Age, growth, and reproduction of black grouper, *Mycteroperca bonaci*, in Florida waters

Roy E. Crabtree
Lewis H. Bullock
Florida Marine Research Institute, Department of Environmental Protection
100 Eighth Avenue SE, St. Petersburg, Florida 33701-5095
E-mail address (for R. E. Crabtree): crabtree_r@epic7.dep.state.fl.us

Abstract.—We examined 1166 black grouper ranging from 155 to 1518 mm TL collected in South Florida waters from 1994 to 1996. Among all black grouper that we sexed, females ranged from 155 to 1310 mm in length (mean= 696, n=834), and males ranged from 947 to 1518 mm (mean=1255, n=54). Ages of 927 black grouper ranging from 155 to 1518 mm TL were estimated from thin-sectioned otoliths (sagittae). Marginal-increment analysis of grouper 1–7 years old suggested that a single annulus was formed each year during May–June. Black grouper appear to reach a maximum age of at least 33 years, but ages of fish older than 7 years are unvalidated. Growth of the black grouper in our study was rapid until an age of about 10 years and then slowed considerably. The von Bertalanffy growth equation for black grouper was $TL = 1306.2(1-e^{(-0.169(Age+0.768))})$. Black grouper are protogynous hermaphrodites. We estimated that 50% of the females in the population had reached sexual maturity by 826 mm and an age of 5.2 years. By a length of 1214 mm and an age of 15.5 years, 50% of the females in our sample had transformed into males. The presence of large females in the population suggests that some females may not transform into males. The scarcity of transitional grouper (n=1) in our sample suggests that transition occurs quickly. Black grouper appear to spawn yearround, but peak spawning occurs during winter and early spring. Vitellogenic oocytes and oocytes in the final stages of maturation were most common during January–March.

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The black grouper, *Mycteroperca bonaci*, is a large, piscivorous epinepheline serranid that inhabits coral reefs and rocky ledges from North Carolina to Florida, in the Gulf of Mexico, off Bermuda, in the West Indies, and from Central America to southern Brazil (Smith, 1971; Bullock and Smith, 1991). The black grouper supports important commercial and recreational fisheries in South Florida. Commercial landings of the species consistently exceed landings of any other grouper in the Florida Keys. Statewide, the estimated value of Florida black grouper commercial landings has decreased from $2.7 million in 1990 to $1.1 million in 1996 (Florida Department of Environmental Protection Marine Fisheries Information System[1]).

Florida imposes both recreational and commercial regulations on black grouper caught in state waters. There is a recreational minimum size limit of 20 inches (508 mm) total length (TL) and an aggregate daily bag limit of five groupers, including black, yellowfin (*Mycteroperca venenosa*), gag (*M. microlepis*), red (*Epinephelus morio*), yellowmouth (*M. interstitialis*), and scamp (*M. phenax*). The commercial minimum size limit is also 20 inches TL. Federal regulations include a 20-inch minimum size limit and an annual quota for the shallow-water grouper complex, which includes the black grouper. In addition, the activities of commercial longline fishermen east of Cape San Blas, Florida (85°22'W), are restricted to depths greater than 20 fathoms.

Even though the black grouper fishery is regulated in both state and federal waters, little is known about the species' biology, and existing data are inadequate to evaluate the effect of fishing mortality on populations. Like most grouper species, black grouper are protogynous hermaphrodites (García-Cagide and García, 1996). Hermaphroditic species may respond differently to fishing mortality than typical gonochoristic species do, and in some situations, protogynous hermaphrodites may be more sensitive to overfishing than gonochorists (Bannerot et al., 1987). For example, size-selective fishing mortality can greatly skew population sex ratios towards females, which may affect spawning success. In addition, many grouper species form large spawning aggregations that can be easily overfished (Sadovy, 1994). Only Manooch and Mason (1987) have specifically addressed black

[1] Florida Dep. Environmental Protection Marine Fisheries Information System. 1997. Florida Marine Research Inst., Florida Dep. Environmental Protection, 100 Eighth Ave. SE, St. Petersburg, Florida 33701-5095.

grouper life history in South Florida waters, but their study was limited because most of the fish examined were eviscerated and could not be sexed. Consequently, they could not describe sex-specific growth rates, length and age at sexual maturity, length and age at sexual transition, or sex ratios. Furthermore, black grouper often attain much larger sizes than the largest fish aged by Manooch and Mason, who acknowledged that black grouper can probably live longer than the oldest fish they aged (14 years). García-Cagide and García (1996) estimated the length at sexual maturity, the length at transition from female to male, and described seasonal spawning patterns of black grouper captured off Cuba, but they did not estimate ages of the fish they examined. Additional information on growth, maturation, and the length and age at transition are needed to fully assess the status of black grouper stocks. Our article describes growth, length and age at maturity, length and age at sexual transition, dichromatism between the sexes, and seasonal patterns of gonad development. In addition, we used marginal-increment analysis to validate the ages of young black grouper.

Methods

Collections

We obtained black grouper from a variety of sources throughout the Florida Keys and Florida's Gulf coast from November 1994 to November 1996. Most of the black grouper in our sample were caught by spear fishermen and landed in the Florida Keys (n=733). Commercial hook-and-line and bottom longline black grouper catches were landed and sampled at fish houses in Pinellas County on Florida's Gulf coast (n=433). Commercial fishermen captured grouper primarily south of 25°N in depths greater than 37 m, but some catches were made north of 25°N along the Florida Gulf coast to the Florida Panhandle in depths of 37–100 m. Most of the black grouper landed in Pinellas County were caught on or near the Tortugas Banks west of the Florida Keys by commercial longline fishermen, whose operations are restricted by federal regulations to depths greater than 20 fathoms (37 m).

Standard length (SL), fork length (FL), and total length (TL) were measured to the nearest millimeter (mm). Total length was measured following Hubbs and Lagler (1964). Fork length was measured as the center-line length from the tip of the lower jaw to the center of the caudal fin. Unless otherwise indicated, all lengths reported are total lengths. Large grouper were weighed to the nearest 50 grams and smaller grouper were weighed to the nearest gram. Otoliths (sagittae) were removed, rinsed in water, and stored dry until sectioned. Otoliths were weighed to the nearest 0.01 mg. Gonad weight (g) was recorded, and gonad samples for histology were removed from the fish and preserved in 10% buffered formalin; they were later soaked in water for one hour and then stored in 70% ethanol.

We examined most grouper to detect color changes that might be associated with the sex of the fish. All of the fish we examined were dead and had been on ice for several hours to a week. The colors of the pectoral, anal, dorsal, and caudal fins were recorded throughout the year during routine sampling of gonads and otoliths. Only grouper for which we had gonad histology samples were selected for differential color analysis.

Age and growth

The left sagitta was usually used for age estimation; however, in cases where the left otolith was broken, lost, or damaged during processing, the right otolith was substituted. We prepared most otoliths by using a Buehler Isomet low-speed saw with a diamond blade to cut three or four thin sections to a thickness of approximately 0.5 mm, one of which was through the otolith core. Sections were then mounted on a microscope slide with Histomount. Initially, we prepared some otoliths for age estimation by embedding them in Spurr, a high-density plastic medium (Secor et al., 1992). A 1- to 2-mm-thick transverse section containing the otolith core was cut. The section was mounted on a microscope slide with thermoplastic glue (CrystalBond 509 adhesive) and polished with "wet/dry" sandpaper (grit sizes ranging from 220 to 2,000) until annuli were visible. Sections were then polished on a Buehler polishing cloth with 0.05-μ gamma alumina powder to remove scratches. There was no consistent difference in the quality of either preparation technique. Mounting the sections in Histomount took less time than embedding them in Spurr, so we adopted it as our standard protocol.

Annuli were counted using compound microscopes equipped with transmitted light and dissection microscopes equipped with reflected light. Two independent readers counted annuli on each otolith section three times each, for a total of six counts for each otolith. Thirty-two otoliths were considered unreadable by one or both readers and were discarded from our analysis before the completion of six readings. Annulus counts for individual otoliths often varied among readings. We established criteria for accepting or rejecting individual otoliths by calculating a coefficient of variation:

$$CV = (SD/\bar{X} \times 100),$$

where SD = the standard deviation of counts for a given otolith; and
$\bar{X}$ = the mean annulus count for a given otolith.

After six readings were completed for all otoliths, otoliths for which the CV>12% were again read independently by each reader without knowledge of previous annulus counts. The count with the greatest difference from the mean of all counts was then discarded and replaced with a new count made by the reader whose reading was discarded. This protocol was repeated twice, and if the CV for a given otolith remained >12%, the otolith was rejected from our analysis.

The von Bertalanffy (1957) growth equation $TL_t = L_\infty(1-e^{(-K(t-t_0))})$ was fitted to observed age-length data with nonlinear regression procedures. Age was estimated as the mean of all six annulus counts. We did not attempt to backcalculate the length at which the last annulus was formed because of the variability among annulus counts for most otoliths. Our estimates of length at age thus include some seasonal growth that occurred after the formation of the final annulus. To estimate mean length at age, we rounded all ages to the nearest integer, but in growth and maturity models the mean age was not rounded to an integer and fractional ages were used. Length-weight regressions were calculated by linear regression of $\log_{10}$-transformed data.

Age validation

Measurements for marginal-increment analysis were made with a digital image-processing system on an axis extending along the sulcal ridge from the otolith's core to the proximal margin of the section. Because many black grouper otoliths were difficult to read, we selected only otoliths for which there was 100% agreement among all readings for marginal-increment analysis. In addition, we restricted measurements to otoliths with from one to seven annuli because it was difficult to measure the more closely spaced outer annuli of older fish. We expressed the distance from the final annulus to the otolith's edge (marginal increment) as a percentage of the distance between the last two annuli formed on the otolith. For all grouper, the distance between the otolith core and the first annulus (r_1) was typically much greater than the distance between the first and second annuli (r_2–r_1). For this reason, we divided the distance between the first and second annuli by the distance between the otolith's core and the first annulus for each otolith measured and then calculated the mean of this number for the entire sample:

$$\frac{\sum_{i=1}^{n}((r_2 - r_1)/r_1)_i}{n} = 0.353. \qquad (SE = 0.0065)$$

We then estimated the expected distance between the first and second annulus for each age-1 black grouper otolith as a function of the distance between the otolith's core and the first annulus. The percent marginal increment for age-1 fish was then calculated as

$$(MI/(0.353 \times r_1)) \times 100,$$

where MI = the marginal increment.

