Preliminary Results on the Reproductive Cycle of the Black Grouper, *Mycteroperca bonaci*, from the Southern Gulf of Mexico

XIMENA RENÁN[1], THIERRY BRULÉ[1], TERESA COLÁS- MARRUFO[1], YAZMÍN HAUYON[2] and CHRISTIAN DÉNIEL[3].

[1]*Centro de Investigación y Estudios Avanzados del IPN, Unidad Mérida, México*

[2]*Institut de Biologie Animale Université de Lausanne, Suisse*

[3]*Université de Bretagne Occidentale Institut Universitaire Européen de la Mer Brest, France*

ABSTRACT

The Black Grouper, *Mycteroperca bonaci*, locally known as "Negrillo" is one of the 17 grouper species found on the Campeche Bank. Its importance relies on being after the *Epinephelus morio*, the largest catching volume specie and a very important source of food for the state of Yucatan, Mexico. In spite of this fact, the *M. bonaci* has not been well studied and little is known about its biology, not only in this particular zone but also throughout all its distribution area. This paper presents results on sex determination, sexuality and sexual maturation of *M. bonaci*. After three years of sampling on the Campeche Bank a gonadic histologycal analysis was done. It included more than 800 samples of individuals between 50 to 130 cm (FL). Sex determination studies included 605 females, 205 males and five individuals in sexual inversion. Results confirm that *M. bonaci* is a protogynous hermaphrodite. Considering the sexual classes and seasonal variation in mean of GSI, gonadal activity was found to be from December to March, with a spawning period clearly identified in January to March.

KEY WORDS: Campeche Bank, hermaphroditism, *Mycteroperca bonaci*.

INTRODUCTION

The Campeche Bank is a continental platform of about 175,000 km^2, which surrounds the Yucatan Peninsula and is limited to 25 km inwards the Gulf of Mexico (García 1980). This Bank is part of the region covered by the Western Central Atlantic Fishery Commission (WECAFC), the international organization which looks after the fisheries management of the Caribbean Sea, the Gulf of Mexico and their Atlantic approaches, form Cape Hatteras to Recife, Brazil (Brulé and Déniel 1996).

This platform is the center of fisheries in the state of Yucatan. Landings of octopus and groupers comprise the most important fisheries in this area. In fact, by 1995 grouper landings comprised 91.5% of the entire national fishing production in Mexico. Based on studies by Colás-Marrufo et al. (in press) and Tuz- Sulub (1999), 17 different species of groupers were identified on the Campeche Bank. Of these species, the most important are the red grouper (*Epinephelus morio*), the black grouper (*Mycteroperca bonaci*) and the gag (*Mycteroperca microlepis*). The entire catch was established from these three species, which contribute the greatest numbers of individuals and biomass. The black grouper contributes 40% of the total weight of the entire state production (Tuz-Sulub 1999).

Black groupers occur off Bermuda and are distributed from southern Florida through the southeastern part of the Caribbean and West Indies to northern South America and throughout the Gulf of Mexico. Its habitat is irregular bottom, such as rocky relief and coral reefs (Cervigón and Velasquez 1966). Young black groupers tend to occur in shallow waters, whereas larger fish are restricted to depths greater than 20 m (Fischer 1978). This specie is reported to have a length of at least a meter and weight more than 65 kg (Böhlke and Chaplin 1968, Manooch and Manson 1987, Heemstra and Randall 1993).

According to Sadovy (1994), evidence exists that the populations of groupers from the west center Atlantic show typical signs of overfishing. There has been an abrupt decrease in the average of size and number of the fishing stock, an increase in the effort required to generate maximum sustainable yield (MSY) and the extinction or disruption of reproductive aggregations. Although none of this evidence appears in grouper populations on the Campeche Bank, leaders of the state government of Yucatan have expressed their concern to the Fisheries Secretary, in particular that no population of groupers nn the Campeche Bank has an established management plan.

Nevertheless, the selection of closure season or minimum size of capture is very difficult due to the fact that the fishery in the Campeche Bank include 17 different groupers species, and information on their biology is needed. This is one of the main objectives of the present study, to provide with essential knowledge on the reproductive biology of the black grouper such as sex determination, sexuality and sexual maturation. This data is required to better understand and manage the grouper stocks of the Campeche Bank.

