function and typically, size at first maturity, 50% maturity, and maximum maturity are estimated (Lmin, L50, Lmax). These definitions of maturity, however, may be less important or less apparent if individuals vary in annual reproductive development and if reproduction is de-coupled to a substantial degree from size or age.

We found evidence of this de-coupling of the reproductive readiness-size relationship among female red grouper when we tried to estimate the traditional measures of maturity. When we used macroscopic and microscopic criteria to discern females who were reproductively active (vitellogenic, hydrated or recently spent) from females who are inactive (ovaries without vitellogenic ova) during the peak 3 months of spawning (March, April, May) we detected a number of females, from a broad size range who were not in active spawning condition (Table 4). While there was a slight trend for larger females to be active, among the larger size-classes (eg. 50 mm size classes from 600-850 mm TL) the fraction of active females ranged from 59 to 73 % (all years combined) leading us to speculate that a substantial number of females were not spawning.

We don't have large enough sample sizes to clearly resolve whether or not there are annual differences in the number of active vs. inactive females but our findings suggest that differences may occur. Comparing the 3-month spawning periods between 1993 and 2001, we observed the lowest fraction of active females in yr-1993 (36%) and the highest in yr-2001 (82%; Figure 6). While March, April and May are peak months in the eastern Gulf, there is evidence by us and others (Figure 3; Moe 1969, Brule et al. 1999, Burgos 2001) that spawning can occur to some degree through much of the year. Thus we cannot eliminate the possibility that females who were large enough but were inactive during the peak period spawned at other times or in other years. In fact, there is some evidence for this.

When we examined the available ovarian histological sections by size-class, we observed that females who were inactive during the peak months, often exhibited atretic material providing evidence of previous spawning. This approach, using histological sections, is often used to distinguish inactive or resting females from truly immature females based on the presence or absence of atretic material. Although there

has been some debate on whether this distinction can be readily made in red grouper, previous workers used this approach to define maturity (Brule et al. 1999, Burgos 2001). When we did the same, we observed increasing proportions of "mature" fish among increasingly larger size-classes similar to these previous workers. Although we have more histological samples to process, we observed that the smallest female considered mature was 381 mm TL (Lmin), the largest was 980 mm TL (Lmax), and approximately 50% of the females were mature upon reaching the 400-500 mm size classes (Table 4). Previous workers note similar estimates for L50 (509 mm FL, Brule et al. 1999, 487 mm TL Burgos 2001) or approximate length at maturity (485 mm FL, Moe 1969). Minimum and maximum sizes at maturity were also similar except for the comparison with Moe 1969. Moe found smaller sizes at first maturity (236 mm FL) than any workers since. This early finding may also be related to the smaller size in general (asymptotic size) reflected in Moe's data from the 1960s compared to more recent work (Lombardi-Carlson et al 2002).

Further work

All previous studies investigating red grouper reproduction noted some difficulty in fully characterizing the maturity pattern among females (e.g., by size). Moe 1969, speculated that perhaps some females were undergoing vitellogenesis for a year or two before actually spawning. Brule et al. (1999), and Burgos (2001) noted the apparent size and reproductive activity gradient occurring across depth but also noted that resting and truly immature females were difficult to impossible to distinguish and that sexual maturation occurred over a broad size range. Burgos (2001) further noted a substantial number of females were considered indeterminate in their maturity status but that resting females were also observed during the spawning season. With the multi-year data available to us, it appeared that there may be annual variation in reproductive readiness and traits usually associated with maturity and frequency of spawning. Red grouper males exhibit small testis and low GSIs characteristic of a pair-spawning species, and red grouper appear not to aggregate but rather to spawn in small groups (Coleman et al. 1996, Brule et al. 1999). Lack of reproductive synchrony among females may make sense if pair-spawning within small groups tends to limit numbers of actively spawning fish. It is clear that further work needs to be accomplished on the question of reproductive variability by size, year and across a habitat or depth gradient. Certainly, we think it is

important that the sensitivity of assessment-model results to reproductive variables be used to focus future research efforts.