We then plotted the percent marginal increments as a function of capture month for 1995–96, the period during which we made regular monthly collections.

Reproduction

Histological sections of gonads from 857 black grouper that ranged in length from 155 to 1518 mm were prepared and assessed for reproductive state. Gonad samples were processed histologically with `,Modification of the periodic acid Schiff's (PAS) stain for glycol-methacrylate sections, with Weigert's iron-hematoxylin as a nuclear stain, and with metanil yellow as a counterstain (Quintero-Hunter et al., 1991).

Oocytes were staged and counted from histological preparations at 100× with a compound microscope attached to a digital image-processing system. Four oocyte stages were recognized in black grouper ovaries: primary growth, cortical alveolar, vitellogenic, and oocytes in the final stages of maturation (Wallace and Selman, 1981). The final stages of oocyte maturation included yolk coalescence, germinal vesicle migration, germinal vesicle breakdown, and hydration. At least 300 oocytes per slide were staged and counted in arbitrarily chosen fields, and frequencies were expressed as a percentage of the total count. We counted all oocytes that had at least 50% of their area visible in a field before moving to the next field.

Gonads were classified on the basis of the maturity criteria of Moe (1969). Female grouper were considered sexually mature if ovaries contained vitellogenic oocytes or contained evidence of widespread atresia consistent with gonadal recrudescence. Mature females included Moe's classes 2, 3, and 4.

Monthly median gonadosomatic indices (GSI) were plotted to show seasonal reproductive patterns. Gonadosomatic indices were calculated for 201 sexu-

ally mature female grouper ranging in length from 565 to 1310 mm and for 43 sexually mature male grouper ranging in length from 947 to 1518 mm as

$$GSI = (GW / (TW - GW)) \times 100,$$

where GW = total gonad weight (g); and
TW = total fish weight (g).

We measured the diameters of whole oocytes from six females whose ovaries contained numerous hydrated oocytes. Samples of ovarian tissue that were preserved in 10% formalin were rinsed briefly in water and transferred to 33% glycerin for measurements. Diameters of at least 300 oocytes for each female were measured with a digital image-prossessing system. Only oocytes larger than 0.2 mm in diameter were measured.

We estimated the length at which 50% of the females in the population reached sexual maturity by fitting a logistic function to the percentage of females that were sexually mature and their respective lengths. A similar function was fitted to the percentage of the population made up by females to estimate the length at which 50% of the females in the population transformed into males. The parameter b in Table 1 is the inflection point of the logistic curves and is the estimate of the length or age at which 50% of the females in the population were sexually mature or the length or age at which 50% of the population had undergone transition from female to male. Sometimes distinguishing between the gonads of sexually immature grouper and the regressed gonads of mature fish was difficult, and we eliminated 29 females that ranged in length from 586 to 894 mm from our analysis of maturation because we were uncertain of their maturity. Hunter et al. (1992) recommended that only fish collected early in the spawning season be used to estimate the length at 50% maturity, but because we found evidence of some spawning during most of the year, we included all months in our analysis.

Results

The 1166 black grouper we examined ranged from 155 to 1518 mm in length. Among black grouper that we sexed, females ranged from 155 to 1310 mm in length (mean=696.3, SD=204.53, n=834) and males ranged from 947 to 1518 mm (mean=1254.9, SD=126.19, n=54; Fig. 1). Our sample contained 276 grouper that were not sexed, usually because the grouper had been eviscerated at sea by fishermen. The sex ratio of our sample was 1:15.4 (male:female); greatly skewed towards females. Black grouper landed in Pinellas County on Florida's Gulf coast by commercial fishermen were much larger than those landed in the Florida Keys (Fig. 2). The male:female sex ratio of the black grouper we sampled that were landed in the Florida Keys was 1:58.7, and the sex

Table 1

The relations between standard length, fork length, and total length, between length and weight, between the percentage of females that were sexually mature and length, and between the percentage of the population made up by females and length of black grouper, *Mycteroperca bonaci*, from South Florida waters. TL = total length (mm), FL = fork length (mm), SL = standard length (mm), WT = whole weight (g), GWT = gutted weight (g), OTOWT = otolith weight (g), and AGE = age (years). The total length range for all length-length regressions was 238–1518 mm and for the length-weight regression was 177–1518 mm; the total weight range for the total weight-gutted weight regression was 467–61,462 g; the age range for the age-otolith weight regression was 1–30.5 years.

			$Y = a+bX$		
Y	X	n	a (1 SE)	b (1SE)	r^2
SL	FL	1,134	-23.712 (0.6852)	0.883 (0.0008)	0.999
SL	TL	1,141	-21.611 (0.7743)	0.858 (0.0009)	0.999
FL	SL	1,134	27.607 (0.7514)	1.131 (0.0011)	0.999
FL	TL	1,150	1.641 (0.5223)	0.973 (0.0006)	0.999
TL	SL	1,141	26.186 (0.8757)	1.164 (0.0012)	0.999
TL	FL	1,150	-1.317 (0.5378)	1.028 (0.0006)	0.999
WT	GWT	638	81.519 (12.9253)	1.056 (0.0014)	0.999
$\log_{10}$WT	$\log_{10}$TL	772	-5.457 (0.0323)	3.218 (0.0115)	0.995
			$Y = a+b\times\sqrt{X}$		
OTOWT	AGE	389	-0.1026 (0.0036)	0.1210 (0.0014)	0.952
			$Y = \left(1/\left(1+e^{(a(X-b))}\right)\right)\times 100$		
% Mature (Females)	TL	782	-0.0194 (0.0017)	826.0 (4.75)	0.592
% Mature (Females)	AGE	617	-1.375 (0.1341)	5.20 (0.078)	0.555
% Females	TL	888	0.016 (0.0013)	1214.4 (5.05)	0.618
% Females	AGE	694	0.355 (0.0287)	15.55 (0.382)	0.551

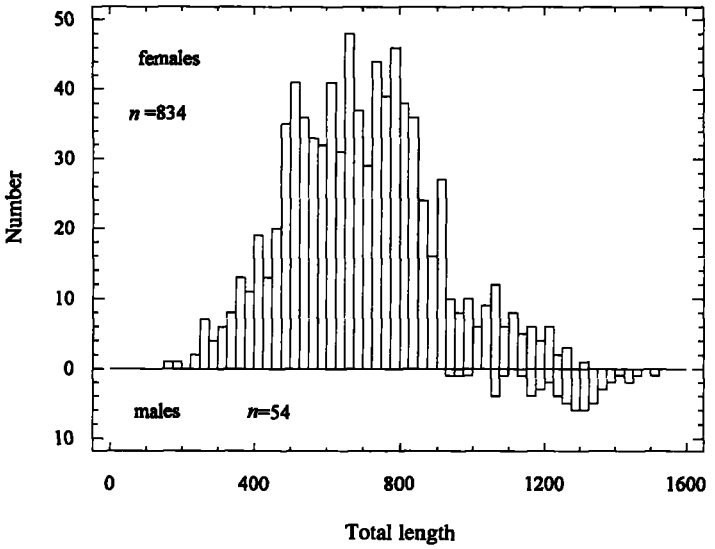

Figure 1

Total lengths (mm) of male and female black grouper, *Mycteroperca bonaci*, sampled from South Florida waters during 1994–96.

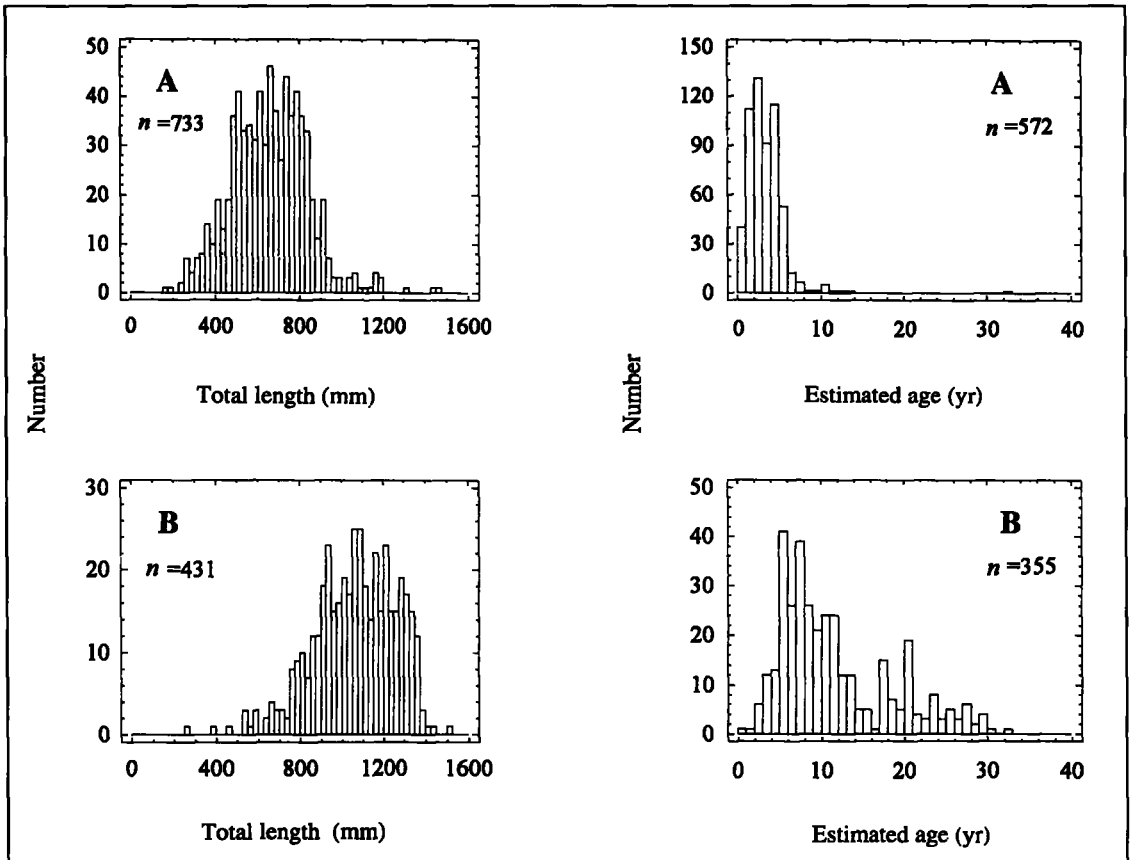

Figure 2

Total lengths (mm) and ages (years) of black grouper, *Mycteroperca bonaci*, landed in the Florida Keys (**A**) and in Pinellas County on Florida's Gulf coast (**B**).

ratio of black grouper landed in Pinellas County by commercial fishermen was 1:3.1. The pooled length-weight equation for sexed and unsexed fish and the relations between SL, FL, and TL are presented in Table 1.

