

Reproductive Biology of the Dusky Grouper *Epinephelus marginatus* (LOWE, 1834)

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ABSTRACT

Investigations were carried out about the reproductive biology of the dusky grouper, *Epinephelus marginatus* (Lowe, 1834) a serranid protogynous hermaphrodite fish inhabitant of rocky bottoms from shallow waters up to 200 m depth. Samples were caught in the islands that form Arvoredo Biological Marine Reserve (SC - Brazil) and the coastal islands near Florianópolis region from March 1998 to May 2000. Data from samples of juvenile groupers on intertidal pools were used to link their occurrence in the pools to the post-spawning season. To describe the histological changes on gonads along the reproductive cycle and to establish maturation scales, 109 groupers were histologically tested for sex determination. First maturation size was determined for females ($L_{50}=470$ mm; $r^2=0.99$). The results of calculated gonadosomatic (GSI) and hepatosomatic (HSI) indexes, showed that maturation occurred along the spring months and the spawning activity during the summer with a peak in December. Liver recovery (HSI elevation) was evident in the autumn, was the post spawning season. The relationship between length and weight was $W = 9 \cdot 10^{-6} \cdot TL^{3.1149}$ ($r^2=0.998$; $n=246$).

Key words: Reproduction, Dusky Grouper, *Epinephelus marginatus*, Santa Catarina, Brazil

INTRODUCTION

The Dusky grouper, *Epinephelus marginatus* (Lowe, 1834) (Serranidae, Epinephelinae), inhabits rocky bottoms, from shallow waters to depths of more than 200 meters and lives in caves of stone blocks (Tortonese, 1986). It is a well-known teleost fish widely distributed along the southern coast of Brazil and also throughout the Mediterranean and the eastern Atlantic from the British Isles to South Africa, Azores, Madeira, Canaries and Cape Verde (Barreiros, 1995). It is a protogynous hermaphrodite. In Europe, it occurs

when individuals are 14-17 years old and about 80-90 cm long (total length) (Bruslé and Bruslé, 1975, 1976; Bou-Ain, 1984; Chauvet, 1991; Garcia, 1996; Barreiros, 1998). Besides its ecological importance, it has an important economic role for artisanal fisheries along Santa Catarina's coast (Medeiros et al. 1997), and is also an emblematic species for spearfishing and scuba diving. The aims of this work were to examine the reproductive biology of the Dusky Grouper and describe the stages of gonad maturation, maturity size in females, and provide information on seasonality of spawning.