METHODS

Samples of the black grouper were taken from April 1996 to June 1998. Four Yucatan commercial fishing boats collected the samples, most of them in the northeastern part of the Campeche Bank, at 29 different fishing sites, using

standard hook and line gear (Figure 1). Data obtained for each sample included collection date, location, fork (FL) and standard length (SL) (cm), total (TW) and eviscerated weight (EW) (g), gonads weight (GW) and liver weight (LW) (g. A small part of the gonad (2 cm^3) was removed and fixed in Bouin´s fluid for the histological study of oogenesis and spermatogenesis. The preserved gonads were embedded in paraffin, thin sectioned at 6 µm, and stained as suggested in Gabe (1968). Gonad sections were examined to determine sex and gonad development. The different stages of the oogenesis and spermatogenesis were taken from the suggested microscopic cellular characteristics established by Moe (1969) and Brulé and Déniel (1996) for *Epinephelus morio*. According to Coleman's formula (Coleman, 1991), an "effective diameter" ($d_e = [ab^2]^{1/3}$, with a: major axis and b: minor axis) was calculated for 100 oocytes of each oocyte stage.

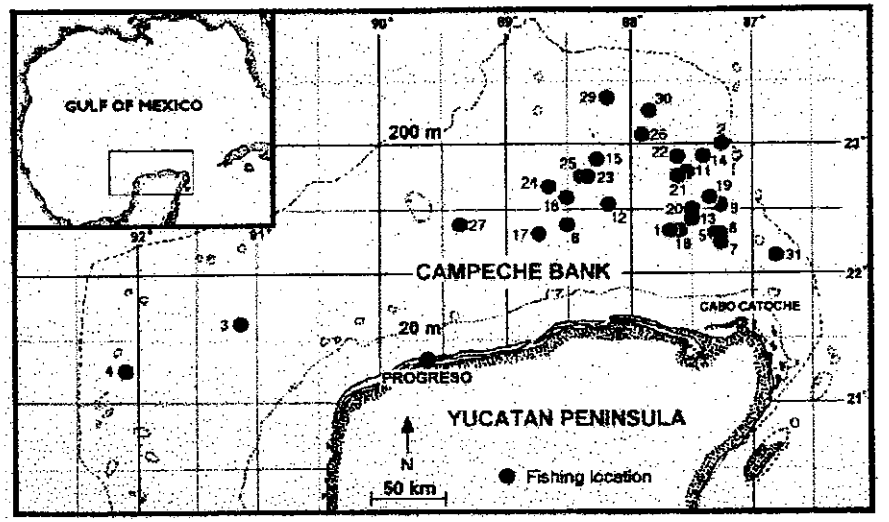

Figure 1. Map of the Campeche Bank and the north coast of the Yucatan Peninsula, showing the 29 fishing sites where black grouper individuals were collected.

The standard of judgement used to distinguish the transitional individuals was taken from five criteria established by Sadovy and Shapiro (1985), Collins et al. (1987) and Shapiro (1987). The distribution of sex in 5 cm FL size groups were compared through a Kolgomorov- Smirnov test, and the mean size per sex were established with a Z test (Scherrer 1984). To ascertain if the sex ratio obtained could be compared to an equilibrated sex ratio (1:1) a Chi- Square test was accomplished (α = 0.05) (Scherrer 1984). For the gonad development, specimens were gathered into nine different classes, which fits in one of the histological conditions of female, transitional and male individuals (Brulé and Déniel, 1996). An accumulated frequency histogram of sexual classes was made for each sex during an annual cycle. The reproductive state of the Black grouper was further examined by calculating a gonadosomatic index (GSI = GW*100/EW) for each individual. Data from the same months for the three years were combined and the results were presented as monthly mean values.

RESULTS

A total of 815 Black Groupers, ranging from 55 cm to 130 cm (FL) and 650 g to 34,500 g (EW) were examined during this study. All of the individuals were captured offshore at different depths in the Campeche Bank, from April 1996 to June 1998. By histological analysis we determined 74% of the captured individuals were females (N = 605), 25% were males (N = 205) and 1% (N = 5) were transitional individuals. Female sizes ranged from 55 cm to 125 cm, males from 85 cm to 130 cm (FL) and individual in sexual inversion ranged from 90 cm to 110 cm (Figure 2). Three criteria were observed:

i) 75.5% (N = 154) males had a lumen inside their testis
ii) 35.5% exhibited spermatic sinuses in the testicular membrane (N = 73).
iii) The presence of five transitional individuals 1% (N = 5) Sadovy and Shapiro (1985). The size frequency distribution of females was different from that of the males (Kolmogorov- Smirnov, D = 0.756). Besides females had a mean of the fork length (96.82 ± 12.23 cm) smaller than the mean fork length of males (105.13 ± 13.98 cm)(Z = 24.88). The mean fork length of transitional individuals was 107.3 ± 9.67 cm.

