CSIRO PUBLISHING

Marine and Freshwater Research
http://dx.doi.org/10.1071/MF13120

Reproductive biology of the dusky grouper (*Epinephelus marginatus*) at the southern limit of its distribution in the south-western Atlantic

Mario V. Condini[A,D], *Luís F. Fávaro*[B], *Antonio S. Varela Jr.*[C] *and Alexandre M. Garcia*[A]

[A]Laboratório de Ictiologia, Instituto de Oceanografia, Universidade Federal de Rio Grande, C.P. 474, CEP: 96.201-900, Rio Grande – RS, Brazil.
[B]Laboratório de Reprodução de Comunidade de Peixes, Departamento de Biologia Celular, Universidade Federal do Paraná, CEP: 81.531-990, Curitiba, PR, Brazil.
[C]Instituto de Ciências Biológicas, Universidade Federal de Rio Grande, CEP: 96.201-900, Rio Grande – RS, Brazil.
[D]Corresponding author. Email: mvcondini@yahoo.com.br

Abstract. We investigated the reproductive parameters of a dusky grouper *Epinephelus marginatus* population at the southern limit of its distribution in the south-western Atlantic. The analysed specimens were captured between January 2008 and April 2011 at Carpinteiro Bank (CB) (32°16′S; 051°47′W), a seamount located at the 20 m isobath ~16 nautical miles from the coast. The gonads of a total of 201 individuals (184 females, 16 males and 1 individual in sexual transition) with a total length (TL, mm) between 278 and 1160 mm were analysed microscopically. Histological analyses of the gonads suggested that the species is a partial spawner with a long spawning period (between November and March) and with a reproductive peak between November and January. The average female length at first maturity (L50) was 496 (TL, mm). We also observed partially spawned ovaries and partially spawned testes, which indicated reproductive activity, and we noted the presence of hyalinised follicles. These findings reveal that CB is the southernmost portion of the south-western Atlantic in which the dusky grouper reproduces. Additionally, an inshore-offshore comparison suggested that deeper (>20 m) rocky bottoms, such as the studied seamount, constitute higher quality habitats for this species when compared with littoral (<5 m) rocky reefs.

Additional keywords: length at first maturity, protogynous hermaphroditism, spawning seasonality.

Received 28 November 2012, accepted 9 July 2013, published online 25 September 2013

Introduction

The groupers (Family: Epinephelidae) are represented by several species of great importance in marine ecosystems and play a fundamental role in maintaining the trophic chain and its equilibrium because they often act as top predators in their ecosystems (Parrish 1987; Reñones *et al.* 2002). The different grouper species exhibit K-strategist species characteristics such as a complex reproductive mode, late sexual maturity and a slow growth rate (Manooch 1987). These characteristics, along with the large commercial value of groupers throughout their range of occurrence (Heemstra and Randall 1993), make these species vulnerable to overfishing (Huntsman *et al.* 1999; Coleman *et al.* 2000).

The dusky grouper *Epinephelus marginatus* (Lowe, 1834) is a monoandric protogynous hermaphroditic species (Marino *et al.* 2001) that belongs to the Epinephelidae family. This species has a wide distribution, occurring in the Mediterranean Sea and along both coasts of the Atlantic Ocean; in the Indian Ocean, it is found along the southern and south-eastern coasts of Africa (Fennessy 2006). On the western coast of the Atlantic Ocean, the dusky grouper has been recorded from Rio de Janeiro to the Patagonia region of Argentina (Figueiredo and Menezes 1980; Irigoyen *et al.* 2005), whereas on the eastern coast, this species occurs from the British Isles to South Africa (Heemstra and Randall 1993). The groupers live on rocky bottoms at a depth of up to 250 m, although they are present in higher densities at depths of up to 50 m (Bruslé 1985; Heemstra and Randall 1993; Harmelin and Harmelin-Vivien 1999). Similar to other species of the Epinephelidae, the dusky grouper is considered 'endangered' (EN a2d) by the International Union for Conservation of Nature (Cornish and Harmelin-Vivien 2004) because of its complex life strategy and increases in fishing pressure.