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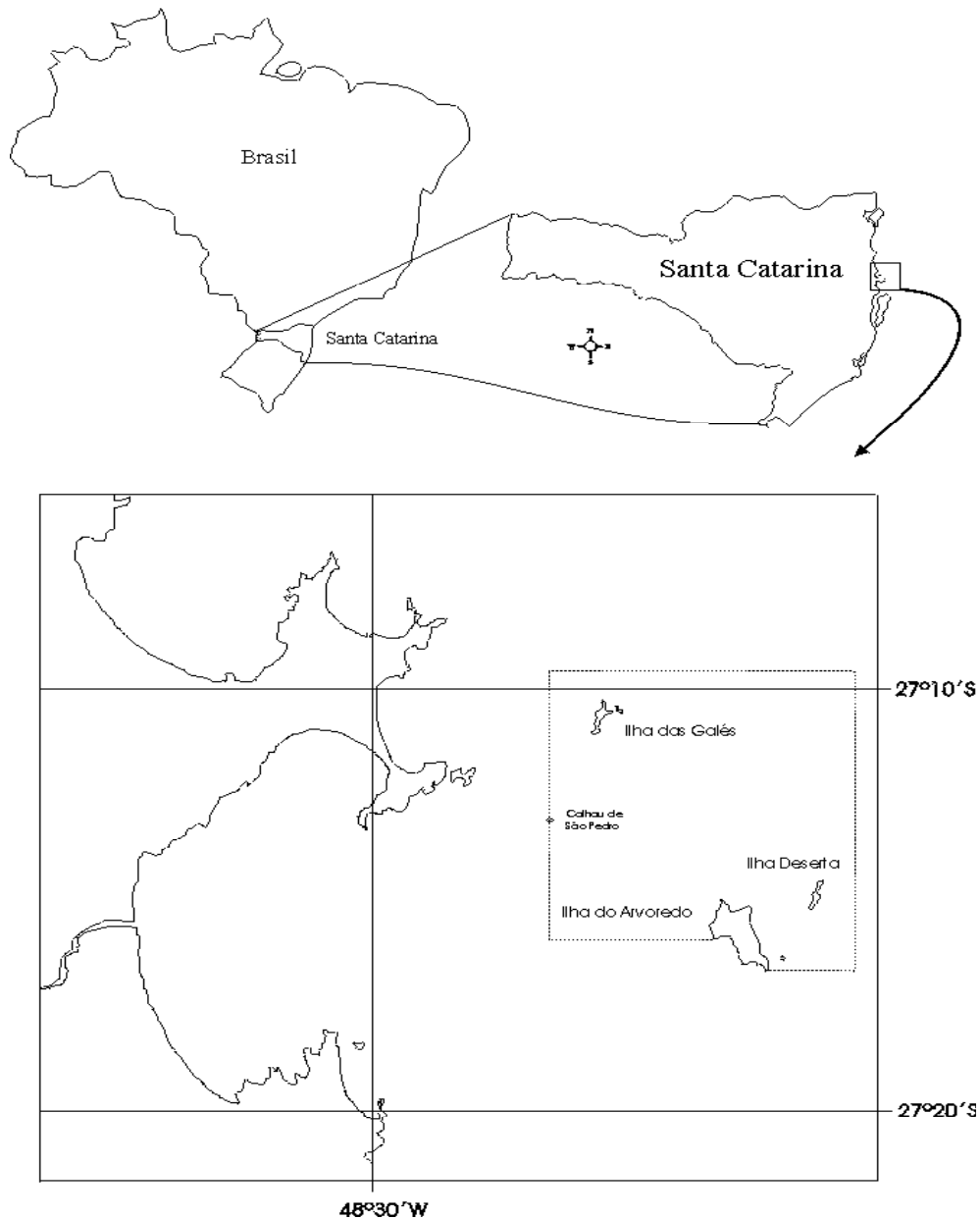


Figure 1 - Localization of Arvoredo Biological Reserve.

MATERIALS AND METHODS

Specimens were collected from March 1998 through May 2000, monthly in the Arvoredo Biological Marine Reserve (Fig. 1), and also along the coastal islets (Xavier, Campeche and Moleques do Norte) of Santa Catarina Island. Samples were collected by spearfishing methods, which are known to make species selection easier (Derbal and Kara, 1996; Barreiros and Santos, 1998).

Some specimens were obtained from fish markets from the long line fleet that operates along Laguna's coast, south of Santa Catarina State.

Groupers in tidal pools were collected using aquarium nets and acetone diluted Chinaldin®, a potent anaesthetic (Gibson, 1967; 1999), to correlate their occurrence with the spawning season.

Specimens were subjected to sex determination, measured for length to the nearest millimeter (Total

length – TL and Standard length - SL), and weighed with a precision of 0.01g. After removing gonads, they were preserved in 10% formaldehyde for a week, and then transferred to 70% alcohol. Sections were taken from the proximal, medial and distal parts of one gonadal lobe, embedded in paraplastic, sectioned transversely at 5-7 μm , and stained with “Harris” hematoxylin and eosin. Each tissue sample was examined microscopically to determine reproductive state. Gonad’s classification was adapted based on the gonad development criteria of Vazzoler (1996), Lourinho (1998) and Silveira (1999). Maturity of individual ovaries was based on the most advanced type of oocyte present, regardless of their abundance. Males were classified based on the male gametogenic stages present in the testis and the development of the sperm duct. Length at which 50% of the females in the population reached sexual maturity was estimated by determining the inflection point of a logistic function fit to the female maturity and length data.

