# Life history of red grouper, (*Epinephelus morio*) off the coasts of North Carolina and South Carolina

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# LIFE HISTORY OF RED GROUPER (*EPINEPHELUS MORIO*) OFF THE COASTS OF NORTH CAROLINA AND SOUTH CAROLINA

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## ABSTRACT

The objective of this research was to update the knowledge on the life history of red grouper *Epinephelus morio* (Valenciennes in Cuvier and Valenciennes, 1828), a commercially important species off North Carolina and South Carolina. A total of 2146 specimens was sampled from commercial catches and obtained with fishery-independent gear between December 1996 and September 1999. Red grouper ranged in total length (TL) and age from 315 to 851 mm and from 2 to 20 yrs. Observed length-at-age was described by the following von Bertalanffy growth equation: TL (mm) = 853(1–exp (–0.209(age (yr)+0.812))). Sex and reproductive state of 2068 red grouper were assessed by histological analysis. Of these, 86.8% were classified as female, 5.9% as transitional, and 7.3 % as male. Age at 50% maturity was 2.4 yrs and TL at 50% maturity was 487 mm. The sex ratio of red grouper was 1 male to 6.6 females. Red grouper was confirmed to be a protogynous hermaphrodite. Age at 50% transition was 7.2 yrs and TL at 50% transition was 690 mm. Spawning in females occurred between mid-February and mid-June. Spawning females were captured on multiple locations off the North Carolina and South Carolina coasts, at depths between 33 and 90 m.

Red grouper, *Epinephelus morio* (Valenciennes in Cuvier and Valenciennes, 1828) is an important commercial species in the Gulf of Mexico and along the Atlantic coast of the southeastern United States (US). In the southeastern US Atlantic waters, red grouper is managed as a single stock. Management regulations are based on biological information collected from the Gulf of Mexico and eastern Florida waters in the 1960s and 1970s (SAFMC, 1983; 1991). Although there is no significant genetic differentiation among populations of red grouper off the southeastern U.S. Atlantic coast and in the Gulf of Mexico (Zatcoff et al., 2004), other evidence suggests that the red grouper stock off the Carolinas is geographically isolated. Red grouper have been observed in North Carolina waters (Chester et al., 1984; Parker, 1990; Parker and Greene, 1999), but not off southern South Carolina and Georgia (Powles and Barans, 1980; Sedberry and Van Dolah, 1984). Few red grouper are landed in Georgia by the commercial fishery (National Marine Fisheries Service, 2003). Furthermore, fishery-independent capture of red grouper off Georgia and southern South Carolina is infrequent (MARMAP program, 1973–2004, South Carolina Department of Natural Resources).

If red grouper off North Carolina and South Carolina constitute an isolated subpopulation, life history parameters may differ from other areas. Yet, there is little information available on the life history of red grouper off the southeastern U.S. Results of the only life history study on red grouper in these waters (Stiles and Burton, 1994) should be considered with some caution, as samples were pooled from North Carolina to the Florida Keys over a long period (1972–1988). Using growth and population parameters from Stiles and Burton (1994), Huntsman et al. (1994) estimated the spawning potential ratio (SPR) for red grouper in the western North Atlantic

to be 41% regionally, with SPR values between 24% and 34% for the "Carolinas subpopulation", suggesting that it was more severely impacted by fishing mortality.

The objective of our study was to fully describe the life history of red grouper from North Carolina and South Carolina. Data on population age structure, growth parameters, analysis of reproductive seasonality, and estimations of sex ratio, size and age at maturity, size and age at transition, and spawning frequency will allow the evaluation of current management regulations and comparisons with future studies to detect signs of overfishing (Harris and McGovern, 1997; McGovern et al., 1998).

## Methods

Sampling.—Fishery-dependent samples were obtained by the North Carolina Department of Environment and Natural Resources from commercial fish houses in Wrightsville Beach and Southport (North Carolina) between December 1996 and September 1999. We obtained additional fishery-dependent samples from fishing houses in Murrells Inlet and Mt. Pleasant (South Carolina), and Calabash (North Carolina). Commercial fishing trips usually lasted 2–3 d, and fish were caught using baited hooks on snapper reels. Fish were packed in ice after capture. Most fish were landed whole, although some were landed gutted with gonads still attached to the mesentery. In some cases (34.3% of specimens), fishermen provided the approximate location and depth of the catch. In general, the whole catch was sampled, although random samples were taken when the catch was large.

Fishery-independent samples along the southeastern United States were obtained during MARMAP program cruises between 1997 and 2000 on randomly selected offshore hard-bottom habitat between Cape Lookout (North Carolina) and Cape Canaveral (Florida) from May through September. Fish were captured with chevron traps (see Collins, 1990) baited with clupeids and soaked for approximately 90 min. Additional samples were obtained using longline and hook-and-line. Only specimens captured north of 32°N were included in this study. All fish were captured during daylight hours, and the location and depth of the sampling sites were recorded. Fish were iced immediately after capture.

Total length (TL, ± 1 mm) and either whole fish weight (FW, ± 0.01 lb) or gutted fish weight (GFW, ± 0.01 lb) were measured for each fish sampled from the commercial fishery. GFW was converted to FW following Goodyear and Schirripa (1993). Total length, fork length (FL) and standard length (SL) were measured (± 1 mm) for all fish collected by MARMAP. Whole weight for groupers < 2.5 kg was measured (± 1 g) with a triple beam balance. Larger groupers were weighed (± 10 g) with an electronic scale.

Because information on the location and depth of samples from the commercial fishery was limited and approximate, an additional 83 samples obtained with various types of fishing gear during MARMAP cruises between 1991 and 1996 were used to analyze the relationship between water depth and size, age, sex, and reproductive state. These specimens were not included in other analyses.

Age and Growth.—Sagittal otoliths were extracted from all fish, rinsed in water, and stored dry. Only the left sagittal otolith was obtained from fish sampled from the commercial fishery, whereas both otoliths were obtained from MARMAP fish. Otoliths were embedded in epoxy resin and sectioned (0.8 mm thick) through the core along a dorso-ventral plane with a low-speed saw equipped with a high-concentration diamond wheel. Otolith sections were mounted on glass slides and examined with a dissecting microscope (30×) using transmitted light. Increments (one opaque and one translucent zone) were counted independently by two readers with no reference to fish length or date of capture. If the readers disagreed on the number of increments in a section, the first reader analyzed the section several months later, without referencing previous readings. If two of the three readings coincided, that age was assigned to the specimen; otherwise, the specimen was eliminated from analyses.

Otolith radius and increment radii were measured only on sections cut through the core for which the readers agreed on the increment count. An image of the section was obtained with a video camera connected to the microscope and a frame digitizer. Images were analyzed on a computer monitor and measurements were made with Optimas® digital image processing software. Otolith radius was measured from the core to the edge of the section, along the ventral edge of the sulcus acousticus (Moe, 1969). The distance from the core to the center of each opaque zone was measured along the same axis. To detect possible bias, the size distribution of fish from which otolith radius and increment radii were measured was compared to the size distribution of all samples using a Kolmogorov-Smirnov (KS) two-sample test (Sokal and Rholf, 1981). For each fish, the marginal increment was calculated as the distance between the last increment and the otolith edge, expressed as a fraction of the distance between the last and the next to last increments.

