

LIFE HISTORY OF THE RED GROUPER (*Epinephelus morio*) OFF THE NORTH CAROLINA
AND SOUTH CAROLINA COAST

by

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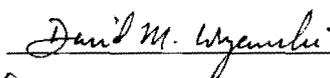
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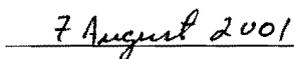
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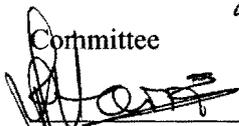
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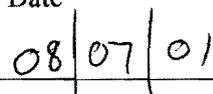
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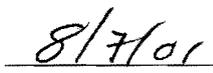
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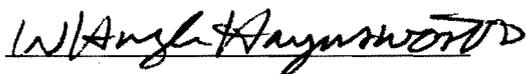
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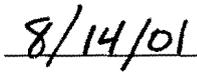
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ABSTRACT

The objective of this study was to update the life history information of the red grouper, *Epinephelus morio*, off the North Carolina and South Carolina coast. A total of 1928 red grouper were sampled from commercial catches off North Carolina between December 1996 and September 1999. An additional 218 specimens were obtained through fishery-independent sampling. Red groupers ranged in total length (TL) and age from 315 to 851 mm TL and from 2 to 20 years. Marginal increment analysis indicated that annulus formation occurred during July and August. TL at age is described by the following von Bertalanffy growth equation: $TL (mm) = 853(1 - \exp(-0.209(\text{age} (y) + 0.812)))$. Sex and reproductive stage of 2068 red groupers were assessed by histological analysis. Of these, 86.8% were classified as females, 5.9% as transitionals, and 7.3% as males. Age at 50% maturity was 2.36 years and TL at 50% maturity was 487.2 mm. The sex ratio of red grouper (6.6:1 females to males) was significantly different from 1:1. Sex ratio differed significantly from 1:1 in favor of females between ages 2 to 6 and when $TL < 680$ mm. Spawning females, recognized by the presence of migratory nucleus oocytes, hydrated oocytes and post-ovulatory follicles, were found between February and June. Female red groupers spawned every 2-3 days, equivalent to a maximum of 42 times each spawning season. Specimens in spawning condition were captured mostly at depths > 40 m. Red groupers are protogynous hermaphrodites. Age at 50% transition was 7.24 years old and TL at 50% transition was 690.4 mm. Catch curve analysis indicated that in 1998 total mortality was 0.75 and that red groupers are fully recruited to the fishery at age 4. A model developed to estimate yield per recruit and sex-specific spawning biomass per recruit including release mortality and sex ratio compensation showed that female SPR was 0.18 and male SPR was 0.22, suggesting that the red grouper population off the Carolinas is overfished.

INTRODUCTION

The red grouper (*Epinephelus morio*, Valenciennes 1828) is a protogynous serranid that occurs in the western Atlantic from Massachusetts (U.S.) to Rio de Janeiro (Brazil), including the Gulf of Mexico and Bermuda. Centers of abundance occur off the west coast of Florida and the north coast of the Yucatan Peninsula on Campeche Bank (Brulé and Déniel, 1996). Adults are associated with rocky reef bottoms (Moe, 1969) and are usually caught in relatively shallow waters (20-70 m).

Red grouper is an important commercial species in the Gulf of Mexico. Since 1983, when grouper landings were first reported by species, the red grouper catch in the Gulf of Mexico waters of the U.S. has fluctuated between 2,200 and 4,000 metric tons (t) per year (National Marine Fisheries Service, NMFS¹). Red grouper landings off western Florida have been an order of magnitude higher than landings of other important commercial species like gag (*Mycteroperca microlepis*) or yellowedge grouper (*Epinephelus flavolimbatus*). Nearly all the commercial landings of red grouper in the U.S. are from western Florida (Goodyear and Schirripa, 1993).

Several studies on red grouper in the Gulf of Mexico have been carried out. Moe (1969) studied the biology of the red grouper from western Florida, including age and growth, reproductive biology, and ecology. Johnson and Collins (1994) described age-size structure, and Schirripa and Burns (1997) estimated growth based on tag and recapture observations. Aspects of the red grouper reproduction and the effect of fisheries on red grouper sex ratio and age at sexual maturity in the Gulf of Mexico were described by Coleman et al. (1996). Goodyear and Schirripa (1993) analyzed the characteristics of the Gulf fishery.

¹ NMFS, Fisheries Statistics and Economics Division, Silver Springs, MD.
<http://www.st.nmfs.gov/st1/commercial/>

The red grouper is the target of the most important finfish fishery on the continental shelf of the Yucatan peninsula (Arreguín-Sánchez et al., 1996); however, the fishery is unregulated. Mexican and Cuban commercial fleets harvest adult fish, while Mexican artisanal fishermen capture juveniles. Descriptions of the red grouper biology (Brulé and Déniel, 1996), reproduction (Brulé et al., 1999), and population dynamics (Arreguín-Sánchez et al., 1996) have been provided for this area.

Richardson and Gold (1993) found low genetic diversity in red grouper off western Florida using mitochondrial DNA (mtDNA) haplotype frequencies. The genetic homogeneity observed by the same authors among red grouper from the Campeche Bank was consistent with the hypothesis of a single stock for the Gulf of Mexico (Richardson and Gold, 1997).

Red grouper landings off the southeastern coast of the U.S. are modest compared with landings from the Gulf of Mexico. North Carolina landings increased from 3.5 t in 1983 to a peak of 72.3 t in 1995 (NMFS¹), and decreased slightly during the next two years. During the same period, catches in South Carolina and Georgia were almost non-existent, although eight tons were reported in 1997 in South Carolina. In eastern Florida, landings have fluctuated between 15 and 30 t during the period 1986-1999.

There is little information available on the life history of red grouper off the southeastern coast of the 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 over a long period (from 1972 to 1988), and life history characteristics could have changed due to the increasing fishing mortality. They also combined samples from North Carolina to the Florida Keys and had a relatively small sample size ($n = 488$).

No genetic studies have been done in the southeastern U.S. and the presence of a separate stock of red grouper along the eastern coast of the U.S. has not been established. Preliminary results from a Marine Fisheries Initiative (MARFIN) study at the South Carolina Department of Natural Resources (SCDNR) indicated low levels of genetic variability along the southeastern U.S.

(Zatcoff²). Nevertheless, some evidence suggests the existence of a geographically isolated red grouper stock off the Carolinas. Chester et al. (1984) analyzed hook and line catches from the Carolinas and concluded that the red grouper is a member of a fish assemblage restricted to the area around Cape Fear (NC) and south along the northern South Carolina coast. Grimes et. al (1982), Parker (1990) and Parker and Greene (1999) reported red grouper in Onslow Bay, whereas Powles and Barans (1980) did not find red grouper in sponge-coral habitats off Charleston, SC, and red grouper were not reported by Sedberry and Van Dolah (1984) off South Carolina and Georgia. Furthermore, few red grouper have been landed in Georgia and South Carolina by the commercial fishery and red grouper were infrequently caught off Georgia and southern South Carolina during fisheries-independent sampling carried out by the MARMAP (Marine Resources Monitoring Research and Prediction), a cooperative project of SCDNR and NMFS.

Red grouper is a member of the fish community commonly known as the snapper-grouper complex. When the Fishery Management Plan (FMP) for some species of this complex was developed in 1983, the red grouper was considered growth-overfished and a minimum size of 12 inches (305 mm) total length (TL) was imposed. Under the definition used by the SAFMC, growth overfishing occurred when a significant increase in yield per recruit (YPR) was predicted if fishing mortality was decreased or the age of first capture was increased (SAFMC, 1983). Yield per recruit analysis for the red grouper, based primarily on parameters estimated in the late 1960s and early 1970s for the Gulf of Mexico, demonstrated that a 12-inch minimum size would increase YPR by 34% (SAFMC, 1983).

In 1990, when the Second Snapper/Grouper Amendment was added to the FMP, the SAFMC adopted a new overfishing criterion based on the spawning potential ratio (SPR) (SAFMC, 1990), defining a minimum SPR of 30% for red grouper (SAFMC, 1991). If the estimated SPR was below this level, immediate actions were to be taken to protect the reproductive potential of the

² Zatcoff, M. 2000. South Carolina Department of Natural Resources, P.O. Box 12559, Charleston, SC 29422. Personal commun.

stock. If the SPR was found to be over 30%, management policies would be directed to obtain an optimum yield. Since estimated values of SPR for red grouper off eastern Florida varied between 11 and 28%, the SAFMC implemented a 20-inch (508 mm) TL minimum size limit, which would provide a SPR of 40% and an increase in YPR (SAFMC, 1991).

Huntsman et al. (1994), using growth and population parameters from Stiles and Burton (1994), estimated YPR and SPR for the red grouper in the western Atlantic from Cape Hatteras, North Carolina, to the Dry Tortugas, Florida. They reported a region-wide SPR of 41% for red grouper, but SPR values between 24% and 34% were estimated for the "Carolinas sub-population", suggesting that it was more severely impacted by fishing mortality. This information has not yet been applied to red grouper management in the southeastern U.S.

As noted above, management regulations for red grouper in the southeastern U.S. have been based on biological information from the Gulf of Mexico and eastern Florida waters. However, red grouper growth and population parameters off the Carolinas may be significantly different, and a 20-inch minimum size may not be adequate for achieving management goals established by the SAFMC. Updated life history information on red grouper from North Carolina and South Carolina is needed to examine the suitability of management regulations. Commercial red grouper landings off North Carolina have increased by a factor of five since 1992. An adequate knowledge of the life history of this species off the Carolinas will allow comparisons with future studies to detect signs of overfishing (Dayton et al., 1995).

MATERIALS AND METHODS

Sampling

Red grouper samples were obtained from commercial fish houses in Wrightsville Beach (NC) and Southport (NC) between December 1996 and September 1999 by personnel of the North Carolina Department Environment and Natural Resources (NCDENR). Additional samples from the commercial fishery were obtained by NMFS from fishing ports along the North and South Carolina coast, including Murrells Inlet and Mt. Pleasant (SC), and Calabash (NC). The whole catch was sampled in most cases, although random samples were taken when the catch was large (Rohde³). Commercial fishing trips usually lasted less than six days, and fish were caught using snapper reels (Wyanski et al., 2000). In some cases, fishermen provided the approximate location and depth of the catch. Obtaining samples only from North Carolina and South Carolina fishing ports, coupled with the short duration of the fishing trips, assured that the fish sampled were captured off North Carolina and South Carolina.

Fishery-independent samples for North Carolina and South Carolina were obtained during the MARMAP program research cruises on the *R/V Palmetto* between 1997 and 2000. MARMAP samples reef fishes at randomly selected areas of hard bottom habitat off the southeastern United States between Cape Hatteras (NC) and Cape Canaveral (FL). Fishes were captured with chevron traps (Collins, 1990) baited with clupeids and soaked for approximately 90 minutes. Some samples were obtained using longline and hook and line. All fish were captured during daylight hours, and the location and depth of the sampling sites were recorded.

Total length (TL, to the nearest mm) and gutted fish weight (± 0.01 lb and converted to kg) were measured in each fish sampled from the commercial fishery. Gutted fish weight (GFW) was

³ Rohde, F. 2000. North Carolina Department Environment and Natural Resources, 127 Cardinal Drive Wilmington, NC 28405. Personal commun.

converted to whole fish weight (FW) using the relationship $FW (kg) = 1.053 \text{ GFW} (kg) - 0.3647$, modified from Goodyear and Schirripa (1993).

For all fish collected by MARMAP, TL, fork length (FL) and standard length (SL) were measured to the nearest mm. Whole weight in small groupers (less than 2.5 kg) was measured to the nearest g with a triple beam balance. Larger groupers were weighed to the nearest 50 g using an electronic scale.

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

The area sampled by MARMAP was divided in three regions: north of 32°N, between 32°N and 30°N, and south of 30°N. For each region, catch per unit effort (CPUE), estimated for the period 1991-2000 was expressed as number of red grouper caught per trap.

Sagittal otoliths were extracted by accessing the base of the cranium through the operculum and scraping the otic bulla with a chisel until the otic chamber was exposed. In most specimens from the commercial fishery, only the left otolith was obtained, whereas both otoliths were obtained from MARMAP fish. Otoliths were rinsed in water and stored dry in paper envelopes. Gonads of specimens from the commercial fishery were extracted, wrapped in cheesecloth, and preserved in 10% seawater formalin. Preserved gonads were weighed to the nearest g, and a sample from the posterior area of the gonad was obtained for histological analysis to determine sex and reproductive stage. The gonads from 18 specimens were weighed both before and after preservation to establish a relationship between fresh and preserved gonad weights, and preserved gonad weights were converted to fresh gonad weights. Gonads obtained from MARMAP sampling were preserved for histological analysis immediately after extraction.

Age and growth

To describe the age, growth and reproductive biology of the red grouper, samples from fishery-dependent and fishery-independent sources were pooled. 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. Sections were mounted on glass slides. Increments (one opaque and one translucent zone) were counted independently by two readers with no reference to fish length or date of capture. Otolith sections were examined with a dissecting microscope (30x) using transmitted light. If the readers disagreed on the number of increments of any specimen, the primary reader analyzed the section several months later, with no reference to 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 and on 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 digital image processing software. Otolith radius was measured from the core to the edge of the section, on the ventral edge of the sulcus acousticus (Fig. 1). The distance from the core to the center of each increment 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 two sample test (KS) (Sokal and Rohlf, 1981).

For each fish, the marginal increment was calculated as

$$MI = \frac{OR - I_L}{I_L - I_{L-1}}$$

where OR is the otolith radius, I_L is the distance between the otolith core and the last increment and I_{L-1} is the distance between the otolith core and the next to last increment. Mean marginal

increment was plotted as a function of the month of capture. If the increments were deposited annually, the monthly mean marginal increment should demonstrate a minimum value. The first day of the month with lowest mean marginal increment was assigned as birth date for all specimens aged.

Length frequency distributions from fishery-dependent and fishery-independent samples were compared using a Kolmogorov-Smirnov 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 commercial and MARMAP samples were compared using Fisher's exact test, following the procedure outlined by 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 α^* is the significance level for n individual tests and α is the desired experimentwise error (Hayes, 1993).

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

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

where TL_i is the total length at time of formation of the i 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 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, thus increasing the apparent otolith radius, were not used either 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 ages in each fish. The NLMIX macro for the SAS software (Littel et al., 1996) was used following the procedure established by Jones (2000).

To facilitate comparison with previous studies, 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, 1990). To reduce the effect of the differences in the sample size in each age class, fish length was weighted by the inverse of the number of fish in each class. Observed and back-calculated mean lengths at age were compared using one-way analysis of variance (ANOVA).

Reproduction

A portion of the posterior area of each gonad was preserved in 10% seawater formalin for 14 d, and then transferred to 50% isopropanol for the same period. Samples were vacuum infiltrated, blocked in paraffin and sectioned with a rotary microtome. Three transverse sections 6 to 8 μm thick were mounted on glass slides, stained with double-strength Gill haematoxylin, and counter-stained with eosin-y.

The sex and reproductive stage of each specimen was assessed independently by two readers using histological criteria (Table 1). In case of disagreement, both readers analyzed the section

simultaneously. If agreement could not be reached, the sample was eliminated from analyses. Females in the developing, spawning, spent or resting stages were considered mature. Individuals undergoing sex transition were considered active males when there was sperm present in the collecting ducts and sinuses. Females with migratory nucleus oocytes, hydrated oocytes or postovulatory follicles were considered in spawning condition.

To verify that immature females and resting females were clearly distinguished, the size distributions of specimens assigned to these two categories were compared with the size distribution of active females. Mature females in the developing, running ripe and spent states were considered active. An overlap in the size distributions of resting and active females indicated that the criteria used were appropriate (Wyanski et al., 2000).

Age at 50% maturity (A_{50}) and length at 50% maturity (L_{50}) were estimated using the PROBIT procedure (SAS, 1990). The model that best fit the maturity data (gompit, logit or probit models) was selected with the LOGISTIC procedure (SAS, 1990). Age at 50% sexual transition and length at 50% sexual transition were estimated in the same manner.

Spawning season was defined as the number of days between the dates of capture of the first and last specimens with hydrated oocytes, migratory nucleus oocytes, or post-ovulatory follicles (Collins et al. 1998). The duration of the spawning season was verified by examining the monthly trend of the gonadosomatic index (GSI), defined as

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

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

The number of days between each spawning event was estimated by dividing the total number of active females by the number of females observed with recent (< 24 h) post-ovulatory follicles (Hunter and Macewicz, 1985). To detect possible biases, 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. Spawning frequency was estimated by dividing the duration of the spawning season by the number of days between each spawning event.

Population dynamics

Total mortality (Z) was estimated using catch curve analysis on fishery-dependent samples collected in 1998. A linear regression was fitted to the descending limb of the natural logarithm of the frequency of samples on each year class (Ricker, 1975). The following relationships between natural mortality and life history parameters were used to obtain a series of estimates of natural mortality:

$$\text{Log}(M) = 0.0066 - 0.279 \cdot \log(L_{\infty}) + 0.6543(K) + 0.4634 \cdot \log(T^{\circ}\text{C}) \quad (\text{Pauly, 1980})$$

$$\text{Ln}(Z) = 1.44 - 0.982 \cdot \text{Ln}(t_{\text{max}}) \quad (\text{Hoenig, 1983})$$

$$M = 0.0189 + 2.06 \cdot K \quad (\text{Ralston, 1987})$$

$$M = 1.63 \cdot K \quad (\text{Jensen, 1996})$$

where M is natural mortality, Z is total mortality, L_{inf} and K are parameters from the von Bertalanffy growth equation, $T^{\circ}\text{C}$ is the average water temperature, and t_{max} is the maximum age observed. An average bottom water temperature of 17.6°C in winter and 24.4°C in summer, reported by Mathews and Pashuk (1986) for Onslow Bay (NC), was used with Pauly's (1980) equation. Because Hoenig's (1983) equation relates total mortality rates to the maximum age observed in the population, using the maximum age observed in an unfished population would provide an estimation of natural mortality. In all cases, I used von Bertalanffy parameters estimated from back-calculated lengths at age. Fishing mortality (F) on completely recruited cohorts was estimated by subtracting natural mortality from total mortality.

A model to estimate YPR and SPR was constructed, following Ricker's (1975) approach and using the algorithm presented by Gabriel et al. (1989). The model followed a theoretical cohort of fish from age 1 to age 15 under different combinations of age at entry and fishing mortality and estimated the total yield in g per recruit (YPR) and the spawning biomass per recruit (SBPR) produced by the cohort during its lifetime. Under equilibrium conditions (constant natural and fishing mortality) these values are equivalent to the YPR and SBPR produced by the population each year.

Fishery managers control the size at entry to the fishery, rather than the age at entry. To incorporate variability in length at age, fish larger than the theoretical length at age of entry were considered fully recruited to the fishery and were allowed to be captured regardless of age. For each age at entry to the fishery considered (t_c) and each age (t), the fraction of each of the cohort available to the fishery $p(t_c, t)$, was estimated by comparing fishery-dependent and fishery-independent age-length keys. It was assumed that fishermen kept the legal sized fish, and released all undersized fish. Yield calculations were based on the number of fish captured and retained.

Age-specific survival rates of released fish were also built into the model. Release survival rates were estimated from depth-specific release survival rates and from the distribution of fish by age and depth from fishery-independent data. Release survival rates observed by Wilson and Burns (1996) for red grouper were used: 95% in water shallower than 45 m, and zero when depth >65 m. Between 45 and 65 m, an intermediate value of 45% was assumed. The CPUE of red grouper by MARMAP was used to provide an age- and depth-specific index of abundance. Age-specific release survival rates were estimated as

$$s_a = \sum_d a_{ad} s_d$$

where s_a is the release survival rate at age a , a_{ad} is the abundance index of fish of age a at depth strata d , and s_d is the observed survival rate at depth strata d .

Fishing mortality, release mortality and natural mortality were assumed constant throughout the year for each model run. Each cohort was assumed to enter the fishing grounds on the birth date at age 1. To estimate the fraction of total mortality that occurs at time of spawning (Gabriel et al., 1989), I assumed that all the spawning activity occurred on the first day of the month that had the highest frequency of spawning individuals.