Age and growth

When viewed with reflected light, black grouper otoliths have narrow, opaque (bright) annuli that alternate with broad translucent (dark) zones (Fig. 3, A and B). Proceeding from the otolith's core towards the otolith's proximal margin, these translucent zones become increasingly opaque in appearance as the otolith grows. In the outer portion of the otoliths from older grouper (>10 years old), the dark translucent zones are narrow. In some individuals, the annuli are indistinct and irregular in appearance, which makes age estimation difficult. In older grouper, the annuli become closely spaced near the edge of the otolith, and this also makes age estimation difficult (Fig. 3C). Often otoliths of older grouper (>15 years) were more easily read under transmitted light and compound microscopes; otoliths from younger fish were often more easily read under reflected light. Overall, the otoliths from black grouper are similar in appearance to those of other grouper species we have examined in our laboratory.

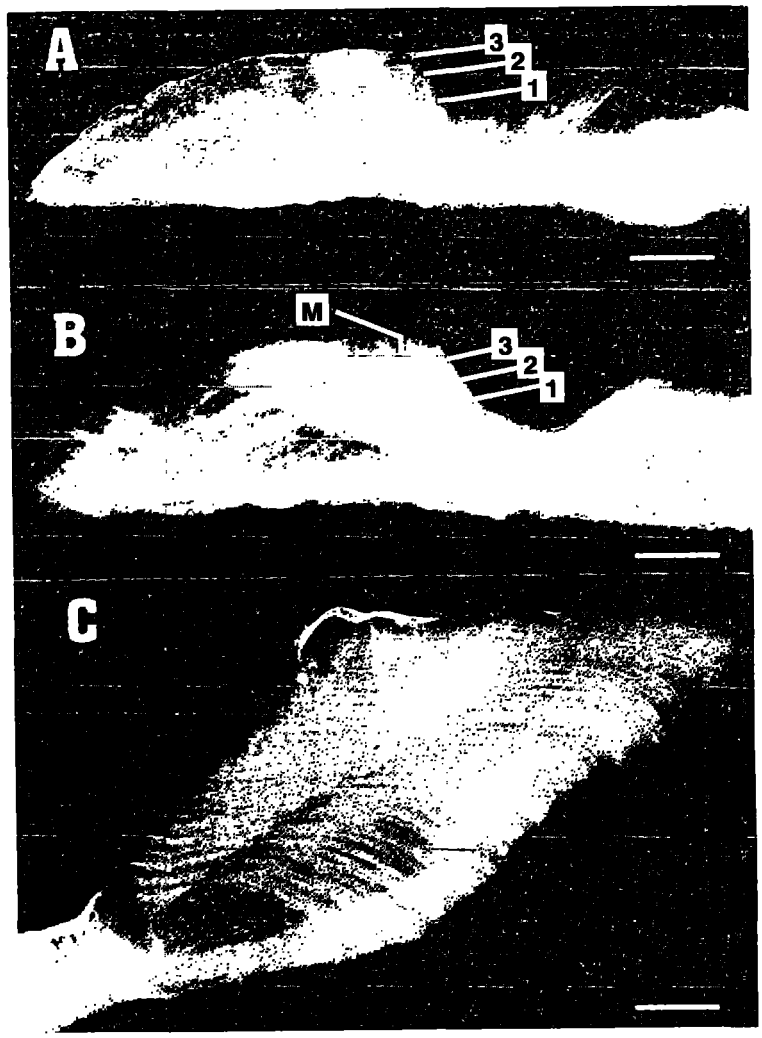

Figure 3

(**A**) Sectioned sagitta from a 3-year-old black grouper, *Mycteroperca bonaci* (508 mm TL), captured in May 1996. The third annulus is on the edge of the otolith. (**B**) Sectioned sagitta from a 3+ year-old black grouper (671 mm TL) captured in January 1996. Note the wide marginal increment (m) subsequent to the third annulus. (**C**) Sectioned sagitta from a 33-year-old male black grouper (1427 mm TL) captured in June 1996. Note the close spacing of annuli that caused difficulty in age estimation. Scale bars = 500 microns.

Marginal-increment analysis of otoliths from grouper 1–7 years old suggested that one annulus had been formed during April–June each year (Fig. 4). Median marginal increments had a consistent seasonal minimum during May–July and a maximum during December–March in both 1995 and 1996. Marginal increments during April–June tended to be either large (>75%) or small (<25%), suggesting that most individuals either had wide margins, as expected just prior to annulus formation, or had just formed an annulus and had either no marginal increment or a narrow margin. By June or July, individuals with wide margins were no longer present, suggesting that annulus formation was completed.

Of 1059 otoliths that we examined, 132 (12.5%) were rejected because of disagreements among readings. We had total agreement among annulus counts for 403 otoliths, but all of these otoliths were from grouper estimated to be less than 10 years old. The length-frequency distribution of fish whose otoliths were rejected because they were unsuitable for age estimation was not significantly different from that of fish whose otoliths were readable (Kolmogorov-Smirnov two-sample test, two-sided test statistic = 1.319, P=0.062), but the otolith weight distribution of fish whose otoliths were unreadable was significantly different from that of fish whose otoliths were readable (Kolmogorov-Smirnov two-sample test, two-sided test statistic=2.017, P<0.001). Otolith weight

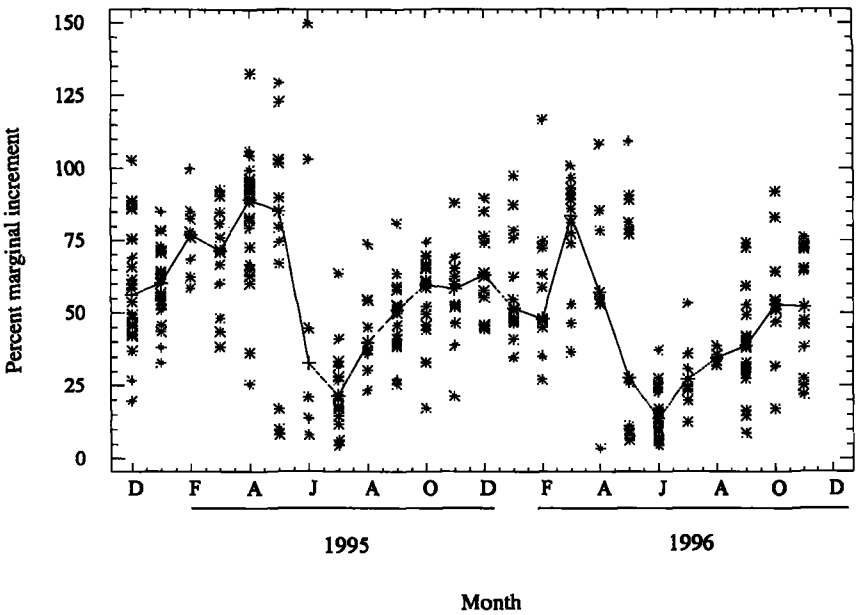

Figure 4

Percent marginal increments (*) and medians (+ and line) for black grouper, *Mycteroperca bonaci*, ages 1–7 captured from South Florida waters (n=330).

was closely related to fish age (r^2=0.952, Table 1, Fig. 5A), and the median weight (0.2437 g) of otoliths rejected as unreadable was significantly greater than that of otoliths that were readable (0.1571 g; Mann-Whitney W test; W=12,492.0, P<0.001).

Black grouper growth was rapid until an age of about 10 years and then slowed considerably (Table 2, Fig. 5B). Most of the fish in our sample were from 2 to 10 years old, and the most abundant age classes were 2–6 years old (Fig. 6). The two oldest black grouper examined were a 33-year-old (1325-mm) fish that was not sexed and a 33-year-old (1427-mm) male (Table 2). The oldest female was a 1275-mm fish estimated to be 23 years old (Fig. 6); the largest female was 1310 mm long (Fig. 1) and was estimated to be 12 years old. Only three female black grouper were estimated to be over 20 years old. The smallest and youngest male was a 947-mm fish estimated to be 6 years old (Fig. 1 and 6). Black grouper landed in Pinellas County on Florida's Gulf coast by commercial fishermen were older overall than those landed in the Florida Keys, where only one fish older than 15 years was examined (Fig. 2). Estimates of von Bertalanffy growth model parameters are presented in Table 3.