The sex ratio (M:F) considered only females and males (N = 810), was 1:3 (Chi- Square = 52.84, DF = 1). A Chi- Square test was accomplished for each of the different size classes established previously (5 cm each), only where males and females were found together (Table 1).

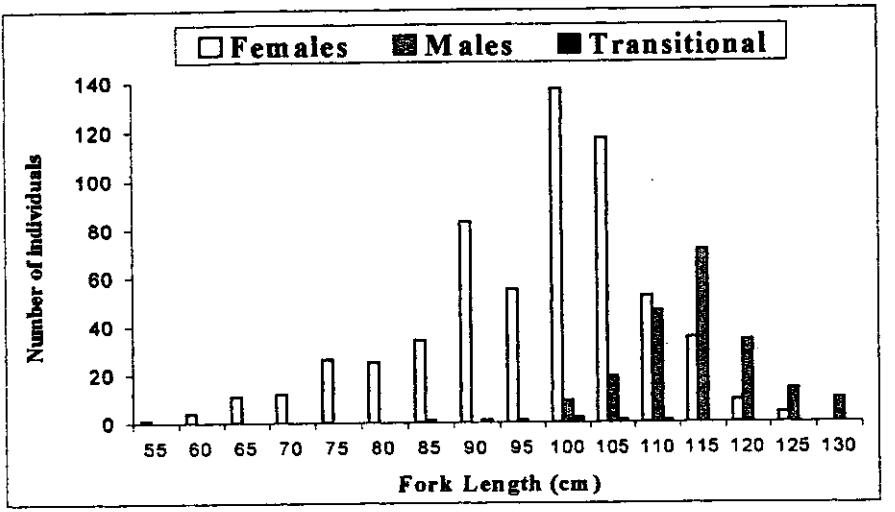

Figure 2. Size frequency distrbution of *M. bonaci* caught on the Campeche Bank (females = 605, males = 205 and transitional = 5 individuals)

Histological analysis established that a number of males displayed remaining oocytes inside their testes (11.25%, N = 27), the other males did not show this remaining cellular condition (88.75%, N = 178). It was established by a Kolgomorov-Smirnov test, that the distributions of males with or without remaining oocytes, differed significantly (D = 0.756). On the other hand, the mean of fork length of males with oocytes was not significantly different from the mean of males without oocytes in their testis (Z = 0.000002) (Figure 3).

Six stages of oogenesis were established through observable cytologic changes during gametogenesis (post-ovulatory follicules and atresic oocytes were not considered as stages). The effective diameter of 100 oocytes in each of the six cellular stages, shows a quantitative characteristic. The mean diameter of the oocytes increased gradually from stage I (9.31 ± 3.82 μm) throughout stage VI (870.84 ± 114.66 μm) (Table 2). Five stages of spermatogenesis were observed.

Table 1. Sex ratio by size classes of *M. bonaci* on the Campeche Bank

Class Distinction	Size Classes	Males	Females	Sex Ratio
55	52.6 - 57.5	0		-
60	57.6 - 62.5	0	4	-
65	62.6 - 67.5	0	11	-
70	67.6 - 72.5	0	12	-
75	72.6 - 77.5	0	26	-
80	77.6 - 82.5	0	25	=
85	82.6 - 87.5	1	34	1:34*
90	87.6 - 92.5	0	83	-
95	92.6 - 97.5	1	55	1:55*
100	97.6 - 102.5	9	137	1:15*
105	102.6 - 107.5	19	117	1:06*
110	107.6 - 112.5	46	52	1:1.13**
115	112.6 - 117.5	71	35	1:0.49**
120	117.6 - 122.5	34	9	1:0.26*
125	122.6 - 127.5	14	4	1:0.28*
130	127.6 - 132.5	10	0	-
	TOTAL	**205**	**605**	**1:2.95***