The number of fish remaining at the end of each year (N_{t+1}) was estimated as

$$N_{t+1} = Nl + Nrs + Ns$$

$$Nl = N_t \cdot p(tc, t) \cdot e^{-M-F}$$

$$Nrs = [N_t \cdot (1 - p(tc, t)) \cdot e^{-M} - N_t \cdot (1 - p(tc, t)) \cdot e^{-M-F}] \cdot ps(t)$$

$$Ns = N_t \cdot (1 - p(tc, t)) \cdot e^{-M}$$

where Nl is the number of legal fish not captured by the fishery, Nrs is the number of surviving sublegal fish that were captured and released, Ns is the number of sublegal fish not captured by the fishery, N_t is the number of fish at the beginning of the year, $p(tc, t)$ is the proportion of fish at age t larger than the theoretical minimum legal length at age of entry tc , $ps(t)$ is the release survival at age t , M is natural mortality rate and F is fishing mortality.

The model recorded the numbers of males and females and estimated spawning biomass for each sex. Based on observations by Coleman et al. (1996), it was assumed that red grouper compensate for the loss of males by initiating sex transition at younger ages and thus keeping a constant sex ratio. To include sex ratio compensation in the model, the percentage of males by age class was approximated to a linear form, following the approach for number-compensated protogyny with fixed maturation described by Huntsman and Schaaf (1994). The slope of the linear regression at each combination of fishing mortality and age at entry was adjusted to keep sex ratio at the observed value of 6.6 females per male. Age at maturity of female red grouper

was considered constant because of the lack of historical maturity data. For comparative purposes, the model was run assuming no release mortality and no sex ratio compensation. YPR and SPR estimations were obtained for F values ranging from 0 to 1, and for age at entry to the fishery from 1 to 10 years old. For each value of t_c , I estimated the fishing mortality that maximizes YPR (F_{max}) and the fishing mortality where the slope of the yield curve is 10% of the initial value ($F_{0.1}$). Sex-specific spawning stock biomass per recruit was calculated by estimating the number of mature individuals of each sex alive at time of spawning. SPRs were expressed as ratios between observed SBPR and the SBPR calculated when $F = 0$.

The sensitivity of the model to changes in the values of natural mortality and von Bertalanffy growth rate was tested in nine different scenarios combining three values of F (0.2, 0.4 and 0.6) and three ages at entry to the fishery (3, 5 and 7 years old). In each scenario, yield per recruit, female SPR and male SPR were calculated with M values of 0.1 and 0.3 and k values 10% higher and 10% lower than the estimated k from back-calculated data. These estimations were compared to YPR and SPR calculated using the estimated value of k and a value of $M=0.2$.

The Q-basic program written to run the model is listed in Appendix 1.

RESULTS

Sampling

A total of 1928 red grouper were sampled from commercial fishermen during December 1996 to September 1999, most of which ($n = 1728$) were captured during 1998. Red grouper ranged in length between 384 and 851 mm TL ($\bar{x} = 562.6$, $SD = 66.70$). Many of the fish sampled (21.9%) were smaller than the minimum legal size of 508 mm TL (20 inches). Commercial fishermen captured red grouper between 32.30°N and 33.57°N, and between 76.56°W and 79.19°W, with depth ranging between 27 and 76 m. An approximate location and depth of capture was reported for 34% of the samples. Based on the short duration of the trips and location of home ports, I assumed that all samples came from the same area.

During the cruises conducted by MARMAP, 218 red grouper were captured during May 1996 to June 2000 with specimens ranging in length from 315 to 779 mm TL (mean 547.0, $SD = 102.93$). Red grouper were captured at depths between 25 and 95 m, and between 32° 08'N and 34° 19'N, and between 76°07'W and 79°18'W (Fig. 2). From 1991 to 2000, red grouper CPUE north of 32°N was 9.1×10^{-2} specimens per trap. During the same period, CPUE between 32°N and 30°N was 0.2×10^{-2} specimens per trap, whereas south of 30°N was 10.7×10^{-2} specimens per trap. Catch per unit effort was significantly lower in the region between 32°N and 30°N than in the other two regions (G test, $P < 0.05$).

Length distributions differed significantly between fishery-independent and fishery-dependent samples (KS, $p < 0.001$). This difference was due mainly to the presence of smaller fish in the fishery-independent samples (Fig. 3). Fish smaller than 500 mm TL were captured in water shallower than 40 m, while larger fish were found distributed evenly with depth (Fig. 4). The relationships between length measurements (mm) and fish weight (g) of fish from both sources were:

$$FL = 0.95 (TL) + 8.72 \quad (n = 452, r^2 = 0.99)$$

$$SL = 0.83 (TL) - 3.59 \quad (n = 450, r^2 = 0.97)$$

$$SL = 0.86 (FL) - 13.30 \quad (n = 354, r^2 = 0.98)$$

$$W = 5.94 * 10^{-6} (TL)^{3.1568} \quad (n = 1911, r^2 = 0.94)$$

Age and growth

Otoliths were obtained from 2110 specimens. Otolith radius and increment radius were measured for 1030 otoliths. Length distribution of fish selected for measurement did not differ significantly from the length distribution of all samples (KS, $P > 0.05$). Marginal increment analysis showed that annual increments were deposited between July and August (Fig. 5), therefore the age assigned to each specimen was equivalent to the number of increments observed. The assigned birth date for red grouper off the Carolinas was July 1.

Initially the two readers agreed on the age of 54% of the red grouper sampled. An additional 38.6% of the counts differed only by one year. After an additional count by the primary reader, an age was assigned to 2030 specimens (95% of the samples). The otoliths of 80 specimens were considered unreadable or an age was not agreed on. Abnormalities observed in otolith sections included crystalline areas and opaque deformities. Aberrant otoliths with calcitic crystallization were present in a small number of specimens ($n = 14$). In some cases this type of crystallization affected only part of the otolith and an age could still be assigned ($n = 5$, Fig. 6A). In one of the specimens it was observed that the left otolith presented calcitic crystallization (Fig. 6B) whereas the right one was normal (Fig. 6C).

The relationship between otolith radius and fish length was approximately linear (Fig. 7) and was described by the following geometric mean regression:

$$TL = 226.076 * OR + 271.637 \quad (n = 770, r = 0.73)$$

The plane of the section in 289 otoliths presented some degree of inclination away from the dorso-ventral plane. These otoliths were not included in the TL-OR regression. Increments in otoliths of fish 13 years and older were closely spaced and difficult to measure, and thus back-calculated lengths were obtained for ages 1-12 (Table 2).

Ages of fish from the commercial fishery ranged from 2 to 20 years, although no fish from 13 to 19 years old were collected. Fish sampled by MARMAP ranged in age between 2 and 10 years old (Fig. 3). 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), $t_0 = -1.278$ (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), $t_0 = -0.812$ (SE = 0.069) (Fig. 8).

Lee's phenomenon, a decreasing trend in the mean length at age back-calculated from specimens of increasing age, was not observed (linear regression, $P > 0.05$, Fig. 9). In addition, except for age 4, no significant differences were observed when comparing mean back-calculated length using all increments against mean back-calculated lengths using only the last increment (Table 3). This indicates that the differences between mean observed and mean back-calculated lengths using all increments (Table 4) are explained by additional growth since the formation of the last increment.

Comparison between age-length keys from fishery-dependent and fishery-independent sources (Tables 5 and 6) yielded no significant differences. Although eight intervals exceeded the 0.05 level (Table 7), the adjusted significance level of 0.0037 was only exceeded in the 561-580 mm TL interval. No significant differences in mean length at age were observed in fish older than five years (Table 8).

Reproduction

Sex and developmental states were assigned to 2068 specimens. Of these, 1796 (86.8%) were classified as females, 121 (5.9%) as transitionals, and 151 (7.3 %) as males. Females ranged from 315 to 739 mm TL and 1 to 10 years old. Transitional individuals ranged from 455 to 744 mm TL and from 3 to 10 years old. Males were 509 to 851 mm TL and 3 to 20 years old. The mean length for females (546.5 mm TL, SD = 60.3) was significantly smaller than that for males (640.8 mm TL, SD = 77.2; KS $P < 0.001$).

The overall sex ratio (6.6:1 females to males and transitionals) was significantly different from 1:1 (G-test, $P < 0.05$). The sex ratio differed significantly from 1:1 in favor of females at ages 2 to 6 (Table 9). In addition, females were significantly more abundant than males when TL < 680 mm (Table 10). Between ages 4 and 7, the mean length of males was significantly higher than the mean length of females (Table 11). Age at 50% maturity was estimated as 2.36 years (probit analysis with normal link function, 95% CI = 1.77-2.74, Fig. 10), and length at 50% maturity as 487.2 mm TL (probit analysis with normal link function, 95% CI = 481.9-491.7).

The overlap in the length distributions of individuals classified as resting females and active females as well as the lack of overlap with immature fish indicated that the criteria used to differentiate resting females from immature females were adequate (Fig. 11). A number of inactive females mostly between 3 and 5 years old ($n = 372$) presented characteristics intermediate between immature and resting females, and their maturity could not be assessed.

Female individuals with hydrated oocytes, migratory nucleus oocytes or postovulatory follicles indicated that red grouper have a protracted spawning season, approximately 115 days long, from mid February to mid June. There was a peak of spawning activity in April (Fig. 12). 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. The distribution of GSI values also

indicated that female red grouper spawn during the spring and early summer. Female GSI ranged from 0.03 to 8.20, while male GSI ranged from 0.04 to 1.25 (Fig. 13).

Similar estimations of spawning frequency (8.8 days, 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 12). Combining the frequencies of hydrated oocytes and migratory nucleus oocytes produced a higher spawning frequency (2.8 days, equivalent to 41 spawning events per season). Red grouper spawning activity tended to occur in waters deeper than 40 m (Fig. 14), where most of the active females, transitional individuals and males were found. In shallower water, most of the individuals captured were immature females, resting females, or inactive females of uncertain maturity (Table 13). Spawning individuals of both sexes were captured along the North Carolina and South Carolina coast, and spawning activity was not limited to any particular location (Fig. 15). Male red grouper have a longer season of reproductive activity than females (November to August, Fig. 12).

Sex transition was found nearly year-round, except in April during peak spawning when no transitionals were observed. Transition was more frequent in November. Transitional red grouper ranged from 3 to 10 years old and from 455 to 744 mm TL. Fifty percent of the females changed sex at 7.24 years old (probit analysis with logistic link function, 95% CI = 6.92-7.66) and at 690.4 mm TL (probit analysis with logistic link function, 95% CI = 678.8-704.1, Fig. 10). No immature females undergoing transition were observed.

Population dynamics

The catch curve on fishery-dependent data obtained in 1998 (Fig. 16) indicated that red grouper were not fully recruited to the fishery until age 4. For ages 4 and older, Z was 0.75. Estimations of natural mortality using Pauly's (1980) equation ranged between 0.18 and 0.21. Using Ralston's (1987) equation, the estimated value of M was 0.37, while Jensen's (1996) equation yielded an estimation of 0.28. If 20 years old is the maximum age of the stock off the

Carolinas, the estimated value of M obtained with Hoenig's (1983) equation is 0.22. For yield per recruit and spawning potential ratio analysis, an intermediate value of 0.2 was used.

Catch per unit effort indicated that older fish tended to be more abundant in deeper waters. Estimated age-specific release survival rates decreased with age (Table 14). One year old fish were only found in water shallower than 45 m, therefore release survival rate at this age was considered to be 95%.

If release mortality was considered negligible, YPR was maximized by delaying harvest until 5 years old and applying a relatively high fishing mortality (Fig. 17). Alternatively, if release mortality was included in the model, red grouper yield per recruit was maximized if age at entry to the fishery (t_c) was 4 years old, equivalent to a minimum legal size of 535 mm TL and fishing mortality rate was 0.4 (Fig. 18). In addition, F_{max} and $F_{0.1}$ were maximized if harvest is delayed until red grouper are 4 year old (Table 15).

Sex-specific SPR plots indicated that if release mortality and sex ratio compensation were not included in the model, male spawning biomass decreased more rapidly than female spawning biomass for any combination of F and t_c (Fig. 19). If release mortality was included, both male and female spawning biomass decreased more rapidly (Fig. 20). Under these conditions, male spawning biomass was particularly vulnerable to fishing mortality. Male and female SPR isopleths bent upward indicating that even at low F and t_c there is a loss of spawning biomass due to release mortality. When release mortality and sex ratio compensation were included, male spawning biomass was more resilient to exploitation than female spawning biomass for any combination of F and t_c (Fig. 21), as individuals changed sex at smaller lengths translating any loss of male spawning biomass into a decrease in female spawning biomass.

The model with release mortality and sex ratio compensation behaved predictably to changes in M and k (Table 16). A lower value of natural mortality ($M = 0.1$) produced higher values of YPR and higher values of female and male spawning biomass per recruit. Female SPR and male SPR tended to decrease rapidly with increasing F and t_c , indicating that the stock is more sensitive

to exploitation at lower values of M . A higher value of natural mortality ($M = 0.3$) had the opposite effect, decreasing YPR and sex specific spawning biomass per recruit while female and male SPR tended to be higher. Increasing or decreasing the value of k by 10% produced similar results.

DISCUSSION

Age and growth

The use of otolith increments for aging red grouper has been previously validated. Schirripa and Burns (1997) compared growth estimations obtained from fish aged using otoliths and a tagging study and found strong agreement, supporting the hypothesis that red grouper form one increment per year. Moe (1969) applied marginal increment analysis as an indirect method to validate the annual formation of increments on 1 to 10 years old red grouper, concluding that fish younger than 4 years deposit one increment between March and May and older fish between May and June. Johnson & Collins (1994) reported that red grouper in the eastern Gulf of Mexico deposit an annual increment between April and July. In the Southeastern U.S., Stiles and Burton (1994) found that the formation of annual increments in red grouper 2 and 3 years old occurred in July and April respectively. In my study, it is suggested that red grouper deposit a new increment between July and August. Ideally, the annual formation of increments should be validated for each age (Beamish and McFarlane, 1983), but the number of samples was high enough to validate the annual formation of increments only for ages 4 and 5. Although unlikely, it is possible that in older fish otolith increments are not annual marks, and thus ages should be considered with some caution.

Red grouper otoliths presented abnormalities similar to those reported in other species. Crystalline areas and opaque deformities noticed in red grouper otoliths were comparable to findings of Wyanski et al. (2000) for snowy grouper (*Epinephelus niveatus*). Aberrant otoliths with calcitic crystallization similar to the ones observed in red grouper also have been reported for pollock (*Pollachius virens*) (Strong et al., 1986). Mugiya (1972) found for three species that only one of the sagittae was aberrant while the other one was normal. A similar situation was observed in red grouper.

In an aging study, it is important to establish that the first increment corresponds to the first year of life. Johnson & Collins (1994) reported a young of the year red grouper that measured 152 mm TL and had no annulus on its sagitta. The sagitta of a 151 mm TL red grouper captured in an estuary near Georgetown (SC) in September 2000 had no annulus either. The otolith radius of this specimen was less than the observed mean radius of the first increment, suggesting that the first increment counted in adult red grouper is the first annulus.

Information about the habits of juvenile red grouper is scarce. Despite extensive sampling efforts in depths from 9 to 338 m by MARMAP, from 1973 to 1999 using several types of gear including trawl nets and traps, no juvenile red grouper were captured (Jennings⁴). My observations of one young-of-the-year red grouper in an aquarium suggest that their cryptic habits make them unavailable to sampling gear utilized by MARMAP. Ross and Moser (1995) reported that red grouper juveniles are common in North Carolina estuaries, while juvenile red grouper are sometimes captured in habitat traps deployed in South Carolina estuaries (Powers⁵), suggesting that they may be estuarine dependent. Moe (1969) found that young-of-the-year red grouper are spread over rocky bottoms in low densities in depths down to 20 fathoms.

At age 2, red grouper are present in the fishing grounds, as evidenced by captures in MARMAP gear, and age-3 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 (Rohde³). This size is smaller than the theoretical mean length at age for one-year-old fish, thus red grouper probably move from estuaries to shallow water reefs during their first year of life.

Moe (1969) reported red grouper as old as 25 years off the west coast of Florida. Off the southeastern U.S., Stiles and Burton (1994) observed fish up to 16 years old, although fish older

⁴ Jennings, J. 2001. Grice Marine Laboratory. 205 Ft. Johnson Rd., Charleston, SC 29412. Personal commun.

⁵ Powers, P. 2000. South Carolina Department of Natural Resources. P.O.Box 12559, Charleston, SC 29422. Personal commun.

than 13 years were rare. In the present study, besides two 20 year olds, no fish older than 12 years were observed. Because of the lack of historical age data for red grouper off the Carolinas, it was not possible to assess if the absence of fish >13 years old reflected the effect of fishing mortality.

The Brody growth coefficient k estimated from observed and back-calculated length at age was slightly lower than in other red grouper studies (Moe, 1969; Stiles and Burton, 1991; Johnson and Collins, 1994), while the asymptotic length obtained from observed and back-calculated length at age was similar to previous studies. The fact that Lee's phenomenon was not observed in this study suggests that the back-calculation method using all increments was appropriate (Ricker, 1992).

The red grouper commercial fishery is clearly size selective. Since the establishment of the minimum legal size of 20 inches TL, fishermen have been capturing larger individuals by using bigger 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 which had not fully recruited to the fishery. For those ages therefore, estimations of mean length at age derived from fishery-dependent data would be biased even if a random sampling design is used (Goodyear, 1995). Alternatively, the chevron traps used by MARMAP for fishery-independent sampling are probably less selective (Dalzell, 1996). Combining fishery-dependent and fishery-independent samples can reduce some of the bias toward larger individuals. Still, it is difficult to assess the extent of the bias, and how the samples obtained are representative of the red grouper population off the Carolinas. The possibility of this bias in growth parameters needs to be considered when estimating biological reference points based on those parameters.

Reproduction

The spawning season observed off the Carolinas (February to July), with a peak during April, was similar to that in other regions. In the Gulf of Mexico, Moe (1969) observed mature active

females in pre-spawning phase between December and June and a peak spawning during April and May, while females in post-spawning phase were observed between May and August. Off the Yucatán Peninsula, ripening females were observed between September and March and running ripe females between January and March (Brulé et al., 1999). In all regions, including the Carolinas, male reproductive activity started earlier and lasted longer than the female spawning season.

A gradient of increasing length with depth was observed for red grouper in several previous investigations (Moe, 1969; Rivas, 1970; Goodyear and Schrippa, 1993). Such a gradient could be the product of size-dependent mortality in shallow water (McPherson and Duarte, 1991), but for red grouper it may indicate the presence of ontogenic migrations. Moe (1969) provided tagging evidence that red grouper resided in shallow water reefs until reaching sexual maturity (400 mm SL, 5 years old), and then migrated towards offshore reefs. Brulé et al. (1999) observed a similar pattern, finding only immature females in inshore collections (7-27 m), and immature and mature females, transitionals and males in offshore collections (30-90 m). My data indicated that red grouper off the Carolinas presented a similar pattern, remaining in shallow waters (<40 m) until reaching sexual maturity and then moving to offshore locations. Most of the inactive females of uncertain maturity were also captured at depths <40 m. These young females could be in resting state but had not yet developed the distinctive histological characteristics of a mature female.

The observed distribution of red grouper suggests the possibility of inshore-offshore migrations. Immature females and inactive females of maturity unknown were only captured in inshore locations. During the spawning season, the majority of mature females sampled at depths < 40 m were in the resting state, while at depths > 40 m most of female red groupers were in developing or spawning states. The presence of resting females during the spawning period have been reported by Moe (1969) and Brulé et al. (1999). Resting females are also present during the spawning season in other grouper species, such as *E. niveatus* (Wyanski et al., 2000). After the spawning season, almost all mature female red grouper were in the resting state, regardless of

depth of capture. In addition, transitionals and males occurred most frequently at depths >40 m. The presence of resting females at depths < 40 m suggests that there is some movement of females from offshore spawning locations toward inshore reefs. The return of females to shallower water after spawning may explain the abundance of females with atretic oocytes in shallow waters in the present study. From 16 females with atretic oocytes for which a depth of capture was recorded, 14 (87.5%) were observed at depths < 40 m. The presence of resting females in inshore locations during the spawning season may also indicate that some females remain inshore and do not spawn. Coleman et al. (1996) observed that some female gag remained inshore during the spawning season, never entering the spawning stock. Transitionals and males were more commonly found at depths > 40 m, suggesting that mature red grouper became permanent residents in offshore reefs after changing sex. Nevertheless, without the conclusive evidence of a tagging experiment this migratory pattern remain hypothetical.