Sexual maturation and transition

Black grouper, like most epinepheline serranids, are protogynous hermaphrodites. Protogyny was suggested by the presence of peripheral sperm-collecting sinuses rather than the centrally located sinuses typical of gonochorists. Furthermore, a membrane-lined central lumen that was not used for sperm transport was present in testes and was a structural remnant of the ovarian lumen (Sadovy and Shapiro, 1987). Additional evidence of hermaphroditism was the presence of atretic vitellogenic oocytes in a transitional gonad containing proliferating testicular tissue (Fig. 7A). A second grouper that we would have considered transitional except for the presence of some mature sperm in peripheral sperm sinuses also contained degenerating vitellogenic oocytes (Fig. 7, B and C); this fish was considered to be a functional male. Transitional grouper also contained numerous PAS-positive melanomacrophage centers (Ravaglia and Maggese, 1995) referred to as "yellow-brown bodies" by Sadovy and Shapiro (1987). When stained with the PAS stain, these structures are brilliant purple. Melanomacrophage centers are thought to be active in degrading atretic oocytes, postovulatory follicles, and residual cells of the spermatogenic cycle (Chan et al., 1967; Ravaglia and Maggese, 1995). Many mature males contained scattered primary growth stage oocytes throughout the testis. We also noted that small amounts of testicular tissue were often present in functional ovaries that showed no evidence of undergoing transition. Finally, the differences in male and female length-frequency distri-

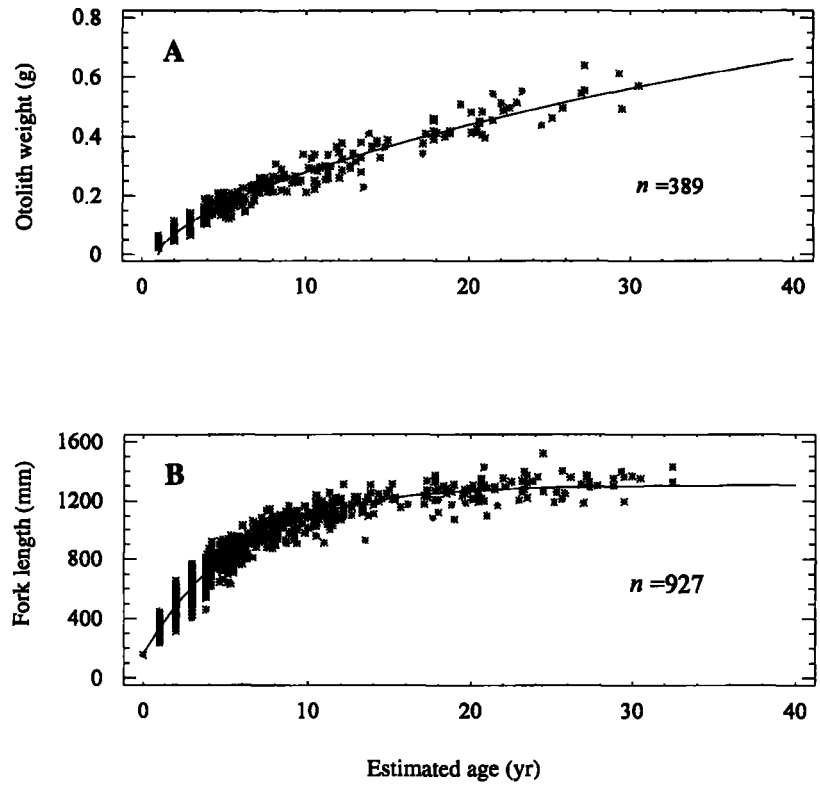

Figure 5

(**A**) The otolith weight–age relation for black grouper *Mycteroperca bonaci*, collected from South Florida waters. The equation for the relation is presented in Table 1. (**B**) Observed and predicted total lengths (mm) from the von Bertalanffy growth model for sexed and unsexed black grouper, *Mycteroperca bonaci*.

butions and the absence of small or young males are consistent with our hypothesis of protogynous hermaphroditism (Fig. 1).

We estimated that 50% of the females in the population reached sexual maturity by 826 mm and an age of 5.2 years (Table 1, Fig. 8). The smallest sexually mature female in our sample was 508 mm, and the youngest sexually mature female was 2 years old. All of the ovaries we examined contained primary growth stage oocytes. Cortical alveolar stage oocytes occurred only in ovaries from grouper larger than about 500 mm and older than 2 years, and they were common only among grouper larger than about 600 mm and older than 3 years (Fig. 9A). Vitellogenic oocytes were found only in ovaries from fish larger than about 600 mm and older than 2 years and were common only among grouper larger than 800 mm and older than 5 years (Fig. 9B). The length and age at which vitellogenic oocytes were commonly found agrees well with our estimate of the length and age at which 50% maturity was reached, suggesting that misclassification of regressed gonads did not greatly bias our estimates.

Transition from female to male was also partially a function of length and age. By a length of 1214 mm and an age of 15.5 years, 50% of the females in the population had transformed into males (Table 1, Fig. 8). We found only one transitional fish (1030 mm long; Fig. 7A) and a second grouper (947 mm long) that appeared to have recently completed transition (Fig. 7, B and C). We did not find any immature males (Moe's class 6) in our sample, suggesting that males become sexually active soon after transition. Most (91%) of the males we examined had ripe testes (Moe's class 9) that contained mature sperm.

Fin pigmentation differed between sexes in black grouper (Fig. 10). Ontogenetic color change independent of sex was ruled out because relatively small males (<1000 mm) displayed the male color phase, but the oldest and largest females (e.g., 1255 mm) did not. The pectoral fins, anal fin, and caudal fin were dark colored in females, but these fins were jet

black in males (Fig. 10, A–C). The jet black pigmentation of the pectoral fin was most apparent on the medial side. The dorsal interspinous membrane of females was yellow to dusky (Fig. 10D). In males, this membrane was usually dark black but was occasionally yellow or yellowish with black tips. Because this membrane can be yellow in both males and females, the coloration of this fin was the least reliable indicator of sex. The distal one-third of the soft dorsal fin was dark black in both sexes and was not useful in determining sex.

Seasonality of gonad development

The frequencies of the four oocyte stages we counted in black grouper ovaries had a seasonal pattern that was repeated in both 1995 and 1996 (Fig. 11, A and B). Vitellogenic oocytes were present in greatest number in January–March in both 1995 and 1996 and were present in all months except September–November 1996. Cortical alveolar stage oocytes were also present in all months except September–November 1996 and were most abundant during December–April. Primary growth stage oocytes were present during all months and made up at least 55% of the total number of oocytes present. Oocytes in the final stages of maturation were not abundant, but they were most common during December–May (Fig. 11B). We examined six females with hydrated ovaries: one was caught during October, one in December, and four in March. The ovaries from these grouper contained a group of vitellogenic oocytes that ranged from 0.5 to 0.7 mm in diameter and a clutch of hydrated oocytes that ranged from 0.8 to 1.2 mm in diameter (Fig. 12). Most of the oocytes <0.2 mm in diameter, which we did not measure, were primary growth stage or cortical alveolar stage oocytes. Postovulatory follicles were not recognized in any female examined.

Females with the greatest GSIs (>5) were captured during February–March (Fig. 13). Male GSIs (range 0.086–0.399, n=43) were generally much less than female GSIs (range 0.032–10.108, n=201) and had no

Table 2

Average observed and predicted total lengths (mm) for sexed and unsexed black grouper, *Mycteroperca bonaci*, and average observed lengths for females and males. The average observed length at age includes some seasonal growth that occurred after the formation of the final annulus. Values in parentheses are standard error and sample size.

	Sexed and unsexed		Females	Males
Age (yr)	Average observed	Predicted	Average observed	Average observed
0	155 (1)	159	155 (1)	
1	338 (8.7;41)	337	339 (8.9; 39)	
2	486 (5.7;113)	487	486 (5.7;112)	
3	606 (5.7;136)	614	607 (5.7;134)	
4	733 (5.5;155)	722	734 (5.7;147)	
5	814 (5.8;113)	813	814 (6.1;97)	
6	871 (7.7;70)	889	857 (10.2;41)	947 (1)
7	972 (9.1;40)	954	966 (13.6;20)	1062 (1)
8	1008 (11.6;39)	1009	998 (20.5;15)	1006 (46.0;2)
9	1038 (12.3;31)	1055	1024 (19.8;14)	1058 (1)
10	1074 (12.3;16)	1094	1078 (14.6;5)	1167 (1)
11	1116 (13.1;33)	1127	1147 (27.0;10)	1130 (22.8;5)
12	1138 (14.4;21)	1155	1131 (29.0;9)	
13	1164 (11.6;11)	1178	1182 (16.0;6)	1172 (1)
14	1175 (30.6;11)	1198	1192 (18.5;2)	1222 (30.5;2)
15	1239 (14.4;7)	1215	1213 (1)	1266 (26.0;3)
16	1165 (10.5;2)	1229		
17	1244 (21.2;6)	1241	1270 (1)	1283 (47.5;2)
18	1252 (30.3;10)	1251		
19	1226 (35.5;6)	1260		1285 (1)
20	1248 (12.9;9)	1267	1220 (1)	
21	1264 (20.2;15)	1273	1195 (1)	1315 (38.7;4)
22	1278 (29.6;5)	1278		
23	1298 (27.3;6)	1283	1275 (1)	1326 (68.5;2)
24	1320 (26.6;5)	1286		1370 (1)
25	1307 (72.2;4)	1289		1390 (128.0;2)
26	1305 (44.5;4)	1292		
27	1309 (23.3;7)	1294		1291 (38.3;4)
28	1300 (1)	1296		
29	1349 (28.3;3)	1298		1349 (28.3;3)
30	1307 (55.9;3)	1299		
31	1350 (1)	1300		1350 (1)
32	1301			
33	1376 (51.0;2)	1302		1427 (1)

Table 3

Parameter estimates for the von Bertalanffy growth model for black grouper, *Mycteroperca bonaci*, collected from the waters of South Florida. Values in parentheses are standard errors.

n	L_∞ (mm)	K	t_0	adjusted r^2
927	1306.2 (8.05)	0.169 (0.0037)	–0.768 (0.0640)	0.941

