Reproduction in the protogynous black grouper (*Mycteroperca bonaci* (Poey)) from the southern Gulf of Mexico

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Abstract—An analysis was made of sexual pattern, spawning season, sizes at sexual maturation, and sex change in black grouper (*Mycteroperca bonaci*) from the southern Gulf of Mexico. Samples were taken between 1996 and 2000, from industrial and small-craft commercial fisheries, in offshore and inshore waters of the continental shelf of the Yucatan Peninsula (Campeche Bank), including the shallow waters of National Marine Park Alacranes Reef. For all collected specimens (n=1229), sex and maturation condition were determined by histological analysis of the gonads. The offshore sample consisted of 75.1% females, 24.3% males, and 0.6% transitional-stage fish. All individuals collected from inshore waters were females. Gonadal structure and population structure characteristics for Campeche Bank black grouper were consistent with the characteristics of monandric protogynous hermaphrodism for a serranid fish. Sexually active males and females were observed year-round, although ripening females, with stage-III, -IV, and -V vitellogenic oocytes in the ovaries, dominated in samples taken between December and March. In addition, peak occurrence of ripe-running females with hyaline oocytes or postovulatory follicles (or both) in the ovaries was recorded in January and February. A few precocious females began spawning in October and November, and others were still in spawning condition in May and June. Fifty percent maturity of females was attained at 72.1 cm fork length (FL). Median size at sexual inversion was 103.3 cm FL, and 50% of the females measuring 111.4 cm FL had transformed into males. The southern Gulf of Mexico grouper fishery was considered deteriorated and lacked a well-defined management strategy. Results of the present study provide helpful information on black grouper reproduction in this area and could help Mexican authorities choose appropriate management strategies for this fishery, such as minimum size limit, closed fishing season, and protection of spawning aggregations.

The black grouper (*Mycteroperca bonaci*) is one of the 20 most commonly sought serranid fishes in the tropical western Atlantic region (Sadovy, 1994). The species ranges from Massachusetts and Bermuda to southeastern Brazil (Böhlke and Chaplin, 1993; Fischer, 1978; Bullock and Smith, 1991; Begossi and Figueiredo, 1995). It is found on irregular bottoms such as coral reefs, drop-off walls, and rocky ledges, in depths from 10 to at least 100 m (Roe, 1977; Manooch and Mason, 1987; Bullock and Smith, 1991; Heemstra and Randall, 1993; Huntsman et al., 1994).

According to Shapiro (1987), the salient feature of grouper reproduction is protogynous hermaphroditism. The first reasonable evidence of protogyny in *M. bonaci* was published by Smith (1959), although there have been other occasional reports on black grouper reproduction (Erdman, 1956; Smith, 1961, 1971, 1972; Naranjo in García-Cagide et al., 1994). Systematic study of sexual pattern and sexual maturation in the species has only been carried out by García-Cagide and García (1996) in Cuban waters and by Crabtree and Bullock (1998) in Florida waters. This grouper has been reported to form spawning aggregations in the Gulf of Mexico and Caribbean Sea (Fine, 1990; Carter and Perrine, 1994; Domeier and Colin, 1997; Eklund et al., 2000).

Black grouper is an important commercial and recreational fin fish resource in Bermuda, southern Florida, Cuba, the southern Gulf of Mexico, and Venezuela (Manooch and Mason, 1987; Cervigon, 1991; Heemstra and Randall, 1993; Claro et al., 1994). In the southern Gulf of Mexico between 1989 and 1999, groupers accounted for 18–30% of the total offshore commercial marine resources harvested from the Campeche Bank (the continental shelf surrounding the northern coast of the Yucatan Peninsula) and resources landed in inshore waters off the state of Yucatán (SEMARNAP, 2000a). At least 18 grouper species are commercially exploited in this region—the most important of these by catch number and weight are red grouper (*Epinephelus morio*), followed by black grouper and gag (*Mycteroperca microlepis*) (Colás-Marrufo et al., 1998). Because grouper landings in
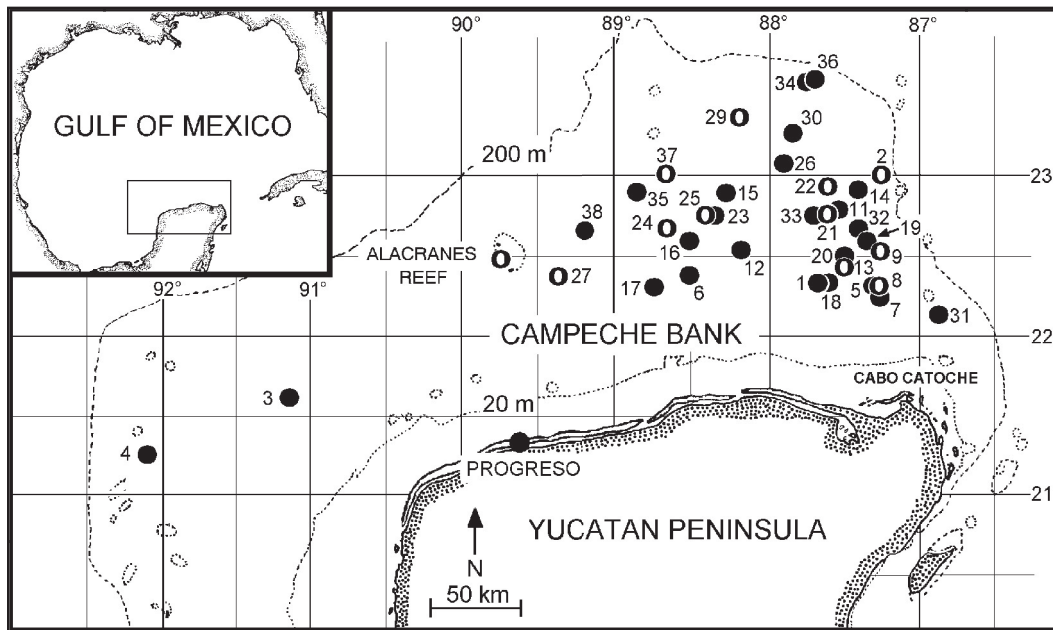
Figure 1

Map of the Campeche Bank, Mexico, showing the geographic distribution of sampling locations (●) for black grouper (*Mycteroperca bonaci*) observed during the period 1996–2000. Samping locations marked (○) are where ripe-running female black grouper were caught. Sample locations: 1 = April 1996; 2 = April and May 1996; 3,4 = May 1996; 5 = November 1996; 6, 7 = December 1996; 8 = January 1997; 9 = January and February 1997; 11 = February 1997; 12 = May 1997; 13 = June 1997; 14 = June and July 1997; 15 = July 1997; 16 = July and August 1997; 17, 18 = August 1997; 19 = September 1997; 20 = September and October 1997; 21 = October and November 1997; 22 = November 1997; 23 = December 1997; 24,25 = January 1998; 26 = January and February 1998; 27 = February and March 1998; 29 = March 1998; 30,31 = June 1998; 32 = July 1998; 33 = August 1998; 34 = August and September 1998; 35 = September 1998; 36 = April 1999; 37,38 = May 1999; Alacranes Reef was sampled November and December 1999, January, February, and August to November 2000. No black grouper were caught in sample locations 10 (22°33′–88°24′W; February 1997) and 28 (22°30′N–89°30′W; March 1998).

the Campeche Bank decreased between 1991 and 1997, the Mexican government proposed management measures to protect the grouper resource, but without considering the biological characteristics and fishery aspects of each exploited species (SEMARNAP, 2000a, 2000b). Given that sustainable resource management is founded on stock assessments and knowledge of the biology of exploited species (Sadovy, 1997), more information on the biology of the most abundant groupers from the southern Gulf of Mexico in general, including Campeche Bank, is necessary to implement and refine management strategies.

This lack of knowledge is especially acute for Campeche Bank black grouper. For example, although growth, feeding, and reproduction of the Yucatan red grouper are well documented, none of this information is available for the black grouper in this region (Brulé and Déniel, 1994; Brulé et al., 1994, 1999). This lack of information is alarming because *M. bonaci* can account for 40% of the grouper catch by weight for some commercial vessels, and if this species is not included in stock monitoring and reproduction studies, effective overall management of the southern Gulf of Mexico grouper fishery could be seriously undermined (Colás-Marrufo et al., 1998).

With the final aim of defining more accurate and efficient management practices for the Campeche Bank grouper fishery, we present analyses of sexual status, sexual cycle, spawning season, size at sexual maturation, and sex change for black grouper from the southern Gulf of Mexico.