At depths >40 m, red grouper in spawning condition were captured throughout the sampled area and were not restricted to any specific location. Sadovy et al. (1994) suggested that in this aspect the spawning behavior of the red grouper and the red hind, *Epinephelus guttatus*, are similar. In comparison, other species like Nassau grouper (*E. striatus*) aggregate in large numbers during the spawning season. Coleman et al. (1996) speculated that red grouper spawn in small polygynous groups distributed over a broad area.

Moe (1969) found no histological or analytical evidence to suggest that red grouper spawn more than once each season, and suggested that vitellogenic oocytes are retained in the gonad for one or two months and that all spawning occurs in May. Alternatively, Koenig (1993, Cited in Goodyear and Schrippa, 1993) analyzed oocyte diameter distributions and concluded that red grouper release batches of oocytes during a protracted spawning season. This conclusion is also supported by Johnson et al. (1998), based on the presence of different oocytes stages and high levels of sex steroids during the spawning season. The presence of developing females with

postovulatory follicles in my study indicates that red grouper spawn more than once during each season.

For species with indeterminate fecundity, an estimation of spawning frequency is required to estimate total and annual fecundity. Spawning frequency estimations are based on the assumption that some histological structures, generally hydrated oocytes or postovulatory follicles, indicate imminent or recent spawning. The rate of postovulatory follicles degradation varies with species and water temperature (Fitzhugh and Hettler, 1995); however, no description of degradation rates in groupers was found in the literature. In this study, I assumed that changes in postovulatory follicle structure were similar to those in skipjack tuna, *Katsuwonus pelamis*, spawning at 25°C (Hunter et al. 1986). No red grouper females with non-degraded postovulatory follicles, corresponding to up to two hours after spawning, were observed. Only five females were observed with <12 h old postovulatory follicles, and five with 12-24 h old follicles. These results are similar to Collins et al. (1998), who found few individuals of gag with postovulatory follicles, and to Crabtree and Bullock (1998) who reported that postovulatory follicles were rare in black grouper (*Mycteroperca bonaci*). Because red grouper were sampled during daylight, the absence of new postovulatory follicles (<2 h old) could indicate that red grouper spawn during the night.

Poor preservation of gonads may also account for the low number of postovulatory follicles observed, as these structures are sensitive to decay (Hunter and Macewicz, 1985, Pashuk⁶). Only 50% of the females with postovulatory follicles were sampled from the commercial catch, even though samples from this source corresponded to 84.5% of the total number of active females sampled. Fish in commercial boats may be kept on ice several days before landed. Gonads of many of the samples obtained from the commercial fishery showed signs of decay. MARMAP samples, on the other hand, were kept on ice <24 h before fixation of gonad tissue. It is likely that

⁶ Pashuk, O. 1999. South Carolina Department of Natural Resources. P.O.Box 12559, Charleston, SC 29422. Personal communication.

my estimation of spawning frequency using postovulatory follicles is biased because most of my samples were obtained from the commercial fishery.

Estimates of spawning frequency can also be based on the frequency of females with hydrated oocytes (Hunter and Goldberg, 1980). Hydration is reported to occur <12h before spawning (Taylor and Murphy, 1992; Fitzhugh et al., 1993; Sullivan et al., 1997). If the duration of the hydration phase is similar in red grouper, using hydrated oocytes as an indication of imminent spawning may underestimate spawning frequency because a specimen captured with no hydration during the morning could start hydration during the afternoon and spawn during the night. In this case, considering migratory nucleus oocytes as an additional indication of imminent spawning could eliminate some of the bias. Thus, spawning frequency estimations based on the combined frequencies of females with hydrated oocytes and migratory nucleus oocytes are probably more reliable than estimations based on hydrated oocytes alone. Nevertheless, until the timing of the spawning activity of the red grouper is determined, spawning frequency estimates in the present study must be considered only an approximation and should be used with caution. In addition, age-specific differences in spawning frequency were not investigated because of the low number of individuals sampled with signs of an imminent or past spawning event. Changes in spawning frequency with age have been reported for several species, including gag (Collins et al., 1998) and are likely to occur also in red grouper.

All the characteristics mentioned as evidence of protogyny by Sadovy and Shapiro (1987) were observed in red grouper sampled off the Carolinas: testes with a central cavity lined by a membrane, transitional individuals, testes with atretic bodies, and sperm sinuses within the ovarian wall. Population characteristics also supported the hypothesis of protogyny: female biased sex ratio, differences in length distributions, lack of males in the younger age classes and in the smaller length classes.

Transitional individuals were observed more frequently than in previous studies. Moe (1969) and Brulé et al. (1999) reported that approximately 1% of the individuals sampled were

transitionals. The higher percentage of transitionals (5.8%) observed in this study can be explained by differences in the definition of the transition stage. Moe (1969) considered transition a short stage, in which only spermatogonia and primary spermatocytes were observed in crypts along the periphery of the ovarian lamellae. Individuals with more advanced stages of spermatogenesis and sinuses in the dorsal musculature of the gonad for the collection of sperm, but in which crypts of seminiferous tissue are still located in the periphery of the ovarian lamellae, were classified as immature males. Individuals with these characters were classified as transitionals in the present study. In addition, a transitional fish was considered male when spermatozoa were observed in collecting ducts and sinuses, meaning that the specimen was capable of spawning. Brulé et al. (1999) considered transitionals as those whose gonads had primarily ovarian tissue with proliferation of small crypts of spermatogonia and spermatocytes. Individuals with gonads in which advanced stages of spermatogenesis had invaded the ovarian lamella were classified as males. Fish with these characteristics would have been classified as a transitional in the present study if there were no spermatozoa present in the collecting ducts. Finally, it is possible that some individuals classified as transitionals, because there were no sperm in sinuses and collecting ducts, were actually spent or resting males. In any case, transitional individuals were counted as males in all calculations because it was assumed that sex transition to male is a non-reversible process.

Seasonal trends in transition differed from previous studies. Moe (1969) reported that most sexual transition occurred from April to June, although if Moe's (1969) immature males are considered transitional individuals there was no clear seasonal trend in sex transition. Brulé et al. (1999) captured transitionals during five months. In the present study, with the exception of the month of peak spawning sex transition was observed year-round with a clear peak of transition in November. The fact that transitional individuals were found year-round supports the hypothesis of Coleman et al. (1996) that male and female red grouper coexist year round; thus, sex ratio assessment and initiation of sex change can occur anytime.

Population dynamics

Red grouper total mortality rate off North Carolina and South Carolina (0.75) is higher than mortality rates reported in previous studies. Moe (1969), using catch curve analysis, estimated that the total mortality rate for red grouper in the Gulf of Mexico was 0.32. Stiles and Burton (1994) estimated a total mortality rate of 0.46. They used samples collected from 1972 to 1988 and during that extended period mortality rates may have changed. If fishing mortality increased, older classes would be over-represented in the catch and the mortality estimates based on catch curve analysis could underestimate the true value of total mortality (Bannerot et al., 1987). Using catch curve analysis, Goodyear and Schripa (1993) obtained a total mortality estimation of 0.50 for red grouper in the Gulf of Mexico for the period 1991-1992. The high total mortality rate observed in my study is consistent with the recent history of the fishery. Since 1992, red grouper landings in North Carolina have increased by a factor of five. Although changes in red grouper biomass are possible, it is likely that some of the increase in landings was the result of increased fishing mortality.

Natural mortality estimated from established relationships between this parameter and life history characters were comparable to estimates in previous studies. Stiles and Burton (1991) used a value of $M = 0.17$ to estimate yield per recruit on the Atlantic Coast. Goodyear and Schripa (1993) assumed that $M = 0.2$, although they considered that it may be too high based on the frequency of older fish off western Florida. Huntsman et al. (1990) considered that using relationships between natural mortality and life history traits to estimate natural mortality tended to produce overestimations. They noted that by using Hoenig's (1983) relationship the estimate of M for gag was 0.2, but only an estimate of approximately 0.1 allowed sufficient historical fishing mortality to explain the large catches and decline of the population size of this species off the southeastern U.S. Given the important role that natural mortality estimates have in establishing reference points and the inherent difficulty in obtaining unbiased estimations (Vetter, 1988), reference points should be estimated for a range of possible values of M .

There is concern that reference points based on SPR may not be adequate for establishing management regulations for protogynous species (Coleman and Koenig, 1999). In general, female spawning biomass is used as a proxy to egg production in such SPR calculations. In protogynous species, males may be selectively removed by the fishery, owing to their large size or behavioral traits (Gilmore and Jones, 1992). For example, between 1976 to 1982 male gag constituted 19.6% of the mature individuals, but in 1995 only 5.5% of mature individuals were males (McGovern et al., 1998). Because of their mode of reproduction, species like gag or Nassau grouper could compensate poorly for the losses of males because males and females co-occur only when forming spawning aggregations and the window of opportunity for sex ratio assessment and the initiation of sex change is relatively short (Coleman et al., 1996). As a result, the number of male individuals in the population could decrease then to a point where a significant proportion of eggs go unfertilized and recruitment is threatened. Theoretical YPR and SPR models that include sex change predict decreases in sex ratio with fishing exploitation (Buxton, 1992; Punt et al., 1993). Alternatively, it has been suggested that some species of protogynous hermaphrodites such as red grouper can compensate for the loss of males by initiating sex transition at smaller lengths. Coleman et al. (1996) reported little change in red grouper sex ratio off the west coast of Florida after 25 years of relatively intense exploitation. They suggested that male and female red grouper co-occur year-round, thereby having greater opportunity for sex ratio assessment and initiation of sex change. Mean length at age of male red grouper is significantly larger than of females, and the presence of a growth spurt after sex change could explain this difference (Garrat et al., 1993). Differences in mean length at age between sexes could also be produced if as a response to the removal of males of a group, the largest female undergoes sex change. This mode of sex change has been described among other serranids (e.g. *Anthias squamipinnis*, Shapiro, 1980). Change of mean length at sex transition as a response to fishing mortality has been reported for red porgy (*Pagrus pagrus*) (Harris and McGovern, 1997). Sex ratio compensation mechanisms in red grouper can make sperm limitation problems more unlikely by maintaining a relatively constant

proportion of males in the population, although the decrease in length at transition would produce a further decrease in the female spawning biomass. In this case, reference points based on SPR may be adequate for protogynous species with sex ratio compensation, as long as these mechanisms are included in the SPR estimations.

The effect of fishing mortality on the sex ratio of a protogynous stock will depend on the type of sex ratio compensation present. Huntsman and Schaaf (1994) explored the effect of four types of sex ratio compensation mechanisms on the reproductive capacity of the graysby (*Epinephelus cruentatus*), a protogynous grouper. Reproductive capacity was measured using a proxy for relative reproductive success equivalent to male SPR. They reported that sex ratio compensation mechanisms allowed a higher reproductive capacity by maintaining higher male SPR than uncompensated protogynous stocks, although the effect of each type of compensation was different. For the model developed in this study it was assumed that red groupers compensate through number-compensated protogyny with fixed maturity, that is sex transition was initiated at younger ages to maintain a constant numeric sex ratio, while age at maturity remained constant. Although observations by Coleman et al. (1996) suggest that there is number compensated protogyny in red grouper, it is not possible to establish if there were changes in age at maturity due to the lack of historical data. The selection of a constant age at maturity produces more conservative estimations of female SPR because the loss of female spawning biomass is not compensated by maturation at earlier ages.

Estimated yield per recruit is comparable to values reported in previous studies. If release mortality is not included in the model, YPR is maximized when delaying harvest until age 5 and then applying a fishing mortality of 0.6 or higher. Huntsman et al. (1994) obtained very similar results, suggesting that YPR for red grouper off the southeastern U.S. is maximized when $t_c = 5$ and F is 0.4 or higher. Stiles and Burton (1994) estimated that YPR would be maximized at a fishing mortality rate of 1.1 and an age of entry to the fishery between 8 and 9 years, although they used data from the period 1972-1988. Alternatively, if release mortality is included in the

model, YPR is maximized when $t_c = 4$ and $F = 0.4$. When considering release mortality my results are similar to those reported by Waters and Huntsman (1986): maximum YPR values tend to be lower than if release mortality is not considered, YPR is maximized at lower fishing mortality rates and younger age at entry (or smaller minimum sizes), and the YPR isopleths close at high fishing mortality rates. Under the present conditions ($F = 0.55$, $t_c = 4$) the commercial red grouper fishery off the Carolinas was approaching 98.4% of the maximum YPR.

Previous estimations of SPR for the southeastern U.S. produced contradictory results. Huntsman et al. (1994) estimated red grouper SPR based on 1988 conditions, assuming that $M = 0.2$ and suggested that the "subpopulation" off the Carolinas was impacted more severely (SPR = 0.24-0.34) than the rest of the southeastern U.S. (SPR = 0.41). They suggested that if $t_c = 5$, SPR would not decrease below 0.30 even at high mortality rates. Huntsman et al. (1992⁷) analyzed data from 1990 assuming that the natural mortality rate was 0.2 and reported that SPR was 0.61 and therefore the population was not approaching overfished status. They considered that the legal size (20 inches) would maintain a SPR of 0.68. Potts et al. (1998⁸) using data from 1996 and assuming $M = 0.2$ estimated a SPR of 0.21 and noted that a reduction in fishing mortality from 0.26 to 0.19 was required to obtain a SPR of 0.30. Apparently, this reduction in fishing mortality has not occurred.

The present results indicated that red grouper population off the Carolinas is probably overfished. If $M = 0.2$, $F = 0.55$ and $t_c = 4$, and sex ratio compensation and release mortality are included in the model, female SPR is 0.18, whereas male SPR is 0.22. A reduction of fishing mortality to 0.29 is required to obtain a female SPR of 0.30. At the present fishing mortality even if $t_c = 10$ female SPR will not be higher than 0.26.

⁷ Huntsman, G.R., J. Potts, R. Mays, R.L. Dixon, P.W. Willis, M. Burton and B.W. Harvey. 1992. A stock assessment of the snapper-grouper complex in the U.S. South Atlantic based on fish caught in 1990. Beaufort Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service.

⁸ Potts, J.C., M.L. Burton and C.S. Manooch, III. 1998. Trends in catch data and estimated static SPR values for fifteen species of reef fish landed along the southeastern United States. National Marine Fisheries Service, Beaufort Laboratory

The model assumed that sex ratio compensation operated at all combinations of F and t_c . This is probably an oversimplification because at high fishing mortality rates mechanisms of sex ratio assessment and sex change initiation would be overridden; however, at moderate fishing mortality rates it is probably a good approximation. The model output is relatively resilient to changes in the value of natural mortality and von Bertalanffy growth parameters. Given the uncertainties in the estimation of the natural mortality parameter, it is advisable to use a conservative value (lower M) when estimating reference points. In addition, a conservative value of k should be used to compensate for biases caused by the use of size selective gear by the commercial fishery.

SUMMARY AND CONCLUSIONS

Between 1996 and 2000, red grouper were obtained from commercial fishermen and fishery independent sampling off the coast of North Carolina and South Carolina. Fish captured by commercial fishermen ($n = 1928$) ranged between 384 and 851 mm TL. Approximately 22% of the fish landed were smaller than the minimum legal size (508 mm TL). Fish sampled by MARMAP ($n = 218$) ranged between 315 and 779 mm TL. The mean length of fish sampled from commercial fishermen was significantly higher (ANOVA $p < 0.05$) than of fish captured by MARMAP.

Otolith marginal increment analysis indicated that red grouper deposit one increment per year, between July and August. Red grouper ages ranged between 2 and 20 years old, although 95% of the fish were 6 years old or younger. Mean length at age was significantly lower in fishery-independent samples 5 years old or younger than in fishery-dependent samples (ANOVA, $p < 0.05$). Age length keys from both sources were compared using Fisher's exact test. Age length keys were not significantly different, allowing for pooling of fishery-independent and fishery-dependent samples for growth and reproduction analyses.

Fish lengths were back-calculated with the body-proportionate hypothesis using a geometric mean regression to describe the relationship between otolith radius and fish length. A von Bertalanffy growth curve was fitted to back-calculated length at age data, using a non-linear mixed model. The parameters estimated are the following: $L_{\infty} = 836.12$, $k = 0.170$, $t_0 = -1.278$. To allow comparison with previous studies, a von Bertalanffy growth curve was fitted to observed length at age. The following parameters were estimated: $L_{\infty} = 853.22$, $k = 0.209$, $t_0 = -0.812$.

Gonad samples were embedded in paraffin, sectioned and stained with double-strength Gill haematoxylin and eosin-y. Sex and developmental stage were agreed upon by two readers for

2068 samples. Using probit analysis, it was estimated that 50% of female red grouper reach sexual maturity at 2.4 years old and 487 mm TL. Overall sex ratio for red grouper (6.6:1 females to males) was significantly different than 1:1. Sex ratio differed significantly from 1:1 in favor of females between ages 2 to 6. In addition, sex ratio was female oriented when TL was less than 680 mm.

Females with hydrated oocytes were found between February and June. A peak of spawning activity was observed in April. Females in late developmental stages were found between January and July. Red grouper have a protracted spawning season, although only a small number of females (about 30% or less) are active (in developing, spawning or spent stages) at any moment. Hydrated oocytes or migratory-nucleus oocytes were found in 30 females, but postovulatory follicles < 12h old were observed in only five specimens, suggesting that red grouper are spawning in the evening or early night. Based on the frequency of specimens observed with hydrated or migratory-nucleus oocytes, it was estimated that red grouper spawned every 2-3 days, equivalent to a maximum of 42 times each spawning season. Most of the spawning females were captured at depths > 40 m.

Red grouper are protogynous hermaphrodites. Transitional fish were found nearly year round. Only in April, during peak spawning, were no transitionals observed. A peak of transition was observed in November. Using probit analysis, it was estimated that 50% of red grouper changed sex at 7.2 years old and at 690 mm TL. Transition was observed in a broad range of ages (3-10 years) and sizes (455-744 mm TL), which indicated that this process is socially mediated, rather than size or age mediated.

The high fishing mortality rate estimated was consistent with the recent history of the red grouper fishery. Between 1991 and 1999, red grouper landings off North Carolina increased five fold. It is unlikely that this increase in landings was caused by an increase in red grouper biomass, but probably reflects an increase in fishing mortality.

The low male and female SPR observed indicates that red grouper off the Carolinas are probably overfished. If the current minimum legal size of 20 inches is maintained, fishing mortality must be reduced to 0.29 in order to increase female SPR to more than 30%. At the present fishing mortality of 0.55, only very large minimum legal sizes will have that effect. Nevertheless, a reduction in fishing mortality may be difficult to achieve. Red grouper is one of the species captured by fishermen targeting the multi-species snapper-grouper complex and minimum legal sizes or bag limits may not be effective in reducing fishing mortality because of high release mortality after incidental capture.

An alternative to increasing SPR is to protect a fraction of the red grouper population from fishing mortality by establishing marine protected areas (MPAs). MPAs not only can protect reef ecosystem structure and the genetic diversity and age structure of reef fish species like the red grouper, but could also export recruits and adult individuals to exploited areas. In addition, given the uncertainties in reef fish stock assessment, MPAs provide a safety net against failures in fishery management.

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Figure 1. Sagittal otolith section of an age-5 red grouper. Section made along the dorso-ventral plane through the core. Line indicates the axis of measurement of otolith radius and increment radius.

Fig. 1

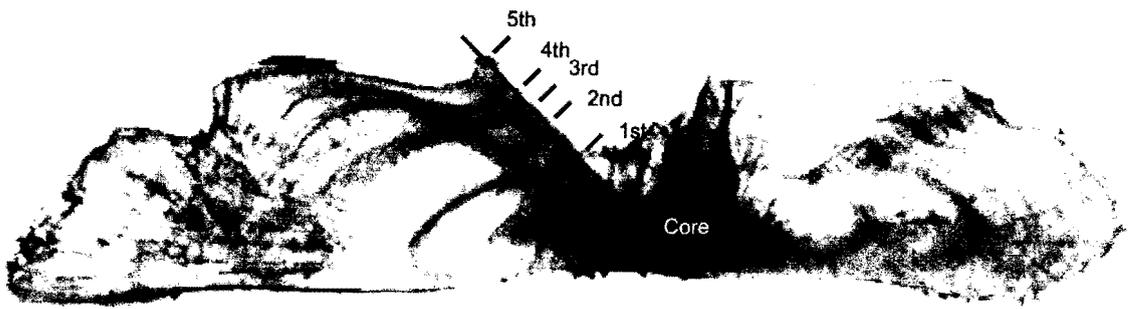


Figure 2. MARMAP sampling locations during 1991-2000, and locations where red grouper were caught.