Length frequency distributions from fishery-dependent and fishery-independent samples were compared using a KS two-sample test. Age-length keys from fishery-dependent and fishery-independent samples were obtained by creating a matrix containing the number of samples by age within 20 mm TL intervals (Ricker, 1975). Age-length keys from fishery-dependent and MARMAP samples were compared using Fisher's exact test, following Hayes (1993). A comparison was made for each 20-mm length interval between 440 and 700 mm TL. To maintain the power of the test, comparisons were limited to length intervals with a sample size greater than six in both age-length keys. The following adjusted significance level was used to compensate for the high number of tests required to compare age-length keys:

$$\alpha^* = 1 - e^{(\ln(1-\alpha)/n)}$$

where $\alpha^*$ is the significance level for $n$ individual tests and $\alpha$ is the desired experimentwise error (Hayes, 1993). Results indicated that there were no significant differences between fishery-dependent and fishery-independent sources; thus, samples from both sources were pooled for all subsequent analyses.

A geometric mean regression was fitted to the total length and otolith radius data, following Ricker (1992). Back-calculated lengths-at-age for individual fish were obtained using the body proportional hypothesis (Francis, 1990):

$$TL_i = \left[(c + dS_i)/(c + dS_c)\right] TL_c$$

where $TL_i$ is the total length at time of formation of the $i^{\text{th}}$ increment, $c$ and $d$ are the intercept and slope of the TL-OR geometric mean regression, $S_i$ is the distance from the otolith core to the $i^{\text{th}}$ increment, $S_c$ is the otolith radius at time of capture, and $TL_c$ is the total length of fish at time of capture. To avoid biases, otolith sections that presented some degree of inclination from the dorso-ventral plane, thereby increasing the apparent otolith radius, were not used to calculate the geometric mean regression, although back-calculated lengths at age were obtained from them. To detect the presence of Lee's phenomenon (Ricker, 1975), linear regression analysis was used to detect trends in mean length-at-age back-calculated from fish of increasing age.

The parameters of the von Bertalanffy growth curve (Ricker, 1975) were estimated by fitting a mixed model design (Lindstrom and Bates, 1990) to all back-calculated lengths at age. This model was selected because of the lack of independence between back-calculated lengths in each fish. The NLMIX macro for the SAS software (Littel et al., 1996) was used following the procedure established by Jones (2000). A second von Bertalanffy growth equation was fitted to the observed age and total length data using the NLIN procedure and Marquardt´s algorithm (SAS Institute Inc., 1990). Fish length was weighted by the inverse of the number of fish at each age class to reduce the effect of differences in sample size among age classes.

Reproduction.—Gonads of specimens from the commercial fishery were preserved in 10% seawater formalin immediately after sample processing at fish houses. Preserved gonads

were weighed (± 1 g) and a sample from the posterior region of the gonads was obtained for histological analysis to determine sex and reproductive state.

Gonads obtained from MARMAP sampling were preserved between 6 and 24 hrs after capture. A portion of the posterior region of each gonad was preserved in 11% seawater formalin for 14 d, and then transferred to 50% isopropanol for the same period. The higher formalin concentration was used to compensate for the water content of the tissue. Samples were vacuum infiltrated, blocked in paraffin and sectioned with a rotary microtome. Three transverse sections, 6–8 μm thick, were mounted on glass slides, stained with double-strength Gill's haematoxylin, and counter-stained with eosin-y.

The sex and reproductive state of each specimen was assessed independently by two readers using histological criteria (Table 1) without reference to age, fish length, and date of capture. In cases of disagreement, both readers analyzed the section simultaneously. If agreement could not be reached, the sample was eliminated from analyses. Females in states 2 through 6 and in state 8 were considered mature. Females in states 2c, 3, and 4 were considered to be in spawning condition, while only males in state 3 were considered to be spawning. Transitional individuals with sperm in lobular lumina and sinuses were considered males for sex ratio analysis. Sex ratio was calculated as the ratio of the number of males and transitionals to the number of females.

To verify that immature females (state 1) and regressed females (state 5) were correctly distinguished, the size distributions of specimens assigned to these two categories were compared with the combined size distribution of active females (i.e., in the developing, running ripe, developing-previous spawn, and spent states). An overlap in the size distributions of regressed and active females and differences in the size distributions of regressed and immature females indicated that the criteria used to distinguish regressed and immature females were appropriate (Wyanski et al., 2000).

Female age at 50% maturity, length at 50% maturity, age at 50% sexual transition, and length at 50% sexual transition were estimated using the PROBIT procedure (SAS, 1990). The model that best fit the maturity data (gompit, logit, or probit model) was selected with the LOGISTIC procedure (SAS, 1990). Individuals of uncertain maturity were not included in these analyses.

Spawning season was defined as the number of days between the dates of capture of the first and last specimens in spawning condition (Collins et al., 1998). The duration of the spawning season was verified by examining the monthly trend of the gonadosomatic index (GSI), defined as

$$GSI = \frac{FGW}{FW - FGW} \times 100$$

where FGW is the fresh gonad weight, and FW is the total weight of the fish.

Postovulatory follicles were assigned ages following Hunter et al. (1986). The number of days between spawning events was estimated by dividing the total number of active females by the number of females observed with recent (< 24 hrs old) postovulatory follicles (Hunter and Macewicz, 1985). To detect possible biases caused by the fact that our samples were collected during daylight hours, the number of days between spawning events was also estimated using the number of females with hydrated oocytes and the number of females with hydrated oocytes or migratory nucleus oocytes, as these structures indicate that spawning is likely to occur in the next 12–24 hrs (Hunter and Macewicz, 1985; Fitzhugh et al., 1993). The number of spawning events per individual was estimated by dividing the duration of the spawning season by the number of days between each spawning event.

Table 1. Histological criteria to assess sex and reproductive state in red grouper based on Moe (1969), Hunter et al. (1986), Wenner et al. (1986), McGovern et al. (1998), and Wyanski et al. (2000).

| Reproductive state | Male | Female |
|---|---|---|
| 0. Uncertain maturity | Inactive testes; unable to assess maturity; state = 1 or 6. | Inactive ovaries with primary growth oocytes only; unable to assess maturity; state = 1 or 6. |
| 1. Immature | No immature males observed. | Primary growth oocytes only, no evidence of atresia. In comparison to resting females, most primary growth oocytes < 80 um, area of transverse section of ovary is smaller, lamellae lack muscle and connective tissue bundles and are not as elongate, oogonia abundant along margin of lamellae, ovary wall is thinner. |
| 2. Developing | Development of cysts containing primary and secondary spermatocytes through some accumulation of spematozoa in lobular lumina and ducts. | 2a. Early developing: Most-advanced oocytes in cortical-alveoli stage.<br>2b. Intermediate developing: Most-advanced oocytes in yolk-granule or yolk-globule stage.<br>2c. Late developing: Most-advanced oocytes in migratory-nucleus stage; partial coalescence of yolk globules possible. |
| 3. Running ripe | Predominance of spermatozoa in lobules and ducts; little or no occurrence of spematogenesis. | Completion of yolk coalescence and hydration in most advanced oocytes; zona radiata becomes thinner. |
| 4. Developing-previous spawn | | 4.1. Vitellogenic oocytes and postovulatory follicles < 12 hrs old<br>4.2. Vitellogenic oocytes and postovulatory follicles 12–24 hrs old<br>4.3. Vitellogenic oocytes and postovulatory follicles > 24 hrs old |
| 5. Spent | No spermatogenesis; some residual spermatozoa in shrunken lobules and ducts. | More than 50% of vitellogenic oocytes with alpha or beta atresia. |
| 6. Regressed | Little or no spermatocyte development; empty lobular lumina and ducts; some recrudescence (spermatogonia through primary spermatocytes) possible at end of state. | Primary growth oocytes only; traces of atresia. In comparison with immature females, most primary growth oocytes > 80 um, area of transverse section of ovary is larger, lamellae have muscle and connective tissue bundles, lamellae are more elongated and convoluted, oogonia less abundant along margin of lamellae, ovarian wall is thicker and exhibits varying degrees of expansion due to previous spawning, melano-macrophage centers and/or foci of inflammatory cells may be present. |
| 7. Transitional | Protogyny: testicular proliferation (mitotic spermatogonial development and possibly limited spermatogenesis) within lamellae of spent or resting ovaries and development of peripheral sinuses in musculature of ovarian wall. | No protandry observed. |
| 8. Mature specimen of unknown state. | Mature, but inadequate quantity of tissue or postmortem histolysis prevents further assessment of reproductive state. | Mature, but inadequate quantity of tissue or postmortem histolysis prevent further assessment of reproductive state. |