Acknowledgements

We would like to acknowledge and extend our gratitude to the efforts of several port agents, fishermen and colleagues who provided red grouper reproductive samples and information on catch for this study, especially, Tim Brandt, Lew Bullock, Debbie Fable, Greg Fairclough, Lisa Hallock, Tom Herbert, Ed Little, Guy Pizzuti, Renee Roman and June Weeks. Captain Eric Schmidt is credited with beginning a cooperative sampling program to gather habitat and site-specific data. Financial support was received in part by the U.S. Department of Commerce Marine Fisheries Initiative Program (MARFIN).

Literature Cited

Brule, T., and C. Deniel 1996. Biological research on the red grouper (Epinephelus morio) from the southern Gulf of Mexico, p. 28-42. *In* F. Arreguin-Sanchez, J. L. Munro, M. C. Balgos and D. Pauly (eds.) Biology, fisheries and culture of tropical groupers and snappers. ICLARM Conf. Proc. 48, 449 p.

Brule, T., C. Deniel, T. Colas-Marrufo, and M. Sanchez-Crespo 1999. Red grouper reproduction in the southern Gulf of Mexico. Trans. Am. Fish. Soc. 128 (3): p. 385-402.

Bullock, L. H., and G. B. Smith 1991. Seabasses (Pisces: Serranidae). Fla. Mar. Res. Inst. (Part II) Vol. VIII, 243 p.

Burgos, J. M. 2001. Life history of the red grouper (Epinephelus morio) off the North Carolina and South Carolina coast. Master's Thesis. University of Charleston, South Carolina.

Coleman, F.C., C.C. Koenig and L.A. Collins. 1996. Reproductive styles of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environmental Biology of Fishes 47:129-141.

Collins, L. A., A. G. Johnson, C. C. Koenig and M. S. Baker, Jr. 1998. Reproductive patterns, sex ratio, and fecundity in gag, Mycteroperca microlepis (Serranidae), a protogynous grouper from the northeastern Gulf of Mexico. Fish. Bull. 96: 415-427.

Hunter, J. R., N. C. H. Lo, and R. J. H. Leong. 1985. Batch fecundity in multiple spawning fishes. Pages 67-77 in: R. Lasker (ed.) An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy*, Engraulis mordax.* NOAA/NMFS Tech. Rep. **36**. 99 p.

Hunter, J. R. and B. J. Macewicz. 1985. Measurement of spawning frequency in multiple spawning fishes. Pages 79-94 in: R. Lasker (ed.) An egg production method for estimating spawning biomass of pelagic fish: an application to the northern anchovy*, Engraulis mordax.* NOAA/NMFS Tech. Rep. **36**. 99 p.

Johnson, A. G., and L. A. Collins 1994. Age-size structure of red grouper (Epinephelus morio) from the eastern Gulf of Mexico. NE Gulf Science. 13 (2): 101-106.

Johnson, A.G., M.S. Baker Jr. and L. A. Collins. 1997. Magnitude and composition of undersized grouper bycatch. Proc. Gulf Carib. Fish. Inst. 49:161-172.

Lombardi-Carlson, L.A., G. R. Fitzhugh, and J.J. Mikulas. 2002. Red grouper (Epinephelus morio) age – length structure and description of growth from the eastern Gulf of Mexico: 1992-2001. National Marine Fisheries Service, Panama City Laboratory, Contribution Series 2002-6.

Moe, M.A., Jr. 1969. Biology of the red grouper (*Epinephelus morio* Valenciennes) from the eastern Gulf of Mexico. Prof. Pap. Ser. Mar. Lab. Fla. No. 10. 95 p.

Wallace, R. A., and K. Selman 1981. Cellular and dynamic aspects of oocyte growth in teleosts. Amer. Zool. 21:325-343.

Table 1. Red grouper gonad maturation stages, adapted from Moe (1969), Wallace and Selman (1981), and Hunter, Lo and Leong (1985).