Materials and methods

Black grouper were collected from commercial catches taken from rocky bottoms in both offshore and inshore waters of the Campeche Bank and in the shallow waters of the Alacranes Reef complex. Alacranes Reef is the most important complex of coral reefs located on the Yucatan continental shelf. Because of its high scientific and economic potential, the Mexican government declared this reef a National Marine Park in June 1994 (Fig. 1). In offshore waters, black grouper (*n*=880) were caught by the long-line industrial fleet from 38 locations mainly situated in the northeastern part of the Campeche Bank, at depths ranging from 40 to 210 m, between April 1996 and May 1999. In inshore waters, some specimens (*n*=39) were obtained

from the small craft-fleet, whose crew captured them using spear guns at depths ranging from 4 to 20 m, in areas close to the port of Progreso, between November 1998 and May 1999. On Alacranes Reef, black groupers (n=206) were captured with spear guns by small-craft fishermen at depths of 10–12 m between November 1999 and February 2000.

Fork and standard lengths (FL, SL), whole and gutted weights (WW, GW), and weight of gonads (gW), were recorded for all collected fish. All lengths reported in the present study are fork length and all weights are gutted weight. In discussion, total length data for Cuba (García-Cagide and García, 1996) and Florida (Crabtree and Bullock, 1998) populations were converted to fork lengths by using the fork-length to total-length relationship calculated by Crabtree and Bullock (1998).

Criteria presented by Sadovy and Shapiro (1987) were used to diagnose sequential hermaphroditism in black grouper of Campeche Bank. Sex by size-frequency distributions for black groupers were compared by using the Kolmogorov-Smirnov nonparametric test, and differences between male and female mean fork lengths were analyzed by using a one-tailed z-test (if n>30) or a one-tailed t-test (if n<30). The male-to-female ratio (M:F), excluding transitional-stage fish (referred to as "transitional fish" in this article), was calculated and Pearson chi-square or Yates's corrected chi-square goodness-of-fit tests were carried out to determine if sex ratio differed significantly from unity (Scherrer, 1984). Significance level, α, was 0.05 in all instances.

Sex-dependent change in fin pigmentation, as described by Crabtree and Bullock (1998), was examined in a subset of fish (n=104) caught from Alacranes Reef between August and November 2000. The colors of the pectoral, anal, dorsal, and caudal fins were recorded and histological sections of the gonads were prepared and assessed for sex identification.

For all the fish sampled in all locations, sex and sexual development were determined by examination of the microscopic structure of the gonads. These were preserved in Bouin's fluid, embedded in Paraplast and sectioned to 6 μm thickness. Ovaries and testes sections were stained in Gabe and Martoja's triple stain for light microscopy (Gabe, 1968). Fish were identified as female, male, or as transitional. Based on red grouper microscopic features for oogenesis (Brulé et al., 1999) and for spermatogenesis (Moe, 1969), six descriptive stages were recognized in black grouper ovaries and five in the testes. Histological sections of the ovaries were also scanned for the presence of postovulatory follicles and atretic oocytes in alpha or beta stages (Lambert, 1970). Using the criteria defined by Smith (1959) and Sadovy and Shapiro (1987), we considered individuals with gonads containing primarily ovarian tissue, degenerating or not, with few clusters of spermatocytes, spermatids, or spermatozoa to be undergoing sexual inversion. According to the sexual classes defined by Brulé et al. (1999) for red grouper, female and male black grouper were classified as resting, ripening, ripe-running, or spent, and fish in the process of sexual inversion were classified as transitional. Using the histological features considered by Shapiro et al. (1993) as sign of prior spawning activity for the red hind (*Epinephelus guttatus*) we were able to distinguish resting mature females from immature (virgin) females that had never spawned.

Reproduction periodicity was evaluated for both sexes by examining seasonal variations in the gonadosomatic index (GSI=100×gW/GW) and in the relative proportion of individuals in each sexual class. Specimens from offshore waters taken during different years were pooled by month, and mean GSI values and percent frequencies of sexual classes were generated monthly for a single year. Immature individuals were discarded from this analysis.

Size at which 50% of females were sexually mature (L_{50}) was determined by using a binary logistic regression (SYSTAT statistical computer package for Windows, version 8.0, SPSS Inc., Chicago, IL). For our analysis, resting mature, ripening, ripe-running, and spent females were considered as sexually mature individuals. Moreover, the minimum size at which females become sexually mature (L_{min}) was recorded, and the percentage of females of maximum length at first maturity, L_{min}/L_{max} with L_{max} = maximum length of females recorded in samples, was determined (Grimes, 1987). Sexual transition was analyzed by using a binary logistic regression to estimate the length at which 50% of the females transformed to males (P_{50}) according to Crabtree and Bullock (1998). The size range and median size at which sex inversion occurs were estimated by following the procedures of Shapiro (1984). Furthermore, the variation in size at sex change was analyzed by using two ratios defined by Shapiro (1987): ratio 1, size range of transitional fish divided by maximum size of fish in samples; and ratio 2, range of overlap in size of males and females divided by maximum size of fish in samples.
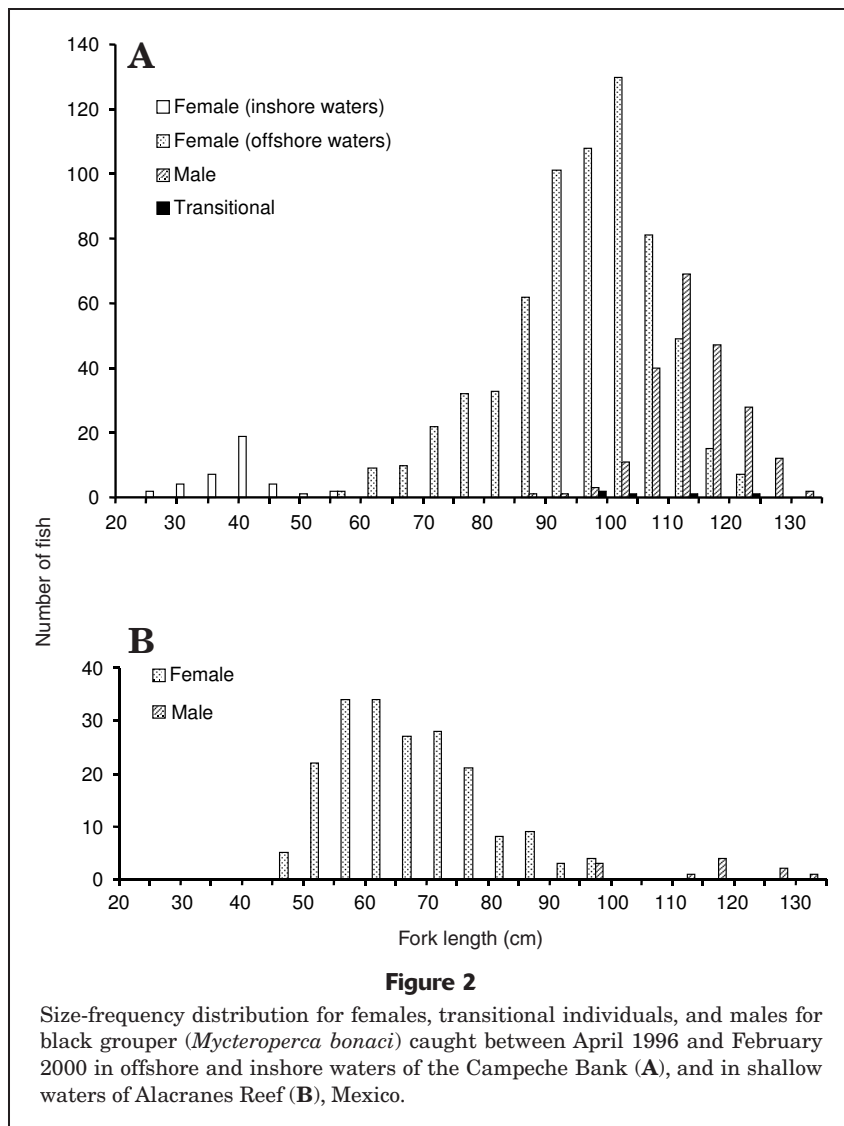
Results

Size-frequency distributions

All individuals collected from inshore waters were females ranging from 25.6 to 58.0 cm in length (Fig. 2). The offshore fish sample, which did not include the Alacranes Reef sample, was composed of 75.1% females, 24.3% males, and 0.6% transitional fish. Females ranged in size from 57.0 to 123.5 cm, males from 86.0 to 132.0 cm, and transitional fish from 99.0 to 121.5 cm. The Alacranes Reef sample was composed of 95% females ranging in size from 46.0 to 100.0 cm and 5% males from 97.0 to 135.0 cm. In the offshore sample, the male size range differed significantly from that of the females (Kolmogorov-Smirnov; n=875; P<0.05), and male mean fork length (114.6 ±7.1 cm; mean ±SD) was greater than female mean fork length (96.6 ±12.1 cm; one-tailed z-test, n=875; P<0.05). Similar results for male and female size ranges (Kolmogorov-Smirnov; n=206; P<0.05) and male (115.7 ±12.9 cm) and female (67.6 ±11.2 cm) mean fork lengths (one-tailed t-test, n=206; P<0.05) were observed for black grouper from the Alacranes Reef sample.