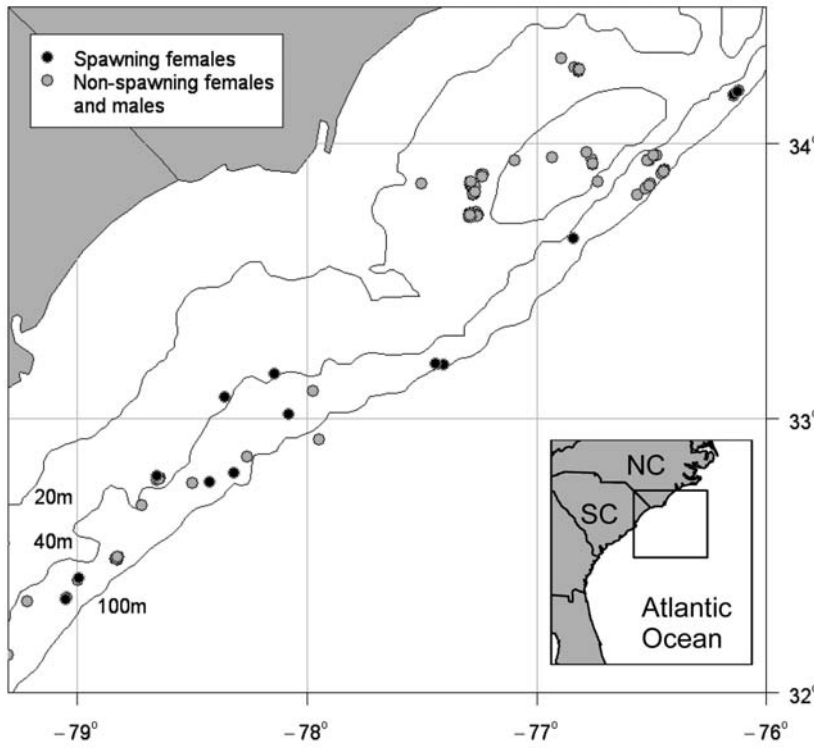


Figure 1. Capture locations of red grouper obtained through fishery-dependent and fishery-independent sampling (1996–2000) off North Carolina (NC) and South Carolina (SC). Spawning females includes females in late developing, running ripe, and developing-previous spawn states.

## Results

A total of 1928 red grouper was sampled from the commercial fishery. Red grouper ranged in length from 384 to 851 mm TL (mean = 563, SD = 66.7). Many of the fish sampled (21.9%) were smaller than the minimum size limit of 508 mm TL. An approximate location and depth of capture was reported for 34% of the specimens. Commercial fishermen captured red grouper between 32°30′N and 33°57′N, at depths of 27–76 m. Based on the short duration of the trips and location of home ports, we concluded that all samples came from waters off North Carolina and South Carolina.

During the MARMAP cruises, 218 red grouper were captured (n = 212 with chevron traps, n = 6 with hook and line). Specimens ranged in length from 315 to 779 mm TL (mean = 547, SD = 102.9) and were captured between 32°08′N and 34°19′N at depths of 25–95 m (Fig. 1).

Length distributions differed significantly between fishery-dependent and fishery-independent samples (K-S: $P < 0.001$), mainly due to the presence of small fish in fishery-independent samples. Fish < 500 mm TL were captured at depths < 40 m,

while larger fish were found at all sampled depths. The relationships among length measurements (mm) and whole fish weight (g) of fish from both sources were:

$$FL = 0.95(TL) + 8.72 \quad (n = 452, r^2 = 0.99)$$
$$SL = 0.83(TL) - 3.59 \quad (n = 450, r^2 = 0.97)$$
$$SL = 0.86(FL) - 13.30 \quad (n = 354, r^2 = 0.98)$$
$$W = 5.94 \times 10^{-6}(TL)^{3.16} \quad (n = 1911, r^2 = 0.94)$$
$$W = 2.18 \times 10^{-6}(TL)^{3.34} \quad (n = 432, r^2 = 0.96)$$

Age and Growth.—We obtained otoliths from 2110 specimens and measured otolith radius and increment radii in 1056 of those specimens. The length distribution of fish selected for measurement did not differ significantly from that for all samples (K-S: $P > 0.05$). Marginal increment analysis showed that annual increments were deposited during July and August; therefore, the age assigned to each specimen was equivalent to the number of increments observed. The two readers agreed on the age of 54% of the red grouper sampled, and an additional 38.6% of the counts differed by one year. After rereading by the primary reader, an age was assigned to 96% of the samples (n = 2038).

The relationship between otolith radius and fish length was approximately linear and was described by the following geometric mean regression: TL = 226.076 * OR + 271.637 (n = 770, $r^2 = 0.53$). Sections from 286 otoliths had some degree of inclination away from the dorso-ventral plane and data from those sections were not included in the regression. Increments in otoliths of fish 13 yrs and older were closely spaced and difficult to measure; thus, back-calculated lengths were obtained only for ages 1–12 (Table 2).

Ages of fish from the commercial fishery ranged from 2 to 20 yrs. No fish 13–19 yrs old were collected. Fish sampled by MARMAP ranged from 2 to 10 yrs old. The von Bertalanffy growth parameters estimated from back-calculated length-at-age were: $L_\infty = 836.1$ mm TL (SE = 7.08), $K = 0.170$ yr$^{-1}$ (SE = 0.003), $t_o = -1.278$ (SE = 0.018), while the parameters obtained from observed length-at-age were: $L_\infty = 853.2$ mm TL (SE = 3.81), $K = 0.209$ yr$^{-1}$ (SE = 0.004), $t_o = -0.812$ (SE = 0.069) (Fig. 2). Lee's phenomenon, a decreasing trend in the mean length-at-age back-calculated from specimens of increasing age, was not observed in ages 1–5 (linear regression: $P > 0.05$, Table 2). The sample size for older ages was not large enough to perform this analysis.

A comparison of age-length keys from fishery-dependent and fishery-independent sources yielded no significant differences in age distribution by length interval. Although test results for eight intervals exceeded the 0.05 level (Table 3), the adjusted significance level of 0.0037 was exceeded only in the 561–580 mm TL interval. No significant differences in mean length-at-age were observed in fish older than 6 yrs between samples from fishery-dependent and fishery-independent sources (Fig. 3). Mean length-at-age was significantly higher in fishery-dependent samples in ages 3–4 yrs (ANOVA: $P < 0.05$). Comparison was not possible in ages 1 and 2 due to low sample size.