Gonadosomatic indexes (GSI) were calculated as $GSI = (GW / (TW - GW)) \times 100$, where GW = gonad weight (g) and TW = total fish weight (g). Hepatosomatic indexes (HIS) were calculated as $HIS = (LW / TW) \times 100$, where LW = liver weight (g) and TW = total fish weight (g).

RESULTS and DISCUSSION

A total of 139 specimens was collected. Samples of gonads from 109 fishes ranging from 197 to 1002 mm (TL) were collected (Fig. 2). Males varied from 800 to 1002 mm (mean=960 mm, SD=75.4, n=5) and were significantly longer than females, which ranged from 175 to 795 mm (mean=423.5 mm, SD=130.0, n=104) ($p < 0.01$). Lengths were divided in classes of 50 mm (considering the higher limit), and a maximum of 23 fishes for the 400 mm class was observed. Juveniles from tidal pools varied from 22 to 146 mm (mean=39 mm, SD=35.2, n=107).

The length/weight relationship obtained for the grouped data of sexed groupers and juveniles from tidal pool was $TW = 9 \times 10^{-6} \times TL^{3.1149}$ ($r^2 = 0.9985$, n=246) (Fig. 3). Although the value of the exponent “b” (3.1149) of the relation above was similar to the ones found by Kara and Derbal (1995) and Chauvet (1981,1988), a value of $b = 2.598$ (probably influenced by the small sample size, n=22) was obtained by Bouchereau et al. (1999) for the groupers in the Natural Marine Reserve of Lavezzi Islands.

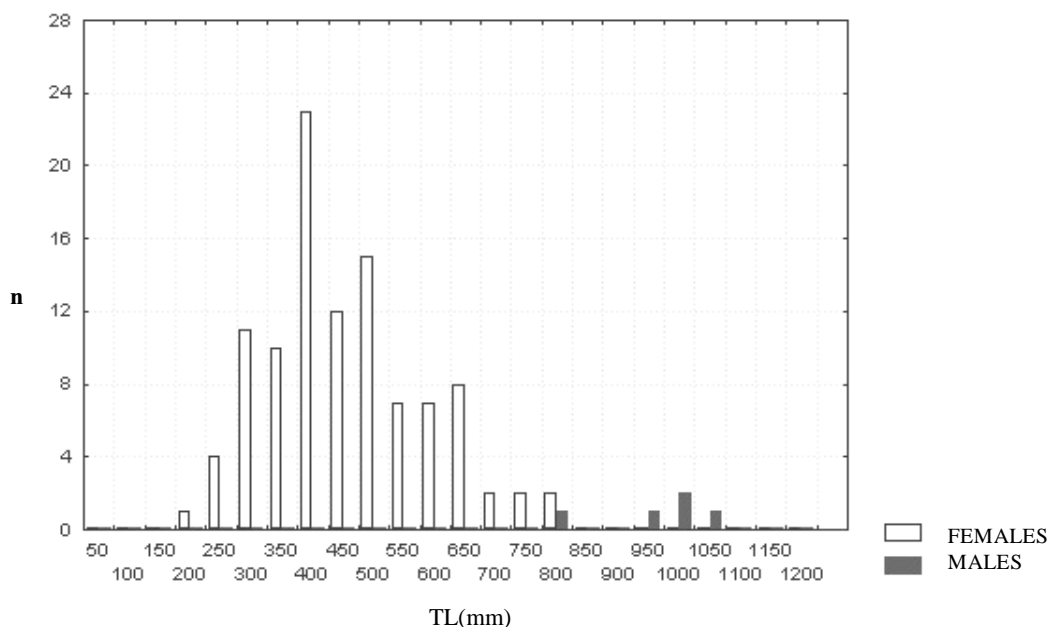


Figure 2 - Length frequencies distribution for the sexed groupers (n=109).