Sex ratio

The male-to-female ratios were calculated for each 5-cm size class from 25.1 to 135.0 cm length (Table 1). The

Figure 2

Size-frequency distribution for females, transitional individuals, and males for black grouper (*Mycteroperca bonaci*) caught between April 1996 and February 2000 in offshore and inshore waters of the Campeche Bank (**A**), and in shallow waters of Alacranes Reef (**B**), Mexico.

sex ratios were female-biased in size classes less than 110.1 cm, did not differ significantly from a 1:1 sex ratio in the 110.1–115.0 cm size class, and were male-biased in size classes larger than 115.0 cm. The overall black grouper sex ratio was 1:4, which differed significantly from unity (χ_1^2=400.8, P<0.05).

Fin pigmentation

Of the 104 black grouper analyzed to detect gender-associated color changes, 98 were females (size range 47.0–99.0 cm), five were males (99.0–115.0 cm), and one, which presented previtellogenic oocytes and nests of spermatocytes and spermatozoa in its gonads, was classified as transitional (99 cm). All males, as well as the transitional specimen, displayed the male color phase with jet black pigmentation on pectoral, anal, and caudal fins. Only 5% of the females (size range 50.0–100.0 cm, n=5) had jet black pigments on their fins.

Gonadal structure

All ovaries presented a central cavity with a germinal epithelium forming the surface layer of a series of projecting ovigerous folds or lamellae of the tunica albuginea.

Of the 225 males assessed histologically, 76% (n=170) presented a membrane-lined central cavity in the testes. This lumen remained unused in the transport of spermatozoa, and sperm ducts or sinuses within the gonadal capsule were observed in 37% of the specimens (n=84) (Fig. 3, A and B). Previtellogenic oocytes (stages I and II) remained in the testes of 13% of the males (n=30), and only one of these (107.0 cm) presented previtellogenic oocytes in degeneration within lamellae in a fully developed testis dominated by crypts of spermatocytes, spermatids, and spermatozoa. Yellow bodies were observed in the testes of 96% of the males.

Internal gonadal structure for the five black groupers classified as transitional was very similar to that of im-

Table 1

Number of sampled fish; proportion of females, males, and transitional-stage fish (transitional fish); and sex ratio by length class for black grouper (*Mycteroperca bonaci*) collected in the inshore and offshore waters of the Campeche Bank and shallow waters of Alacranes Reef, México, between April 1996 and February 2000. Alacranes collection = collection at the Alacranes Reef.

	Females			Total collected		Transitional fish / Offshore collection		Males				Sex ratio (male: female)
Fork length class (cm)	Inshore collection	Offshore collection	Alacranes collection	n	(%)	n	(%)	Offshore collection	Alacranes collection	n	(%)	for total collected
25.1–30.0	2	0	0	2	100.0	0		0	0	0		
30.1–35.0	4	0	0	4	100.0	0		0	0	0		
35.1–40.0	7	0	0	7	100.0	0		0	0	0		
40.1–45.0	19	0	0	19	100.0	0		0	0	0		
45.1–50.0	4	0	5	9	100.0	0		0	0	0		
50.1–55.0	1	0	22	23	100.0	0		0	0	0		
55.1–60.0	2	2	34	38	100.0	0		0	0	0		
60.1–65.0	0	9	34	43	100.0	0		0	0	0		
65.1–70.0	0	10	27	37	100.0	0		0	0	0		
70.1–75.0	0	22	28	50	100.0	0		0	0	0		
75.1–80.0	0	32	21	53	100.0	0		0	0	0		
80.1–85.0	0	33	8	41	100.0	0		0	0	0		
85.1–90.0	0	62	9	71	98.6	0		1	0	1	1.4	1:71
90.1–95.0	0	101	3	104	99.0	0		1	0	1	1.0	1:104
95.1–100.0	0	108	4	112	93.3	2	1.7	3	3	6	5.0	1:18.67
100.1–105.0	0	130	0	130	91.5	1	0.7	11	0	11	7.7	1:11.82
105.1–110.0	0	81	0	81	66.9	0		40	0	40	33.1	1:2.03
110.1–115.0	0	49	0	49	40.8	1	0.8	69	1	70	58.3	1:0.70*
115.1–120.0	0	15	0	15	22.7	0		47	4	51	77.3	1:0.29
120.1–125.0	0	7	0	7	19.4	1	2.8	28	0	28	77.8	1:0.25
125.1–130.0	0	0	0	0		0		12	2	14	100.0	
130.1–135.0	0	0	0	0		0		2	1	3	100.0	
Total	39	661	195	895	79.6	5	0.4	214	11	225	20.0	1:3.98

* Value did not differs significantly from 1:1 sex ratio (χ_1^2; $P>0.05$).

mature or resting females. Stage-I and -II oocytes, yellow bodies, and sometimes bundles of muscle and connective tissue were present within the lamellae. Intermixed with the female tissue, these gonads contained a few nests of spermatogonia, spermatocytes, or spermatozoa, although degeneration of female germinal tissue was not observed (Fig. 3C). These transitional specimens were captured in September, November, and December 1997 and in January and March 1998.

Sexual cycle

Females captured from inshore waters during January, February, March, May, November, and December were immature and had low individual GSI values (GSI range 0.01–0.18%), and only oogonia and previtellogenic oocytes were observed in their ovaries.

Mean GSI for mature females caught in offshore waters began to increase in December (0.5%), reached a maximum value in February (2.2%), and declined to a near minimum level in March (0.7%) and April (0.6%) (Fig. 4). Highest individual GSI values for females were observed in October (4.9%), December (6.0%), January (6.7%), and February (9.6%). Mean GSI for males caught in offshore waters increased in December (0.13%) and January (0.14%)—reaching a maximum value in February (0.22%) and declining from March (0.12%) to August (0.11%) (Fig. 4). Highest individual GSI values for males were observed in September (0.43%) and February (0.39%).

Ripening females, with stage-III, -IV, and -V vitellogenic oocytes in their ovaries, were observed year-round, but dominated in collections made between December and March (42–56% of females) (Fig. 5). Advanced vitellogenic oocytes undergoing final oocyte maturation were noted only for some females captured between January and March (Fig. 6A). Ripe-running females, with hyaline oocytes or postovulatory follicles (or with both) in their ovaries, were recorded between October and June, and peaked in occur-