Sex	Stage	Gonad Maturation Stage	Description of most-advanced oocytes or sperm
Female	1	Immature/resting	Primary growth oocytes (resting ovaries may have some large atretic oocytes, immature ovaries have none)
Female	2	Early developing	Yolk vesicles (cortical alveoli) present
Female	3	Vitellogenic	Vitellogenic oocytes < .400 mm in diameter (yolk globules present)
Female	4	Early hydration	Some late vitellogenic oocytes > .400 mm in diameter have migrating nucleus
Female	5	Hydrated oocytes	Yolk plate formation is ~complete; oocytes > .600 mm in diameter are amoeboid in shape
Female	6	Spent	Over 50% of the large oocytes are atretic
N/A	N/A	Transitional	Female tissue degenerating; male tissue proliferating
Male	1	Immature/resting	Primary spermatocytes
Male	2	Early developing	Secondary spermatocytes
Male	3	Late developing	Spermatids
Male	4	Ripe	~Large pools of spermatozoa (tailed sperm)

Table 2. A comparison of sex, assigned by macroscopic/microscopic method to sex subsequently assigned after viewing histological section. Percent of row total is indicated in parentheses.

	Histological sex			
	Female	Male	Transitional	Total #
Macro/micro sex				
Female	304 (83%)	54	7	365
Male	3	44 (94%)		47
Transitional	11	20	1 (3%)	32

TABLE 3. Spawning frequency estimates for female red grouper from the eastern Gulf of Mexico and the Atlantic Ocean off North Carolina and South Carolina (in Burgos 2001). Active females include vitellogenic, migratory nucleus (MNO), hydrated (HO), and spent stages (stages 3-6 in Table 1.). Length of the spawning season was 89 days in 1996 and 140 days in 2001 for the present report, and 115 days in Burgos 2001. The underlined values for spawning frequency estimates in 2001 and late 1990s are thought to be the most accurate for the two different areas and years. Sample number = n. POF is postovulatory follicle.

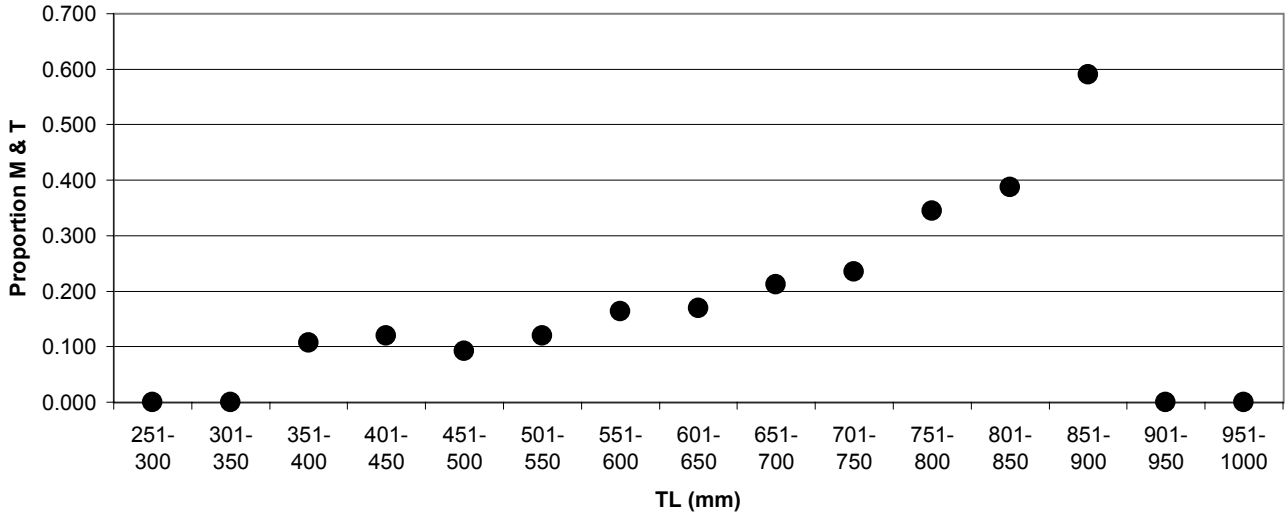
	1996					2001					Late 1990s (Burgos 2001)				
Month	**n Active**	**HO**	**MNO**	**HO & MNO**	**New POF**	**n Active**	**HO**	**MNO**	**HO & MNO**	**New POF**	**n Active**	**HO**	**MNO**	**HO & MNO**	**New POF**
Feb	0*	-	-	-	-	4	0	4	4	0	9	0	2	2	0
March	1	0	1	1	0	7	3	3	6	1	6	1	3	4	1
April	30	2	27	29	1	50	12	33	45	15	17	4	8	12	1
May	13	6	5	11	1	40	5	34	39	4	19	2	0	2	3
June	9**	0	4	4	0	6	0	1	1	0	37	3	9	12	5
Total	53	8	37	45	2	107	20	75	95	20	88	10	22	32	10
Spawning periodicity (no. of days between spawns) for each method		6.6	1.4	1.2	26.3	-	5.4	1.4	1.1	5.4	-	8.8	(4.0) ***	2.8	8.8
Spawning frequency (number of spawns per female for the year) for each method		14	62	75	3	-	**<u>26</u>**	98	124	**<u>26</u>**	-	(13) ****	(29)***	**<u>42</u>**	(13) ****