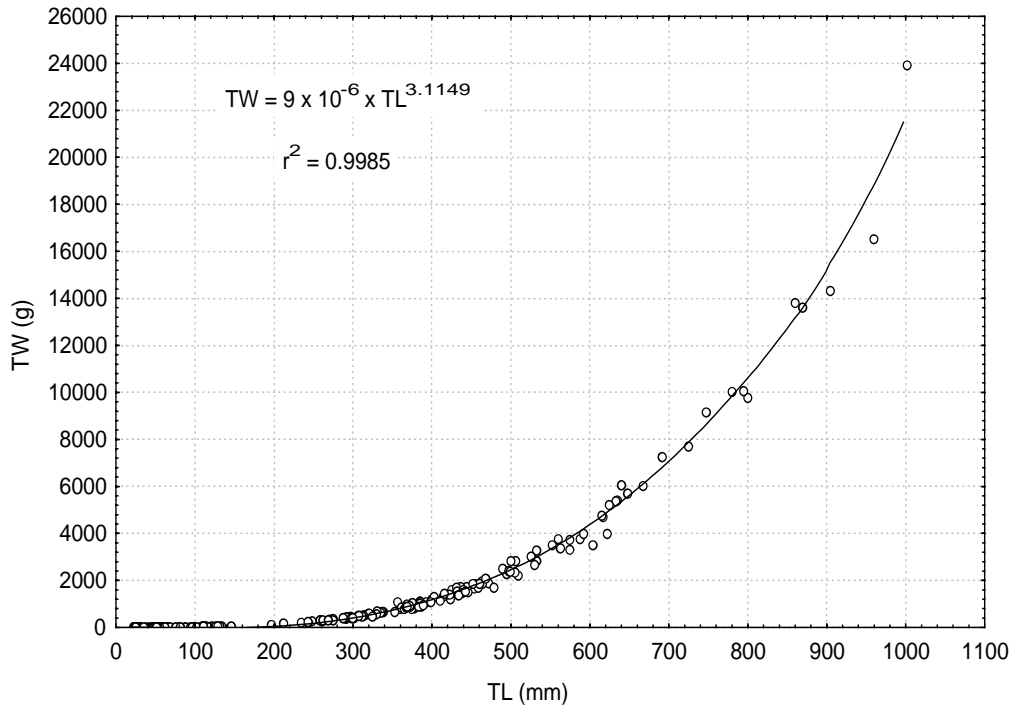


Figure 3 - Length/weight relationship for *E. marginatus*.