Reproduction.—Sex and reproductive state were assigned to 2073 specimens. Of these, 1801 (86.8%) were classified as females, 121 (5.9%) as transitionals, and 151 (7.3 %) as males (Table 4). Females ranged from 315 to 739 mm TL and from 1 to 10 yrs old. Transitional individuals ranged from 455 to 744 mm TL and from 3 to 10

Table 2. Mean back-calculated total length (mm)-at-age for red grouper off North Carolina and South Carolina. Samples were from fishery-dependent and fishery-independent sources, 1996–2000. Only specimens whose otolith was sectioned through the core were included (n = 1,056).

| Age (yr) | n | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 2 | 226.1 | 310.3 | | | | | | | | | | |
| 3 | 71 | 268.8 | 359.6 | 423.4 | | | | | | | | | |
| 4 | 471 | 266.4 | 360.0 | 425.7 | 485.8 | | | | | | | | |
| 5 | 364 | 268.2 | 365.0 | 436.6 | 493.6 | 551.4 | | | | | | | |
| 6 | 68 | 279.4 | 370.9 | 443.0 | 503.0 | 563.8 | 616.1 | | | | | | |
| 7 | 49 | 272.4 | 364.6 | 430.4 | 491.2 | 552.4 | 606.4 | 652.1 | | | | | |
| 8 | 15 | 272.0 | 355.8 | 418.9 | 477.5 | 541.2 | 593.2 | 639.6 | 686.0 | | | | |
| 9 | 8 | 272.1 | 359.0 | 428.9 | 496.5 | 550.9 | 607.6 | 659.4 | 691.4 | 725.0 | | | |
| 10 | 5 | 236.5 | 316.0 | 379.6 | 437.0 | 503.9 | 545.3 | 580.7 | 618.1 | 656.9 | 681.9 | | |
| 11 | 0 | | | | | | | | | | | | |
| 12 | 3 | 219.5 | 320.5 | 391.7 | 469.1 | 527.2 | 567.1 | 611.2 | 645.3 | 679.9 | 707.3 | 732.4 | 765.8 |
| Average: | | 268.0 | 362.1 | 430.3 | 489.8 | 552.2 | 606.7 | 644.5 | 672.5 | 695.2 | 691.4 | 732.4 | 765.8 |

yrs old. Males were 509–851 mm TL and 3–20 yrs old. The mean length for females (546 mm TL; SD = 60.3) was significantly smaller than that for males and transitionals combined (640 mm TL; SD = 77.2; K-S: $P < 0.001$). Gonad weights were obtained from 1589 specimens.

The overall sex ratio (1 male: 6.6 females), which included females of all reproductive states, was significantly different from 1:1 (G-test: $P < 0.05$), favoring females at ages 3–6 (Table 4). In addition, the sex ratio of mature individuals was 1 male: 4.5 females, whereas the sex ratio of spawning individuals was 1 male: 0.9 females. Females were significantly more abundant than males in size intervals $\leq$ 660 mm TL

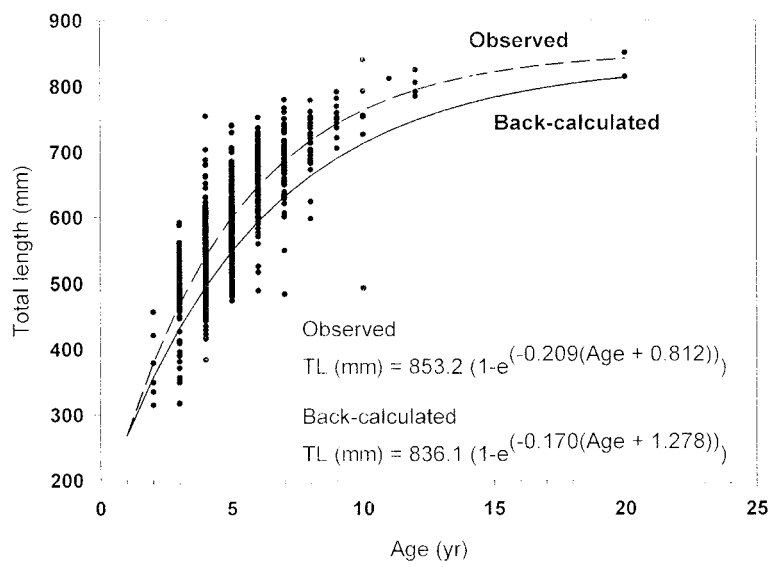


Figure 2. Observed age and total length of red grouper off North Carolina and South Carolina, and von Bertalanffy growth curves fitted to observed and back-calculated length at age.

Table 3. Comparison of age-lengths keys from fishery-dependent and fishery-independent samples of red grouper using Fisher's exact test. *P < 0.05, **P < 0.0037 (adjusted significance level).

| Size interval (mm TL) | P |
|---|---|
| 441–460 | 0.117 |
| 461–480 | 0.007* |
| 481–500 | 0.045* |
| 501–520 | 0.029* |
| 521–540 | 0.848 |
| 541–560 | 0.027* |
| 561–580 | 0.001** |
| 581–600 | 0.364 |
| 601–620 | — |
| 621–640 | 0.048* |
| 641–660 | 0.302 |
| 661–680 | 0.390 |
| 681–700 | 0.028* |
| 701–720 | 0.026* |

(Table 5). Between ages 4 and 7, males were significantly larger than females (Table 6). The smallest mature female was 405 mm TL and 3 yrs old, whereas the largest immature female was 641 mm TL and 6 yrs old (Table 5). Age at 50% maturity was estimated as 2.4 yrs (probit model, 95% CI = 1.77–2.74), and length at 50% maturity as 487 mm TL (probit model, 95% CI = 482–492). All individuals 7 yrs and older were mature, as well as all individuals in size classes 661–680 mm TL and larger (Tables 4 and 5).

The overlap in the length distributions of individuals classified as regressed females and active females as well as the minimal overlap with that for immature fish indicated that the criteria used to differentiate regressed and immature females were adequate (Fig. 4). A number of inactive females (n = 372), mostly between 3 and

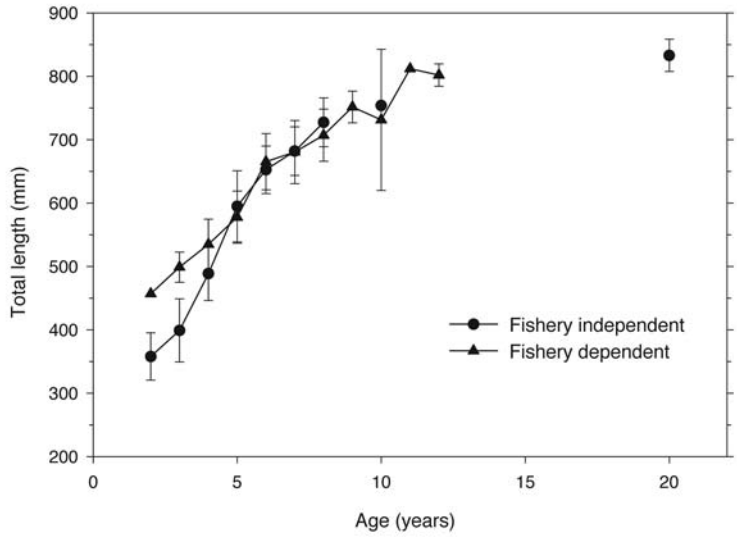


Figure 3. Length-at-age of red grouper from fishery-dependent and fishery-independent sources, 1996–2000. Error bars are +/– 1 standard deviations.