* Of three females sampled in February 1996, none were active.
** Of 23 females sampled in July and August 1996, only one was active: a 921mm TL, 24 year old stage 4 with a large ovary that was sampled on August 25.
*** Spawning frequency estimated by the "MNO" method was calculated from data presented in Burgos 2001 and are shown in parentheses
**** Burgos (2001) had an apparent miscalculation for spawning frequency from the HO and POF methods. We calculated an estimate of 13 rather than 10.6

Table 4. Females sampled during the peak spawning months of March, April and May were categorized as percent active (vitellogenic) using macroscopic/microscopic method and percent mature (vitellogenic and including evidence of previous spawning) based on histological sections. Percent active and percent mature were only calculated for n>10.

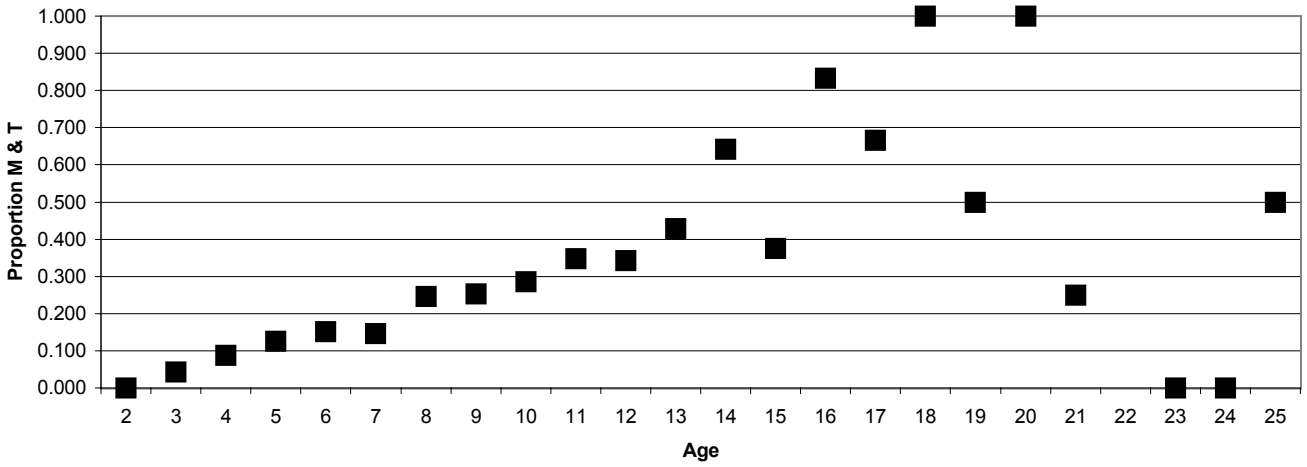
	Macroscopic/microscopic				Histological			
Size class	**active**	**inactive**	**total**	**%**	**mature**	**immature**	**total**	**%**
351-400	7	3	10		7	3	10	
401-450	13	17	30	43	15	13	28	54
451-500	30	26	56	54	14	15	29	48
501-550	83	67	150	55	37	11	48	77
551-600	90	38	128	70	33	5	38	87
601-650	69	42	111	62	20	2	22	91
651-700	54	38	92	59	23	1	24	96
701-750	48	27	75	64	12	1	13	92
751-800	22	10	32	69	2		2	
801-850	19	7	26	73	4		4	
851-900	6		6					
901-950	1	1	2					
951-1000	2		2					