* Statistically different **Not statistically different

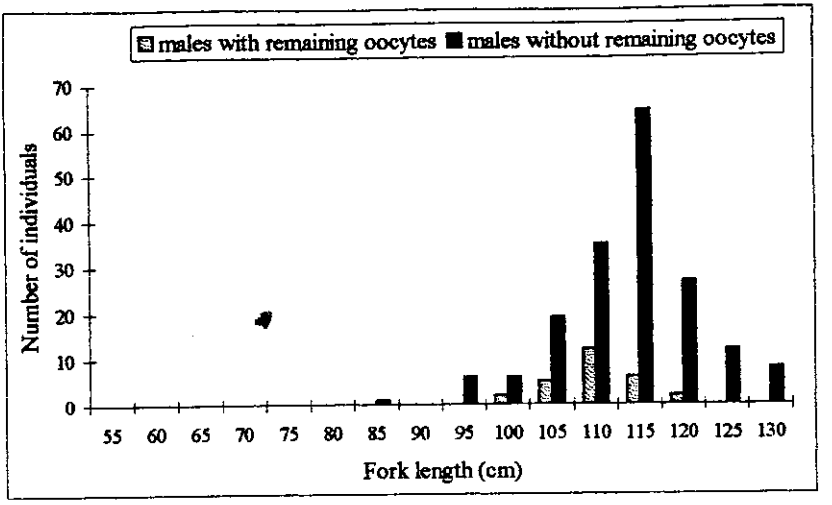

Figure 3. Frequency distribution of *Mycteroperca bonaci* males with and without remaining oocytes in the tests.

Table 2. Effective diameter calculated for the oocytes and their nucleus. (Size range for each stage in parenthesis).

Cellular Stage	Cellular diameter (mm)	Nuclear diameter (mm)
I Primary oocyte	15.062 ± 5.23 (1.745 - 19.195)	2.769 ± 2.076 (0.0769 - 10.32)
II Immature oocyte	23.186 ± 6.637 (9.096 - 56.726)	10.111 ± 3.636 (2.672 - 23.287)
III Primary vitellogensis	59.649 ± 16.562 (19.1 - 77.591)	22.136 ± 6.73 (6.6060 - 26.747)
IV Early Secondary vitellogenesis	161.762 ± 41.463 (34.085 - 192.316)	34.44 ± 7.858 (9.125 - 29.372)
V Late secondary vitellogenesis	262.961 ± 66.958 (199.483 - 576.988)	45.862 ± 8.188 (13.152 - 43.561)
VI Hyaline oocyte*	870.84 ± 114.66 (193.60 - 883.0)	

* Value obtained by measuring, under a binocular microscope, fresh hyaline oocytes (N = 100) samples from ripe ovaries

Changes in the monthly mean gonadosomatic indexes confirmed the histological observations of spawning and resting periods. The relative weight of ovaries increased slowly from October to November and rapidly from January to February. The GSI of males did not show great variations throughout the year. The GSIs reached maximum values in January (female: 1.4%; males: 0.14%) and February (female: 2.2%; males 0.18%) indicating ripening. Relative weight began to decrease in March to reach its minimum values in May for females (0.12%) and on March for males (0.11%) indicating that gametes had been shed (Figure 4).