Table 4. Frequency of red grouper by sex category and age. Sex ratio is estimated as number of males and transitionals per number of females. The hypothesis of a sex ratio of 1:1 was tested for each age using a G-test. Values of G were adjusted using William's correction (Sokal and Rohlf, 1981). * indicates $G > \chi^2_{0.05,\,1}$

| Age (yr) | Immature females (stage 1) | Females, uncertain maturity (stage 0) | Mature females (stages 2–6 and 8) | Transitionals | Males | Sex ratio (male: female) | G |
|---|---|---|---|---|---|---|---|
| 2 | 6 | 0 | 0 | 0 | 0 | | |
| 3 | 26 | 125 | 60 | 2 | 1 | 1:70.3 | 264.49* |
| 4 | 136 | 167 | 561 | 37 | 16 | 1:16.3 | 861.90* |
| 5 | 34 | 64 | 399 | 52 | 38 | 1:5.5 | 310.52* |
| 6 | 2 | 2 | 75 | 18 | 37 | 1:1.4 | 4.31* |
| 7 | 0 | 0 | 32 | 5 | 20 | 1:1.3 | 0.85 |
| 8 | 0 | 0 | 12 | 1 | 10 | 1:1.1 | 0.04 |
| 9 | 0 | 0 | 4 | 0 | 7 | 1:0.6 | 0.79 |
| 10 | 0 | 1 | 2 | 1 | 3 | 1:0.8 | 0.13 |
| 11 | 0 | 0 | 0 | 0 | 1 | | |
| 12 | 0 | 0 | 0 | 0 | 4 | | |
| 20 | 0 | 0 | 0 | 0 | 2 | | |
| No age | 9 | 13 | 71 | 5 | 12 | | |
| Total | 213 | 372 | 1,216 | 121 | 151 | 1:6.6 | 1,256.49* |

5 yrs old, exhibited characteristics intermediate between immature and regressed females; therefore, their reproductive state and maturity could not be assessed. Sex and maturity could not be determined in five specimens. Three males and four females were considered mature, but of unknown reproductive state (state 8).

The occurrence of females with hydrated oocytes, migratory-nucleus oocytes or postovulatory follicles, as well as the distribution of female GSI values, indicated that red grouper has a protracted spawning season (~115 d), from mid-February to mid-June, with a peak in April (Fig. 5A). Postovulatory follicles were observed in females captured during March and June. Atretic oocytes were more common toward the end of the spawning period (May through July), although females with atretic oocytes were observed during most of the year. Female GSI ranged from 0.03 to 7.05, with the highest values observed between April and June (Fig. 6A).

Spawning males were observed between November and August, although they occurred more frequently between January and March (Fig. 5B). Male GSI ranged from 0.06 to 0.59, with the highest values observed between April and July (Fig. 6B). Females in spawning condition were captured by MARMAP between 32°20′N and 34°11′N, at depths between 33 and 90 m (Fig. 1). Running ripe males were observed at the same latitude range, at depths between 33 and 86 m. Red grouper spawning activity appears to primarily occur in waters deeper than 40 m, where most of the active females, transitional individuals, and males were found. In shallower water, most of the females captured were immature, regressed, or inactive with uncertain maturity.

Similar estimations of spawning frequency (8.8 d, equivalent to 13 spawning events per season) were obtained if hydrated oocytes or postovulatory follicles were used as evidence of imminent or recent spawning (Table 7). Combining the frequencies of specimens with hydrated oocytes and migratory nucleus oocytes, which as-

Table 5. Frequency of red groupers by sex and total length intervals. Sex ratio was estimated as number of males and transitionals over number of females. A G-test was used in each length interval to test the hypothesis of a sex ratio of 1:1. Values of G were adjusted using William's correction (Sokal and Rohlf, 1981). * indicates $G > \chi^2_{0.05, 1}$

| Total length (mm) | Immature females (stage 1) | Females, uncertain maturity (stage 0) | Mature females (stages 2–6 and 8) | Transitionals | Males | Sex ratio (male: female) | G |
|---|---|---|---|---|---|---|---|
| 301–400 | 17 | 0 | 0 | 0 | 0 | | |
| 401–420 | 4 | 0 | 2 | 0 | 0 | | |
| 421–440 | 7 | 1 | 0 | 0 | 0 | | |
| 441–460 | 14 | 3 | 1 | 1 | 0 | 1:18.0 | 18.03* |
| 461–480 | 35 | 57 | 16 | 1 | 0 | 1:108.0 | 139.09* |
| 481–500 | 57 | 113 | 76 | 4 | 0 | 1:61.5 | 303.59* |
| 501–520 | 46 | 97 | 121 | 3 | 2 | 1:52.8 | 322.55* |
| 521–540 | 20 | 47 | 182 | 11 | 2 | 1:19.2 | 259.28* |
| 541–560 | 8 | 27 | 202 | 14 | 2 | 1:14.8 | 228.46* |
| 561–580 | 2 | 16 | 175 | 14 | 8 | 1:8.8 | 155.72* |
| 581–600 | 0 | 4 | 157 | 18 | 10 | 1:5.8 | 102.11* |
| 601–620 | 0 | 4 | 99 | 15 | 16 | 1:3.3 | 40.65* |
| 621–640 | 1 | 0 | 51 | 8 | 11 | 1:2.7 | 15.83* |
| 641–660 | 1 | 1 | 41 | 6 | 13 | 1:2.3 | 9.46* |
| 661–680 | 0 | 1 | 33 | 14 | 11 | 1:1.4 | 1.37 |
| 681–700 | 0 | 0 | 25 | 4 | 16 | 1:1.3 | 0.55 |
| 701–720 | 0 | 0 | 19 | 6 | 23 | 1:0.7 | 2.08 |
| 721–740 | 0 | 0 | 3 | 1 | 12 | 1:0.2 | 6.53* |
| 741–760 | 0 | 0 | 6 | 1 | 10 | 1:0.6 | 1.45 |
| 761–780 | 0 | 0 | 2 | 0 | 4 | 1:0.5 | 0.63 |
| 781–800 | 0 | 0 | 2 | 0 | 3 | 1:0.7 | |
| 801–860 | 0 | 0 | 0 | 0 | 7 | | |
| No length | 1 | 1 | 3 | 0 | 1 | | |
| Total | 213 | 372 | 1,216 | 121 | 151 | 1:6.6 | 1,256.49* |

sumed that these specimens would have spawned on the same day, produced a higher spawning frequency (2.8 d, equivalent to 42 spawning events per season).

Sex transition was found nearly year-round, except in April during peak spawning. The percentage of transitionals peaked in November. Age at 50% transition was 7.2 yrs (probit model, 95% CI = 6.9–7.7) and size at 50% transition was 690 mm TL (probit model, 95% CI = 679–704). No immature females undergoing transition and no primary males were observed.