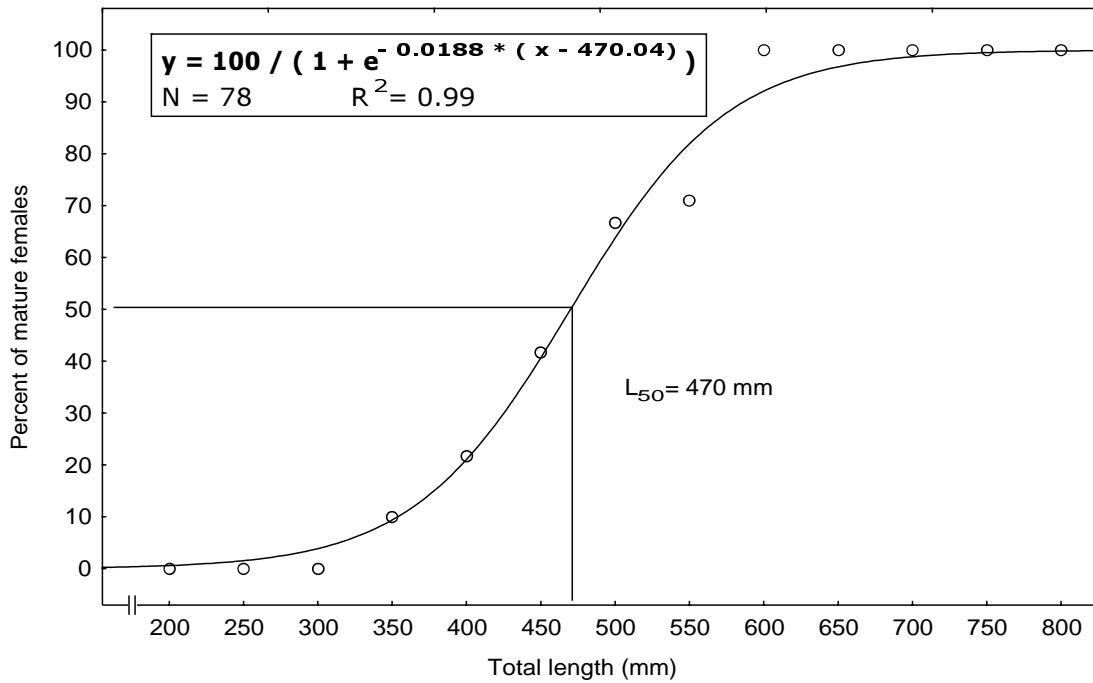


Figure 4 - First maturation curve for *E. marginatus*.

First maturation curve presented a characteristic shape typical of species that reached higher sizes, higher longevity and slower growth rate, as was the case of *E. marginatus*. Its maturation process occurred along a vast amplitude of lengths (350 to 600 mm). The estimated length at which 50% of the females in the population reached sexual maturity was 470 mm (Fig. 4), which corresponded to a grouper weighing approximately 2 kg (Fig. 3). The value of $L_{50}=470$ mm was within the amplitude found for Tunisian groupers by Chauvet

(1988), where the population studied reached sexual maturity between 450 and 550 mm total length, with an average age of 5 years.

Size of sexual inversion was not calculated due to an insufficient sample size. Tunisian and Azorean groupers had an inversion size between 800 and 900 mm (Chauvet, 1988; Barreiros, 1998). In our samples the highest female total length was 795 mm total length and the smallest male was 800 mm.

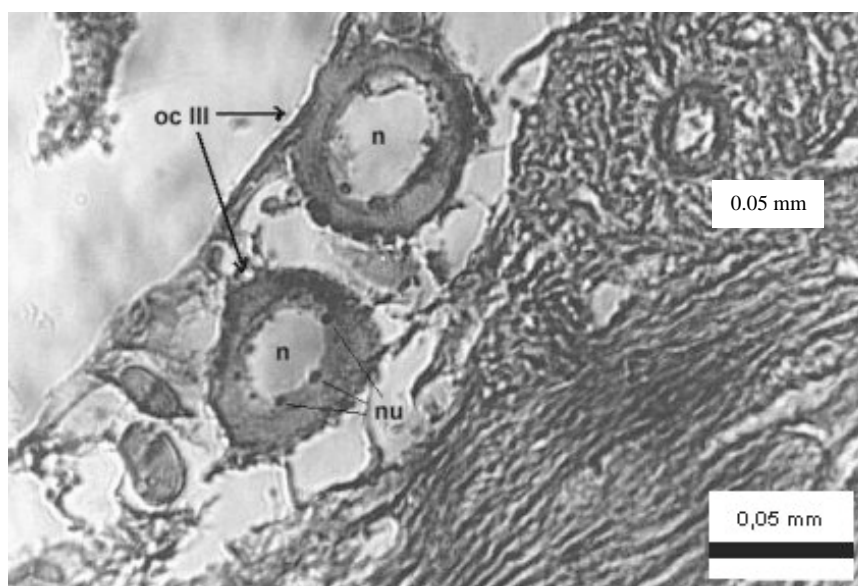


Figure 5 - oocyte 3 (oc III) -advanced perinucleolar stage, n - nucleus, nu – nucleolus. 400x.