Fig. 2

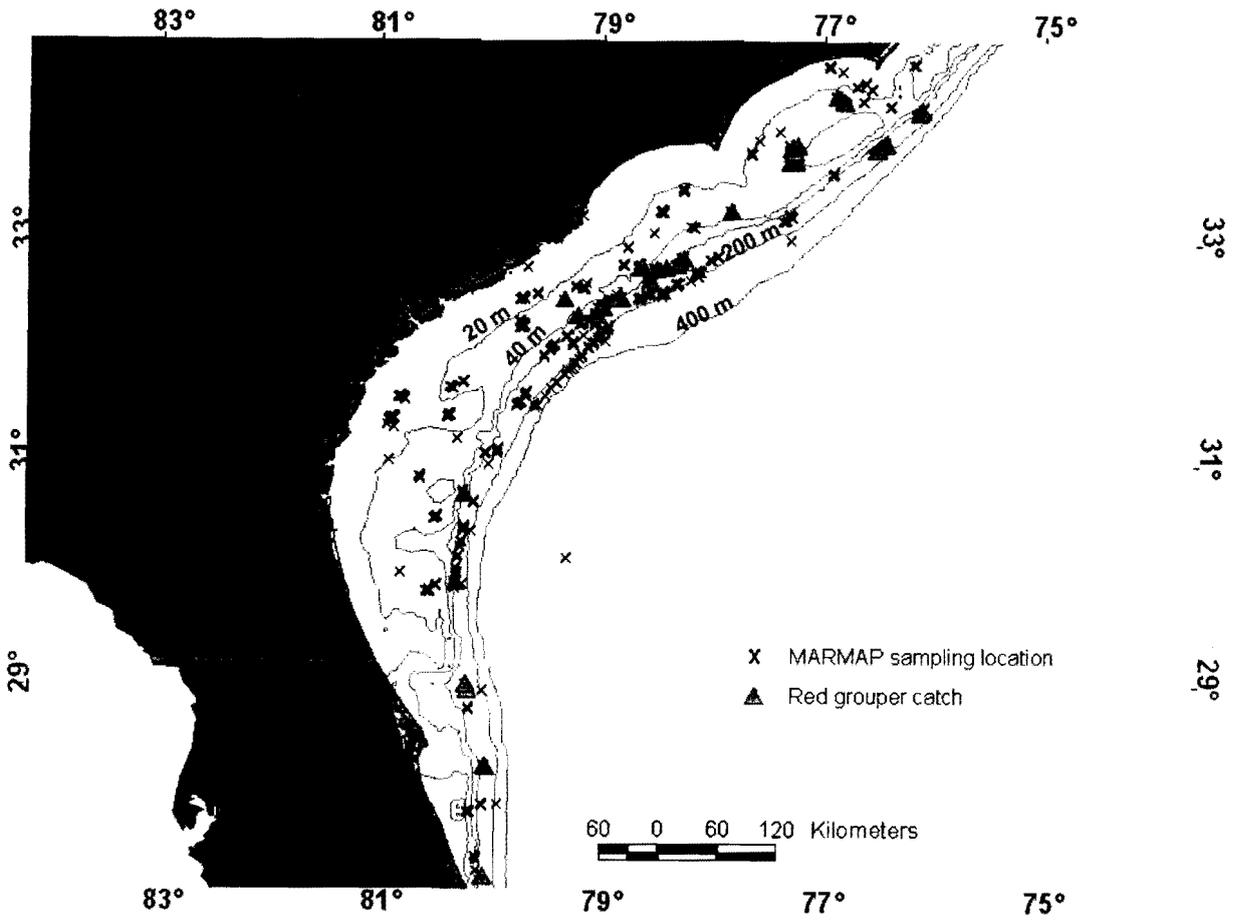


Figure 3. Length and age frequencies of red grouper sampled from the commercial fishery (A) and obtained through the MARMAP fishery-independent sampling program (B). Note the difference in vertical scales. Two 20-year-old specimens sampled from the commercial fishery are not included in the age plots.

Fig. 3

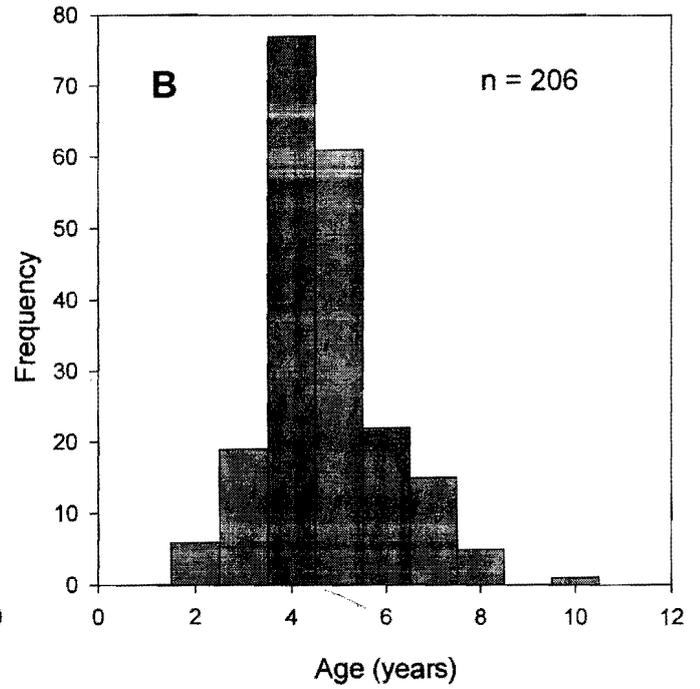
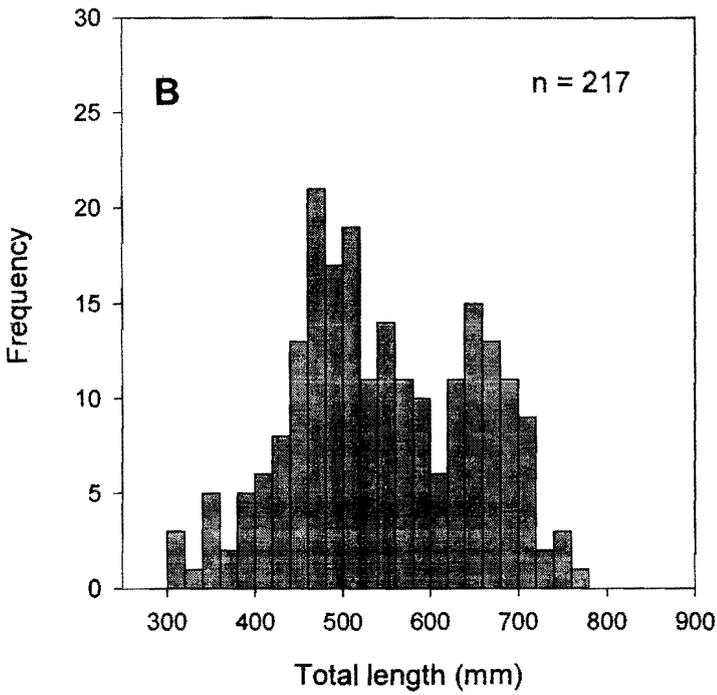
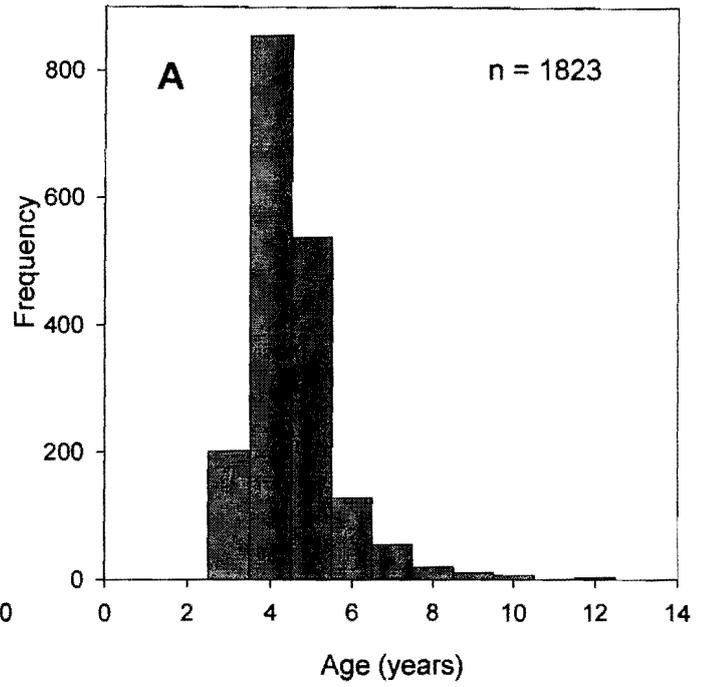
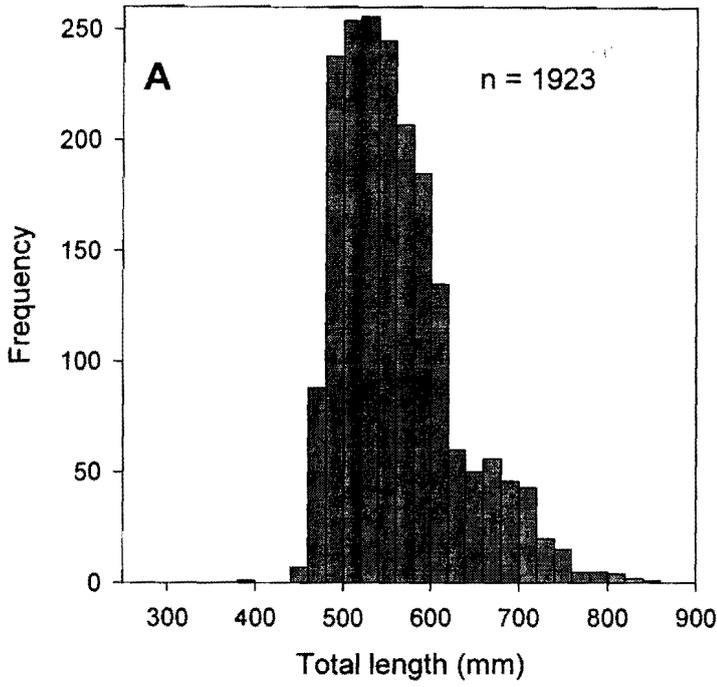


Figure 4. Relationship between total length and depth of capture for red grouper captured by the MARMAP program off North Carolina and South Carolina (1991-2000).

Fig. 4

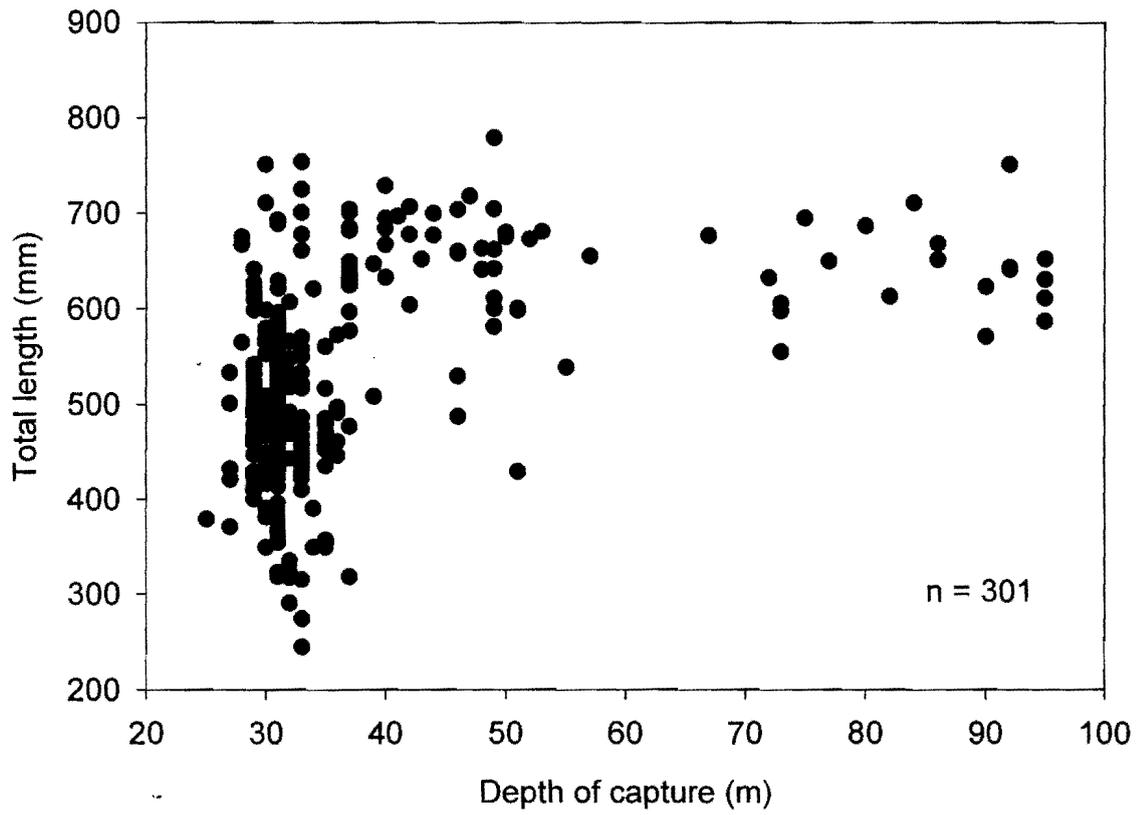


Figure 5. Mean marginal increment on otoliths of 1059 red grouper ages 2-12. Bars indicate standard deviation.

Fig 5

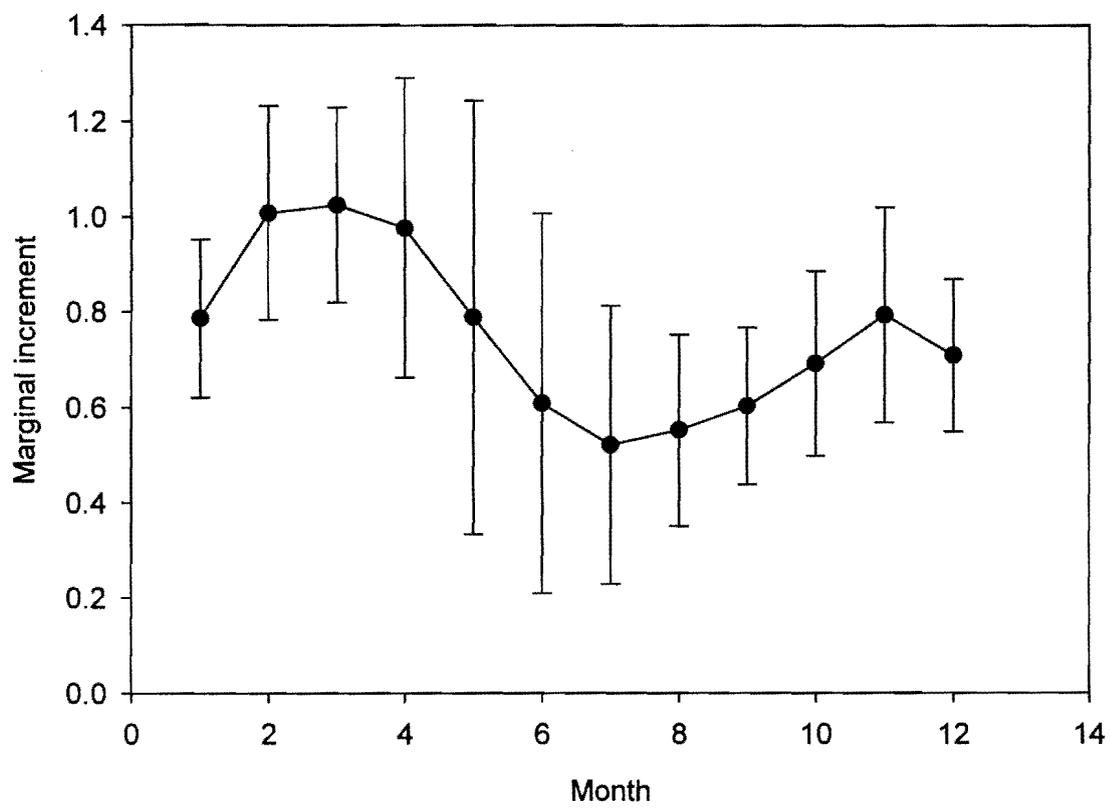


Figure 6. A. Left sagittal otolith with aberrant crystallization from an age-5 red grouper. B. Right otolith of the same specimen, with normal crystallization. C. Otolith of an age-4 red grouper with partial aberrant crystallization.

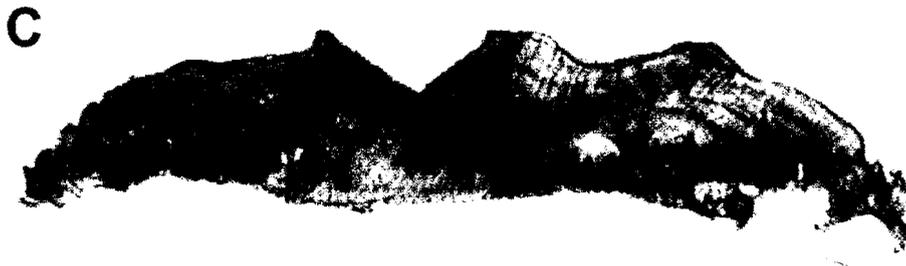


Figure 7. Relationship between otolith radius and total length for red grouper off North Carolina and South Carolina from fishery-dependent and fishery-independent sources, 1996-2000. Line indicates the geometric mean regression fitted to the data.

Fig. 7

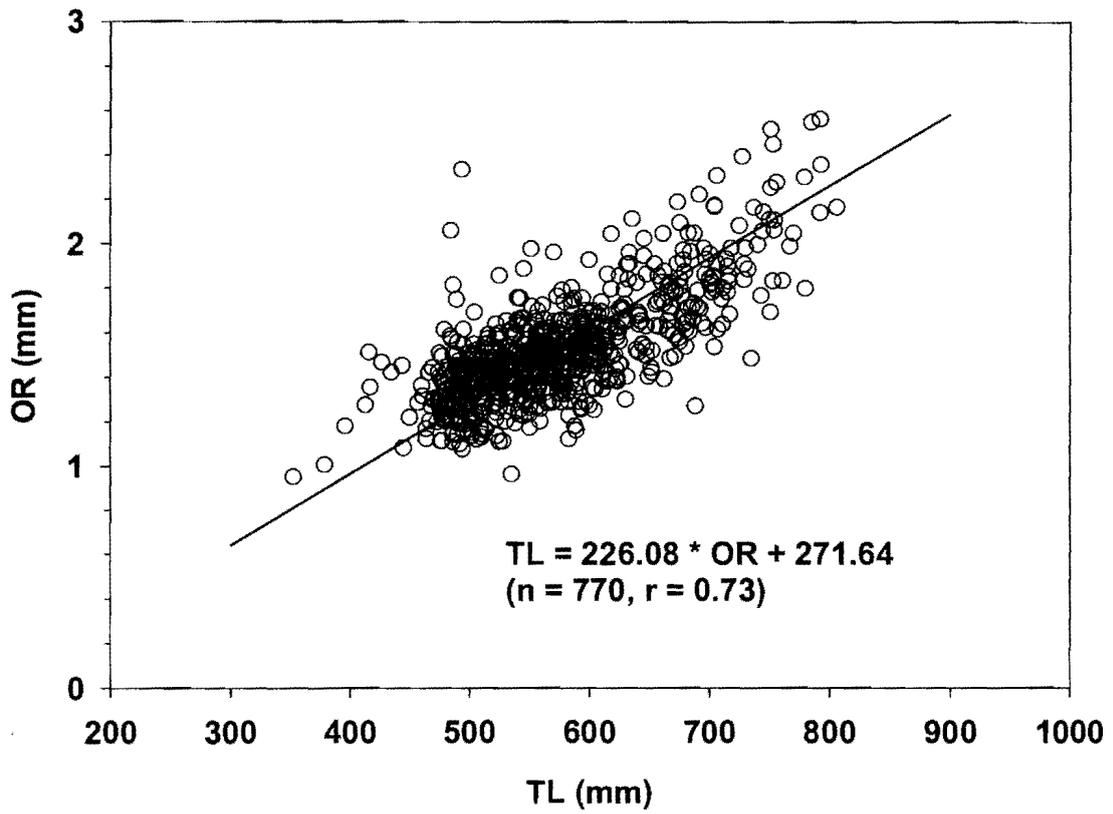


Figure 8. Observed age and total length of red grouper off North Carolina and South Carolina, and von Bertalanffy growth curves fitted to observed and back calculated length at age.