## Discussion

The use of annual otolith increments for aging red grouper has been previously validated by marginal increment analysis (Moe, 1969; Johnson and Collins, 1994; Stiles and Burton, 1994; ) and tagging (Schrirripa and Burns, 1997). We found that red grouper deposit a new opaque zone in July or August. Although annual increment depositions should be validated for each age (Beamish and McFarlane, 1983), the number of samples per month in our study was sufficiently high (> 10) to validate

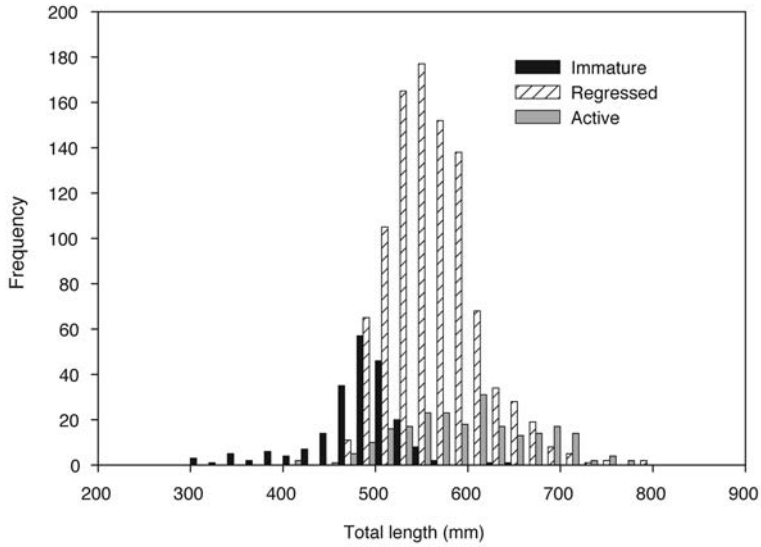


Figure 4. Comparison among length frequencies of immature, resting, and active female red groupers. Active females include females in reproductive states 2, 3, 4, and 5.

annual depositions for only ages 4 and 5. Although unlikely, it is possible that otolith increments in younger or older fish are not deposited annually.

It is likely that the first otolith increment in red grouper corresponds to the first year of life. Johnson and Collins (1994) reported a young-of-the-year (YOY) red grouper that measured 152 mm TL and had no annulus on its sagittae. Likewise, a 151 mm TL red grouper captured near Georgetown, South Carolina, in September 2000 also had no annulus (P. Mikell, South Carolina Department of Natural Resources, pers. comm.) and its otolith radius was less than the observed mean radius of the first increment in other specimens.

Red grouper are long-lived, reaching ages of 25 yrs (Moe, 1969) in the eastern Gulf of Mexico and 16 yrs along the Atlantic coast of the southeastern U.S. (Stiles and Burton, 1994). In the present study, only two specimens (age 20) were older than 12 yrs. Due to poor historical age records for red grouper off the Carolinas, it is impossible to assess if the absence of fish > 12 yrs old reflects the effect of age-specific fishing mortality.

Table 6. Comparison between male and female mean observed length-at-age with one-way ANOVA. Standard error uses a pooled estimate of error variance. * = $P < 0.05$.

| Age | Females (mm) | SE | n | Males (mm) | SE | n | P |
|---|---|---|---|---|---|---|---|
| 3 | 489 | 2.68 | 211 | 513 | 22.43 | 3 | 0.2838 |
| 4 | 528 | 1.39 | 864 | 567 | 5.61 | 53 | < 0.0001* |
| 5 | 574 | 1.84 | 497 | 608 | 4.33 | 90 | < 0.0001* |
| 6 | 646 | 4.65 | 79 | 680 | 5.63 | 55 | < 0.0001* |
| 7 | 665 | 8.58 | 32 | 695 | 9.70 | 25 | 0.0202* |
| 8 | 762 | 12.32 | 12 | 745 | 9.32 | 11 | 0.3087 |
| 9 | 695 | 14.77 | 14 | 725 | 20.90 | 7 | 0.2577 |
| 10 | 680 | 63.45 | 3 | 769 | 54.95 | 4 | 0.3350 |

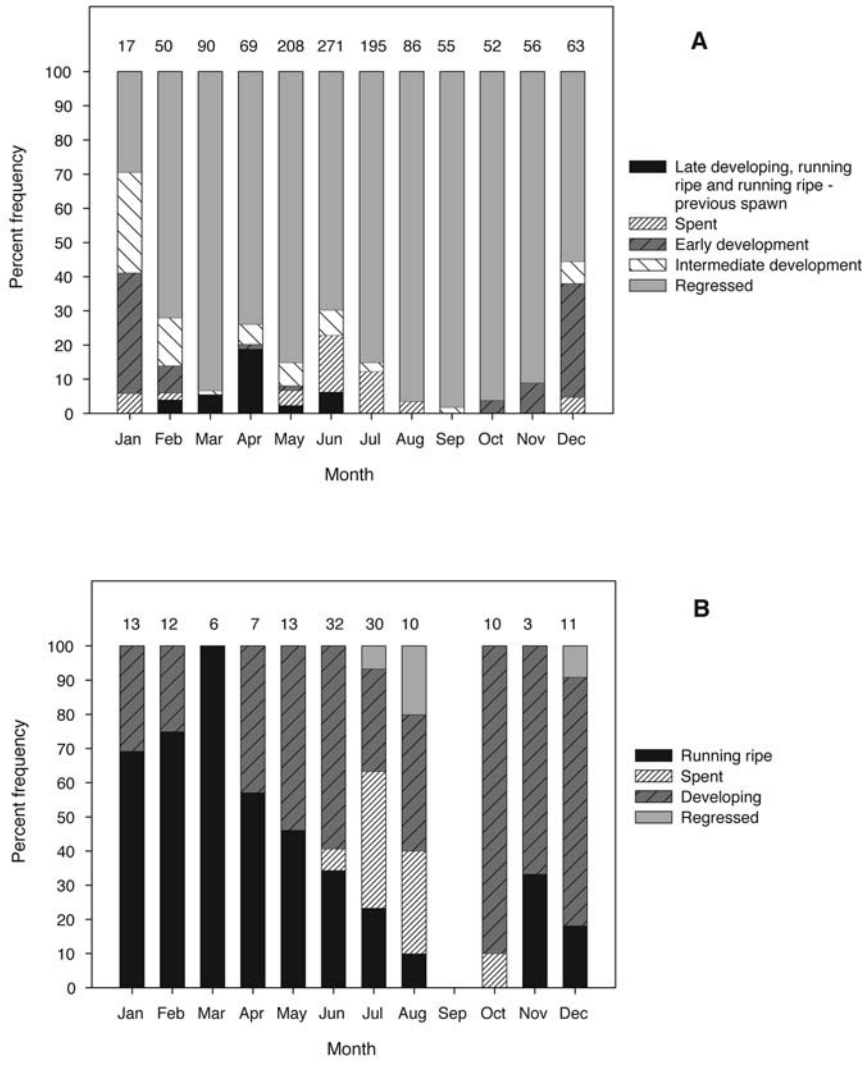


Figure 5. Spawning season for (A) female and (B) male red grouper, 1996–2000. The number above each bar indicates the sample size. Individuals in stage 8 (mature, unknown reproductive state) were not included.

The parameters of the von Bertalanffy equation are comparable to those in other red grouper studies (see Moe, 1969; Johnson and Collins, 1994; Stiles and Burton, 1994; Schirripa and Burns, 1997; Lombardi-Carlson et al., 2002). The absence of Lee's phenomenon suggests that the differences observed in growth parameters estimated from back-calculated vs observed length-at-age can be explained by the growth that occurred between the deposition of the last increment and the moment of capture. The absence of Lee's phenomenon in our data also suggests that the back-calculation method using all increments was appropriate (Ricker, 1992).