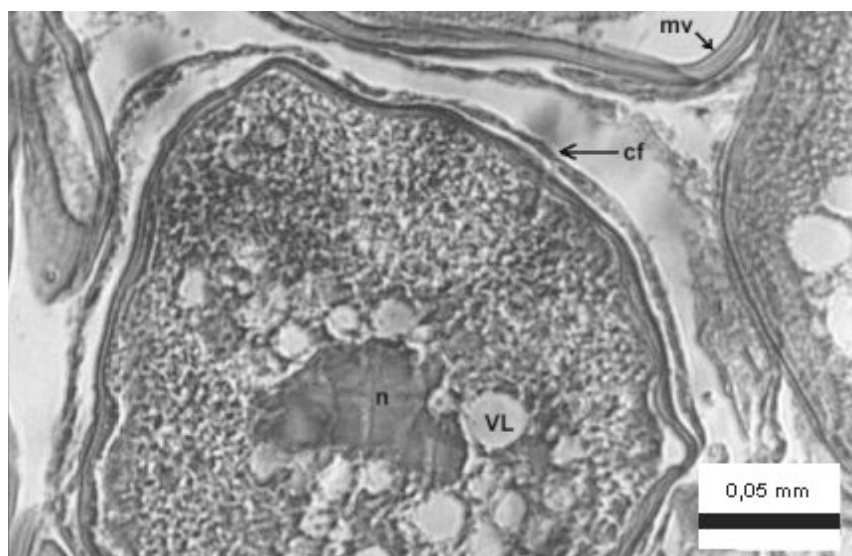


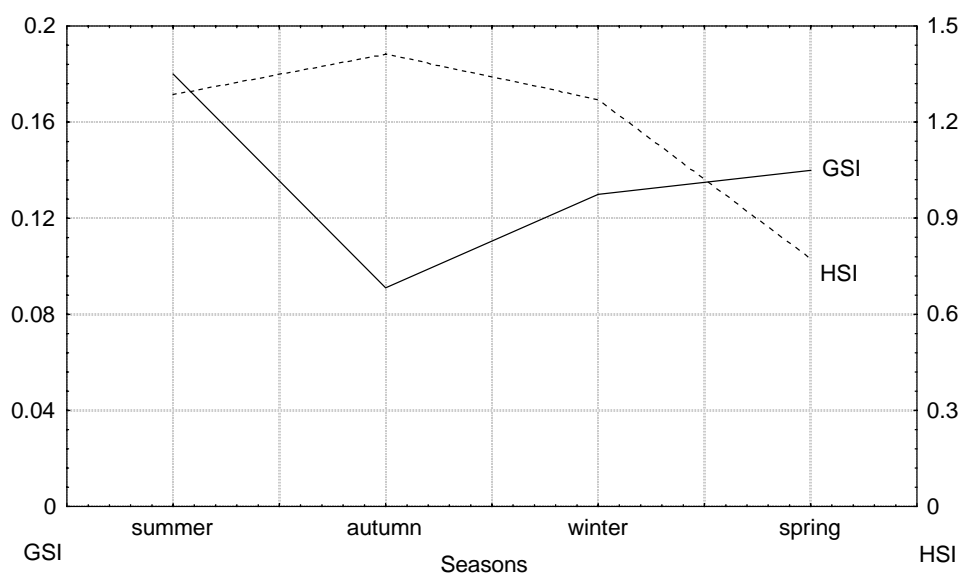
Figure 6 - oocyte 5 - advanced vitellogenic stage. n - nucleus, VL yolk vesicle, cf - follicular layer, mv - zona radiata 400x.

Table 1 - Reproductive classes of *E. marginatus* gonads defined by characteristic histological criteria.