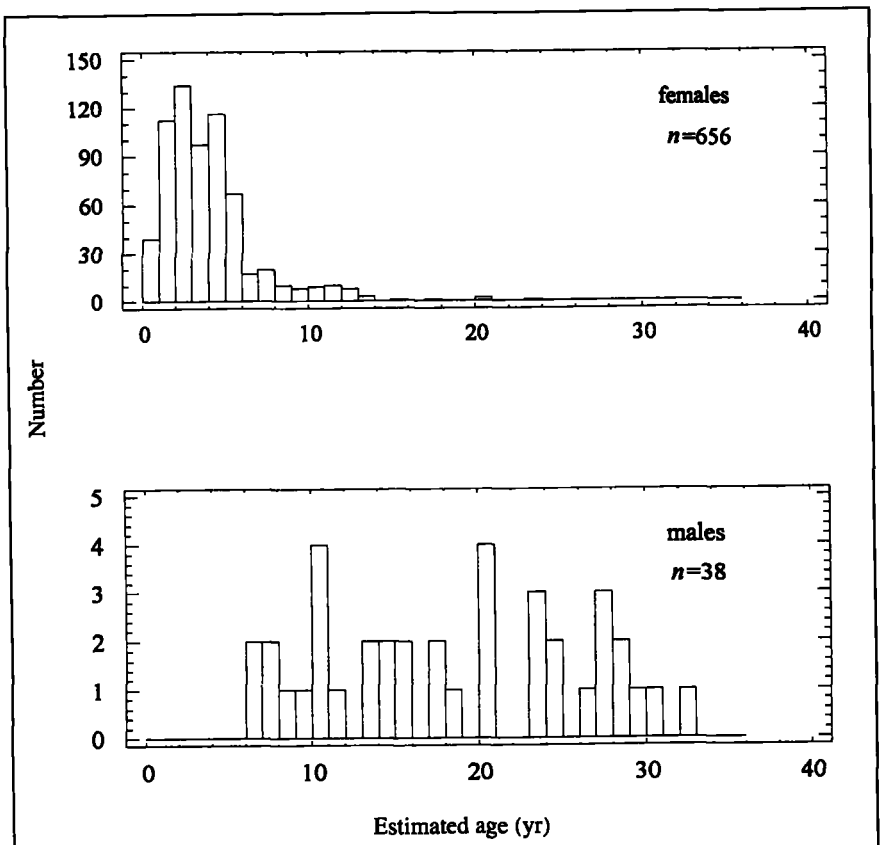

Figure 6

Age-frequency distributions for male and female black grouper, *Mycteroperca bonaci*, from South Florida waters.

seasonal trend (Fig. 13). Testes from sexually mature males ranged in weight from 14 to 197 g and were much smaller overall than ovaries from sexually mature females, which ranged in weight from 1 to 1354 g.

Discussion

We obtained black grouper from a variety of fishery-dependent and fishery-independent sources, and it is difficult to assess the extent to which the length and age structure of our sample reflects that of the population or the degree to which different fishing gears biased the various samples. Although we sampled grouper that were landed in two geographically separate locations, the Florida Keys and in Pinellas County on Florida's Gulf coast, most of the grouper landed in both locations were caught in the waters off the Florida Keys. Most of the large (>1000 mm) fish and most of the males that we examined came from commercial fish houses on Florida's Gulf coast, and most of these grouper were caught on the Tortugas Banks west of the Florida Keys. Fish obtained from Keys headboats, fish houses, and spearfishermen were smaller and younger overall than grouper sampled from fish houses in Pinellas County (Fig. 2). Most black grouper landed in Pinellas County fish houses were caught with commercial longlines in depths greater than 37 m (20 fathoms); most of the fish landed in the Keys were captured by spear fishermen, principally in water 6–50 m deep. The greater depths fished and the greater distances traveled by Gulf-coast commercial fishermen to presumably more remote fishing areas probably account for the differences between the two samples.

Age and growth

Black grouper appear, on the basis of marginal-increment validation for grouper 1–7 years old, to form a single annulus each year in late spring or early summer. We were unable to validate that annuli are annual marks in older grouper, and the accuracy of our age estimates for older fish is unknown. The annuli we counted on otolith sections from older grouper were similar in appearance to validated annuli;

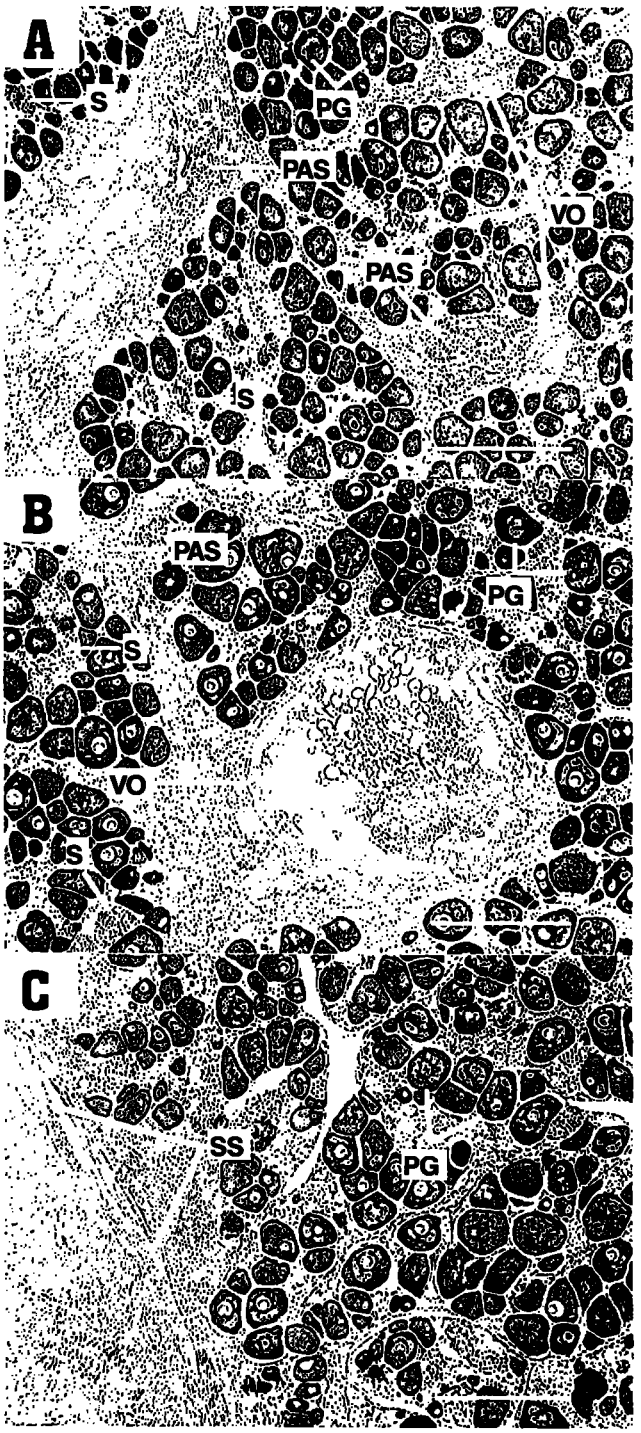

Figure 7

(**A**) A transitional gonad from a 1030-mm-TL black grouper, *Mycteroperca bonaci*, captured in September 1994 with proliferating spermatogenic tissue in sperm cysts (S), primary growth stage oocytes (PG), degenerating vitellogenic oocytes (VO), and PAS-positive melanomacrophage centers (PAS). (**B**) A 947-mm-TL black grouper captured in November 1995 with proliferating spermatogenic tissue in sperm cysts (S), primary growth stage oocytes (PG), degenerating vitellogenic oocytes (VO), and PAS-positive melanomacrophage centers (PAS). (**C**) The same ovary as in B showing the presence of mature sperm in peripheral sperm sinuses (SS). Scale bars = 200 microns.

however, as annuli became progressively more closely spaced, age estimation became more difficult. Additional work is needed to assess the accuracy of our age estimates for older grouper; consequently, our age estimates predicted lengths at age, and growth model parameters should be used with caution. From their analyses of marginal increments, Manooch and Mason (1987) also reported that black grouper from the Florida Keys form a single annulus each year. They found that annuli were usually formed during March–May, about a month earlier than we estimated. This difference is probably a result of differences in interpretation of the appearance of an annulus present on the otolith margin. Other congeners also form annuli during late spring and early summer. Collins et al. (1987) reported that gag, *M. microlepis,* off the southeastern U.S. coast form annuli during late spring to late summer, and Hood and Schlieder (1992) suggested that gag form annuli during summer in the eastern Gulf of Mexico. Bullock and Murphy (1994) reported that yellowmouth grouper, *M. interstitialis*, form opaque bands during late summer and early fall. Matheson et al. (1986) found that scamp, *M. phenax*, in North Carolina waters form an annual mark during April or May.

We rejected 12.5% of the otoliths we sectioned as unreadable, and this is a possible source of bias to our growth model parameters. Otolith weight is a useful predictor of age of black grouper (r^2=0.952), and the otoliths that we rejected were generally heavier and thus probably older than most of those that we considered readable. In addition, although the length-frequency distribution of fish whose otoliths were rejected was not significantly different from that of all fish whose otoliths were readable, the significance level of the test (P=0.062) was close enough to 0.05 to cause us to suspect that the distributions could have been different. If so, we may have rejected as unreadable more otoliths from large grouper than from small grouper. We may also have tended to exclude slower-growing older grouper from our sample of aged fish, and this could have biased our growth models. However, when we recalculated growth models and included all fish regardless of CV, the resulting growth-parameter estimates were all within one standard error of those in Table 3. Thus any bias to the growth model caused by our rejection of otoliths from older grouper appears to be negligible. Our choice of a threshold CV of 12% was arbitrary, but growth model parameters did not appear to be sensitive to the choice of CV thresh-