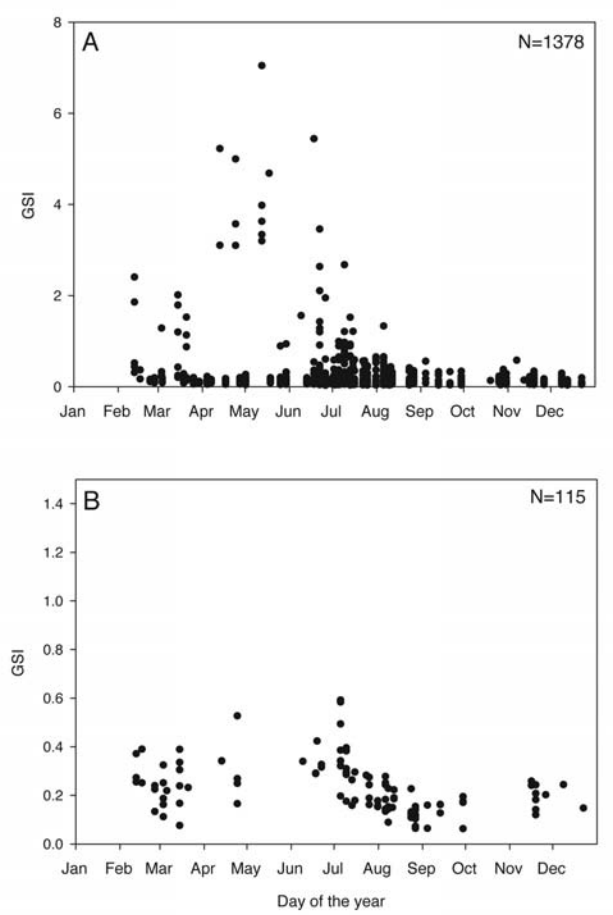


Figure 6. Values of gonadosomatic index (GSI) for (A) female and (B) male red grouper, 1996–1999. GSI values for transitional individuals are not shown.

Between ages 2 and 5 yrs, size-at-age of specimens from fishery-dependent samples was significantly larger than that of specimens from fishery-independent samples. This is largely due to the size limit, which resulted in fishermen using larger hooks, avoiding relatively shallow waters where smaller groupers live, and discarding or releasing undersized fish (Goodyear and Schrippa, 1993). As a result, samples obtained from the commercial fishery were biased toward larger individuals, particularly for younger age classes that had not fully recruited to the fishery. For those ages, therefore, estimates of mean length-at-age derived from fishery-dependent data would be biased even if a random sampling design was used (Goodyear, 1995). Fishery-independent sampling using chevron traps is probably less selective (Dalzell, 1996), so combining fishery-dependent and fishery-independent samples reduces some of the bias toward larger individuals. Even so, it is difficult to assess the extent of the bias and, therefore, the degree to which samples are representative of the red grouper population off the Carolinas. The possibility of bias in growth parameters needs to be considered when estimating biological reference points based on these parameters.

Table 7. Estimation of red grouper spawning frequency. Active females include stages 2b, 2c, 3, and 4. The number of spawning events per season was calculated assuming that females spawn continuously during a 115-d long spawning season. HO = number of females with hydrated oocytes, MNO = females with migratory nucleus oocytes, POF = females with postovulatory follicles.

| Month | Active females | HO | MNO | HO + MNO | < 24-hrs old POF |
|---|---|---|---|---|---|
| Feb | 9 | 0 | 2 | 2 | 0 |
| Mar | 6 | 1 | 3 | 4 | 1 |
| Apr | 17 | 4 | 8 | 12 | 1 |
| May | 19 | 2 | 0 | 2 | 3 |
| Jun | 37 | 3 | 9 | 12 | 5 |
| Total | 88 | 10 | 22 | 32 | 10 |
| Spawning frequency | | 8.80 | | 2.75 | 8.80 |
| Number of spawning events per season | | 13.1 | | 41.8 | 13.1 |

Information about the habitat requirements of juvenile red grouper is scarce. No juvenile red grouper were captured by MARMAP from 1973–2003 despite extensive sampling efforts with several types of gear including trawl nets and traps at depths of 9–338 m. Personal observations of YOY red grouper in an aquarium suggest that their cryptic behavior makes them unavailable to sampling gear utilized by MARMAP. Juvenile red grouper are common in North Carolina estuaries (Ross and Moser, 1995) and are captured occasionally in habitat traps deployed in South Carolina estuaries (P. Mikell, South Carolina Department of Natural Resources, pers. comm.), suggesting that they are estuarine-dependent. Juveniles may use inshore reefs as nursery areas (Moe, 1969, Sluka et al., 1994). By age 2 yrs, red grouper are present in the fishing grounds, as evidenced by captures in MARMAP gear, and 3 yr old red grouper are routinely captured by the fishery. Before the minimum size was established in 1993, the commercial fishery landed red grouper as small as 254 mm TL (F. Rohde, North Carolina Department of Environment and Natural Resources, pers. comm.), smaller than the theoretical mean length-at-age 1 estimated from von Bertalanffy growth curves. Thus, red grouper may move from estuaries to shallow offshore reefs during their first year of life.

A gradient of increasing length with depth has been observed for red grouper (Moe, 1969; Rivas, 1970; Goodyear and Schrripa, 1993). Such a gradient could be the product of size-dependent mortality in shallow water (McPherson and Duarte, 1991), but it is more likely indicative of an ontogenic migration. Tagging data demonstrate that red grouper reside in shallow water reefs off western Florida until reaching sexual maturity (400 mm SL, 5 yrs old), and then migrate towards offshore reefs (Moe, 1969). A similar pattern occurs off the Yucatán Peninsula, where only immature females appear in inshore collections (7–27 m), and immature and mature females, transitionals, and males appear in offshore collections (30–90 m, Brulé et al., 1999). Our data indicate that red grouper off the Carolinas also remain in shallow waters (< 40 m) until reaching sexual maturity and then move offshore to spawn. Transitionals and males were more commonly found at depths > 40 m, suggesting that mature red grouper became permanent residents on offshore reefs after changing sex. Our estimate of size at 50% maturity (487 mm TL) is comparable to the values reported in other studies (50.9 cm FL in Brulé et al., 1999; 400–500 mm TL in Collins et al., 2002), even though methodologies differed.

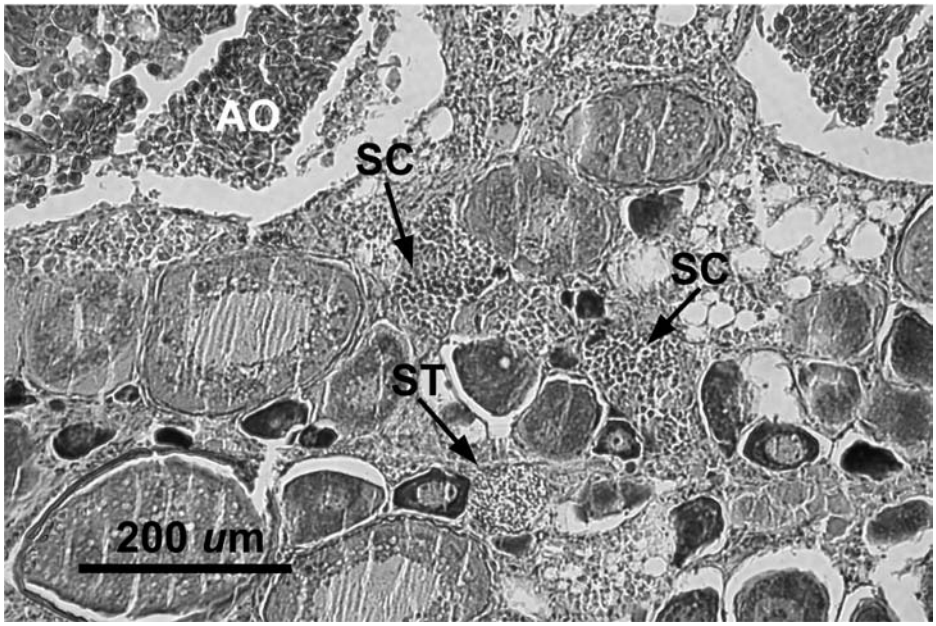


Figure 7. Transverse section of the gonad of a transitional red grouper (TL = 607 mm) captured in May 1997. Labels indicate an atretic vitellogenic oocyte in alpha stage (AO), a crypt of spermatids (ST), and crypts of spermatozoa (SC).