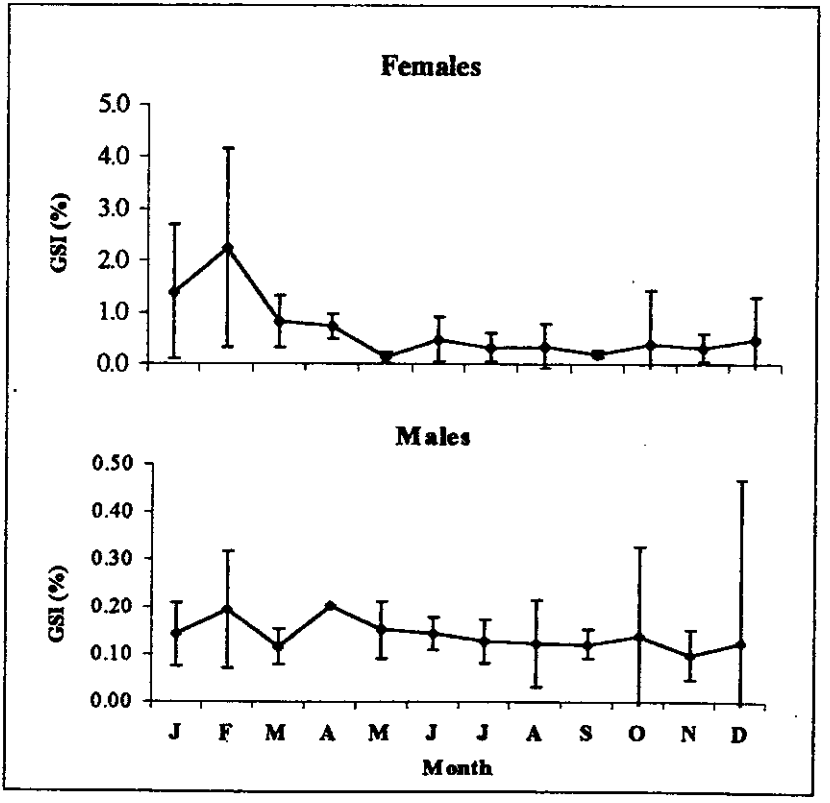

Figure 4. Seasonal variations in mean gonadosomatic index (%) for females and males of *M. bonaci* from Campeche Bank. Vertical bars indicate standard deviation.

Vitellogenesis started in September, for the most precocious females, and continued throughout April. The highest percent of ripening occured during December to April and for ripe females from January to April. In May only immature and atretic vitellogenic oocytes remained in the ovaries, indicating that females have entered the spent-resting period. However, immature females were found throughout the annual cycle (Figure 5).

Ripening males were observed throughout the annual cycle with the exception of April, during which only one ripe individual was found. Riep males were observed every month of the year, many entering a spent period from January through July (Figure 5).

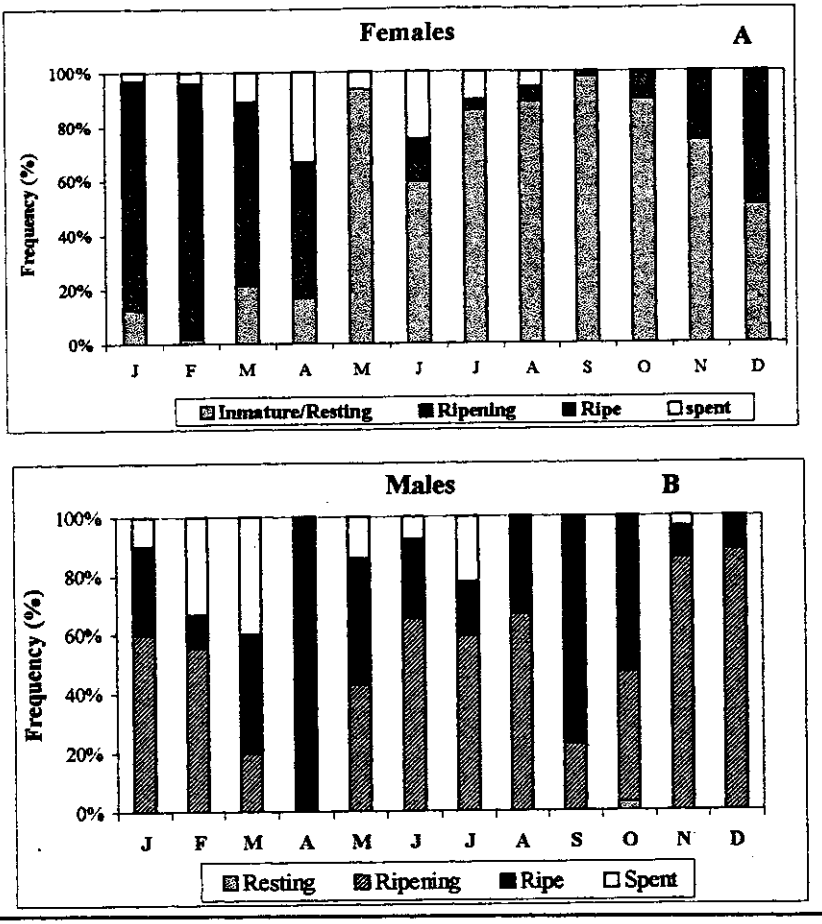

Figure 5. Percent frequency distribution of gonad development classes by month during an annual cycle of *Mycteroperca bonaci* (A: females; B: males)