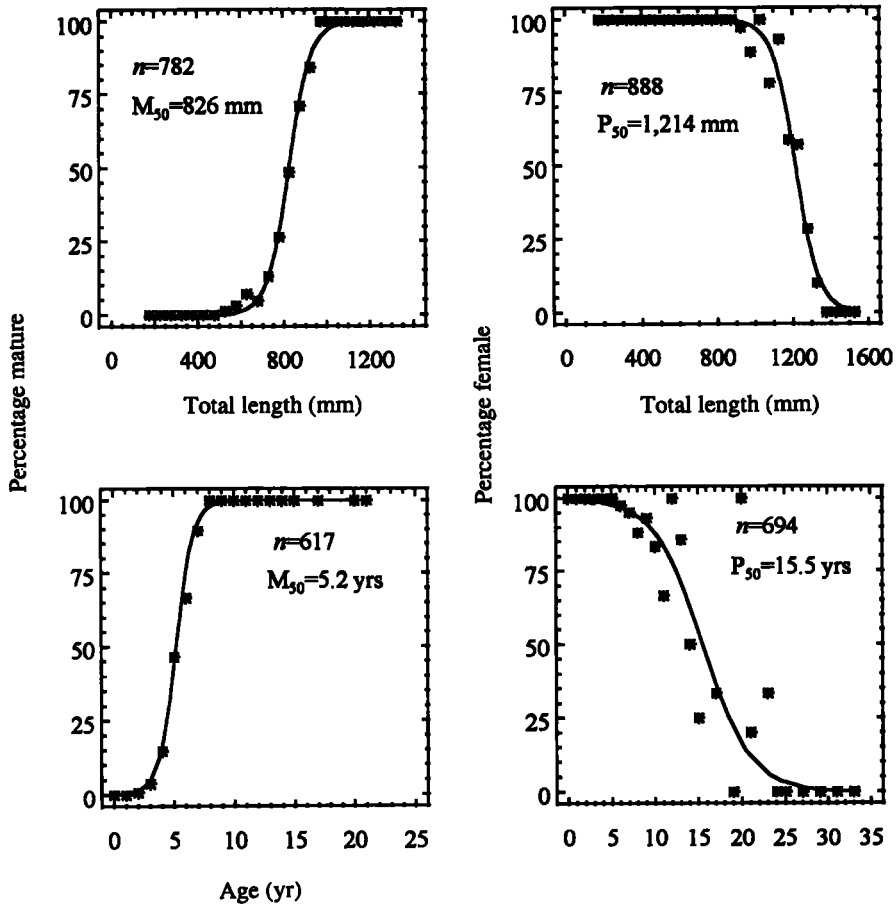

Figure 8

Logistic curves describing the percentage of female black grouper, *Mycteroperca bonaci*, collected from South Florida waters that were sexually mature and the percentage of our black grouper Sample that was made up by females as a function of both total length (mm) and age (years). M_{50} is the length or age predicted by the logistic regression at which 50% of the female black grouper were sexually mature, and P_{50} is the length or age predicted by the logistic regression at which 50% of the black grouper in our sample were females.

olds. When we recalculated growth models with CV thresholds of 10% and 5%, our estimates of L_∞ and K remained within two standard errors of the estimates at a CV of 12%, but the numbers of otoliths eliminated from our analysis increased substantially at lower CV thresholds.

The two oldest black grouper in our sample were both estimated to be 33 years old, considerably older than Manooch and Mason's (1987) oldest grouper (14 years). Manooch and Mason sampled hook-and-line-caught black grouper from Keys headboats during 1978–85 and speculated that the maximum age attained by black grouper was probably about 19 years. The presence of older fish in our sample is probably because our sample contained black grouper that were larger (largest 1518 mm) than those examined by Manooch and Mason (largest aged 1180 mm; largest measured 1259 mm). We measured 125 black grouper longer than 1200 mm and aged 94 of these. Of the 98 grouper that we estimated to be older than 14 years, 84% were longer than 1200 mm and were longer than any fish aged by Manooch and Mason. Most of the large black grouper in our sample were caught by commercial longliners and landed in Pinellas County; the lengths and ages of the grouper in our sample that were landed in the Keys were similar to those of Manooch and Mason (1987).

Our estimates of length at age are similar to those of Manooch and Mason (1987), and the predicted growth curves from both data sets are in general agreement (Fig. 14). In some cases, such as age classes 5–9, our estimates of mean length at age are

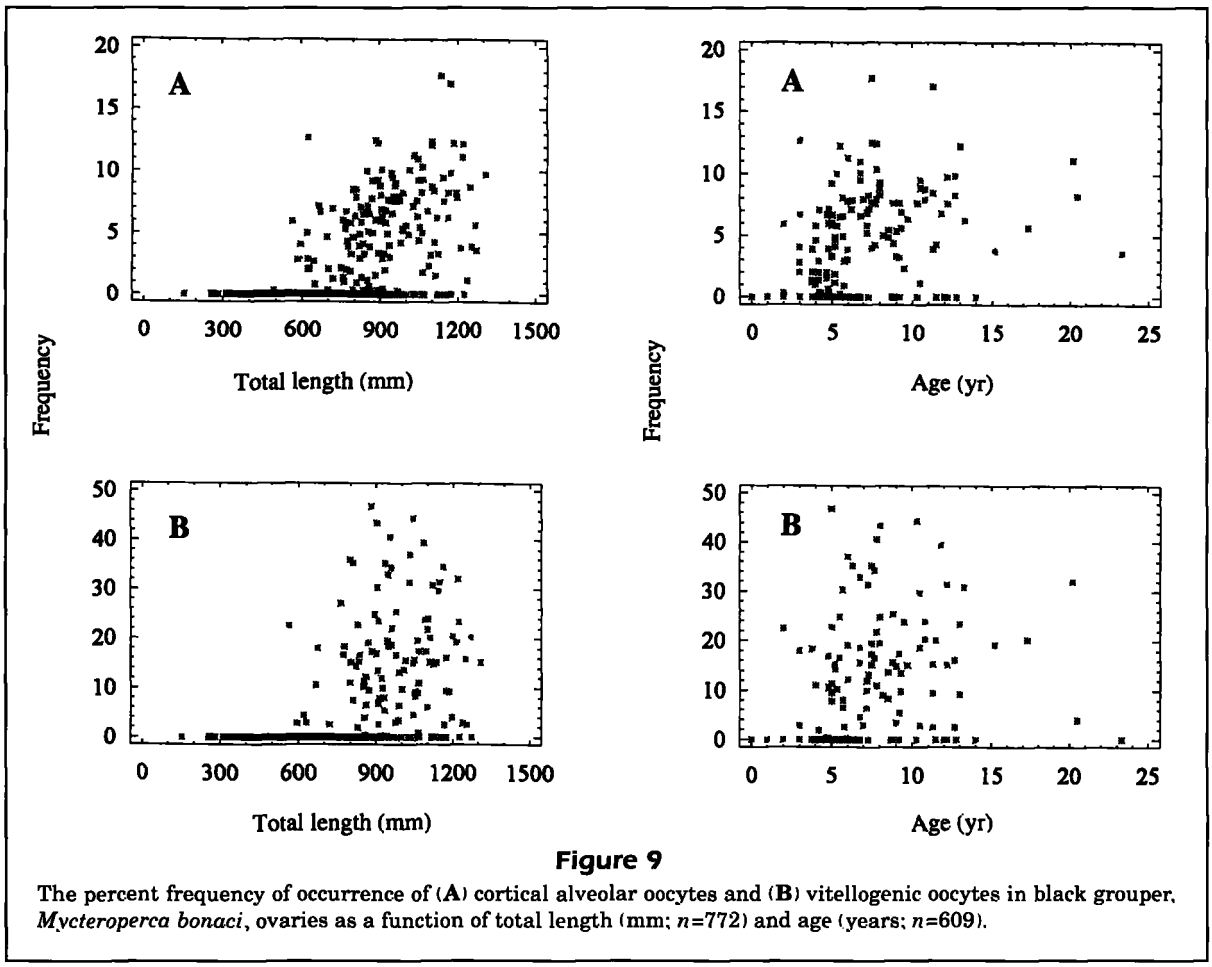

Figure 9

The percent frequency of occurrence of (**A**) cortical alveolar oocytes and (**B**) vitellogenic oocytes in black grouper, *Mycteroperca bonaci*, ovaries as a function of total length (mm; n=772) and age (years; n=609).

greater than those of Manooch and Mason, but their estimates for these age classes fall below not only our predicted lengths but also their predicted lengths. The von Bertalanffy growth model parameters estimated by Manooch and Mason were L_∞ = 1,352 mm, greater than our estimate of L_∞ = 1,306 mm, and K = 0.1156, less than our estimate of K = 0.169.

Black grouper, though not as large as jewfish or warsaw grouper, are among the largest species of grouper, and it is not surprising that they attain ages over 30 years. Estimates of the longevity of other grouper species are similar to our estimates for black grouper. Hood and Schlieder (1992) and Collins et al. (1987) estimated a maximum age of 21–22 years for gag, Bullock et al. (1992) estimated a maximum age of 37 years for jewfish (*E. itajara*), Bullock and Murphy (1994) estimated a maximum age of 28 years for yellowmouth grouper, Manooch and Mason (1987) reported that warsaw grouper (*E. nigritus*) reach 41 years, and Moe (1969) reported a red grouper (*E. morio*) 25 years old. The growth rate (K=0.169/year) of black grouper is similar to that estimated for gag (K=0.166/year, Hood and Schlieder, 1992), but is greater than the growth rates estimated for many other grouper species: jewfish K = 0.126/year (Bullock et al., 1992), yellowmouth grouper K = 0.08/year (Bullock and Murphy, 1994), and warsaw grouper K = 0.054/year (Manooch and Mason, 1987).