Figure 9. Mean back calculated total length (TL) between ages 1 to 5 as a function of age at capture. The lack of a significant trend (simple linear regression, $P > 0.05$) indicates the absence of Lee's phenomenon.

Fig. 9

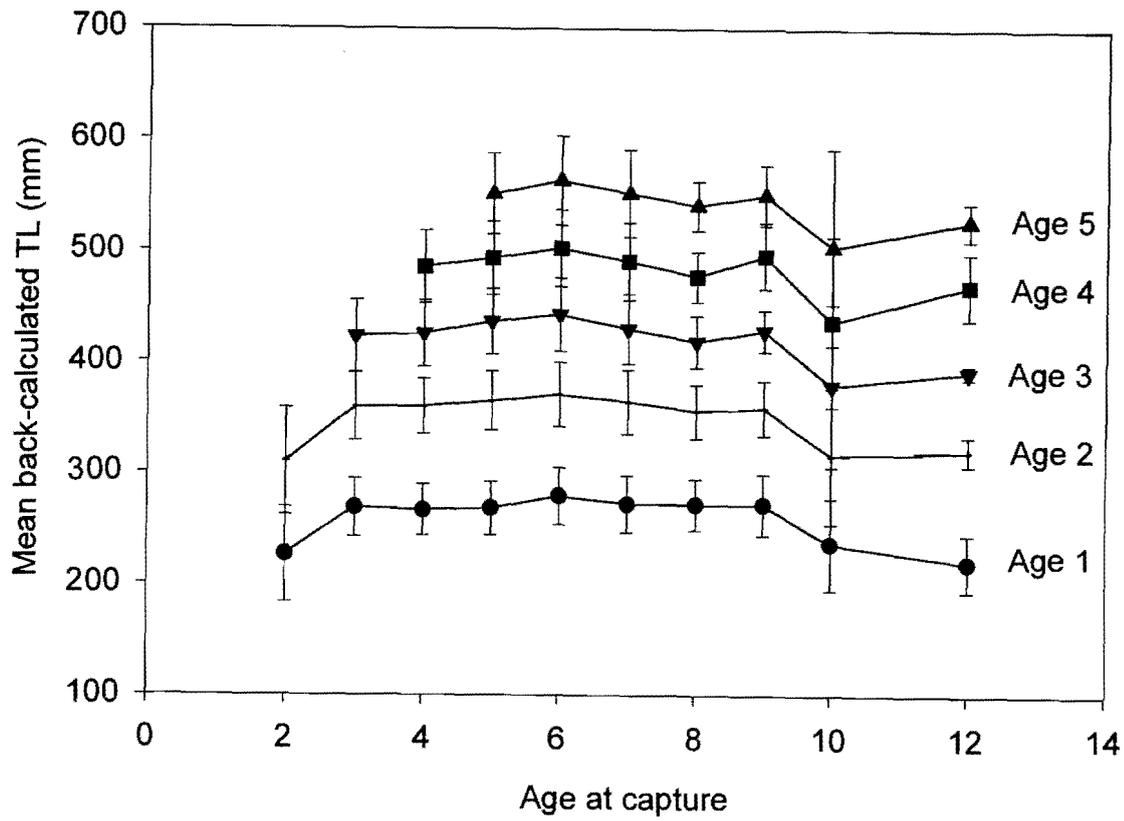


Figure 10. Maturity and transition ogives by age and total length (TL). Transition ogives include transitional and males.

Fig. 10

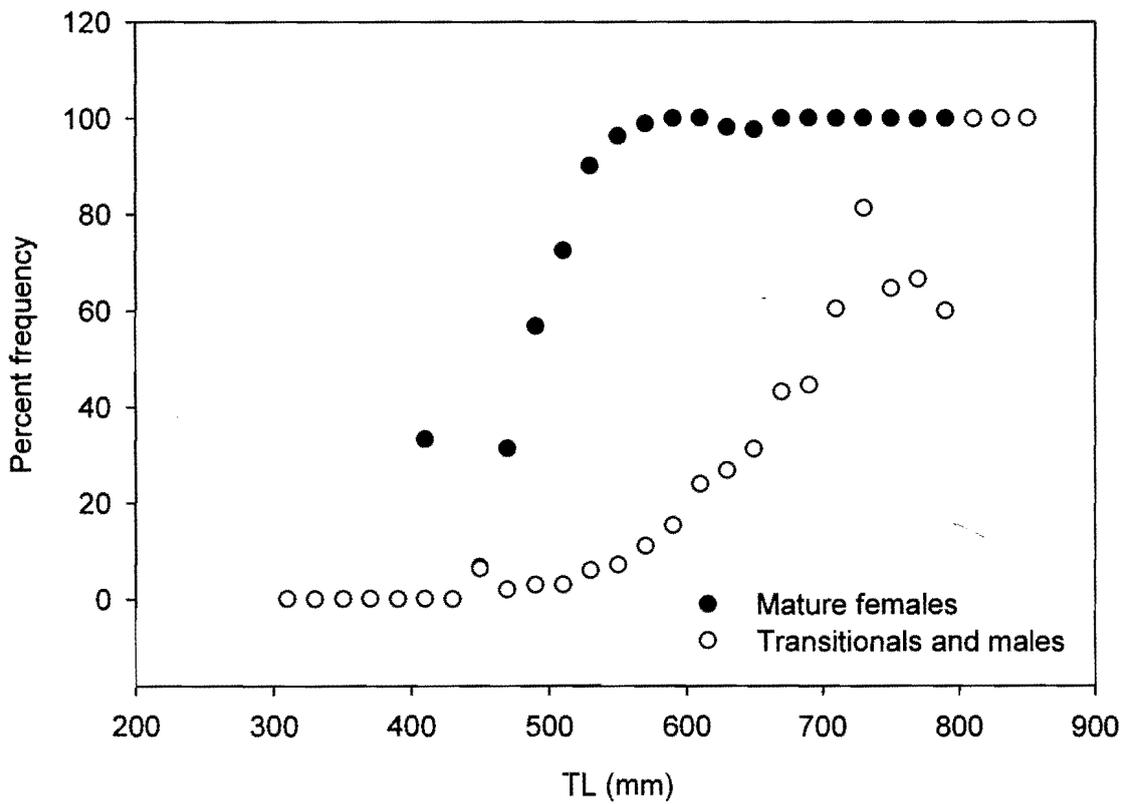
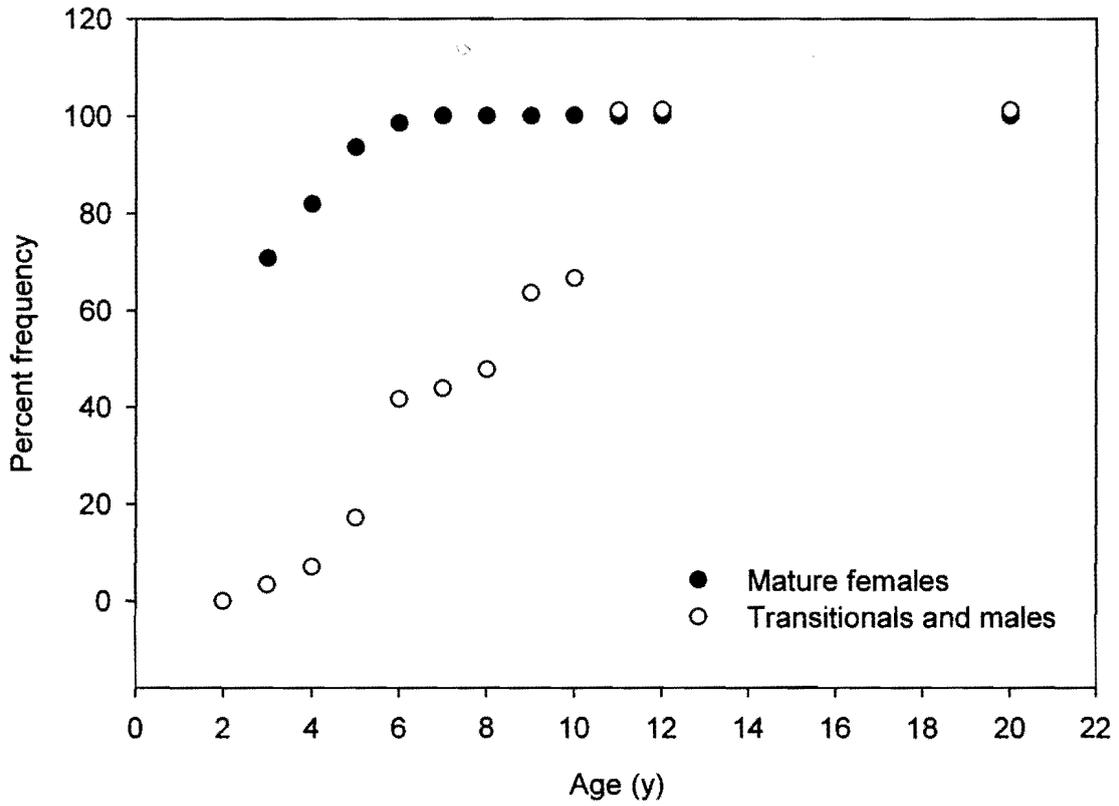


Figure 11. Comparison between length frequencies of immature red grouper with length frequency of resting females and active females. Active females includes developing, spawning or spent females.

Fig. 11

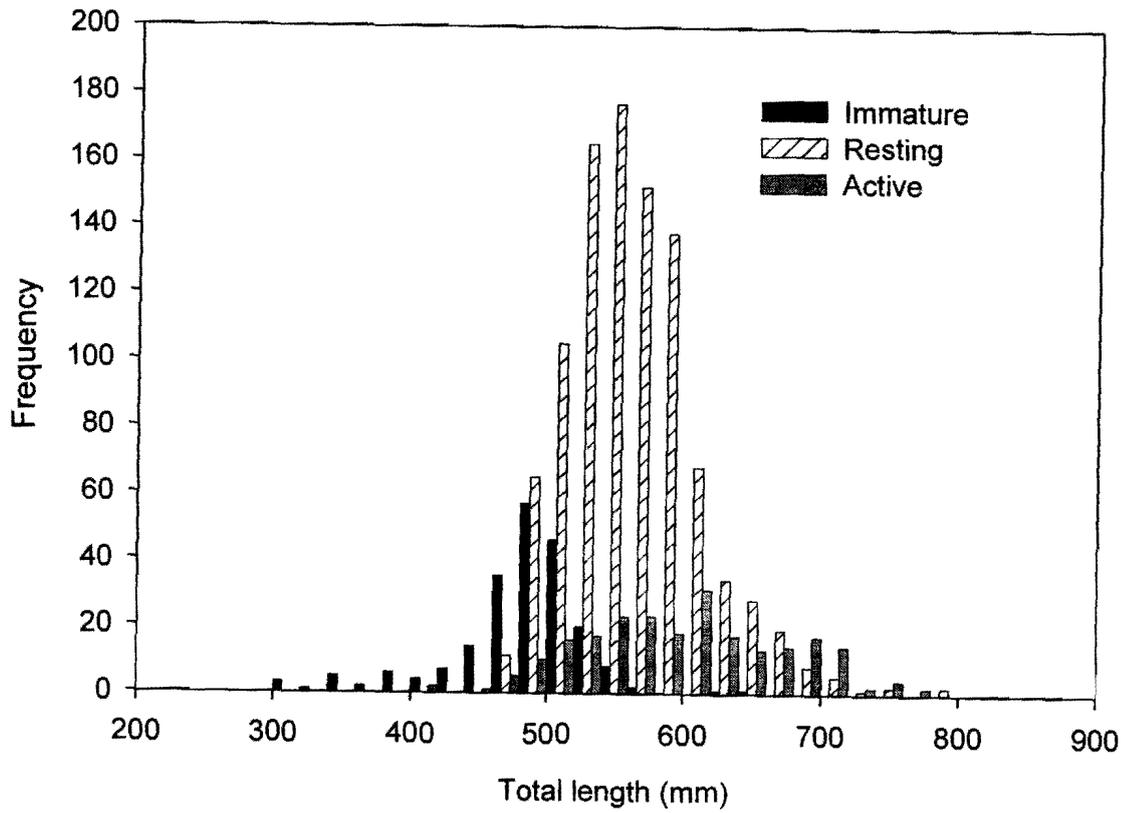


Figure 12. Spawning season for female (A) and male (B) red grouper off North Carolina and South Carolina, 1996-2000. Number above each bar is the sample size.

Fig. 12

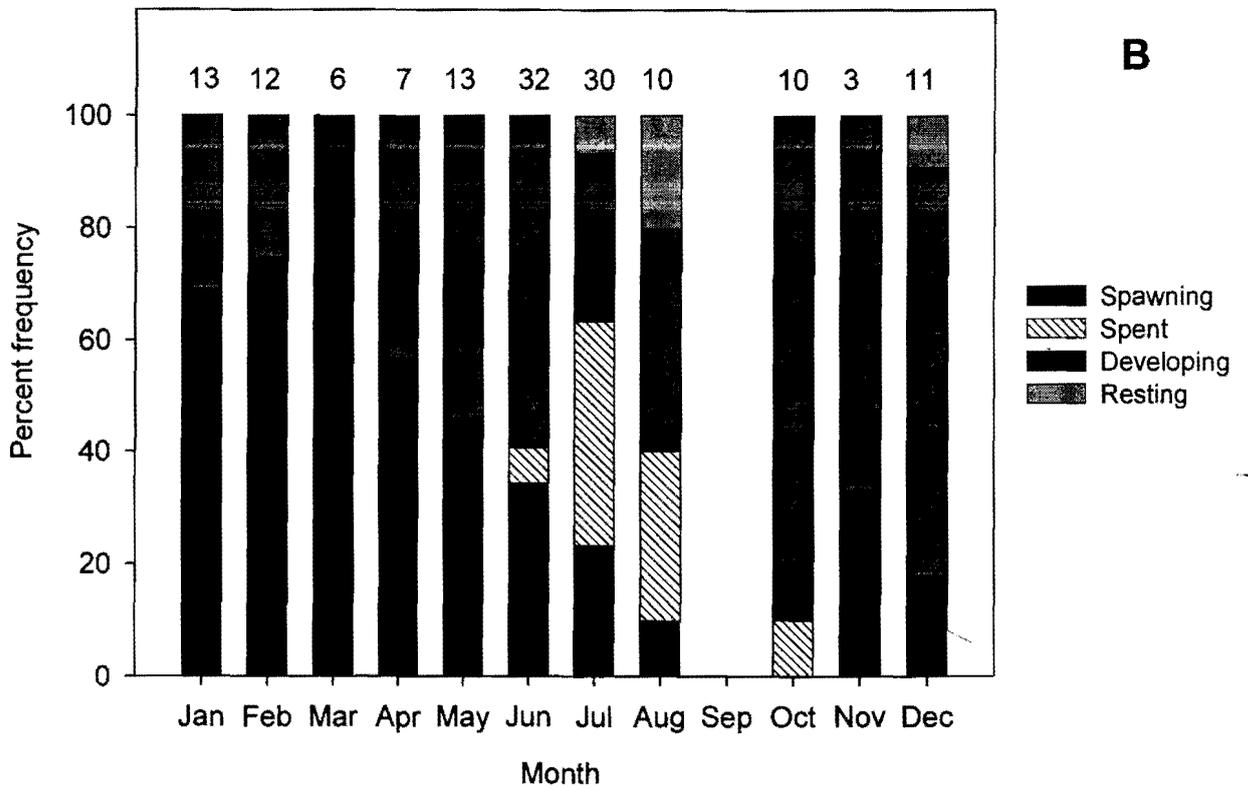
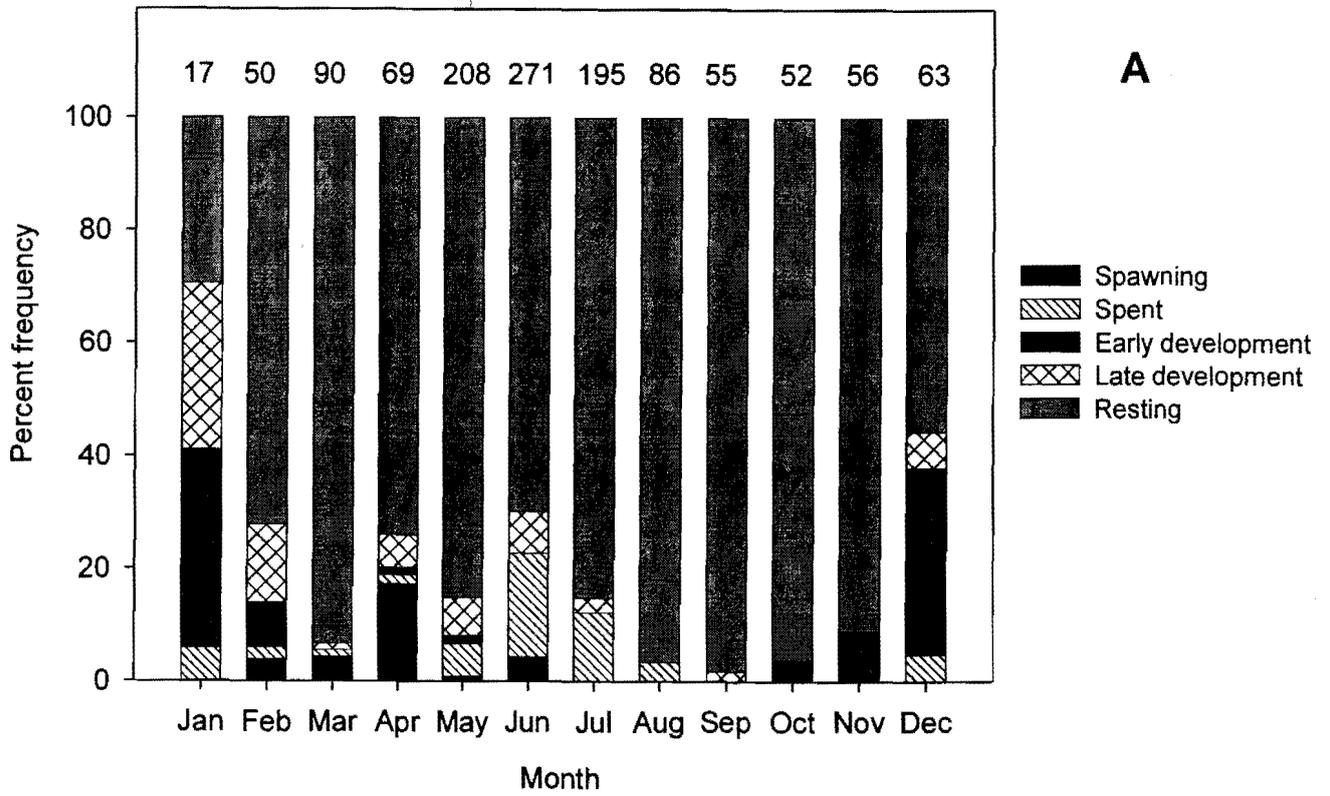


Figure 13. Male and female mean gonadosomatic index (GSI). Bars indicate standard deviation.