DISCUSSION

The results indicate that *Mycteroperca bonaci* is a protogynous hermaphrodite; smaller individuals are females and older individuals become actively reproductive males (Smith 1959, Collins et al., 1987, Shapiro 1985, Shapiro 1987 y García-Cagide y García 1996). Three of the five criteria suggested by Sadovy and Shapiro (1985) for the classification of a protogynous hermaphrodites were identified. Males had a lumen inside their testis, present spermatic sinuses into the testicular membrane, and five individuals were established to be transitional individuals with an ovarian lumen along with spermatogonian and spermatocites groups. This is the most important criterion to established hermaphroditism in any specie. According to Shaprio (1987), one of the several difficulties of defining the time of sex change form the occurrence of transitionals in monthly samples, is the relatively small number of transitional gonads found. One reason for finding small numbers of transitional individuals is that sex change may be completed very quickly (Brulé and Déniel 1996). Transitional black groupers were found during five months of the year, including one month of the peak spawning. Nevertheless, García-Cagide and García (1996) established that the black grouper is a monadric specie, and this corroborates with the results obtained in this study, as the presence of the ovaric lumen inside the testis of 75% of the males. However, more information about juveniles of the population is needed in order to establish the sex of young individuals, and therefore conclude if all adults males would have previously been females.

Additional criteria for the classification of protogynous hermaphrodite are the bimodal population size distribution and sex ratio. For instance, in size distribution bimodal histograms, females tend to appear at smaller size classes (55 - 125 cm) and males were found in bigger size classes (85 - 130 cm).

The sex ratio was 1:3 (M:F)(N = 810) clearly showing bias towards females, as expected, since *M. bonaci* is a protogynous hermaphrodite. This sex ratio is different from the one obtained by García-Cagide and García (1996) for *M. bonaci* in Cuba (1: 30.3) (N = 209), which is the only study about the reproduction of the black grouper. The results suggest that for the smaller size classes the sex ratio was biased towards the females (85 - 105 cm) and in the upper size classes (120 - 130 cm) biased towards the males .

Another criterion of protogynous hermaphroditism, is the presence of remaining cells as oocytes inside the mature testis. 11.25% of males did not have remaining oocytes (100 - 120 cm FL) and 88.75% did present this feature (130 - 189 cm FL). This criterion is the least reliable because it could indicate other characteristics, such as a bisexual period or juvenile female phase and its found even in some gonocoric species (Sadovy and Shapiro 1987).

Mature males and females were found in all size classes from 60 – 130 cm FL. These agree with results found in other studies, such as García–Cagide and García (1996) in which mature females and males were found at 85 and 100 cm (FL), respectively. In addition, Valdés Muñoz (1980) found on the Cuban platform five mature females between 57.5 and 64.5 cm (FL) and three males with 62.3 – 67.8 cm (FL). Similarly, Smith (1959) found fourteen mature females of about 45.2 cm and seven mature males with 107.5 cm (FL).

Although some grouper species are known to spawn over 6 - 8 months, most spawn over 1- 5 months, and many spawn primarily during 1 - 2 months (Shapiro 1987, Bullock and Smith 1991). Histological observations of the changes in the gonad development and seasonal variation in mean GSI, indicated a gonadal activity from September to February, with a peak spawning activity in January to March and a resting period from May to September. García-Cagide and García (1996) reported that the spawning period of the black grouper happens during winter with a peak in November and February. Smith (1961) reported some mature black grouper individuals on Campeche Bank from July to August and in the Bahamas in January. Erdman (1956) observed mature individuals in February at Puerto Rico. These results agree with the present study, and it could be concluded that black groupers spawn in winter, primarily in January to March. Even though there is no evidence that black groupers form spawning aggregations on the Campeche Bank, Domeier and Colin (1997) established that *M. bonaci* aggregates in the same sites as *E. striatus* in Belize (January and February) and in Honduras (January), although these spawning aggregations have not been studied.

The monthly samplings of *Mycteroperca bonaci* were homogeneous since all size classes were established between the same rank (females: 55 - 125 cm; males: (85 - 130 cm). This could be achieved because the fishing method was selective and it was included in the same fishing plan. There are no fishing regulations for black grouper in the southeastern part of the Gulf of Mexico, with the exception of 3,900 tons that the Cuban fleets are allowed to catch (Brulé y Colás, 1997). One proposal made to the Yucatan Government for the regulation of groupers is to establish a minimum catch size over 35 cm (FL). Of the 815 samples of black grouper captured during this study, all individuals were beyond this limit. It has been established that adult groupers, including black grouper, live in deep reef zones, were as the juveniles live in coastal zones (Jory and Iversen 1989, Parrish 1987, Sluka and Sullivan 1996). Therefore, we could conclude that most of the individuals captured were adults based on the vertical distribution of most groupers present. One of the main purposes of this study was to contribute with reproductive data of one of the most important fishing specie on the Campeche Bank, and therefore help to establish a regulation program for this resource.

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