Females	
(1) Immature	Chromatin nucleolar stage (1) (small cells with large nucleus, 1 or 2 nucleolus), and initial perinucleolar stage (2) (bigger oocytes with more than 2 nucleolus) oocytes present. Well-organized aspect.
(2) Mature resting	Similar to the previous stage, but with a less organized aspect.
(3) Developing	Advanced perinucleolar stage (3) (bigger oocytes with many nucleolus), initial vitellogenic stage (4) (yolk vesicles appear in cytoplasm), advanced vitellogenic stage (5) (appearance of yolk proteins, irregular nucleus shape) oocytes present. Not well-organized aspect.
(4) Developed	Completed vitellogenic stage oocytes (6) (yolk vesicles almost not seen). In this stage occurs the peripheral migration of the nucleus and dissolution of its membrane. These oocytes turn into hydrated oocytes (7), by a rapid increase in the size due to hydration of oocytes. Other oocytes are less abundant.
(4) Spent	All seven oocyte stages present, little of stage 7. Atresia may be found. (post-spawning)
Males	
(1) Immature	Crypts with spermatogonia
(2) Developing	Crypts with spermatocytes 1 and 2
(3) Late Mature	Spermatids and spermatozoa in the lumen of lobules
(4) Ripe	Sperm duct filled with spermatozoa

Table 2 - Indexes results with standard deviation (SD).

season	N	GSI	HSI	SD GIS	SD HSI
summer	53	0.180	1.286	0.209	0.788
autumn	43	0.091	1.412	0.081	0.694
winter	4	0.130	1.270	0.048	0.354
spring	9	0.140	0.775	0.472	0.238

**Figure 7** - Gonadosomatic index (GSI) and hepatosomatic index (HSI) for *E. marginatus* along the seasons.

Microscopic gonad stages are presented in Table 1. Some oocytes stages are presented in Fig. 5 and 6. Close attention should be given to male classification, not only because its finer classification was difficult and subjective, but also because of our low sample size.

For a better resolution of the indexes results, data were grouped in four seasons (Table 2). The number of sexed groupers, mean TL and standard deviation were respectively: in summer (n=53, mean TL=423.6, SD=127.9), autumn (n=43, mean TL=451.5, SD=149.5), winter (n=4, mean TL=740.5, SD=262.9) and spring: (n=9, mean TL=617.7, SD=259.2). Median gonadosomatic index (GSI) showed the values in summer, decreasing in autumn and getting higher along winter and spring highest. The hepatosomatic index (HSI) plotted showed an inverse pattern of these values in comparison to GSI indexes (Fig. 7).

This behavior suggested that *E. marginatus* matured in spring and spawned in early summer, with a peak in December. It showed an evident liver recovery (high HSI level) in autumn. Data on juvenile grouper in intertidal areas were found to occur in tidepools from December up to March (Bertoncini et al., 1999), with TL's ranging from 20 to 30 mm, peaking in February and March. Azevedo et al. (1995) suggested that these intertidal pools have an important role as nursery areas for *E. marginatus*. Heemstra AND Randall (1993) reported that *Epinephelus septemfasciatus*, a species reaching sizes similar to *E. marginatus*, reached 30.8 mm in 60 days after spawning in laboratory conditions. These data reinforced the results showed by the indexes presented above.

Hermaphrodite fishes present age and sexual inversion rates, which are characteristic of a specific geographic region. Chauvet (1988) studying *E. marginatus* growth in the Tunisian coast stands out the necessity in developing qualitative studies gathering grouper stocks from different geographic origins. The presence of big females in a population suggested that not all the females inverted to males. Such big females were not observed in our samples in Santa Catarina, but Barreiros [unpublished results] reported that in 2000, big *E. marginatus* females were caught in the Azores, weighing over 18 kg (approximately 950 mm).

The reproductive characteristics of other three hermaphrodite grouper species studied by Coleman et al. (1996), and Sluka AND Sullivan, (1988) showed that these characteristics may

affect stocks sustainability. Nowadays it is known that some protogynous stocks may be far more vulnerable to fishing than are comparable gonochoristic stocks (Schaaf and Huntsman, 1994; Musick et al., 2001; Coleman et al., 2001).