Fig 13

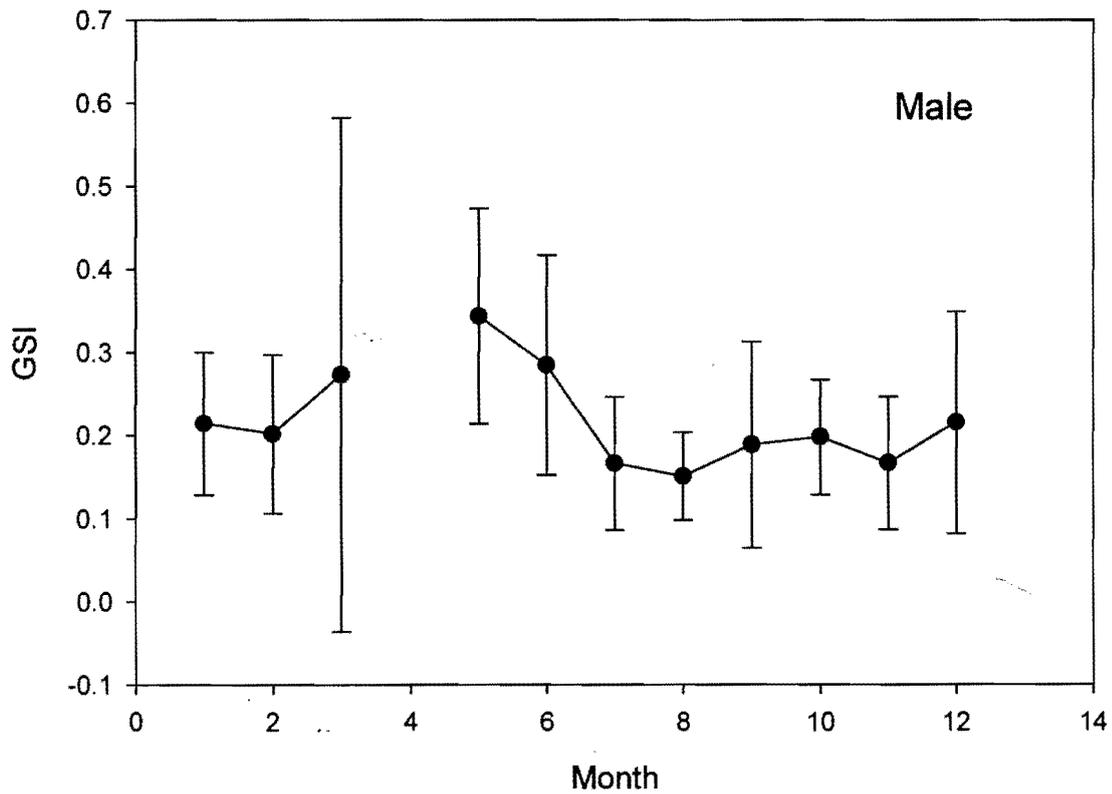
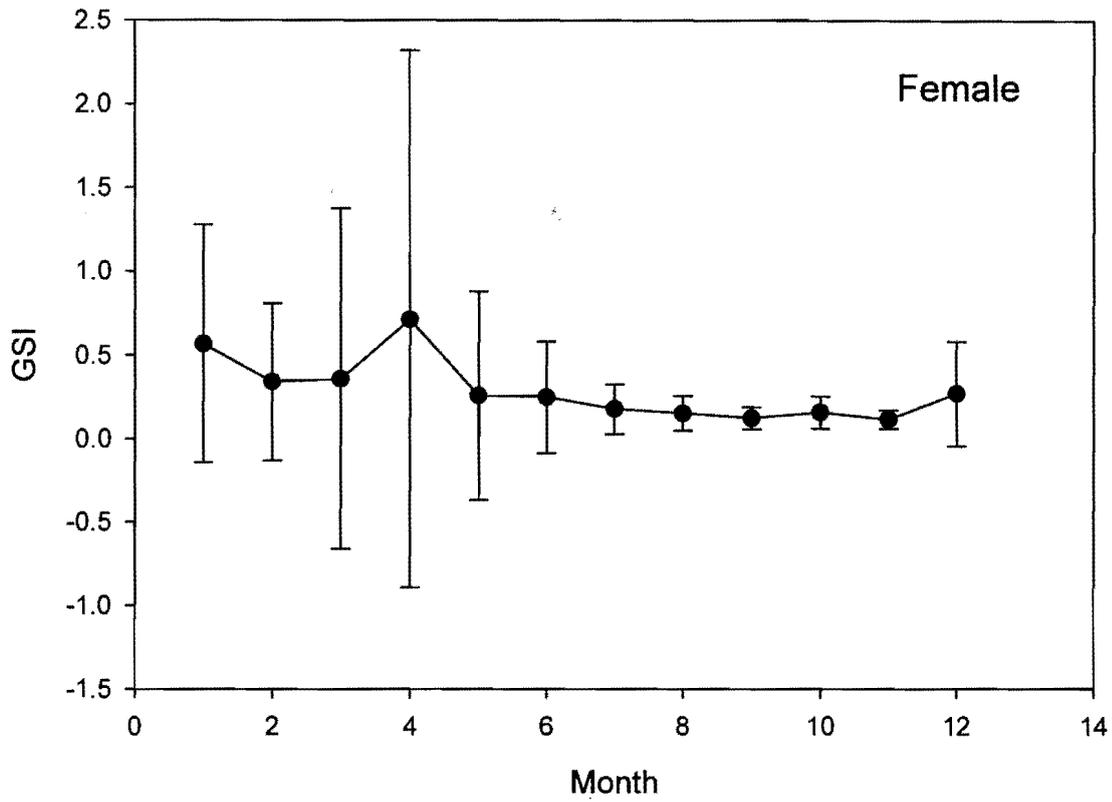


Figure 14. Distribution of sex and maturity stages by depth based on fishery-independent samples, 1991-2000. Active females included developing, spawning or spent females. Inactive females are non-spawning females whose maturity could not be determined. Numbers on the right indicate sample size.

Fig. 14

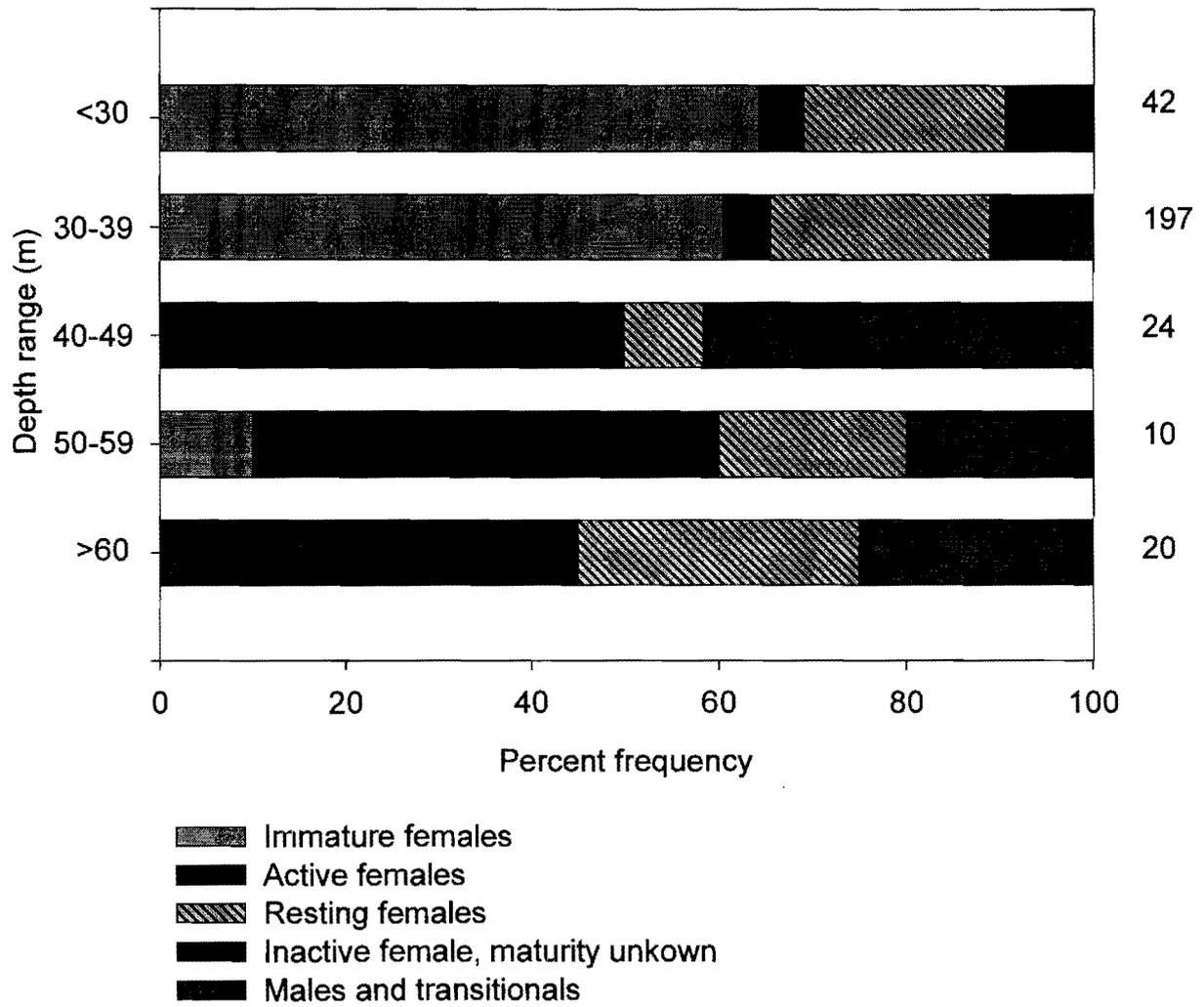


Figure 15. Locations where male and female red grouper in spawning condition were captured, suggesting possible spawning locations, 1996-2000. Based on fishery-dependent and fishery-independent samples.

Fig. 15

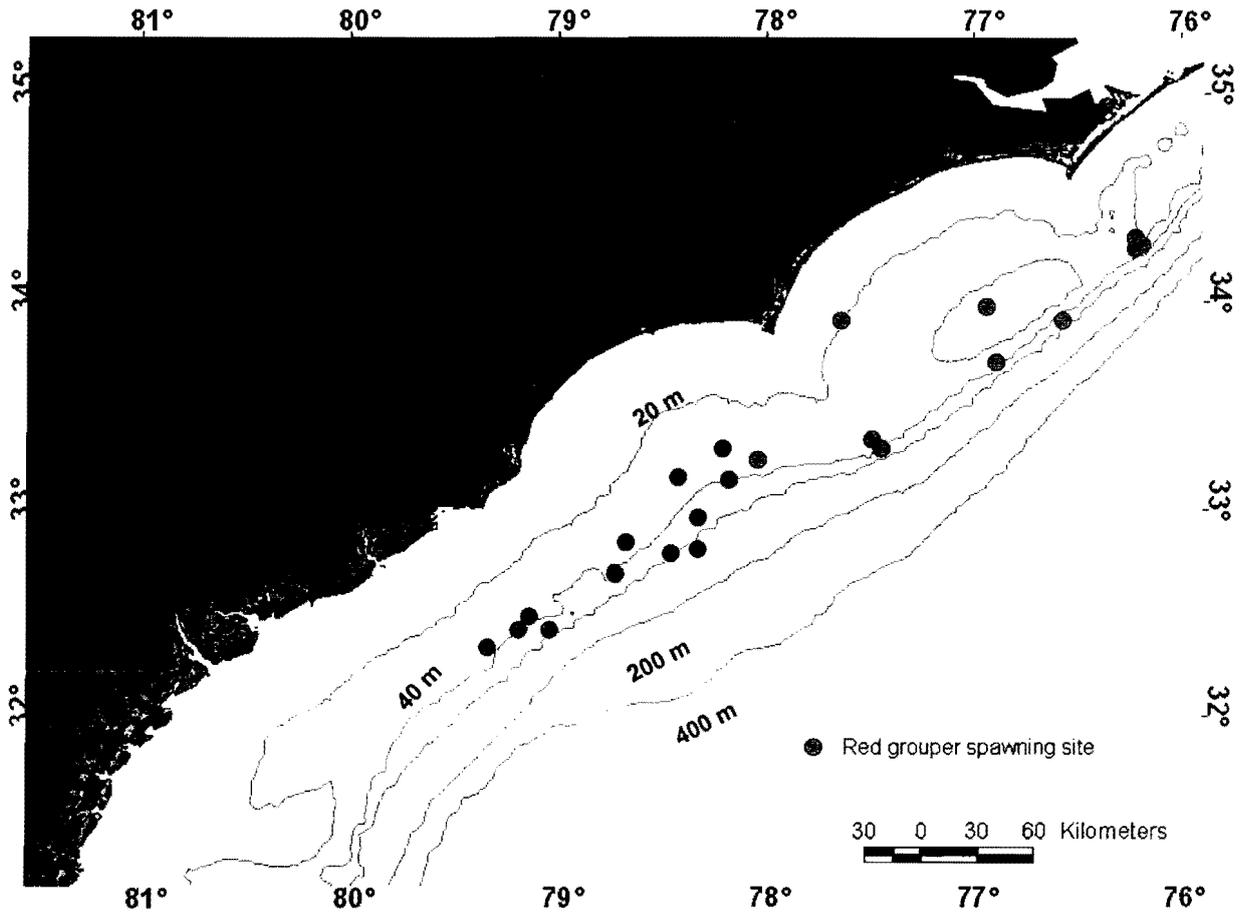


Figure 16. Red grouper catch curve. Based on fishery-dependent data from North Carolina in 1998.

Fig. 16

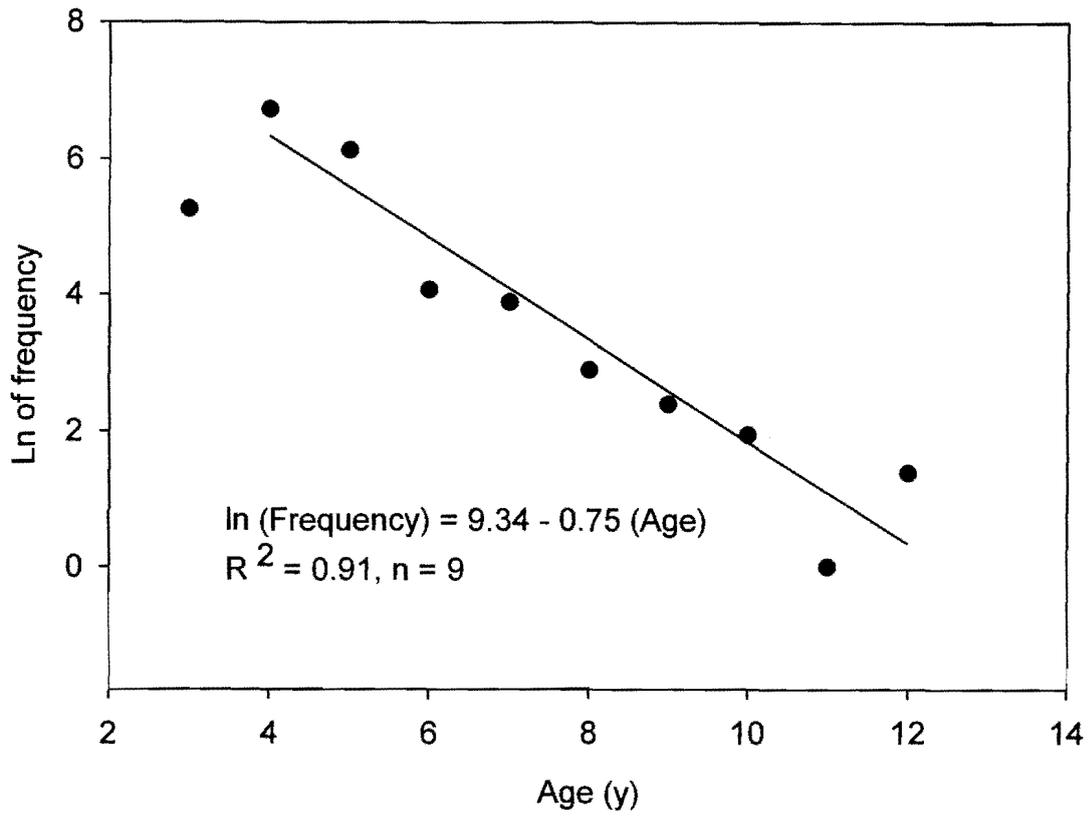


Figure 17. Yield per recruit for red grouper off North Carolina and South Carolina assuming no release mortality.

Fig. 17

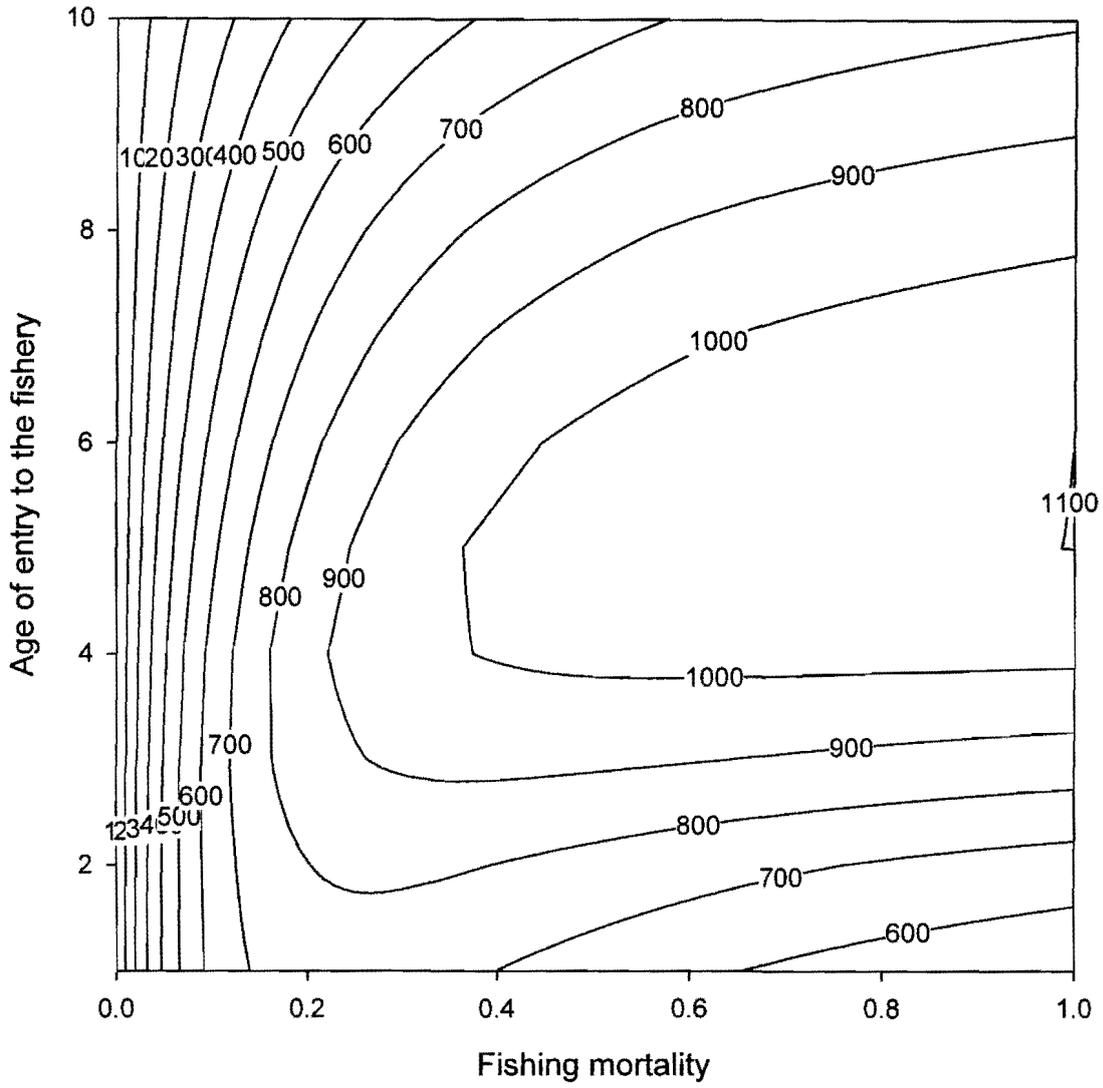


Figure 18. Yield per recruit for red grouper off North Carolina and South Carolina incorporating age-specific release mortality.