Thus, because of the precarious conservation status of the species, many countries have developed greater interest in studying the ecology of the dusky grouper in recent years with

the aim of establishing fishing management measures (Marino *et al.* 2001; Fennessy 2006; Reñones *et al.* 2010), commercial captive breeding (Marino *et al.* 2003; Sanches *et al.* 2007; Cunha *et al.* 2009) and population restocking in degraded areas (La Mesa *et al.* 2008;). The dusky grouper population in the Mediterranean Sea, in particular, has been intensively studied with regard to trophic ecology (Reñones *et al.* 2002; Linde *et al.* 2004; López and Orvay 2005), age structure and growth (Chauvet 1988; Kara and Derbal 1995; Bouchereau *et al.* 1999; Reñones *et al.* 2007). Researchers have shown particular interest in studies of reproductive biology, which are considered very important because they provide information that is crucial for the conservation of this species. This information includes the location of spawning aggregations (Zabala *et al.* 1997*a*), the length of the spawning period (Kara and Derbal 1999; Marino *et al.* 2001; Reñones *et al.* 2010), the length of females at first maturity and the record of individuals undergoing sexual transition (Marino *et al.* 2001; Reñones *et al.* 2010). However, studies of the dusky grouper in the Atlantic Ocean are scarce (Machado *et al.* 2003; Machado *et al.* 2008), and only a few studies have been undertaken to examine the reproductive biology of this species (Andrade *et al.* 2003; Seyboth *et al.* 2011).

The only published study on dusky grouper reproductive biology in the extreme south-western Atlantic (above 30°S) was performed by Seyboth *et al.* (2011) at the rocky jetties locally known as 'Molhes da Barra de Rio Grande' (32°09′S, 52°05′W). By means of microscopic analysis of the gonads, these authors observed that the individuals occurring in this location are either immature females or in sexual resting, leading to the conclusion that this coastal habitat (depth <5 m) functions as a growth and feeding area (Condini *et al.* 2011). Seyboth *et al.* (2011) speculated that, in the extreme south of Brazil, this species would reproduce in deeper rocky bottoms (>15 m) along the coast. Many fish species exhibit a depth-related habitat shift as they become larger and older (Helfman *et al.* 2009). The juvenile groupers tend to occur more frequently in nearshore habitats compared with the adults, and larger adults tend to occupy deeper water compared with smaller adults (Thompson and Munro 1978; Sluka *et al.* 2001). For example, serranid species use mangrove systems as growing sites for their juveniles; after sexual maturation, they move to coral reefs where they reproduce (Frias-Torres 2006; Craig *et al.* 2011). However, this reproductive migration pattern is much less understood in subtropical latitudes of the south-western Atlantic, where mangroves and coral reefs are absent. In such latitudes (32°S), offshore banks ~10 to 30 nautical miles off the coast at depths between 15 and 50 m (Cardoso and Haimovici 2011) could be potential deeper water habitats for grouper reproduction. These banks are submersed reefs formed by beach rocks and superficially colonised by bryozoans, sponges, crustaceans and polychaetes (Buchmann *et al.* 2001). In the present study, we evaluated the hypothesis that an offshore bank located ~16 nautical miles off the coast (32°16′S, 051°47′W) constitutes a suitable habitat for dusky grouper reproduction in the extreme south-western Atlantic.

Materials and methods

Specimens of the dusky grouper *Epinephelus marginatus* were captured at Carpinteiro Bank (CB) (32°16′S, 051°47′W), which is composed of beach rocks with a high level of cementation by recrystallised calcium carbonate. The CB is located at the 20 m isobath, ~16 nautical miles from the coastal region (Buchmann *et al.* 2001; Abreu and Calliari 2005) (Fig. 1).

The studied individuals were obtained between January 2008 and April 2011 at the CB (Fig. 1) by local commercial fishers using hand lines. In the laboratory, the total length (TL) in millimetres (mm) and the total weight (TW) in grams (g) of each specimen were measured. After a ventral incision, the liver and gonads were extracted from the specimens. The weights of the liver and gonads (in g) were recorded. Subsequently, the gonads were fixed in 10% formalin.