Sexual maturation and transition

Our histological analysis of black grouper gonads is consistent with the diagnostic criteria of Sadovy and Shapiro (1987) for a monandric protogynous hermaphrodite. Furthermore, the absence of small males and a sex ratio highly skewed towards females are consistent with our diagnosis of protogynous hermaphroditism. Although the length and age distributions of males and females overlapped, males occupied the largest and oldest length and age classes and were unrepresented in smaller and younger length and age classes (Figs. 1 and 6). This is an important difference between black grouper and Nassau grouper (*Epinephelus striatus*), which has recently been diagnosed as a gonochorist with potential for sex change (Sadovy and Colin, 1995). In

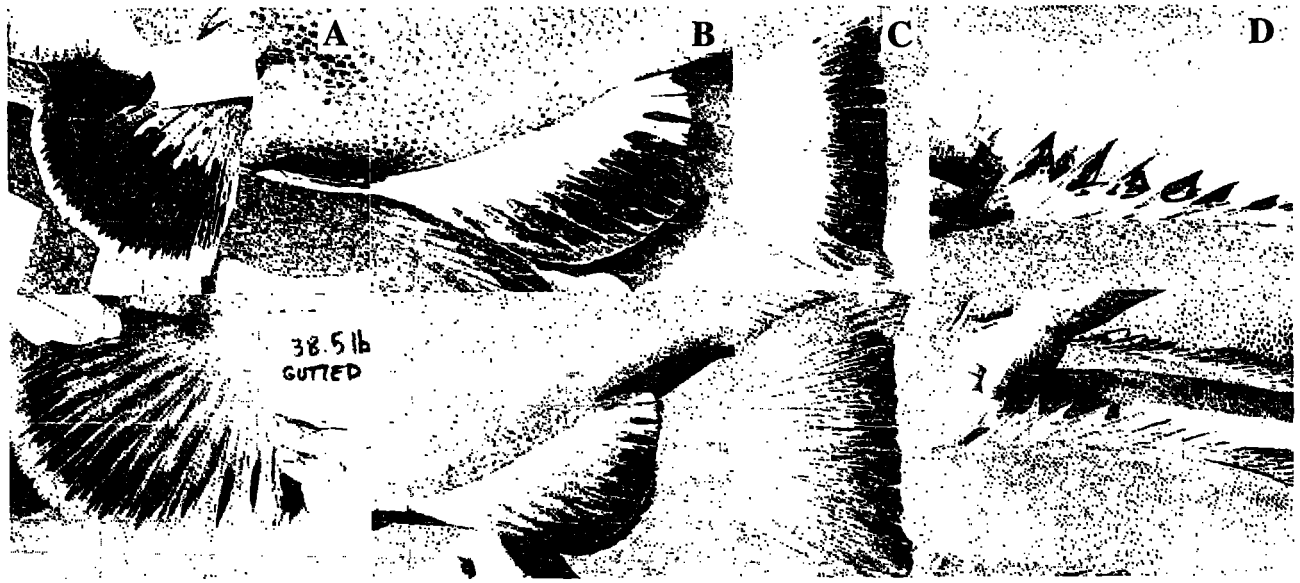

Figure 10

Differences in fin pigmentation in male (above) and female (below) black grouper, *Mycteroperca bonaci*. (**A**) Medial surface of pectoral fin, (**B**) anal fin, and (**C**) caudal fin jet black in males. (**D**) Dorsal interspinous membrane yellow or dusky in females, usually black in males.

Nassau grouper, both males and females can develop directly from juveniles and there is little difference in the lengths of males and females. Furthermore, there is little difference in the length at which sexual maturation occurs in both sexes, in contrast to black grouper. The scarcity of transitional black grouper and the absence of immature males in our samples suggest that transition occurs quickly and that testes become active soon after transition. The presence of large females in the population suggests that some females may not transform into males, but even the largest females we observed were considerably smaller and younger than the largest and oldest males (Figs. 1 and 6).

The current 20-inch (508-mm) minimum size limit is well below our estimate of the size at which 50% of females reach sexual maturity (826 mm and 5.2 years) and is not adequate to allow females to reproduce before recruiting to the fishery. Similar estimates of length at maturity were made by García-Cagide and García (1996) who reported that the smallest sexually mature female black grouper they examined from Cuban waters was 570 mm and that most mature females were 850–1100 mm. Huntsman et al. (1994) used an estimate of 5.04 years as the age at sexual maturity in yield per recruit and spawning stock per recruit models for black grouper. They did not present data to support this estimate, but it is within two standard errors of ours.

Female black grouper reached sexual maturity at a relatively large size compared to other grouper species. Female gag reach 50% sexual maturity at 600–650 mm and 3–4 years (Hood and Schlieder, 1992). Female yellowmouth grouper mature between 400 and 450 mm and 2–4 years (Bullock and Murphy, 1994). Yellowedge grouper, *E. flavolimbatus*, reach 50% maturity at 568 mm (Bullock et al., 1996). Larger grouper such as jewfish reach sexual maturity as females at even larger lengths of 1200–1350 mm and greater ages of 6–7 years than black grouper do (Bullock et al., 1992).

The length and age at which 50% of our sample consisted of females was 1215 mm and 15.5 years. García-Cagide and García (1996) reported that the smallest male black grouper from Cuban waters was 980 mm long and that most males were 1000 to 1100 mm long, similar to our findings even though their sample appeared to contain fewer large grouper than ours did. Our estimates of the length and age at transition for black grouper are both larger and older than estimates for other grouper species. Eastern Gulf of Mexico gag populations are 50% male at about 1050 mm and age 11 (Hood and Schlieder, 1992). Gag in the South Atlantic Bight undergo transition from female to male at 750–950 mm and age 5–11 (Collins et al., 1987). Transitional yellowmouth grouper examined by Bullock and Murphy (1994) were 505–643 mm and 5–14 years old. Yellowedge grouper popula-

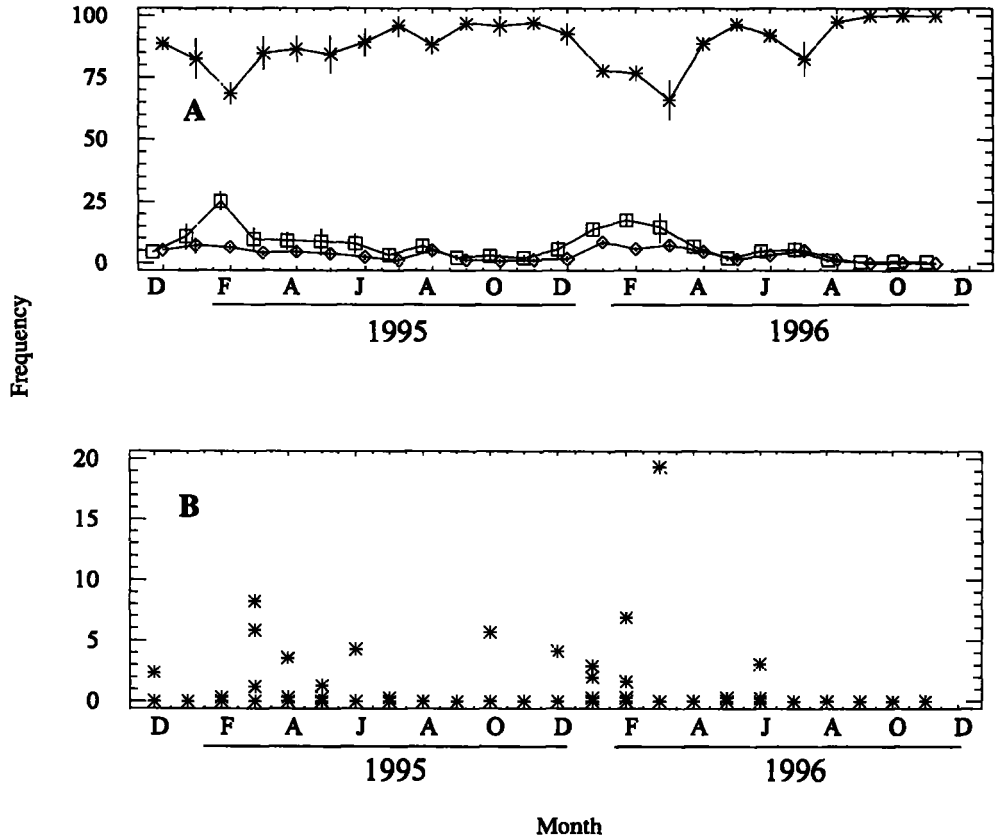

Figure 11

(**A**) Monthly mean percent frequency of occurrence and standard errors of oocyte stages in black grouper, *Mycteroperca bonaci*, ovaries (n=774) for 1995–96. * = primary growth stage oocytes, = cortical alveolar oocytes, and = vitellogenic oocytes. (**B**) The percent frequency of occurrence of oocytes in the final stages of maturation as a function of month in black grouper ovaries.

tions are 50% females at 817 mm (Bullock et al., 1996). Other large grouper such as warsaw grouper and jewfish have not been confirmed to be protogynous hermaphrodites.

The sex ratio of our sample (1:15.4, male:female) may not resemble that of the population because many black grouper that we examined, especially large fish, were eviscerated and could not be sexed. Most of these large fish were probably males, so we may have underestimated their numbers. We are not able to assess the extent of this bias. Furthermore, sex ratios appear to vary widely depending on the depth and location sampled. It is possible that fishing mortality has reduced the numbers of large males in the population, as has been reported for gag from the eastern Gulf of Mexico, where male:female sex ratios as extreme as 1:76.6 have been reported (Coleman et al., 1996). Unfortunately, there are no historical estimates of black grouper sex ratios with which to compare our estimates. García-Cagide and García (1996) reported a male:female sex ratio of 1:30.3 for black grouper from Cuban waters, more skewed towards females than our overall sex ratio but less skewed than the sex ratio of the black grouper we sampled that were landed in the Florida Keys (1:58.7).

Hydrated black grouper oocytes ranged from 0.8 to 1.2 mm in diameter and are similar in size to the hydrated oocytes reported for other groupers (Moe, 1969; Colin et al., 1987; Carter et al., 1994; Sadovy et al., 1994b). We usually did not know the time of day when fish were caught, so we could not estimate the time at which hydration began. Vitellogenic oocytes reached a diameter of about 0.6 mm before under going the final stages of maturation. A distinct group of vitellogenic oocytes with a mean diameter of about 0.6 mm, along with a clutch of larger hydrated oocytes, was present in most of the six grouper for which we measured oocytes. This is consistent with a group-synchronous mode of reproduction (Wallace and Selman, 1981).