Fig. 18

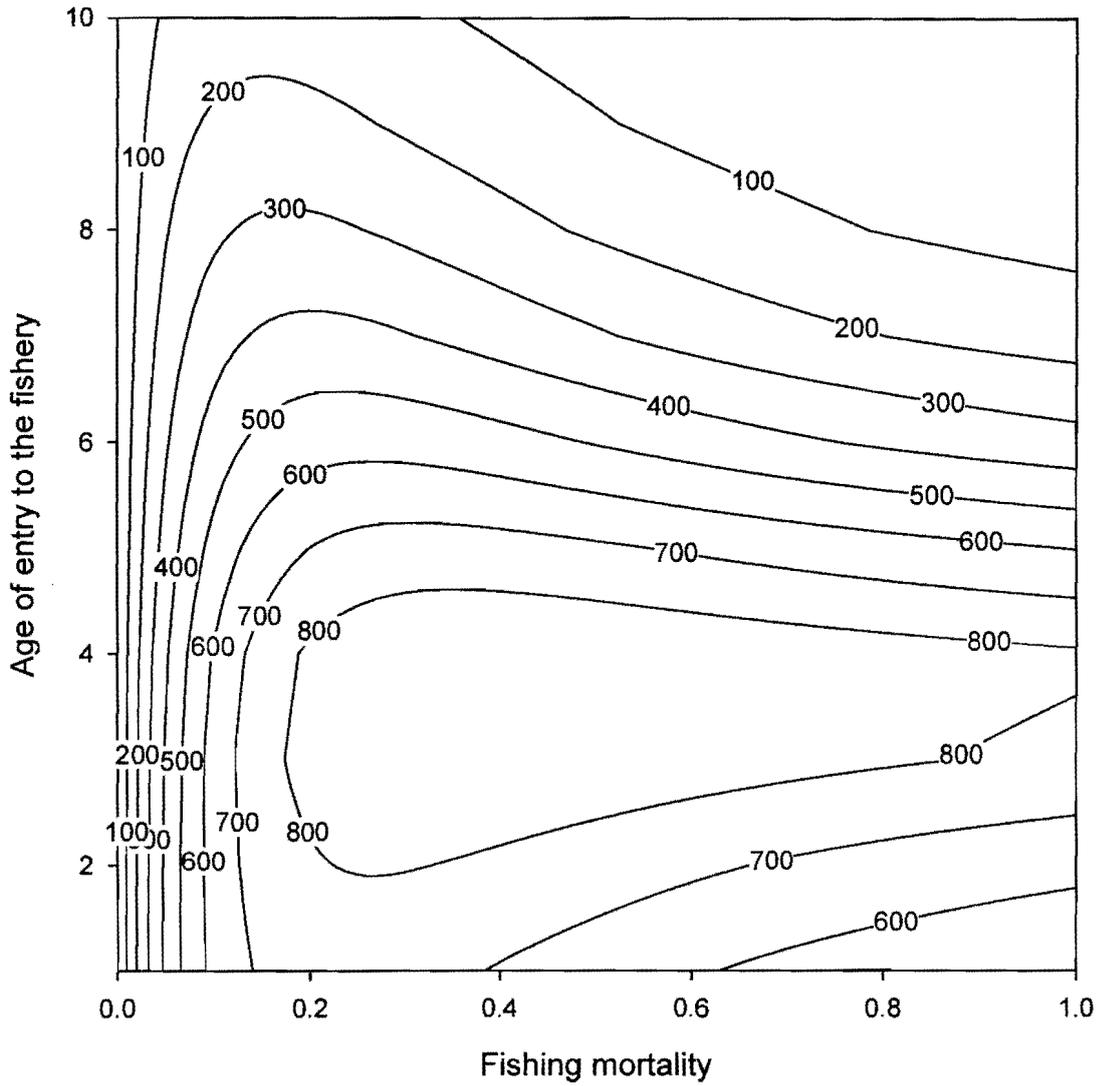


Figure 19. Sex-specific spawning potential ratio for different combinations of fishing mortality (F) and age of entry to the fishery (t_c). Calculations do not include release mortality or sex ratio compensation.

Fig. 19

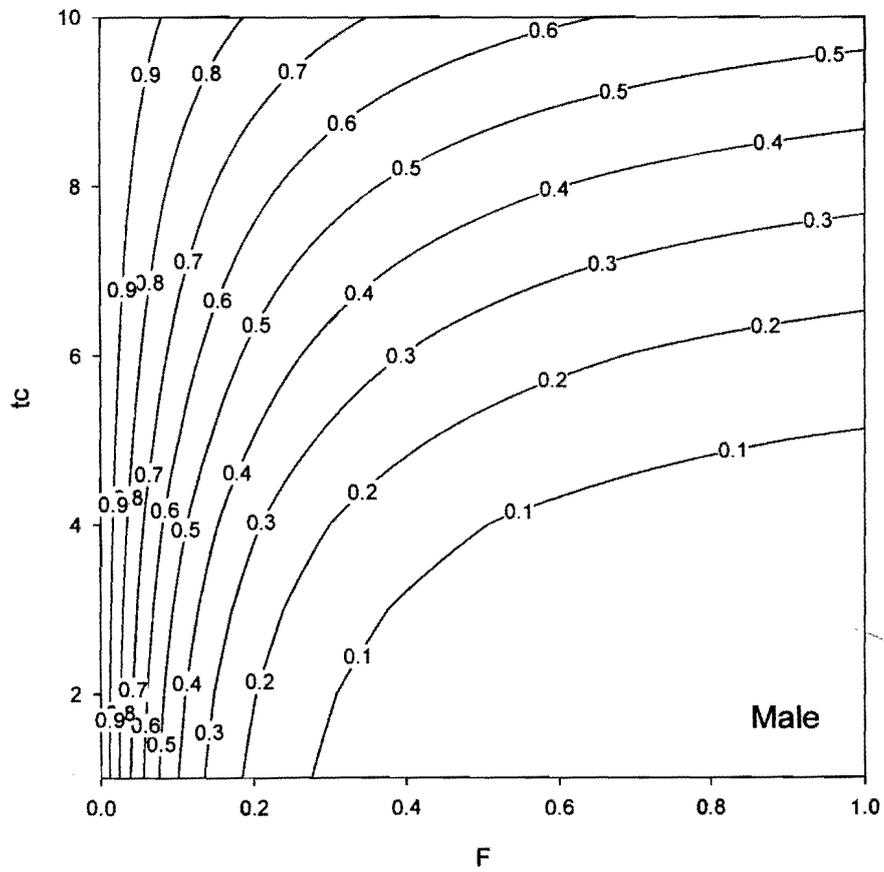
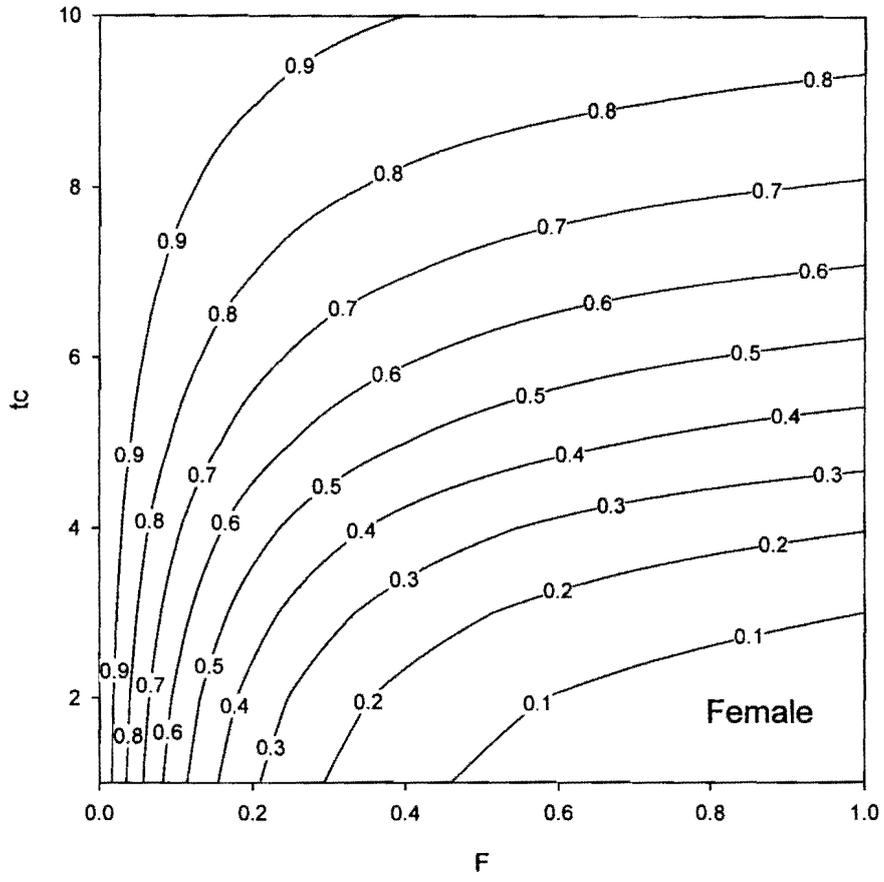


Figure 20. Sex-specific spawning potential ratio for different combinations of fishing mortality (F) and age of entry to the fishery (t_c). Calculations include release mortality but do not include sex ratio compensation.

Fig-20

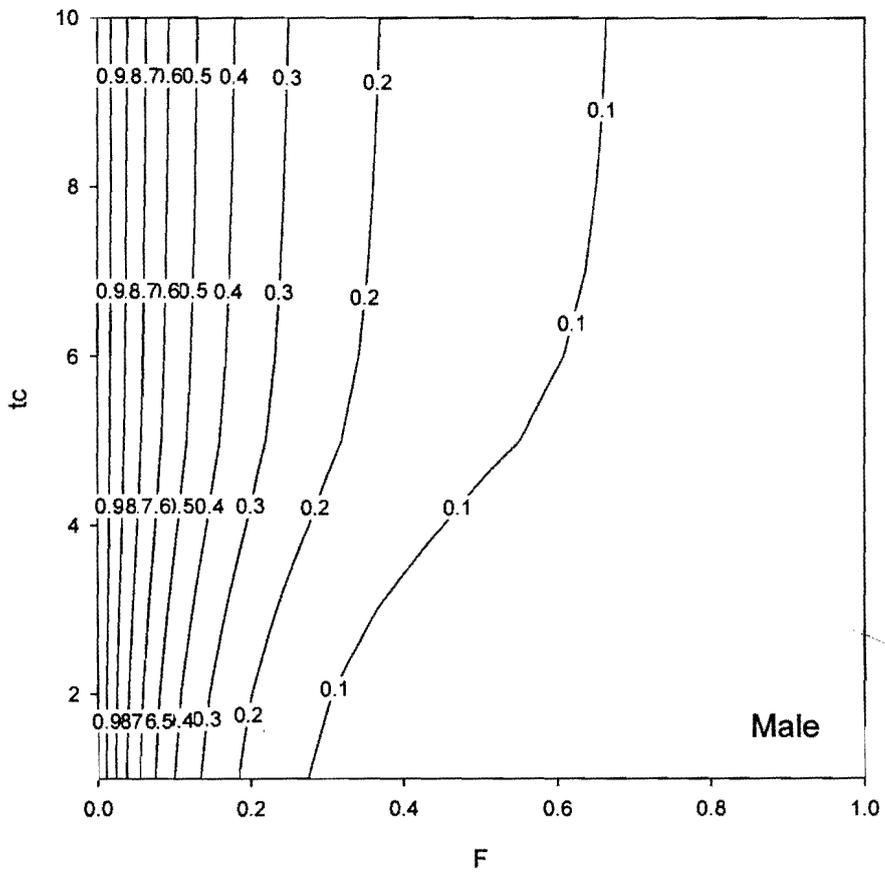
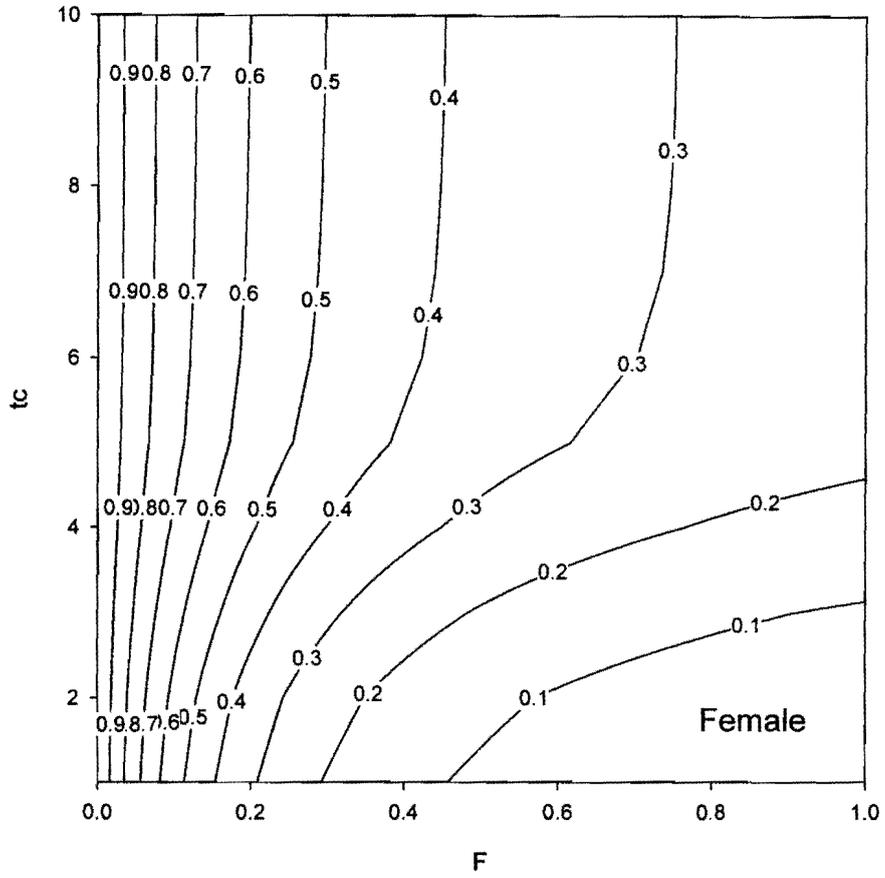


Figure 21. Sex-specific spawning potential ratio for different combinations of fishing mortality (F) and age of entry to the fishery (t_c). Calculations include release mortality and sex ratio compensation.

Fig. 21

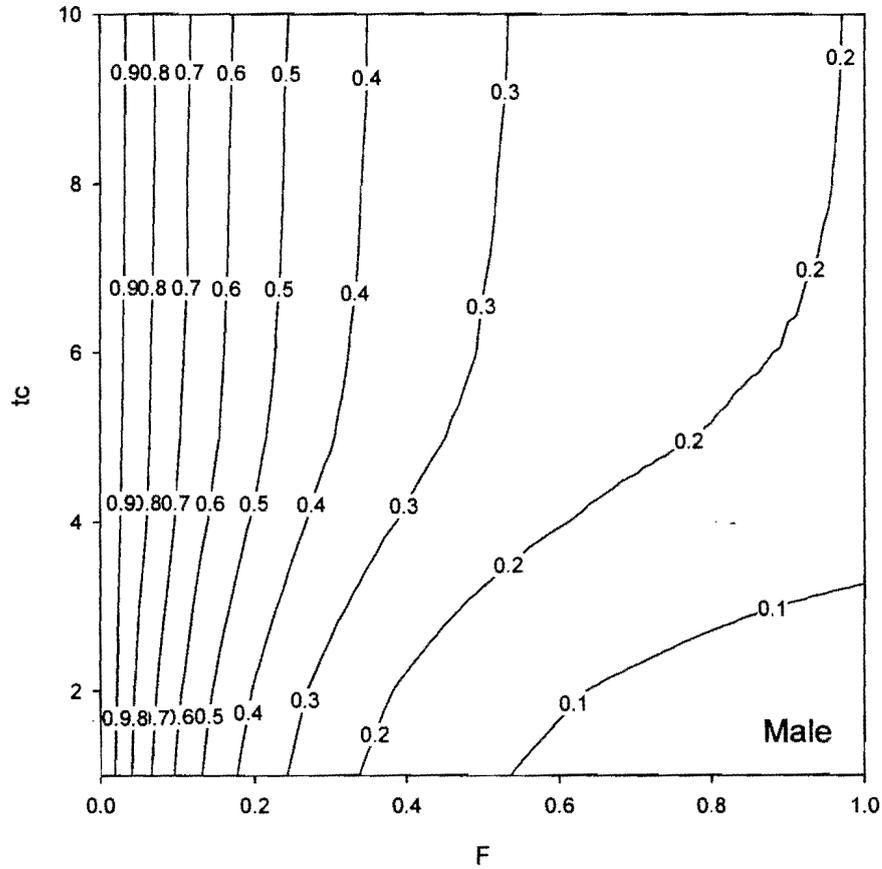
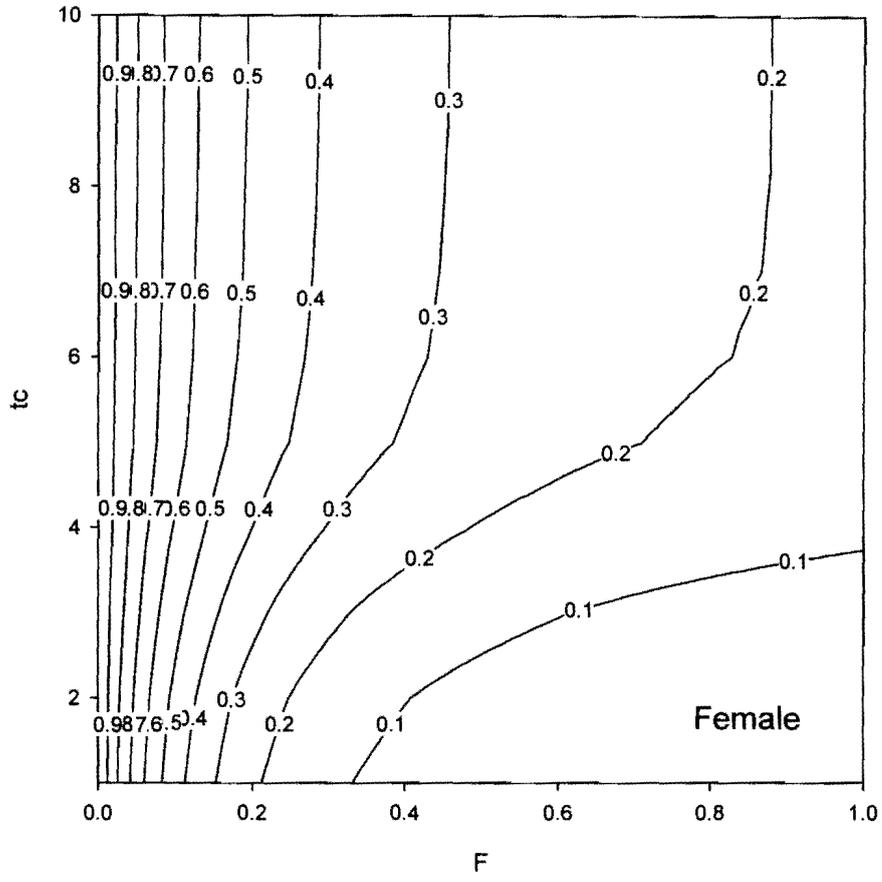


Table 1. Histological criteria to assess sex and reproductive state in red grouper, *Epinephelus morio*, based in Moe (1969), McGovern et al. (1998) and Wyanski et al. (2000).

Reproductive state	Male	Female
Uncertain maturity	Inactive testes; unable to assess maturity.	Inactive ovaries with primary growth oocytes only; unable to assess maturity.
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.
Developing	Development of cysts containing primary and secondary spermatocytes through some accumulation of spermatozoa in lobular lumina and ducts.	Predominance of oocytes with cortical alveoli formation through late vitellogenesis.
Developing, previous spawn		Developing stage as described above plus postovulatory follicles.
Running ripe	Predominance of spermatozoa; little or no occurrence of spermatogenesis.	Completion of yolk coalescence and hydration in most advanced oocytes; zona radiata becomes thinner.
Spent	No spermatogenesis; some residual spermatozoa in lobules and ducts.	More than 50% of vitellogenic oocytes with alpha or beta atresia.

Table 1. (Continued)

Resting	Little or no spermatocyte development; empty lobules and sinuses.	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, melanomacrophage centers and/or foci of inflammatory cells may be present.
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.	
Mature specimen, state unknown.	Mature, but inadequate quantity of tissue or postmortem histolysis prevent further assessment of reproductive state.	Mature, but inadequate quantity of tissue or postmortem histolysis prevent further assessment of reproductive stage.
Unknown	Postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive state.	Postmortem histolysis or inadequate quantity of tissue prevent assessment of reproductive state.

Table 2. Mean back-calculated total lengths (mm) at age for red grouper.

Mean back calculated lengths at successive increments													
Age	N	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
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
n		1056	1054	983	983	512	148	80	31	16	13	3	3
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

Table 3. Mean length at age. Comparison between back calculated lengths using all increments vs. using last increment. ANOVA one way. Standard error uses a pooled estimate of error variance. * = $P < 0.05$.