The observed distribution of female red grouper also suggests the possibility of annual migrations between offshore spawning locations and inshore reefs. During the spawning season, the majority of mature females sampled at depths < 40 m were in the regressed state, while at depths > 40 m most females were in the developing, running ripe, developing-previous spawn, or spent states. After the spawning season, almost all mature females were in the regressed state, regardless of depth of capture. Brulé et al. (1999) reported a similar distribution for female red grouper on Campeche Bank. In addition, 14 of the 16 females with atretic oocytes, for which a depth of capture was recorded, occurred at depths < 40 m, which suggests that females return to shallower water after spawning. The presence of regressed females at inshore locations during the spawning season also suggests that some females do not spawn annually (Collins et al., 2002). At depths > 40 m, red grouper in spawning condition were captured throughout the sampled area and were not restricted to any specific location, indicating that red grouper spawn in small groups distributed over broad areas (Coleman et al., 1996).

The spawning season observed off the Carolinas, mid-February to mid-June with a peak during April, was similar to that in other regions. Off the western coast of Florida, red grouper spawn between January and May (Moe, 1969, Collins et al., 2002), while off the Yucatán Peninsula, running ripe females were observed between January and March (Brulé et al., 1999). In all regions, including the Carolinas, males were in spawning condition for a longer period than were females. Although Moe (1969) found no histological or analytical evidence to suggest that red grouper spawn more than once each season, oocyte diameter distributions (Koenig, 1993) and the presence of different oocyte states and high levels of sex steroids during the spawning season (Johnson et al., 1998) suggest the opposite. The presence of developing

females with postovulatory follicles and vitellogenic oocytes in our study indicates that red grouper spawn more than once during each season.

In batch spawners like red grouper, an estimation of spawning frequency is required to estimate annual fecundity (Hunter and Macewicz, 1985). Spawning frequency estimations are based on the frequency of occurrence of specimens with histological criteria, generally hydrated oocytes or postovulatory follicles, indicative of imminent or recent spawning. Because there are no published descriptions of degradation rates in groupers, we assumed that changes in postovulatory follicle structure during degradation were similar to those in skipjack tuna *Katsuwonus pelamis* (Linnaeus, 1758) spawning at 23–24 °C (Hunter et al., 1986). We observed no female red grouper with non-degraded postovulatory follicles, five females with < 12 hrs old postovulatory follicles, and five with 12–24 hrs old follicles. The low frequency of specimens with postovulatory follicles is an indication that these structures degrade rapidly (Fitzhugh and Hettler, 1995). Because red grouper specimens were collected during daylight, the absence of non-degraded postovulatory follicles could indicate that spawning occurs during the night. Poor preservation of gonads may also account for the low number of postovulatory follicles observed, as these structures are sensitive to postmortem histolysis and freezing (Hunter and Macewicz, 1985; O. Pashuk, South Carolina Department of Natural Resources, pers. comm.). Most of our samples were obtained from the commercial fishery, where fish may be kept on ice several days before being landed. Thus, our estimation of spawning frequency using postovulatory follicles could be negatively biased.

An alternative method to estimate of spawning frequency is based on the frequency of females with hydrated oocytes (Hunter and Goldberg, 1980). Hydration is reported to occur < 12 hrs before spawning in swordfish (*Xiphias gladius* Linnaeus, 1758; Taylor and Murphy, 1992), black drum (*Pogonias cromis* (Linnaeus, 1766); Fitzhugh et al., 1993), and striped bass (*Morone saxatilis* (Walbaum, 1792); Sullivan et al., 1997). If the duration of the hydration phase in red grouper is similar to that in other warm water species, using hydrated oocytes as an indication of imminent spawning may underestimate spawning frequency because a specimen with no hydrated oocytes during the morning could start hydration during the afternoon and spawn at the night. In this case, considering migratory nucleus oocytes as an additional indication of imminent spawning could eliminate some of the bias, making spawning frequency estimations based on the combined frequencies of females with hydrated oocytes and migratory nucleus oocytes more reliable than estimations based on hydrated oocytes alone. In addition, hydrated oocytes and migratory nucleus oocytes are less sensitive to postmortem histolysis, reducing possible bias due to poor preservation of fishery-dependent samples.

The sex ratio observed in this study (1 male: 4.5 females) for the sexually mature population was similar to that of Moe (1969) [1 male and transitional: 5.3 females; reported in Coleman et al. (1996)], but differed from Coleman et al. (1996; 1 male: 2.2–3.5 females), Brulé et al. (1999; 1 male and transitional: 3.2 females), and Collins et al. (2002; 1 male and transitional: 2.3 females). The variation in sex ratio among these studies could reflect a sampling bias such as non-representative sampling of the larger individuals or differences in sampling design, as well as regional differences in exploitation rates. Between 1987 and 2000, red grouper landings in North Carolina and South Carolina increased by a factor of 17. Changes in sex ratio and other life history characteristics caused by fishing mortality have been reported for

other exploited reef fish species off the southeastern United States (Harris and McGovern, 1997; McGovern et al., 1998). The lack of historical data does not allow us to assess this hypothesis.

Red groupers off the Carolinas are protogynous hermaphrodites, as evidenced by the presence of transitional individuals, testes with a central cavity lined by a membrane, atresia of yolked oocytes within testes (Fig. 7) and sperm sinuses within the gonadal wall (see Sadovy and Shapiro, 1987). Transitional individuals were observed more frequently (5.9%) than in previous studies (1%: Moe, 1969, and Brulé et al., 1999; 0%–0.4%: Coleman et al., 1996), possibly due to differences in the definition of the transition state. Specimens with advanced states of spermatogenesis, but in which crypts of seminiferous tissue were restricted to the periphery of ovarian lamellae, were considered immature males by Moe (1969) but were classified as transitionals in the present study. Brulé et al. (1999) classified as males specimens in which advanced states of spermatogenesis had invaded the ovarian lamella. Fish with these characteristics and no spermatozoa present in the collecting ducts would have been classified as transitional in the present study.

Seasonal trends in red grouper sex transition differed from previous studies. Moe (1969) reported that transition occurs from April to June; however, if immature males are considered transitional individuals, there is no clear seasonal trend in sex transition. Brulé et al. (1999) also observed transitionals year-round. In the present study, with the exception of the month of peak spawning (April), sex transition was observed year-round with a clear peak in November. The occurrence of transitional individuals year-round is consistent with the hypothesis of Coleman et al. (1996) that male and female red grouper co-occur year-round; thus, sex ratio assessment and initiation of sex change can occur anytime. The observed trend of males being larger than females at each age suggests that the largest females tend to change sex. This mode of sex transition has been reported for other serranids (e.g., *Anthias squamipinnis* (Peters, 1855); Shapiro, 1980).

The results of the present study will allow the calculation of updated biological reference points required to monitor the status of the red grouper stock off North Carolina and South Carolina, and to evaluate the outcome of stock recovery plans currently in place. In addition, the results of our study will serve as a baseline against which future studies can be compared to detect signs of overfishing. Several aspects of red grouper life history are still unknown and should be the target of future research. Examples of these are spatial and temporal patterns of reproductive activity, including further identification of spawning areas, assessment of cross-shelf migration, and a description of the dynamics of sex transition.

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