rence in January (28%) and February (52%)(Fig. 6B). The gonads of 50 ripe-running females caught between January and April, and during June and November, contained both postovulatory follicles and stages III–V vitellogenic oocytes without sign of degeneration (Fig. 6C). Spent fe-
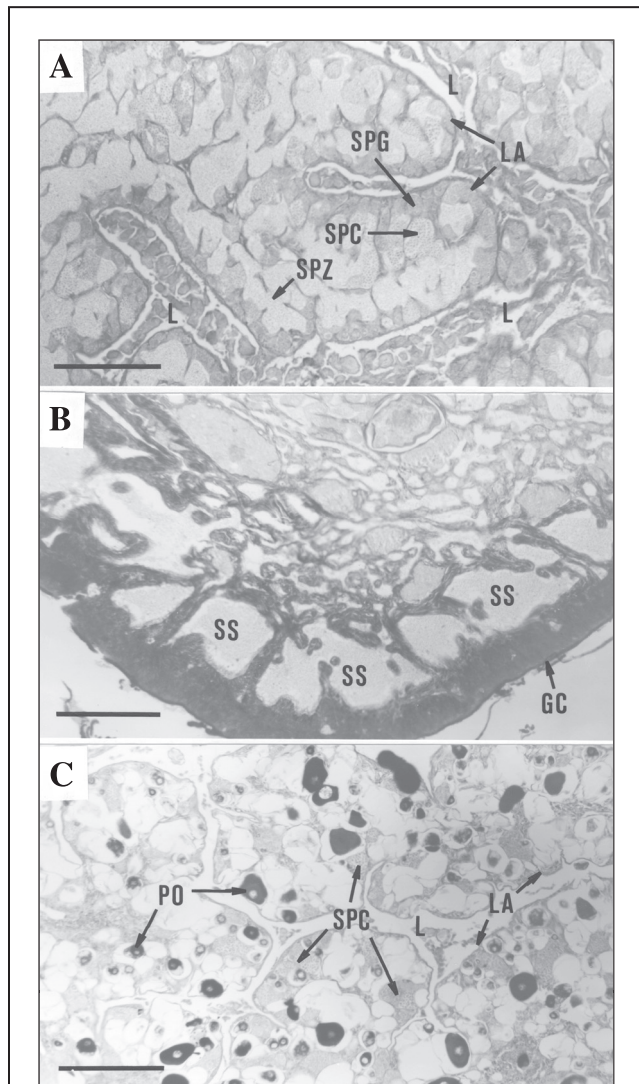
Figure 3
Photomicrographs of histological sections from male and transitional black grouper (*Mycteroperca bonaci*) gonads collected from Campeche Bank, Mexico. (**A**) Section from a 114-cm-FL ripening male captured in July 1997, showing lamellae, lumina, spermatogonia, and spermatocyte cysts, and lamellae sinuses full of spermatozoa. (**B**) Section from a 122-cm-FL ripe-running male captured in September 1997, with sperm sinus full of spermatozoa in gonadal capsule. (**C**) Section from a 113-cm-FL transitional fish captured in January 1998, showing previtellogenic oocytes (stages I) and scattered spermatocyte cysts. GC = gonadal capsule; L = lumen; LA = lamellae; PO = previtellogenic oocyte; SPC = spermatocyte; SPG = spermatogonia; SPZ = spermatozoa; SS = sperm sinus. Scale bars = 200 microns.

males, with atretic and remaining vitellogenic oocytes in their gonads, were caught between January and August (3–29%). Resting mature females, with stages -I and -II oocytes, bundles of muscle, and yellow bodies in their ovaries were abundant in samples taken from May to November (54–98%). Ripening or ripe-running males were recorded year-round and spent males were observed in November (4%), from January to March (10–40%), and from May to July (8–22%).

Various females from the Alacranes Reef were ripening in November (GSI range: 0.03–4.44%, $n=12$), December (GSI range: 0.22–7.18%, $n=9$) and February (GSI range: 0.10–6.61%, $n=25$). In February, some of them were ripe-running, with postovulatory follicles in ovaries (GSI=1.77% and 1.91%, $n=2$) and others were spent (GSI=0.74% and 0.88%, $n=2$). Alacranes Reef males were ripening or ripe-running in November, December, and February (GSI range: 0.03–0.44%, $n=11$).

Location and timing of spawning

Between April 1996 and February 2000, 61 ripe-running females were caught at 11 offshore fishing locations situated in the northeastern part of the Campeche Bank (depth range: 51–68 m), and from shallow waters of the Alacranes Reef (8–10 m) (Fig. 1). All had vitellogenic oocytes (stages-III–V) with hyaline oocytes or postovulatory follicles (or with both) in their ovaries. Most of these females were caught during, or close to, the new moon phase (Table 2).

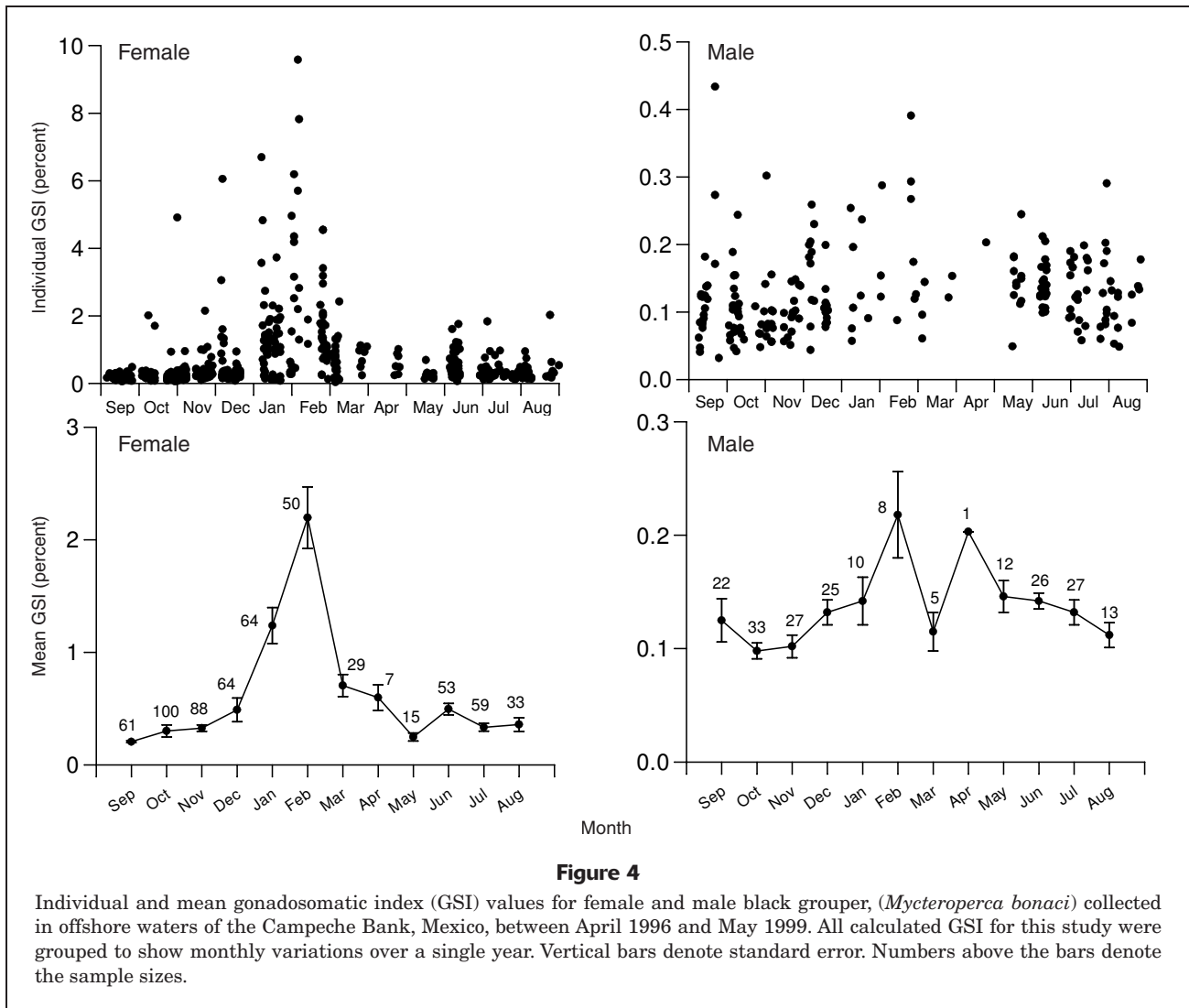### Sizes at maturity and at sexual transition

The smallest mature female ($L_{min}=58.0$ cm) was caught in shallow waters of the Alacranes Reef and had stage-III oocytes in its ovaries. Fifty-percent maturity of females was attained at 72.1 cm in size and all females larger than 95.1 cm were mature (Fig. 7). Because the largest female observed in samples was 123.5 cm (L_{max}), the percentage of females at maximum length at first maturity was $L_{min}/L_{max}= 47\%$.

Females changed sex between 85.5 and 125.0 cm in length (the overlap zone between male and female sizes) and the median size of sexual inversion was 103.3 cm. By the time they attained a length of 111.4 cm, 50% of the females in the sample had transformed into males (Fig. 8). Size range of transitional fish (99.0–121.5 cm) was 17% of maximum fish size (135.0 cm) (ratio 1, see "Materials and methods"section), and sex change occurred over 29% of the maximum size observed for the species (ratio 2). Immature males were not observed during the study.