Many reef fishes are known to aggregate in large numbers at specific times and places to reproduce. Grouper stocks, as in other serranids, were typically the first reef fish stocks to collapse in response to increasing fishing pressure (Johannes, 1998). In the case of groupers, spawning aggregations have been completely obliterated by overfishing at a number of locations in the Atlantic and the Pacific oceans (Johannes et al., 1999). *E. marginatus* aggregation sites are known at a few places, such as the Medes Islands Marine Reserve, Spain (Zabala et al., 1997a, 1997b), off Lampedusa Island, Mediterranean Sea (Marino et al., 2001), and the National Park of Port-Cros, France (Chauvet AND Francour, 1991).

In Brazil, these aggregation sites are supposed to exist based on information obtained from fishermen, in their reports of large catches, up to 400 kg [pers. com.] in a few days of diving in the same area. Little information is known about such important sites in Santa Catarina. Diving centers, operating in the Arvoredo Biological Marine Reserve along the year reported a higher abundance of *E. marginatus* in a single summer week at a diving point north of Deserta Island. Efforts should focus to discover the aggregation sites at Santa Catarina's coast, once mature, juveniles and post-larvae of *E. marginatus* are found in the studied area. In the Mediterranean area, specific fishery statistics for this species are not available (Marino et al., 2001), and *E. marginatus* is believed to be overexploited in most Mediterranean areas (Bruslé and Bruslé, 1976; Chauvet, 1991; Zabala et al., 1997b). To avoid future stock depletion of this important resource in Santa Catarina's waters, the detection of aggregation sites is vital for the development of a management plan considering spawning aggregations (which should be taken into consideration when delineating marine protected areas) and also groupers minimum capture-size. Alternatively, establishing fishing closures during the brief annual spawning aggregation periods might be an effective approach to protect this valuable resource.

RESUMO

Este trabalho trata sobre a biologia reprodutiva da garoupa verdadeira, *Epinephelus marginatus* (Lowe, 1834) um peixe teleosteo pertencente à família Serranidae, hermafrodita protogínico e característico do litoral rochoso, que vive desde águas rasas até os 200 m de profundidade. As amostragens foram realizadas nas ilhas que compõem a Reserva Biológica Marinha do Arvoredo (SC) e ilhas costeiras próximas da região de Florianópolis entre março de 1998 e maio de 2000. Dados de coletas de juvenis em poças de marés foram utilizados visando relacionar a sua ocorrência nestes ambientes com o período de pós-reprodução. Procedeu-se à análise histológica de 109 exemplares, a partir da qual foram descritas as alterações histológicas nas gônadas ao longo do seu ciclo sexual e foram estabelecidas as escalas de maturação. Desta forma determinou-se um tamanho de primeira maturação para as fêmeas ($L_{50}=470$ mm; $r^2=0,99$). O comportamento dos valores dos índices calculados (Índice Hepatosomático (IHS) e Índice Gonadossomático (IGS)) elucidou um período reprodutivo com maturação das gônadas ao longo dos meses de primavera, vindo a desovar na estação do verão, sendo nítida a recuperação do fígado (elevação do IHS) no outono, pós reprodução. Calculou-se também a relação peso comprimento $P = 9 \cdot 10^{-6} \cdot CT^{3,1149}$ ($r^2=0,998$; $n=246$).

ACKNOWLEDGMENTS

This paper is dedicated in memory of Gabriel Fonseca Lima. A special thank to Thalassar Diving group for logistics and homepage: www.cttmar.univali.br/~garoupa We would like to thank Mr. Vitor da Silva Dutra, Manager of Arvoredo Biological Marine Reserve, Mr. Ten. William Wallace, Cb. Murilo and Sd. Vanilson, from the Environmental Police, who kindly provided logistics in Arvoredo Island. We are also grateful to all the Laboratório de Ciências Ambientais staff for support in field and in the laboratory. A special thanks to Paula Lourinho, DOP's staff for data analysis and Susana Mendes for the final revision of the paper.

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Received: July 06, 2001;

Revised: October 22, 2001;

Accepted: September 11, 2002.