Figure 1. Proportions of red grouper classified as male and transitional by size based on all data available; macroscopic/microscopic and histological observations.



Size bin range	Size Bin	Females	Males	Trans	Males & Trans	Total	Proportion M & T
201-250	250	0	0	0	0	0	0.000
251-300	300	1	0	0	0	1	0.000
301-350	350	4	0	0	0	4	0.000
351-400	400	25	2	1	3	28	0.107
401-450	450	66	1	8	9	75	0.120
451-500	500	168	15	2	17	185	0.092
501-550	550	391	47	6	53	444	0.119
551-600	600	316	51	11	62	378	0.164
601-650	650	255	49	3	52	307	0.169
651-700	700	223	56	4	60	283	0.212
701-750	750	153	44	3	47	200	0.235
751-800	800	76	39	1	40	116	0.345
801-850	850	49	29	2	31	80	0.388
851-900	900	9	13	0	13	22	0.591
901-950	950	3	0	0	0	3	0.000
951-1000	1000	2	0	0	0	2	0.000
	Col. Sums=	1741	346	41	387	2128	

Figure 2. Proportions of red grouper classified as male and transitional by age based on all data available; macroscopic/microscopic and histological observations.



Age Bin	Females	Males	Trans	Males & Trans	Total	Proportion M & T
2	6	0	0	0	6	0.000
3	22	0	1	1	23	0.043
4	167	7	9	16	183	0.087
5	321	38	8	46	367	0.125
6	366	54	11	65	431	0.151
7	286	47	2	49	335	0.146
8	172	50	6	56	228	0.246
9	83	27	1	28	111	0.252
10	60	23	1	24	84	0.286
11	32	17	0	17	49	0.347
12	23	12	0	12	35	0.343
13	16	11	1	12	28	0.429
14	5	9	0	9	14	0.643
15	5	3	0	3	8	0.375
16	1	5	0	5	6	0.833
17	1	2	0	2	3	0.667
18	0	2	0	2	2	1.000
19	1	1	0	1	2	0.500
20	0	2	0	2	2	1.000
21	3	1	0	1	4	0.250
22	0	0	0	0	0	
23	1	0	0	0	1	0.000
24	1	0	0	0	1	0.000
25	1	1	0	1	2	0.500
26	0	0	0	0	0	
27	0	0	0	0	0	
28	1	0	0	0	1	
Col. Sums=	1574	312	40	352	1926	

Figure 3. Gonadosomatic index (GSI) values for individual fish including males, females and transitionals.