## Discussion

Sexual pattern

Previous research strongly suggests that sex reversal occurs in *M. bonaci* (Smith, 1959, 1961; García-Cagide and García, 1996; Crabtree and Bullock, 1998). Observations on gonadal and population structure characteristics for black

Figure 4

Individual and mean gonadosomatic index (GSI) values for female and male black grouper, (*Mycteroperca bonaci*) collected in offshore waters of the Campeche Bank, Mexico, between April 1996 and May 1999. All calculated GSI for this study were grouped to show monthly variations over a single year. Vertical bars denote standard error. Numbers above the bars denote the sample sizes.

grouper from the Campeche Bank are consistent with the description of monandric protogynous hermaphroditism previously used for this species. During the present study, three of the five criteria suggested by Sadovy and Shapiro (1987) for identifying protogyny in hermaphroditic fishes were identified in black grouper: membrane-lined central cavities in testes; sperm sinuses in the gonadal wall; and transitional individuals. The five black grouper specimens considered as transitional individuals (0.6% of sampled offshore fish) did not have degenerating ovarian tissue in their gonads. For protogynous species, the degeneration of ovarian tissue should logically accompany proliferation of testicular tissue for the specimen to be termed "transitional." However, according to Sadovy and Shapiro (1987), the paucity of reported cases providing such descriptions raises doubts as to whether transitional gonads display this kind of histological profile. These observations also may be a function of the fact that the incidence of transitional fish in field collections is generally relatively low, as shown by the single transitional specimen (0.1% of fish

collection) identified by Crabtree and Bullock (1998) in a sample of the Florida black grouper population. Precocious spermatocyte or sperm cysts in immature or functional ovaries as observed by Smith (1964; 1965) and Bullock et al. (1996) in coney (*Cephalopholis fulva*), graysby (*Cephalopholis cruentata*), and yellowedge grouper (*Epinephelus flavolimbatus*) were not found in black groupers ovaries during our study.

Other aspects of population structure indicating monandric protogynous hermaphrodism were also seen in *M. bonaci* from the Campeche Bank. These included bimodal size-frequency distributions, where males were larger than females, female-biased sex ratios in size classes less than 110.1 cm, and male-biased ratios in size classes larger than 115.0 cm. No male smaller than 86.0 cm was identified in any of the samples. Despite these biases, the overall male-to-female sex ratio calculated in our study (1:4) was less skewed towards females than those reported by García-Cagide and García (1996) (1:30.3) and Crabtree and Bullock (1998) (1:15.4). Notwithstanding, the sex ratio for black
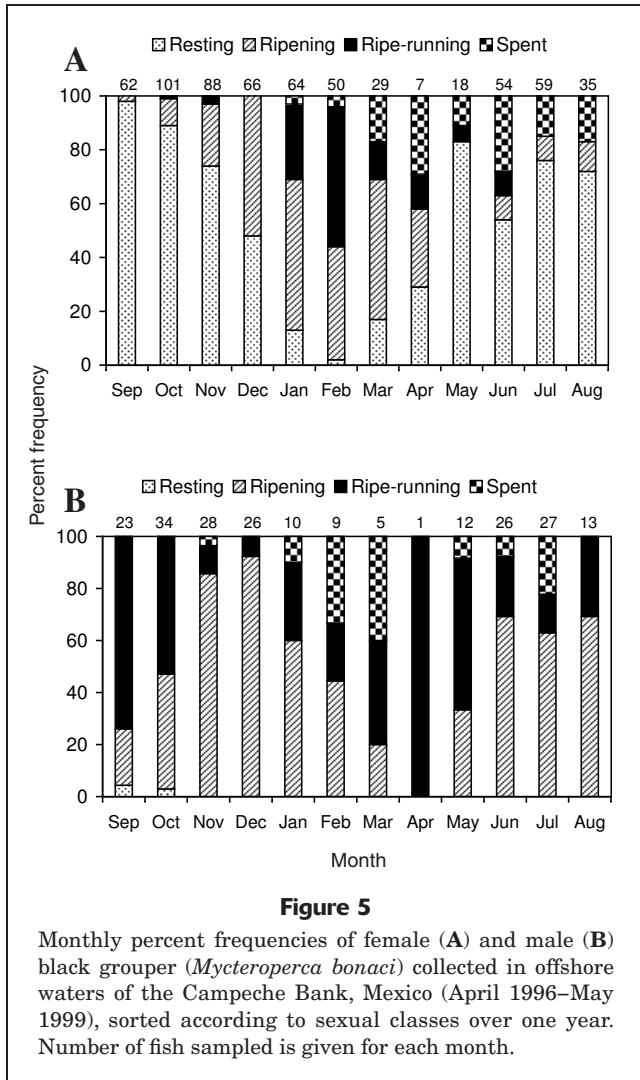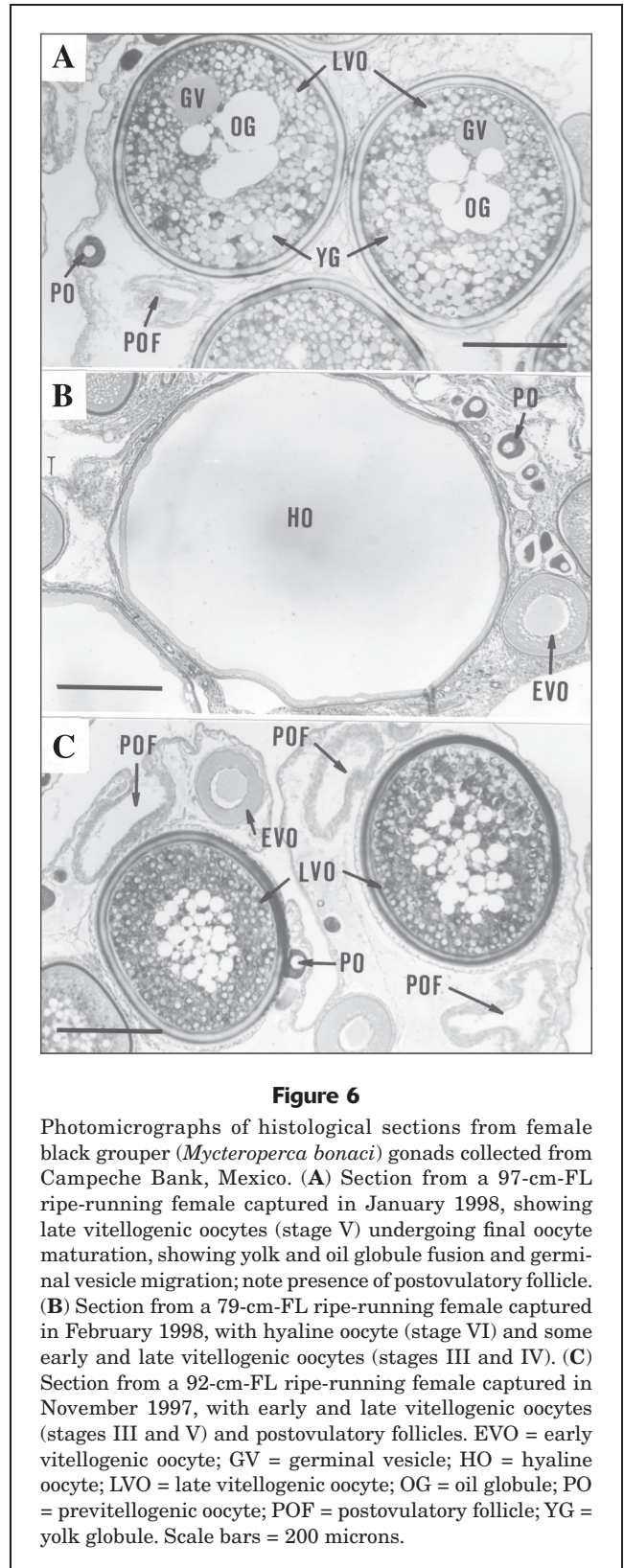
Figure 5

Monthly percent frequencies of female (**A**) and male (**B**) black grouper (*Mycteroperca bonaci*) collected in offshore waters of the Campeche Bank, Mexico (April 1996–May 1999), sorted according to sexual classes over one year. Number of fish sampled is given for each month.