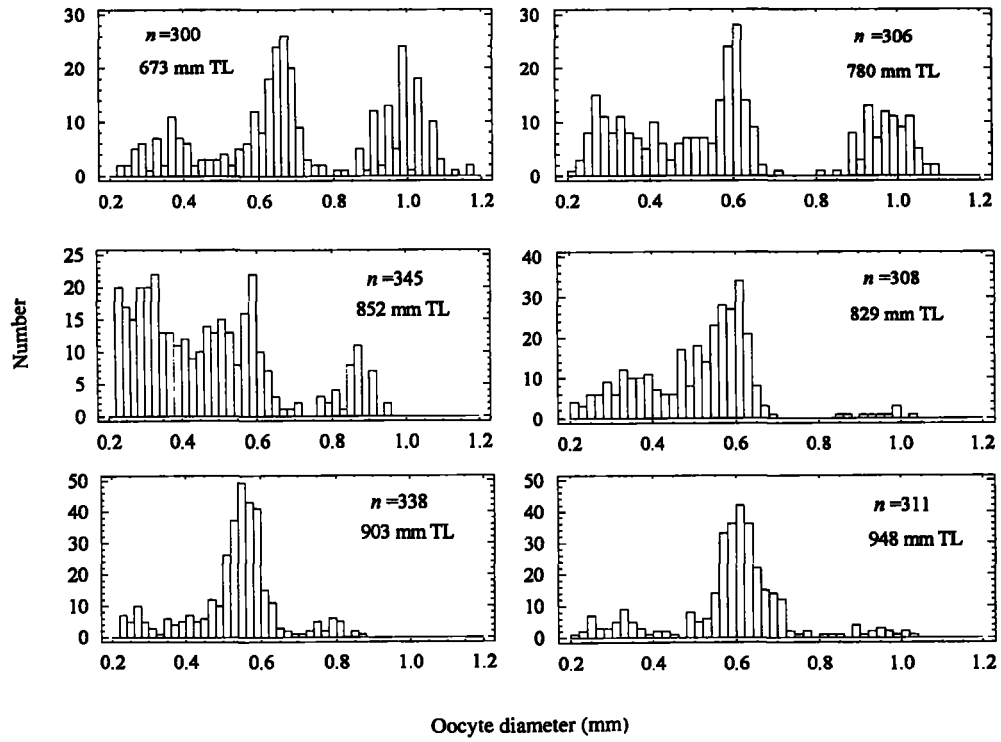

Figure 12

Oocyte diameter (mm) frequency distributions for six hydrated female black grouper, *Mycteroperca bonaci*, from South Florida waters. Hydrated oocytes were 0.8–1.2 mm in diameter. Only oocytes with diameters larger than 0.2 mm were measured. n = the number of oocytes measured.

Seasonality of gonad development

Reproduction in black grouper was seasonal, with peak gonad development during December–March, but females with vitellogenic oocytes were present during all months and females with elevated GSIs occurred in most months. In Cuban waters, García-Cagide and García (1996) also found that black grouper spawn during winter and spring. Other eastern Gulf of Mexico grouper species have similar seasonal patterns of gonad development, and in several species, the spawning season appears to be prolonged. In gag, gonad development occurs during December–May, and peak gonadal activity occurs during February and March (Hood and Schlieder, 1992). Yellowmouth grouper gonads are active throughout the year, and peak gonadal activity occurs during April–May (Bullock and Murphy, 1994). Jewfish have developed gonads from June–December, with peak activity during July–September (Bullock et al., 1992). Yellowedge grouper gonads are developed during January–October, with peak activity during May–September (Bullock et al., 1996).

Many grouper species are known to form seasonal spawning aggregations at specific times and locations each year (Sadovy, 1994). Aggregations of black grouper have been reported off Central America during January and February (Carter, 1989; Fine, 1990; Carter et al., 1994), the same time of year that we observed peak gonadal development and the greatest incidence of oocytes in the final stages of maturation. Among other species of *Mycteroperca*, aggregations of gag and scamp have been reported (Gilmore and Jones, 1992), and spawning by an aggregation of tiger grouper, *M. tigris*, was reported by Sadovy et al. (1994a). Spawning aggregations of black grouper have not been documented in Florida waters, but it is clear from the presence of oocytes in the final stages of maturation in our sample that spawning black grouper are landed in South Florida. Whether black grouper form spawning aggregations in Florida waters and the extent of these aggregations is unknown.

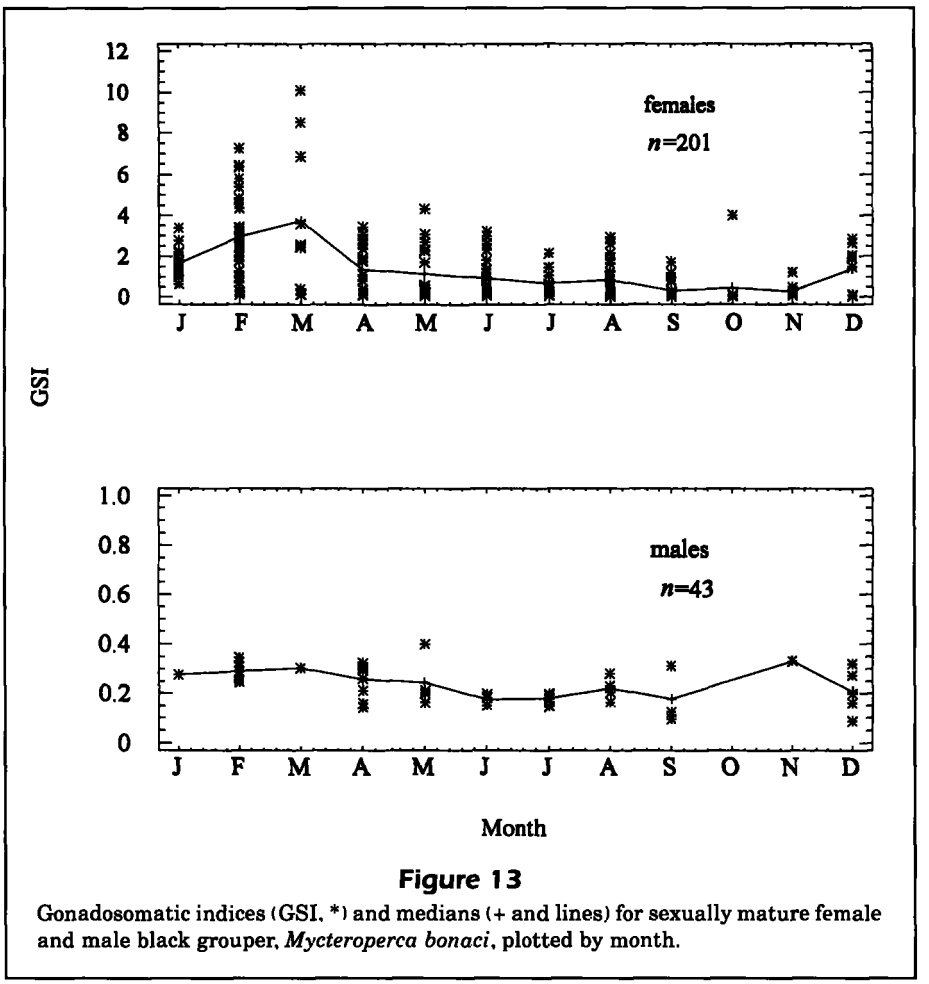

Figure 13

Gonadosomatic indices (GSI, *) and medians (+ and lines) for sexually mature female and male black grouper, *Mycteroperca bonaci*, plotted by month.

Conclusions

The status of black grouper stocks is unclear. Our sample contained many old and large grouper, most of which came from the commercial longline fishery. Large grouper are apparently still abundant in some areas, and their continuing presence in the commercial fishery could reflect the expansion of fishing effort into deeper and more remote areas. In the shallow waters adjacent to the Florida Keys, large and old black grouper were rare, probably a consequence of fishing mortality. The current minimum size does not protect sexually mature females, and the fishery could substantially reduce spawning success in black grouper. In addition, size-selective fishing mortality may selectively remove males from the population. Sex ratios are currently skewed towards females, and it is unknown if populations can compensate by reducing the size of transition as males are selectively removed. Finally, additional research is needed to document the existence of black grouper spawning aggregations in the eastern Gulf of Mexico and in the waters off the Florida Keys and to evaluate the extent to which these aggregations have been affected by fishing mortality.

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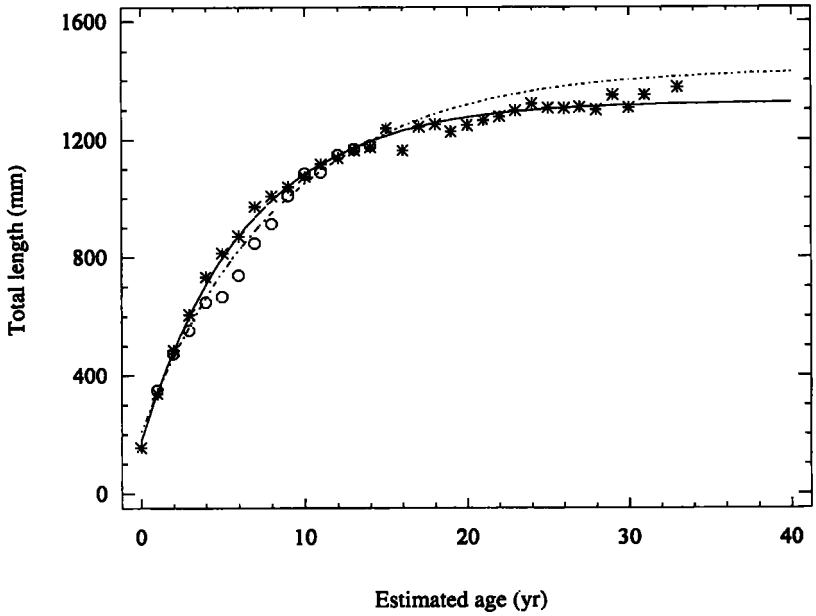

Figure 14

Mean length at age and von Bertalanffy growth curves for black grouper, *Mycteroperca bonaci*, based on data from our study (* and solid line) and from Manooch and Mason (1987; ○ and broken line).

Florida Panhandle. J. Leiby, R. McBride, M. Murphy, J. Quinn, and D. Winkelman made helpful comments that improved the manuscript. Lastly, we thank C. Manooch and M. Burton for providing us with the data described in Manooch and Mason (1987). This project was funded by a grant from the National Marine Fisheries Service, U.S. Department of Commerce, Award NA57FF0060, L.H. Bullock and R.E. Crabtree, principal investigators.

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