The gonads were processed manually, dehydrated in a series of increasing alcohol concentrations, diaphanised in xylol, impregnated and embedded in Paraplast Xtra (Sigma – P3808). Pieces that were 5 µm in width were sectioned using

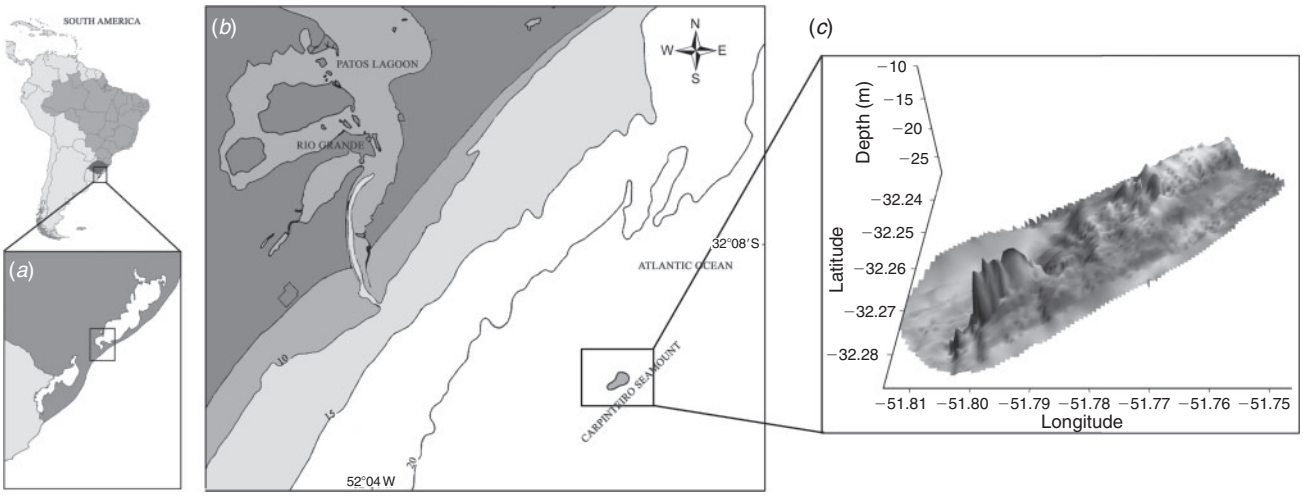

Fig. 1. Patos Lagoon (10 360 km^2) and its estuarine zone in southern Brazil (*a*), showing the location of the Carpinteiro Bank in the adjacent sea area (*b*) and a 3D graphic representation of its topography (*c*). The lines along the coast represent the isobaths of 10, 15 and 20 m.

a rotational microtome and subsequently stained with hematoxylin-eosin (HE). Histological analysis enabled the determination of gonadal development on the basis of a scale proposed by Marino *et al.* (2001) that was adapted for the present study (Table 1). Using this classification, we considered females with gonads in stages F2 to F5 and all males to be 'adults' (Table 1).

The following features were used to determine the developmental stage of the ovaries: the development of ovarian follicles, occurrence of empty follicles and follicular atresia and the quantity of stroma in the lamellae (Oliveira and Fávaro 2011). For the individuals undergoing sexual transition, the tissue organisation and the degree of development of the ovarian and testicular gonadal tissues were analysed (Hastings 1981; Sadovy and Shapiro 1987; Webb and Kingsford 1992). An analysis of testicular development was performed considering the presence or absence and the quantity of the different types of male germline cells (Gomes and Araújo 2004). To evaluate the potential seasonality of spawning, we plotted the monthly frequencies of occurrence of each gonadal stage for both sexes.

The reproductive cycle was determined from the percentage frequency distribution of the gonadal development stages, as obtained from the histological analysis, and from the temporal trends in the monthly gonadosomatic index ($GSI = (GoW/TW - GoW) \times 100$), where TW is the total weight (g) of the individual and GoW is the weight of the gonad (g). Because the GSI data did not show a normal distribution or homoscedasticity, temporal differences in the mean values of these indices were evaluated using the non-parametric Kruskal–Wallis test followed by the Mann–Whitney post-hoc test (Sokal and Rohlf 1995). Only the adult (mature) individuals were used to determine the spawning period of the dusky grouper. The difference in average TL between males and females was examined using a *t*-test and corroborated by the Kolmogorov–Smirnov non-parametric test (Sokal and Rohlf 1995).

The length at first maturity (L50) corresponds to the length class in which 50% of the individuals are sexually mature. In the present study, the L50 was determined for the females and was estimated from a curve that relates the relative frequency of adult individuals to the midpoint of the length classes. The following equation was used to fit the curve: $Fr = 1 - (\exp - aLt_b)$, where (Fr) is the relative frequency of adult individuals, (exp) refers to the natural logarithm base, 'a' and 'b' are coefficients estimated by a least-squares linear regression of the transformed variables and (Lt) is the midpoint of the total length classes (King 1995).