Figure 6

Photomicrographs of histological sections from female black grouper (*Mycteroperca bonaci*) gonads collected from Campeche Bank, Mexico. (**A**) Section from a 97-cm-FL ripe-running female captured in January 1998, showing late vitellogenic oocytes (stage V) undergoing final oocyte maturation, showing yolk and oil globule fusion and germinal vesicle migration; note presence of postovulatory follicle. (**B**) Section from a 79-cm-FL ripe-running female captured in February 1998, with hyaline oocyte (stage VI) and some early and late vitellogenic oocytes (stages III and IV). (**C**) Section from a 92-cm-FL ripe-running female captured in November 1997, with early and late vitellogenic oocytes (stages III and V) and postovulatory follicles. EVO = early vitellogenic oocyte; GV = germinal vesicle; HO = hyaline oocyte; LVO = late vitellogenic oocyte; OG = oil globule; PO = previtellogenic oocyte; POF = postovulatory follicle; YG = yolk globule. Scale bars = 200 microns.

grouper sampled from Florida waters may not resemble that for the entire population. Crabtree and Bullock (1998) stated that they probably underestimated the number of males in their sample because the large black grouper examined were eviscerated and could not be sexed. .

As observed for the first time by Crabtree and Bullock (1998) for black grouper from Florida waters, sexual dimorphism was displayed by the Campeche Bank population. Notwithstanding, a low proportion of females possessed fin pigmentation. Furthermore, it is also possible that individuals undergoing transition from female to male display the male color phase. However, conclusions based on differences in fin pigmentation in male, female, and transitional black grouper from the Campeche Bank are limited by the small number of specimens examined for this purpose.

Spawning season

Sexually active black groupers from the Campeche Bank (ripening females and ripening or ripe-running males) were observed year-round. The monthly relative propor-

tion of individuals in each sex class and mean gonadosomatic indices showed a more consistent annual cycle for
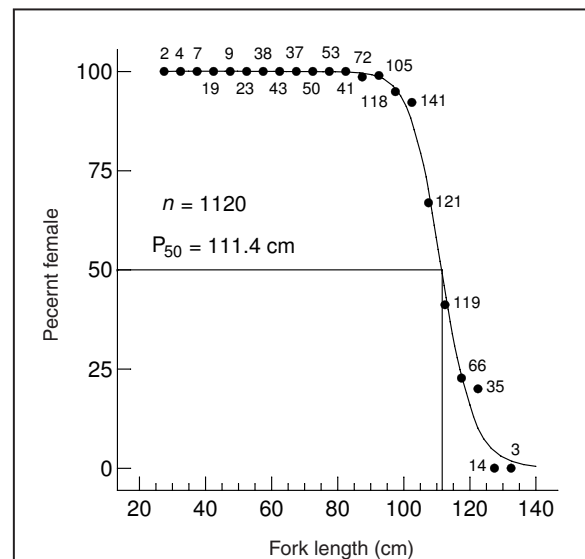
Table 2

Description of samples of ripe-running female black grouper (*Mycteroperca bonaci*) collected in the offshore waters of the Campeche Bank and shallow waters of Alacranes Reef, México, between April 1996 and February 2000. Numbers refer to map locations in Figure 1. HO = hyaline oocyte; POF = postovulatory follicle. WW = whole weight.

Sampling dates		Fishing location	Depth of capture (m)	Date of full and new moon			n	Histological feature of ovaries	Size range of females	
									FL (cm)	WW (kg)
31	Oct 1997	21	51–55	15	1, 31	Oct	1	HO	101.0	17.2
21, 22, 27	Nov 1997	22	68	14	29	Nov	3	POF	75.0–91.5	5.3–11.2
6–12	Jan 1997	8	—	23	8	Jan	6	HO or POF	85.0–108.5	8.6–16.5
8–21	Jan 1998	24	59	12	28	Jan	10	POF or HO and POF	91.0–105.5	11.8–17.3
29, 30	Jan 1998	25	59	12	28	Jan	2	POF	94.0; 101.0	12.4;14.8
1–5	Feb 1997	9	—	22	7	Feb	9	HO or POF	88.0–110.0	11.0–20.2
22–28	Feb 1998	27	55	11	26	Feb	17	HO and POF	76.0–117.0	6.4–25.1
3, 6	Feb 2000	Alacranes reef	8–10	19	5	Feb	2	HO and/or POF	89.0; 88.0	8.0; 10.0
2, 8	Mar 1998	27	55	12	27	Mar	2	POF	76.0; 84.0	6.2; 8.0
25	Mar 1998	29	—	12	27	Mar	2	POF	78.0; 94.0	5.4; 9.1
24	Apr 1996	2	55	17	3	Apr	1	POF	75.0	6.3
16	May 1999	37	67	30	15	May	1	POF	90.0	10.2
4–11	Jun 1997	13	64	20	5	Jun	5	OH or POF	69.5–97.0	4.7–13.9
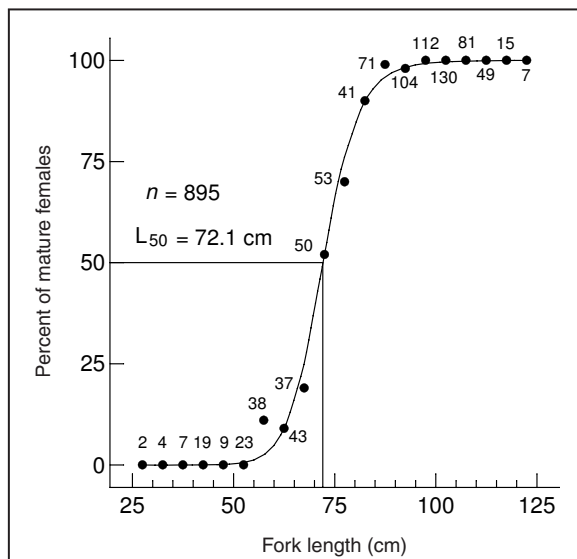
Figure 7

Percent of mature females at length for black grouper (*Mycteroperca bonaci*) from inshore and offshore waters of the Campeche Bank and shallow waters of Alacranes Reef, Mexico (April 1996–February 2000). Proportion of sexually mature females within each size class is plotted with a binary logistic regression. Line indicates length at 50% maturity (L_{50}). Number of fish sampled is given for each size class.

Figure 8

Percentage of female black grouper (*Mycteroperca bonaci*) as a function of fork length. Samples were taken between April 1996 and February 2000 in inshore and offshore waters of the Campeche Bank and shallow waters of Alacranes Reef, Mexico. P_{50} is the length predicted by binary logistic regression at which 50% of the sampled black grouper are female. Number of fish sampled is given for each size class.

females than for males. According to Sadovy (1996), ovaries best reflect duration of fish spawning activity. Under this assumption, the length of the black grouper spawning season was evaluated by using Sadovy's criteria (1996) as defined to assess the duration of annual spawning in reef fish species. Spawning season for black grouper from the Campeche Bank was shown to extend from December to March—a spawning month being defined as one in which

50% or more of the sampled females have yolked oocytes. Peak spawning occurred in February when 50% or more of the sampled females had hyaline oocytes or postovulatory follicles (or both) in their ovaries. In the case of spawning seasonality evaluated by GSI data, peak spawning activity was assigned to the months of January and February in which the mean GSI of female attained was 50% or more of the maximum mean female GSI recorded during the study (2.20% in February). A very few precocious females started to spawn in October (1% of sampled fish) and November (3%) and some were still in spawning condition during May (4%) and June (8%). These results are consistent with previous reports of the *M. bonaci* spawning season in Puerto Rico, the Bahamas, Cuba, and Florida (Erdman, 1956; Smith, 1961, 1971, 1972; García-Gagide et. al., 1994; García-Gagide and García, 1996; Crabtree and Bullock, 1998). Spawning in Bermuda appears to be anomalous, with reproductive activity extending from about early May to early August (Smith, 1971).

Spawning pattern

The authors did not observe spawning aggregations for black grouper from the Campeche Bank as defined by Domeier and Colin (1997) for tropical reef fishes. Black grouper is reported to form spawning aggregations between January and February in Belize, Honduras, and Florida (Carter, 1989; Fine, 1990; Carter and Perrine, 1994; Eklund et al., 2000). Only indirect methods of establishing spawning occurrence, based on female gonadal condition, were used in the present study. Ripe-running females were caught at 11 offshore locations on the Campeche Bank and in the shallow waters of National Marine Park Alacranes Reef, during eight months of the year. The species seemed to spawn preferentially at or around the new moon phase. However, more information, such as direct observations of spawning behavior and data on fish densities at aggregation sites during nonreproductive and reproductive periods, is necessary to know precisely where and when adult *M. bonaci* gather for spawning in the southern Gulf of Mexico. The presence of oocytes at various stages of vitellogenesis in the ovaries of ripening females and the co-existence of postovulatory follicles and vitellogenic oocytes in those of ripe-running females suggest that some individuals may spawn more than once during the spawning season.