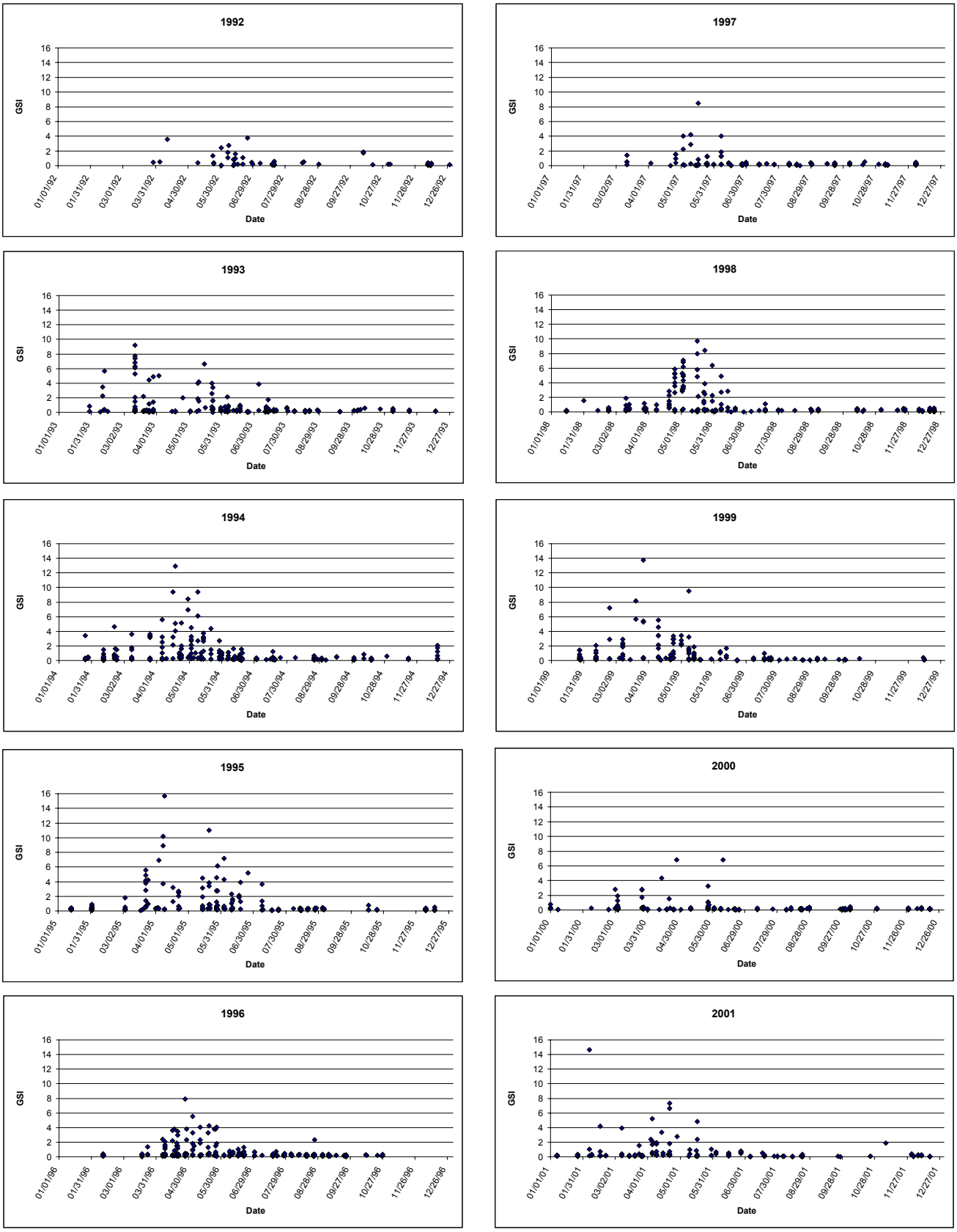


Figure 4. Average gonadosomatic index values for female red grouper