To estimate the sex ratio between males and females as well as between males and adult females only, Pearson's Chi-square test was used ($\alpha = 0.05$) (Sokal and Rohlf 1995) with an expected proportion of 1 : 1.

Table 1. Microscopic description of the developmental stages of the ovaries and testes of the dusky grouper (*Epinephelus marginatus*) in the south-western Atlantic and average values of the gonadosomatic (GSI) and hepatosomatic (HSI) indices for the different gonad stages (± s.e.)

Sex	Stages	Histological description
Females	Immature (F1)	Ovary with small ovigerous lamellae positioned close together, filled with oogonia and pre-vitellogenic ovarian follicles, characterized by the absence of cytoplasmic inclusions (cytoplasmic vesicles and yolk granules). GSI = 0.07 (0.006); HSI = 1.28 (0.044).
	Developing (F2)	Ovigerous lamellae containing pre-vitellogenic ovarian follicles and ovarian follicles in vitellogenesis. Earlier in this stage, vitellogenic follicles occur in cytoplasmic vesicles in the oocyte. As the ovary develops, oocytes containing yolk granules are noted in the cytoplasm, increasing in number until the next phase is reached. GSI = 0.51 (0.054); HSI = 1.73 (0.238).
	Mature (F3)	The ovary exhibits pre-vitellogenic ovarian follicles associated with ovarian follicles in vitellogenesis. Oocytes with yolk granule-filled cytoplasm predominate at this stage, although oocytes containing cytoplasmic vesicles and follicles in atresia are also observed to a lesser extent. Subsequently, spawning hydrated oocytes can be observed. GSI = 5.96 (0.658); HSI = 1.87 (0.244).
	Partially spawned (F4)	Ovaries with pre-vitellogenic follicles are observed, and at different stages of vitellogenesis, the ovaries are associated with empty follicles. This stage indicates reproductive activity and spawning. GSI = 2.54 (0.569); HSI = 1.93 (0.329).
	Resting (F5)	Ovigerous folds with a large amount of connective tissue are associated with muscle bundles with few vitellogenic oocytes among many atretic vitellogenic follicles. Enlarged blood vessels are present. Primary germ cells, oogonia and small oocytes in the previtellogenic stage and nuclear chromatin in the perinucleolar stage are often located on the periphery of the folds of the ovary. GSI = 0.12 (0.013); HSI = 1.56 (0.057).
Transitional	Sexual transition	Ovarian tissue is associated with testicular tissue. At this stage of the life cycle, the forming male gonad still displays the ovarian lumen.
Males	Maturing (M1)	Testicular lobes exhibit different male cell types (spermatogonia, spermatocytes and spermatids), and sperm are occasionally present. GSI = 0.27; HSI = 1.64.
	Mature (M2)	The testes presents lobes that are predominantly filled with testicular spermatozoa.
	Partially spawned (M3)	The testes that were previously filled by sperm have now become partially emptied, demonstrating the elimination of some of the contents. The average value of GSI is similar to that observed during the maturation stage; however, the constitution and structure of the cell lobes are different. GSI = 0.32 (0.043); HSI = 1.34 (0.091).
	Spent (M4)	The depleted testes have lobes with emptied spaces and tissue evident. There is low spermatogenic activity and an abundance of spermatogonia. GSI = 0.15 (0.008); HSI = 1.26 (0.065).