Sexual maturity and sex change

The size at which 50% of females were sexually mature (L_{50}) was lower for black grouper from the Campeche Bank (72.1 cm) than for those from Florida (82.6 cm) or Cuban (84.4–108.7 cm) waters. The L_{min}/L_{max} ratio indicated that the females from the Campeche Bank reached first maturity at a higher proportion of maximum length (47%) than those from Florida waters (40%). This spatial variation in size at first sexual maturity has also been observed in female red grouper from southern and eastern Gulf of Mexico (Brulé et al., 1999).

The size at which 50% of the females transformed to males (P_{50}) was lower for black grouper from the Campeche

Bank (111.4 cm) than for those from Florida (119.9 cm). Notwithstanding, the size range in which males overlapped with females and the ratio-2 results were almost identical for black grouper from Campeche Bank (39.5 cm and 29%) and south Florida (39 cm and 26%) waters (Crabtree and Bullock, 1998). According to Shapiro (1987), these data are more consistent with a mechanism for behavioral induction of sex change than with the idea that this process occurs at a characteristic size or age for all members of a population.

Fishery characteristics and fishery management

As members of the warm-temperate and tropical reef fish complexes, groupers have consistently proven highly vulnerable to anything other than light levels of fishing pressure (Sadovy, 1997). Because of their biological characteristics, these species must be conservatively managed to avoid rapid overfishing and stock collapse (Sadovy, 1997; Coleman et al., 2000). Some groupers from the western Atlantic are even considered endangered and threatened species (IUCN/SSC[1]). Assessment of Morris et al. (2000) and Musick et al. (2000) led to the classification of black grouper as a vulnerable species, that is, not critically endangered, endangered, or threatened severely, but facing a high risk of extinction in the wild in the medium-term future.

The trend in grouper catches in the state of Yucatán has been one of progressive decline from an historical maximum of 13,993 metric tons (t) in 1991, to 8556 t in 1997, followed by an increase to 11,045 metric tons in 2000 (SAGARPA, 2001). According to Monroy-García et al. (2001), catch increase reflects an increase in fishing effort during the last three years. Recent assessments of red grouper population from the Campeche Bank indicate that the current biomass of exploited stock is well below that of maximum biological productivity and that the fishery is considered deteriorated (SEMARNAP, 2000a, 2000b). In response to the multiple threats facing groupers in the Gulf of Mexico, the U.S. and Mexican governments have implemented regulations designed to either reduce or contain effective fishing effort (input controls), or to restrict total catch (output controls) to predefined limits. Notwithstanding, grouper fishery regulations used in Mexican waters are less restrictive than those imposed in U.S. waters. The U.S. regulations currently consist of the following: an annual commercial quota of 4445 t for the shallow-water grouper complex, which includes the black grouper; commercial and recreational minimum size limits of 61.0 cm TL and 55.9 cm TL, respectively; a seasonal closure on commercial harvest and prohibition on sale of this species from 15 February to 15 March; and a recreational aggregate daily bag limit of five groupers per person (Gulf of Mexico Fishery Management Council[2]). The Mexican regulations include license limita-

[1] 2001. IUCN/SSC (International Union for the Conservation of Nature and Natural Resources/Species Survival Commission). SSC Red List Programme IUCN/SSC UK Office, 219c, Huntingdon Road, Cambridge CB3 ODL, United Kingdom. Web page: http://www.redlist.org

[2] 2001. Gulf of Mexico Fishery Management Council. The Commons at Rivergate, 3018 U.S. Hwy. 301 N., Suite 1000 Tampa, Florida 33619-2266. Web page: http://www.gulfcouncil.org

tion and a minimum legal total length of 30 cm for the Mexican fleet, and an annual catch quota of 3900 t for the Cuban fleet (SEMARNAP, 2000b). As can be seen, commercial and recreational exploitation of the Campeche Bank grouper resource still lacks a well-defined management strategy. Many of the obstacles noted by Huntsman and Waters (1987) in developing snapper-grouper management plans for the Gulf of Mexico and U.S. South Atlantic are seen in the southern Gulf of Mexico. For instance, grouper landings are not identified to the species level in Mexican fisheries statistic, but all species are reported in the "mero" (grouper) category, which includes eighteen species of the genera *Cephalopholis, Epinephelus,* and *Mycteroperca* (Colás-Marrufo et al., 1998). Information on grouper recreational catches is lacking, and biological data on the Campeche Bank species, especially on reproduction, are either scarce or nonexistent. Moreover, the 30-cm-TL minimum size limit was applied to prevent the marketing of fish considered too small and was only related to the growth overfishing problem.

Although landing trends by species for the southern Gulf of Mexico grouper fishery remain undetermined, black grouper along with red grouper and gag appear to be the most abundant serranid fishes off the northern coast of the Yucatán Peninsula. Colás-Marrufo et al. (1998) reported black grouper to be second to red grouper in total number (12%) and weight (40%) of grouper catches taken from the Campeche Bank by some commercial fishing boats between 1996 and 1998. If a decrease in commercial grouper landings from Mexican waters is confirmed in the near future, protection measures such as regulation of specific catch will be required for each of these three grouper species.

Results from the present study may aid in better estimating and thus maintaining reproductive output in the black grouper population from the Campeche Bank. With these data, fishing regulations can be based on reproductive aspects. This information will make it possible to propose a minimum size limit for this species near the size at which 50% of females are sexually mature (72 cm FL) (output control) and a closed season during peak spawning in February (input control)—both of which would help prevent recruitment overfishing. Furthermore, if it is confirmed that black grouper from the Campeche Bank spawn in the shallow waters of the Alacranes Reef complex, it should be easy to temporarily ban fishing in the spawning area(s) through enforcement of the regulations protecting this National Marine Park.

Acknowledgments

Literature cited

Begossi, A., and J. L. de Figueiredo.
    1995.   Ethnoichthyology of southern coastal fishermen: cases from Búzios island and Sepetiba bay (Brazil).   Bull. Mar. Sci. 56:710–717.
Böhlke, J. E., and C. G. H. Chaplin.
    1993.   Fishes of the Bahamas and adjacent tropical waters, 2nd ed., 771 p.   Univ. Texas Press, Austin, TX.
Brulé, T., and C. Déniel.
    1994.   Exposé synoptique des données biologiques sur le mérou rouge *Epinephelus morio* (Valenciennes, 1828) du golfe du Mexique.   FAO Synopsis sur les pêches 155, 39 p.   FAO, Rome.
Brulé, T., C. Déniel, T. Colás-Marrufo, and M. Sánchez-Crespo.
    1999.   Red grouper reproduction in the southern Gulf of Mexico.   Trans. Am. Fish. Soc. 128:385–402.
Brulé, T., D. Ordaz Avila, M. Sánchez Crespo, and C. Déniel.
    1994.   Seasonal and diel changes in diet composition of juvenile red grouper (*Epinephelus morio*) from Campeche Bank. Bull. Mar. Sci. 55:255–262.
Bullock, L. H., M. F. Godcharles, and R. E. Crabtree.
    1996.   Reproduction of yellowedge grouper, *Epinephelus flavolimbatus*, from the eastern Gulf of Mexico.   Bull. Mar. Sci. 59:216–224.
Bullock, L. H., and G. B. Smith.
    1991.   Seabasses (Pisces: Serranidae).   Mem. Hourglass Cruises, vol..8, part 2, 243 p.
Carter, J.
    1989.   Grouper sex in Belize.   Nat. Hist. 10:61–68.
Carter, J., and D. Perrine.
    1994.   A spawning aggregation of dog snapper, *Lutjanus jocu* (Pisces: Lutjanidae) in Belize, Central America.   Bull. Mar. Sci. 55:228–234.
Cervigon, F.
    1991.   Los peces marinos de Venezuela, vol. 1, 2nd ed., 423 p. Fundación Científica Los Roques, Caracas.
Claro, R., J. Baisre, and J. P. García-Arteaga.
    1994.   Evolución y manejo de los recursos pesqueros.   *In* Ecología de los peces marinos de Cuba (R. Claro, ed.), p. 435–456.   Centro de Investigaciones de Quintana Roo (CIQRO), Chetumal, Quintana Roo.
Colás-Marrufo, T., T. Brulé, and C. Déniel.
    1998.   Análisis preliminar de las capturas de meros realizadas a través de unidades de la flota mayor en el sureste del Golfo de México.   Proc. Gulf Caribb. Fish. Inst. 50:780–803.
Coleman, F. C., C. C. Koenig, G. R. Huntsman, J. A. Musick, A. M. Eklund, J. C. McGovern, R. W. Chapman, G. R. Sedberry, and C. B. Grimes.
    2000.   Long-lived reef fishes: the grouper-snapper complex. Fisheries 25 (3):14–21.