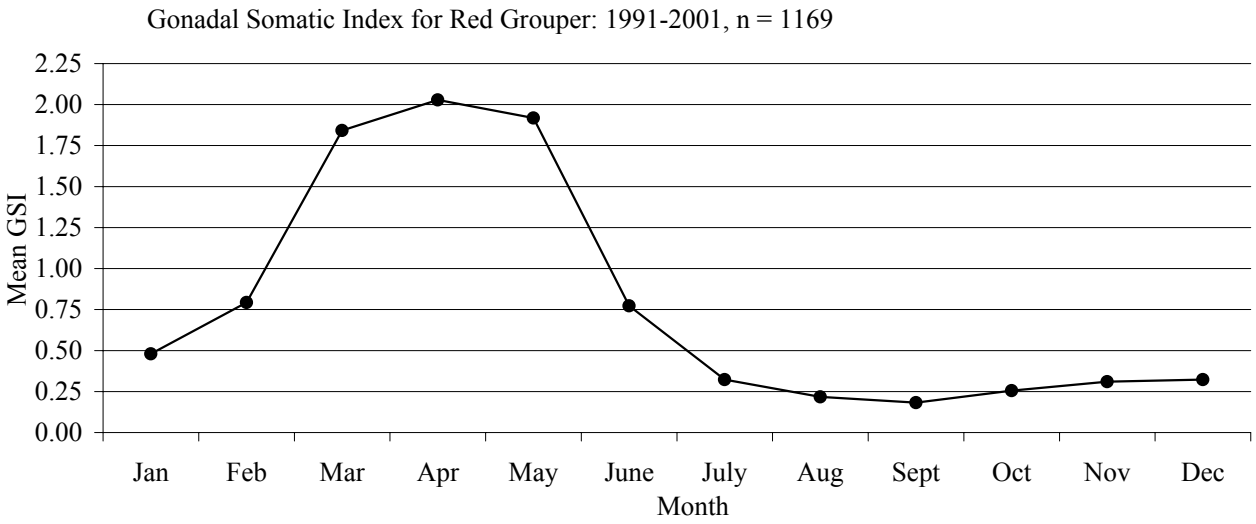
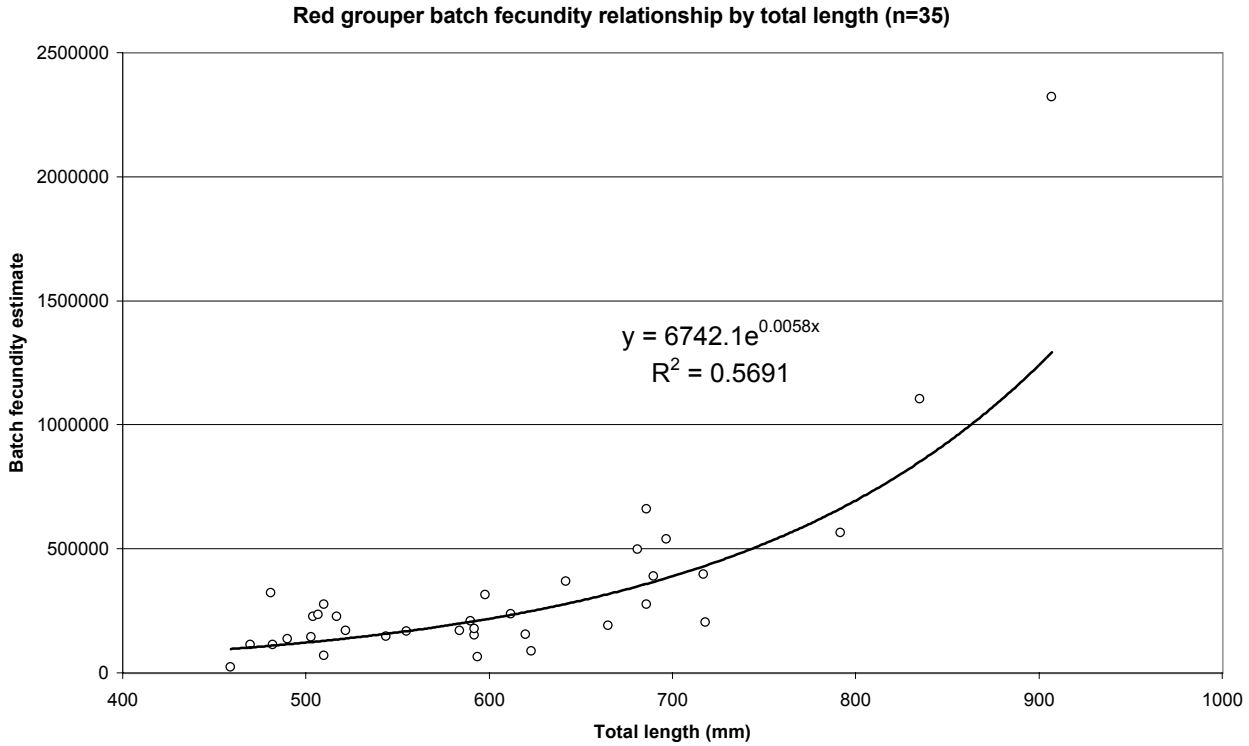