Age	Back-calculated using all increments (standard error)		n	Back-calculated using last increment (standard error)		n	P
3	430.3	(1.00)	1054	423.4	(3.71)	71	0.0747
4	489.8	(1.21)	983	485.8	(1.54)	471	0.0333 *
5	552.2	(1.80)	512	551.4	(1.95)	364	0.7484
6	606.8	(3.66)	148	616.1	(5.47)	68	0.1570
7	644.5	(5.35)	80	652.1	(6.78)	49	0.3777
8	672.5	(8.62)	31	686.0	(12.06)	15	0.3635
9	695.2	(13.99)	16	725.0	(20.89)	8	0.2577
10	691.4	(34.27)	8	681.9	(45.19)	5	0.8719

Table 4. Mean length at age. Comparison between back-calculated lengths using all increments vs. observed lengths. One way ANOVA. Standard error uses a pooled estimate of error variance.

* = $P < 0.05$.

Age	Back-calculated using all increments (SE)		n	Observed (SE)		n	P
3	430.3	(1.00)	1054	490.2	(2.19)	221	<0.0001 *
4	489.8	(1.21)	983	531.0	(1.26)	930	<0.0001 *
5	552.2	(1.80)	512	579.4	(1.67)	598	<0.0001 *
6	606.8	(3.66)	148	663.4	(3.65)	149	<0.0001 *
7	644.5	(5.35)	80	680.8	(5.72)	70	<0.0001 *
8	672.5	(8.62)	31	711.2	(9.60)	25	0.0040 *
9	695.2	(13.99)	16	751.6	(16.87)	11	0.0164 *
10	691.4	(34.27)	8	734.1	(34.27)	8	0.3932

Table 5. Age length keys for red grouper from fishery-dependent samples (1996-2000). Age 12+ is a combination of ages 12-20.

Length class (mm TL)	Number	Age (years)										
		2	3	4	5	6	7	8	9	10	11	12+
301-320	3	0.33	0.67	0	0	0	0	0	0	0	0	0
321-340	1	1	0	0	0	0	0	0	0	0	0	0
341-360	5	0.4	0.6	0	0	0	0	0	0	0	0	0
361-380	2	0.5	0.5	0	0	0	0	0	0	0	0	0
381-400	4	0	1	0	0	0	0	0	0	0	0	0
401-420	6	0	0.67	0.33	0	0	0	0	0	0	0	0
421-440	8	0.13	0.13	0.75	0	0	0	0	0	0	0	0
441-460	12	0	0.08	0.92	0	0	0	0	0	0	0	0
461-480	20	0	0.10	0.90	0	0	0	0	0	0	0	0
481-500	16	0	0.06	0.94	0	0	0	0	0	0	0	0
501-520	17	0	0	0.65	0.35	0	0	0	0	0	0	0
521-540	10	0	0	0.70	0.30	0	0	0	0	0	0	0
541-560	14	0	0	0.21	0.79	0	0	0	0	0	0	0
561-580	11	0	0	0	0.91	0.09	0	0	0	0	0	0
581-600	10	0	0	0.20	0.70	0.10	0	0	0	0	0	0
601-620	5	0	0	0	0.40	0.60	0	0	0	0	0	0
621-640	12	0	0	0.17	0.33	0.17	0.33	0	0	0	0	0
641-660	13	0	0	0	0.62	0.38	0	0	0	0	0	0
661-680	12	0	0	0	0.42	0.42	0.17	0	0	0	0	0
681-700	10	0	0	0	0.30	0.20	0.30	0.20	0	0	0	0
701-720	9	0	0	0	0.22	0.22	0.56	0	0	0	0	0
721-740	2	0	0	0	0	0.50	0	0.50	0	0	0	0
741-760	3	0	0	0	0	0	0.33	0.33	0	0.33	0	0
761-780	1	0	0	0	0	0	0	1	0	0	0	0
Total	206											

Table 6. Age length keys for red grouper from fishery-independent samples (1996-2000). Age 12+ is a combination of ages 12-20.

Length class (mm TL)	Number	Age (years)										
		2	3	4	5	6	7	8	9	10	11	12+
301-320	0	0	0	0	0	0	0	0	0	0	0	0
321-340	0	0	0	0	0	0	0	0	0	0	0	0
341-360	0	0	0	0	0	0	0	0	0	0	0	0
361-380	0	0	0	0	0	0	0	0	0	0	0	0
381-400	1	0	0	1	0	0	0	0	0	0	0	0
401-420	0	0	0	0	0	0	0	0	0	0	0	0
421-440	0	0	0	0	0	0	0	0	0	0	0	0
441-460	7	0.14	0.29	0.57	0	0	0	0	0	0	0	0
461-480	85	0	0.45	0.53	0.02	0	0	0	0	0	0	0
481-500	230	0	0.38	0.55	0.05	0	0	0	0	0	0	0
501-520	243	0	0.19	0.67	0.13	0	0	0	0	0	0	0
521-540	241	0	0.05	0.69	0.25	0	0	0	0	0	0	0
541-560	232	0	0.05	0.59	0.35	0	0	0	0	0	0	0
561-580	190	0	0.01	0.50	0.48	0.01	0	0	0	0	0	0
581-600	174	0	0.02	0.39	0.57	0.02	0	0.01	0	0	0	0
601-620	132	0	0	0.24	0.69	0.05	0.02	0	0	0	0	0
621-640	55	0	0	0.07	0.65	0.18	0.07	0.02	0	0	0	0
641-660	49	0	0	0.04	0.37	0.43	0.16	0	0	0	0	0
661-680	52	0	0	0.06	0.15	0.52	0.25	0.02	0	0	0	0
681-700	43	0	0	0.07	0.05	0.6	0.19	0.09	0	0	0	0
701-720	37	0	0	0.03	0	0.57	0.24	0.14	0.03	0	0	0
721-740	18	0	0	0	0.11	0.22	0.28	0.22	0.11	0.06	0	0
741-760	15	0	0	0.07	0.07	0.07	0.07	0.20	0.33	0.20	0	0
761-780	5	0	0	0	0	0	0.60	0.20	0.20	0	0	0
781-800	5	0	0	0	0	0	0	0	0.40	0.20	0	0.4

Table 6. Continued.

801-820	3	0	0	0	0	0	0	0	0	0	0.33	0.67
821-840	2	0	0	0	0	0	0	0	0	0.50	0	0.50
841-860	1	0	0	0	0	0	0	0	0	0	0	1
Total	1828											

Table 7. Comparison of age-lengths keys from fishery-dependent and fishery-independent samples using Fisher's exact test.

Size interval (mm TL)	P values
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	0.008 *
621-640	0.048 *
641-660	0.302
661-680	0.390
681-700	0.028 *
701-720	0.026 *

* $P < 0.05$

** $P < 0.0037$ (adjusted significance level)

Table 8. Comparison of mean size (TL) at age between fishery dependent and fishery independent samples. One way ANOVA.

Age	Fishery independent		Fishery dependent		One way ANOVA
	Mean TL (mm)	n	Mean TL (mm)	n	
1	349.00	1	-		
2	352.83	6	457.00	1	
3	396.80	15	500.08	199	P<0.0001
4	490.22	83	535.15	853	P<0.0001
5	594.15	55	577.54	537	P = 0.03
6	650.87	23	661.07	127	P = 0.206
7	684.56	16	676.78	58	P = 0.9068
8	711.00	6	706.94	17	P = 0.3310
9	-		758.21	14	
10	754.00	1	704.80	5	
11	-		812.00	1	
12	-		802.00	4	
13	-		755.00	1	
20	-		833.00	2	

Table 9. Frequency of red groupers by sex and age. Sex ratio is estimated as number of females over number of males and transitionals. Values of G adjusted using William's correction (Sokal and Rohlf, 1981). (*) indicates $G > X^2_{0.05[1]}$

Age	Immature females	Females, uncertain maturity	Mature females	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	558	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	69	5	12		
Total	213	372	1211	121	151		

Table 10. Frequency of red groupers by sex and total length intervals. Sex ratio is estimated as number of females over number of males and transitionals. Values of G adjusted using William's correction (Sokal and Rohlf, 1981). (*) indicates $G > X^2_{0.05[1]}$

Total length (mm)	Immature females	Females, uncertain maturity	Mature females	Transitionals	Males	Sex ratio	G
301-320	3	0	0	0	0		
321-340	1	0	0	0	0		
341-360	5	0	0	0	0		
361-380	2	0	0	0	0		
381-400	6	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	75	4	0	1 : 61.3	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	200	14	2	1 : 14.7	228.46 (*)
561-580	2	16	175	14	8	1 : 8.8	155.72 (*)
581-600	0	4	156	18	10	1 : 5.7	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

Table 10. Continued.

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781-800	0	0	2	0	3	1 : 0.7	0.18
801-820	0	0	0	0	4		
821-840	0	0	0	0	2		
841-860	0	0	0	0	1		
No length	1	1	2	0	1		
Total	213	372	1211	121	151		

Table 11. Comparison between male and female mean length at age. One way ANOVA. Standard error uses a pooled estimate of error variance. * = $P < 0.05$.

Age	Females (standard error)	n	Males (standard error)	n	P
3	489.1 (2.68)	211	513.3 (22.43)	3	0.2838
4	528.1 (1.39)	862	567.8 (5.61)	53	<0.0001 *
5	574.0 (1.84)	496	608.2 (4.33)	90	<0.0001 *
6	646.1 (4.65)	79	680.8 (5.63)	54	<0.0001 *
7	664.5 (8.58)	32	695.4 (9.70)	25	0.0202 *
8	762.3 (12.32)	4	745.6 (9.32)	7	0.3087
9	695.2 (14.77)	16	725.0 (20.90)	8	0.2577
10	680.0 (63.45)	3	769.5 (54.95)	4	0.3350

Table 12. Spawning frequency estimations. Active females include late development, spawning and spent females. The number of spawning events per season was calculated assuming that females spawn continuously during a 115 day long spawning season.

Month	Active females	HO	MNO	HO+MNO	<24 h 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		10.6		41.8	10.6

Table 13. Distribution of sex and reproductive state by depth and season, and percentage of mature females by reproductive state. Data from fishery-dependent (n = 641, 1996-1999) and fishery-independent (n = 293, 1991-2000) sources.

Depth < 40 m

	Spawning season				Non-spawning season				
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Jan
Immature females	0	0	0	80	63	58	3	7	0
Inactive females, maturity unknown	20	27	0	34	31	13	6	2	0
Developing females	0	3	2	13	5	0	1	0	5
Spawning females	0	0	1	0	0	0	0	0	0
Spent females	0	0	1	11	16	0	0	0	0
Resting females	35	51	3	79	132	13	48	9	0
Transitionals	2	0	0	2	14	0	6	0	0
Males	0	0	8	9	27	0	0	0	3
Total mature females	199				224				
Developing females	9.1%				2.7%				
Spawning females	0.5%				0%				
Spent females	6.0%				7.1%				
Resting females	84.4%				90.2%				

Depth > 40 m

	Spawning season				Non-spawning season				
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Jan
Immature females	0	0	1	0	0	0	0	0	0
Inactive females, maturity unknown	0	0	0	0	0	0	0	0	0
Developing females	2	3	6	9	0	0	0	0	0
Spawning females	5	11	1	1	0	0	0	0	0
Spent females	0	0	0	8	1	0	0	0	0
Resting females	0	0	0	6	2	1	1	0	0
Transitionals	1	0	1	1	2	2	1	0	0
Males	6	7	3	6	3	0	0	0	0
Total mature females	52				5				
Developing females	38.5%				0%				
Spawning females	34.6%				0%				
Spent females	15.4%				20%				
Resting females	11.5%				80%				

Table 14. Estimation of age-specific release survival rates. Relative catch per unit effort (CPUE) is the fraction of the age-specific CPUE in each depth range. Depth- and age-specific survival rates are obtained multiplying age-specific CPUE by the depth-specific survival rates.

Age	Depth range (m)		
	<45	45-65	>65
	Effort (number of traps)		
	1986	907	112
2	10	0	0
3	27	1	0
4	97	1	2
5	54	11	7
6	14	3	5
7+	12	3	6

Age	Relative CPUE		
2	1.00	0.00	0.00
3	0.92	0.08	0.00
4	0.72	0.02	0.26
5	0.27	0.12	0.61
6	0.13	0.06	0.81
7+	0.10	0.05	0.85

Age	Depth specific release survival rate			Age-specific survival rate
	0.95	0.45	0	
2	0.95	0.00	0.00	0.95
3	0.88	0.03	0.00	0.91
4	0.68	0.01	0.00	0.69
5	0.25	0.05	0.00	0.31
6	0.12	0.03	0.00	0.15
7+	0.09	0.02	0.00	0.11

Table 15. Yield per recruit based reference points. F_{\max} = fishing mortality that maximizes yield per recruit at age of entry (t_c). $F_{0.1}$ = fishing mortality where the slope of the yield curve is 0.1 of the original value.

t_c	Release mortality		No release mortality	
	F_{\max}	$F_{0.1}$	F_{\max}	$F_{0.1}$
1	0.23	0.15	0.23	0.16
2	0.27	0.17	0.28	0.18
3	0.35	0.20	0.39	0.21
4	0.40	0.22	0.67	0.25
5	0.33	0.21	1.00	0.31
6	0.26	0.18	1.00	0.35
7	0.21	0.16	1.00	0.40
8	0.18	0.14	1.00	0.46
9	0.16	0.13	1.00	0.53
10	0.14	0.12	1.00	0.62

Table 16. Sensitivity analysis of the yield per recruit (YPR) and spawning potential ratio (SPR) model. Values indicate percent change on the output values as compared with YPR, sex-specific spawning biomass per recruit (SBPR) and SPR calculated with $M=0.2$ and $k=0.1697$

Percent change on the output values						
M=0.1						
F	tc	YPR	Female SBPR	Male SBPR	Female SPR	Male SPR
0	0		118.54	192.96		
0.2	3	89.76	111.90	73.92	-3.04	-40.63
0.2	5	118.20	113.10	73.96	-2.49	-40.62
0.2	7	152.48	114.45	75.52	-1.87	-40.09
0.4	3	65.46	79.70	56.02	-17.77	-46.74
0.4	5	95.24	83.38	58.02	-16.09	-46.06
0.4	7	130.64	86.71	59.19	-14.57	-45.66
0.6	3	52.78	62.26	45.19	-25.75	-50.44
0.6	5	81.99	71.21	44.19	-21.66	-50.78
0.6	7	116.20	74.72	46.23	-20.05	-50.09

$\tau=0.5$

F	tc	YPR	Female SBPR	Male SBPR	Female SPR	Male SPR
0	0		-58.72	-44.28		
0.2	3	-42.85	-48.99	-38.31	23.57	10.71
0.2	5	-51.16	-49.16	-38.81	23.14	9.82
0.2	7	-58.37	-49.75	-38.79	21.73	9.85
0.4	3	-36.48	-41.09	-32.96	42.69	20.30
0.4	5	-46.60	-43.44	-32.49	37.01	21.16
0.4	7	-55.03	-44.53	-32.83	34.37	20.54
0.6	3	-32.43	-37.27	-27.54	51.94	30.03
0.6	5	-43.52	-39.41	-29.17	46.76	27.11
0.6	7	-52.52	-40.86	-29.62	43.25	26.30

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Table 16. Continued.

Percent change on the output values						
k increased 10%						
F	tc	YPR	Female SBPR	Male SBPR	Female SPR	Male SPR
	0	0	14.11	9.62		
	0.2	3	15.60	17.40	2.89	2.85
	0.2	5	12.99	16.96	2.50	2.59
	0.2	7	10.73	16.78	2.34	2.46
	0.4	3	17.57	19.09	4.37	5.46
	0.4	5	14.39	18.37	3.73	4.86
	0.4	7	11.74	18.11	3.51	4.60
	0.6	3	18.81	19.98	5.15	7.34
	0.6	5	15.32	19.16	4.42	6.39
	0.6	7	12.50	18.86	4.16	6.02
k decreased 10%						
F	tc	YPR	Female SBPR	Male SBPR	Female SPR	Male SPR
	0	0	-14.49	-10.73		
	0.2	3	-15.68	-17.12	-3.08	-2.98
	0.2	5	-13.65	-16.79	-2.69	-2.73
	0.2	7	-11.78	-16.64	-2.52	-2.60
	0.4	3	-17.23	-18.40	-4.57	-5.59
	0.4	5	-14.83	-17.86	-3.95	-5.02
	0.4	7	-12.68	-17.67	-3.72	-4.77
	0.6	3	-18.17	-19.05	-5.34	-7.37
	0.6	5	-15.59	-18.45	-4.64	-6.51
	0.6	7	-13.33	-18.23	-4.38	-6.16

APPENDIX

```

CLS
DIM p(10, 15), mf(15), mm(15), ps(15), wf(15), wm(15)
DIM fishnum(15), cfish(15), propmale(15), spawning(15)
DIM sppropmale(15)

GOSUB Variables
GOSUB Readdata
GOSUB Vonbert
GOSUB Openfile
GOSUB Main
GOSUB Closefile
END

*****
Main:
PRINT #1, "f,tc,ypr,ssb fem,ssb male,sexratio"

FOR f = 0 TO 1 STEP .01      'Fishing mortality loop
z = f + m
FOR tc = 1 TO 10           'Age at entry loop
fish = 1000                'Number of recruits
FOR t = 1 TO 15           'Age loop

*****
'Calculates number of fish at beginning of age (t)

fishnum(t) = fish          'Keeps track of the number of fish at the beginning of
                           'each age
kfish = fish * p(tc, t)    'Number of legal fish
nkfish = fish * (1 - p(tc, t)) 'Number of sub legal fish
cfish(t) = f / z * (1 - EXP(-z)) * kfish 'Number of fish captured and kept
afish = nkfish * EXP(-m)   'Number of sub legal fish if all survive release
ufish = nkfish * EXP(-m - f * u) 'Number of sub legal fish if none survive release
rsfish = (afish - ufish) * ps(t) 'Number of surviving released fish

fish = ufish + kfish * EXP(-z) + rsfish

'Calculate number of fish at time of spawning
safish = nkfish * EXP(-m * ee)
sufish = nkfish * EXP(ee * (-m - f * u))
srsfish = (safish - sufish) * ps(t)
spawning(t) = sufish + kfish * EXP(-z * ee) + srsfish

```

NEXT t

'Searches for new slope of % of males by age so sex ratio is 6.63

dd = .065

GOSUB sexratio

totalfish = 0: totalmales = 0

'Asigns new % of males per age

FOR x = 1 TO 15

pm = cc + dd * x

spm = cc + dd * (x + E)

IF pm > 1 THEN pm = 1

IF pm < 0 THEN pm = 0

IF spm > 1 THEN spm = 1

IF spm < 0 THEN spm = 0

propmale(x) = pm

sppropmale(x) = spm

NEXT x

'Estimates yield and spawning biomass

FOR x = 1 TO 15

yield = yield + cfish(x) * propmale(x) * wm(x) + cfish(x) * (1 - propmale(x)) * wf(x)

ssbm = ssbm + spawning(x) * sppropmale(x) * mm(x) * wm(x)

ssbf = ssbf + spawning(x) * (1 - sppropmale(x)) * mf(x) * wm(x)

totalfish = totalfish + fishnum(x)

totalmales = totalmales + fishnum(x) * propmale(x)

NEXT x

sexratio = (totalfish - totalmales) / totalmales

PRINT #1, f, ";", tc, ";", yield / 1000, ";", ssbf / 1000, ";", ssbm / 1000, ";", sexratio

yield = 0: ssbf = 0: ssbm = 0: totalfish = 0: totalmales = 0

NEXT tc

NEXT f

RETURN

Readdata:

PRINT "Reading data"

FOR x = 1 TO 10

FOR y = 1 TO 15

READ p(x, y)

NEXT y


```

Closefile:
PRINT "Closing file"
CLOSE #1
RETURN

```

```

*****

```

```

Variables:
m = .2           'Natural mortality
Linf = 836.123  'Von Bertalanffy parameters
k = .1697
t0 = -1.2776
a = .00000594#  'TL-fish weight relationship parameters
b = 3.1568
cc = -.22819    'Intercept of %males by age
ee = .7479      'Fraction of the year at spawning
RETURN

```

```

*****

```

```
sexratio:
```

```

totalmales = 0
FOR x = 1 TO 15
  propmale = cc + dd * x
  IF propmale < 0 THEN propmale = 0
  IF propmale > 1 THEN propmale = 1
  totalfish = totalfish + fishnum(x)
  totalmales = totalmales + fishnum(x) * propmale
NEXT x
sr = INT((totalfish - totalmales) / totalmales * 100)
IF sr < 663 THEN RETURN
dd = dd + .0005
totalfish = 0: totalmales = 0
GOTO sexratio

```