Crabtree, R. E., and L. H. Bullock.
    1998.   Age, growth, and reproduction of black grouper, *Mycteroperca bonaci*, in Florida waters.   Fish. Bull. 96:735–753.
Domeier, M. L., and P. L. Colin.
    1997.   Tropical reef fish spawning aggregations: defined and reviewed.   Bull. Mar. Sci. 60:698–726.
Eklund, A. M., D. B. McClellan, and D. E. Harper.
    2000.   Black grouper aggregation in relation to protected areas within the Florida Keys National Marine Sanctuary.   Bull. Mar. Sci. 66:721–728.
Erdman, D. S.
    1956.   Recent fish records from Puerto Rico.   Bull. Mar. Sci. Gulf Caribb. 6:315–340.
Fine, J. C.
    1990.   Groupers in love: spawning aggregations of Nassau grouper in Honduras.   The Explorers Journal, Fall 1990, 131–134.
Fischer, W.
    1978.   FAO species identification sheets for fisheries purposes.   Western Central Atlantic (fishing area 31), vols. 1–7, var. pag.   FAO, Rome.
Gabe, M.
    1968.   Techniques histologiques, 1113 p.   Masson, Paris.
García-Cagide, A., R. Claro, and B. V. Koshelev.
    1994.   Reproducción.   *In* Ecología de los peces marinos de Cuba (R. Claro, ed.), p. 187–261.   Centro de Investigaciones de Quintana Roo (CIQRO), Chetumal, Quintana Roo.
García-Cagide, A., and T. García.
    1996.   Reproducción de *Mycteroperca bonaci* y *Mycteroperca venenosa* (Pisces: Serranidae) en la plataforma cubana.   Rev. Biol. Trop. 44:771–780.
Grimes, C. B.
    1987.   Reproductive biology of the Lutjanidae: a review.   *In* Tropical snappers and groupers: biology and fisheries management (J. J. Polovina and S. Ralston, eds.), p. 239–294.   Westview Press, Boulder, CO.
Heemstra, P. C., and J. E. Randall.
    1993.   FAO species catalogue, vol. 16: Groupers of the world (Family Serranidae, Subfamily Epinephelinae).   An annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known to date.   FAO Fisheries Synopsis 125, 382 p.   FAO, Rome.
Huntsman, G. R., J. Potts, and R. W. Mays.
    1994.   A preliminary assessment of the populations of seven species of grouper (Serranidae, Epinephelinae) in the Western Atlantic Ocean from Cape Hatteras, North Carolina to the Dry Tortugas, Florida.   Proc. Gulf Caribb. Fish. Instit. 43: 193–213.
Huntsman, G. R., and J. R. Waters.
    1987.   Development of management plans for reef fishes-Gulf of Mexico and U.S. South Atlantic.   *In* Tropical snappers and groupers: biology and fisheries management (J. J. Polovina and S. Ralston, eds.), p. 533–560.   Westview Press, Boulder, CO.
Lambert, J. G. D.
    1970.   The ovary of the guppy, *Poecilia reticulata*. The atretic follicle, a corpus atreticum or a corpus luteum praeovulationis.   Z. Zellforsch. Mikrosk. Anat. 107:54–67.
Manooch, C. S., and D. L. Mason.
    1987.   Age and growth of the Warsaw grouper and black grouper from the southeast region of the United States.   Northeast Gulf Sci. 9:65–75.
Moe, M. A.
    1969.   Biology of the red grouper *Epinephelus morio* (Valenciennes) from the eastern Gulf of Mexico.   Fla. Dep. Nat. Resour. Mar. Res. Lab. Prof. Pap. Ser. 10, 95 p.

Monroy-García, C., R. Burgos-Rosas, V. Moreno-García, and E. Gimenez-Hurtado.
    2001.   Informe de investigaciones conjuntas México-Cuba sobre el mero (*Epinephelus morio*, Valenciennes, 1828) en el Banco de Campeche. Convenio de pesca Mxico-Cuba, 42 p.   Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (México) y Ministerio de la Industria Pesquera (Cuba).   Instituto Nacional de la Pesca, Progreso, Yucatán.
Morris, A. V., C. M. Roberts, and J. P. Hawkins.
    2000.   The threatened status of groupers (Epinephelinae).   Biodivers. Conserv. 9:919–942.
Musick, J. A., M. M. Harbin, S. A. Berkeley, G. H. Burgess, A. M. Eklund, L. Findley, R. G. Gilmore, J. T. Golden, D. S. Ha, G. R. Huntsman, J. C. McGovern, S. J. Parker, S. G. Poss, E. Sala, T. W. Schmidt, G. R. Sedberry, H. Weeks, and S. G. Wright.
    2000.   Endangered Species-Marine, estuarine, and diadromous fish stocks at risk of extinction in North america (exclusive of pacific salmonids).   Fisheries (Bethesda) 25(11): 6–30.
Roe, R. B.
    1977.   Distribution of snappers and groupers in the Gulf of Mexico and Caribbean Sea as determined from exploratory fishing data.   *In* Proceedings of the Colloquim on snapper-grouper fisheries ressources of the western Central Atlantic Ocean (H. R. Bullis and A. C. Jones, eds.), p. 129–164.   Florida Sea Grant Program, Report 17, State University System of Florida Sea Grant Program, Gainesville, FL.
Sadovy, Y.
    1994.   Grouper stocks of the western central Atlantic: the need for management and management needs.   Proc. Gulf Caribb. Fish. Inst. 43:43–64.
    1996.   Reproduction of reef fishery species.   *In* Reef fisheries (N. V. C. Polunin and C. M. Roberts, eds.), p.15–59.   Chapman and Hall, London.
    1997.   Problems of sustainability in grouper fisheries.   Proc. Fourth Asian Fish. Forum, p. 321–324.   China Ocean Press, Beijing.
Sadovy, Y., and D. Y. Shapiro.
    1987.   Criteria for the diagnosis of hermaphroditism in fishes.   Copeia 1987:136–156.
Scherrer, B.
    1984.   Biostatistique, 850 p.   Gaëtan Morin Editeur, Boucherville, Québec.
SAGARPA (Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación).
    2001.   Anuario estadístico de pesca 2000, 268 p.   SAGARPA, México.
SEMARNAP (Secretaría de Medio Ambiente, Recursos Naturales y Pesca).
    2000a.   Anuario estadístico de pesca 1999, 271 p.   SEMARNAP, México.
    2000b.   Sustentablilidad y pesca responsable en México.   Evaluación y manejo 1997–1998, 691 p.   SEMARNAP-IPN, México.
Shapiro, D. Y.
    1984.   Sex reversal and sociodemografic processes in coral reef fishes.   *In* Fish reproduction: strategies and tactics, 3rd ed. (G. W. Potts and R. J. Wootton, eds.), p. 103–118.   Academic Press, London.
    1987.   Reproduction in groupers.   *In* Tropical snappers and groupers: biology and fisheries management (J. J. Polovina and S. Ralston, eds.), p. 295–327.   Westview Press, Boulder, CO.

Shapiro, D. Y., Y. Sadovy, and M. A. McGehee.
    1993. Periodicity of sex change and reproduction in the red hind, *Epinephelus guttatus*, a protogynous grouper. Bull. Mar. Sci. 53:1151–1162.

Smith, C. L.
    1959. Hermaphroditism in some serranid fishes from Bermuda. Pap. Mich. Acad. Sci. 44:111–119.
    1961. Synopsis of biological data on groupers (*Epinephelus* and allied genera) of the Western North Atlantic. FAO Fisheries Biology Synopsis 23, 61 p. FAO, Rome.

    1964. Hermaphroditism in Bahamas groupers. Bull. Mus. Nat. Hist., N.W. 73:42–47.
    1965. The patterns of sexuality and the classification of serranid fishes. Am. Mus. Novit. 2207:1–20.
    1971. A revision of the American groupers: *Epinephelus* and allied genera. Bull. Am. Mus. Nat. Hist. 146:67–242.
    1972. A spawning aggregation of Nassau grouper, *Epinephelus striatus* (Bloch). Trans. Am. Fish. Soc. 101:257–261.