Figure 5. Batch fecundity relationships for female red grouper, A. by size and B. by age.

A



B

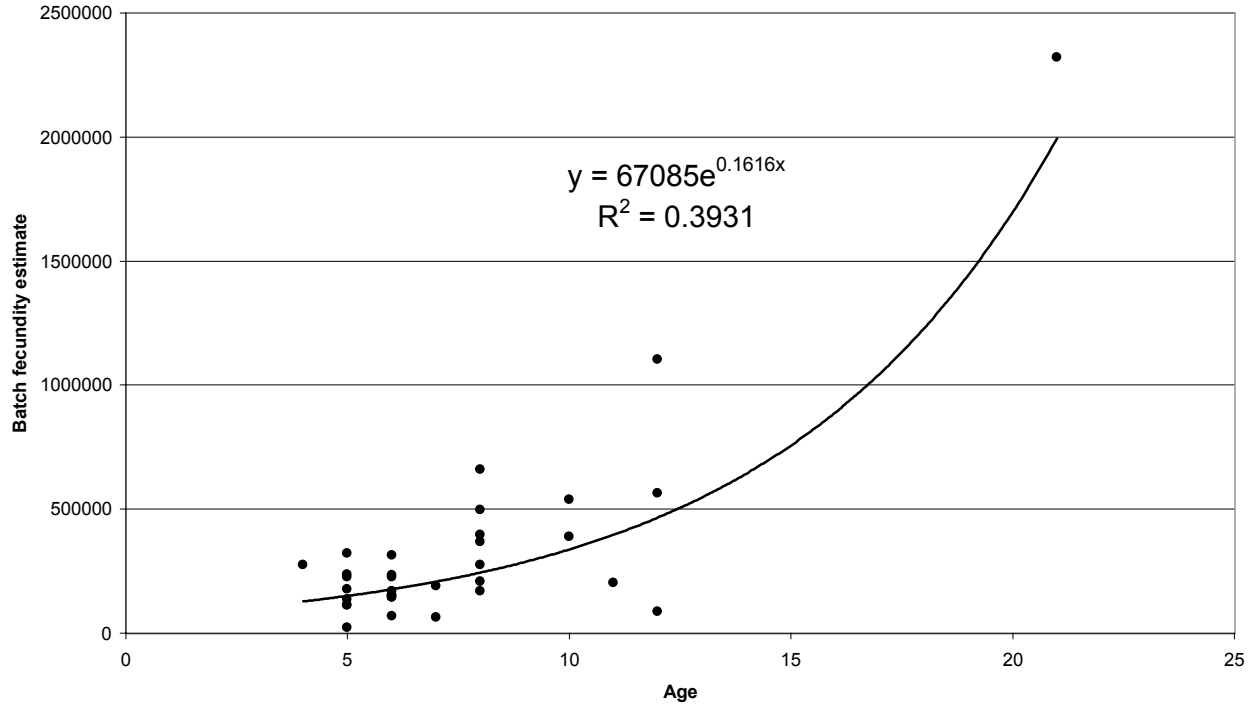


Figure 6. Percent of active (vitellogenic) females detected during the peak spawning months (March, April, May) for years 1993-2001. Sample sizes of females from each annual 3-month period are denoted in parentheses.