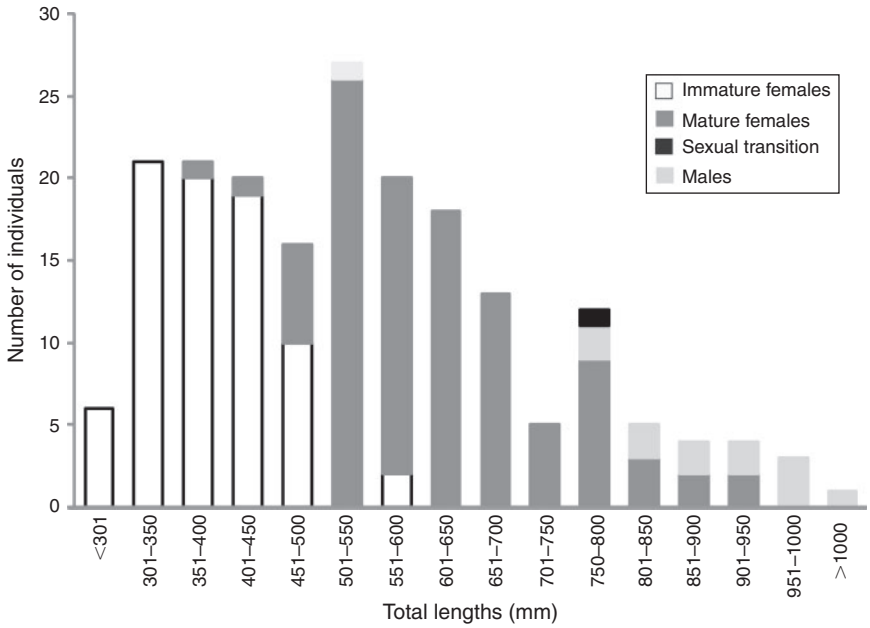

Fig. 2. Size frequency distributions (total length (TL), mm) of immature females, mature females, individuals in sexual transition and males of the dusky grouper (*Epinephelus marginatus*) obtained at the Carpinteiro Bank (CB) in southern Brazil. $n = 201$.

Results

Histological analysis of the gonads revealed that among the 201 individuals analysed, 184 were females, 16 were males and 1 was in sexual transition. On average, males were significantly larger (878.2 mm TL) than females (517.2 mm TL) (*t*-test: $P < 0.001$). Females ranged from 278 to 933 mm TL, with adult females being significantly larger (617.5 mm TL, $n = 106$) than immature females (383.5 mm TL, $n = 78$) (*t*-test: $P < 0.001$) (Fig. 2).

The sex ratio (male : female) in the sampled population was 1 : 11.5, which is significantly different from parity (Pearson's χ^2 test: $n = 200$, $\chi^2 = 1\,811\,500$, df = 11, $P < 0.0001$). When only adult females were considered, this ratio decreased by approximately half to 1 : 6.6, which is still significantly different from parity (Pearson's χ^2 test: $n = 122$, $\chi^2 = 7\,940\,500$, df = 11, $P < 0.0001$).

An examination of the female gonads revealed five ovarian stages: immature (F1), developing (F2), mature (F3), partially spawned (F4) and resting (F5) (Fig. 3). Immature females were characterised by small ovaries containing only oogonia and pre-vitellogenic ovarian follicles. The appearance of ovarian follicles showing cytoplasmic vesicles and/or yolk granules characterised the ovaries in development (F2), whereas a predominance of oocytes with yolk granules and/or hyalinised oocytes indicated mature ovaries (F3). The partially spawned stage (F4) was characterised by post-ovulatory follicles associated with vitellogenic follicles in different stages. After the spawning period, ovaries were found with disorganised ovigerous lamellae containing few vitellogenesis follicles but large amounts of pre-vitellogenic follicles and connective tissue, which together characterised stage F5. The occurrence of empty and post-ovulatory follicles suggests that the dusky grouper utilised the studied area for its reproduction. In contrast, an examination of the male gonads revealed four testicular stages: maturing (M1), mature (M2), partially spawned (M3) and spent (M4) (Fig. 4). Maturing (M1) (Fig. 4*a*) testes were characterised by several different groups of male germ cells (spermatogonia, spermatocytes and spermatids) in development, with occasional occurrence of sperm. Mature testes (M2) (Fig. 4*b*) were predominantly filled with sperm. At M3, the testes were partially filled, suggesting emptying of sperm during reproduction. Depleted testes were observed at stage M4 (Fig. 4*c*), which was characterised by seminiferous tubules with emptied spaces and the presence of spermatogonia. The gonad of the individual in sexual transition contained male germ cells and ovarian lumen.

Monthly frequencies of occurrence of gonads at different developmental stages indicated that developing ovaries began to occur in June, whereas maturing testes (M1) began to appear only in July. Spring, the period in which no male individuals were captured, was the season in which the highest frequency of females with mature ovaries was observed, whereas females with partially spawned gonads and males with partially spawned gonads were observed in greater abundances in the summer. Additionally, immature females and females with post-spawning gonads (the resting stage) were found in large numbers throughout the study period (Fig. 5).

The analysis of the mean monthly GSI corroborated the results of the distribution of gonadal stages (Fig. 6). The females showed statistically significant differences (K-W, $P < 0.05$) among the mean monthly GSI values during the study period, with smaller mean GSI values during the autumn and winter months, peaking between November and January (Fig. 6). The males presented the same pattern as that observed for the

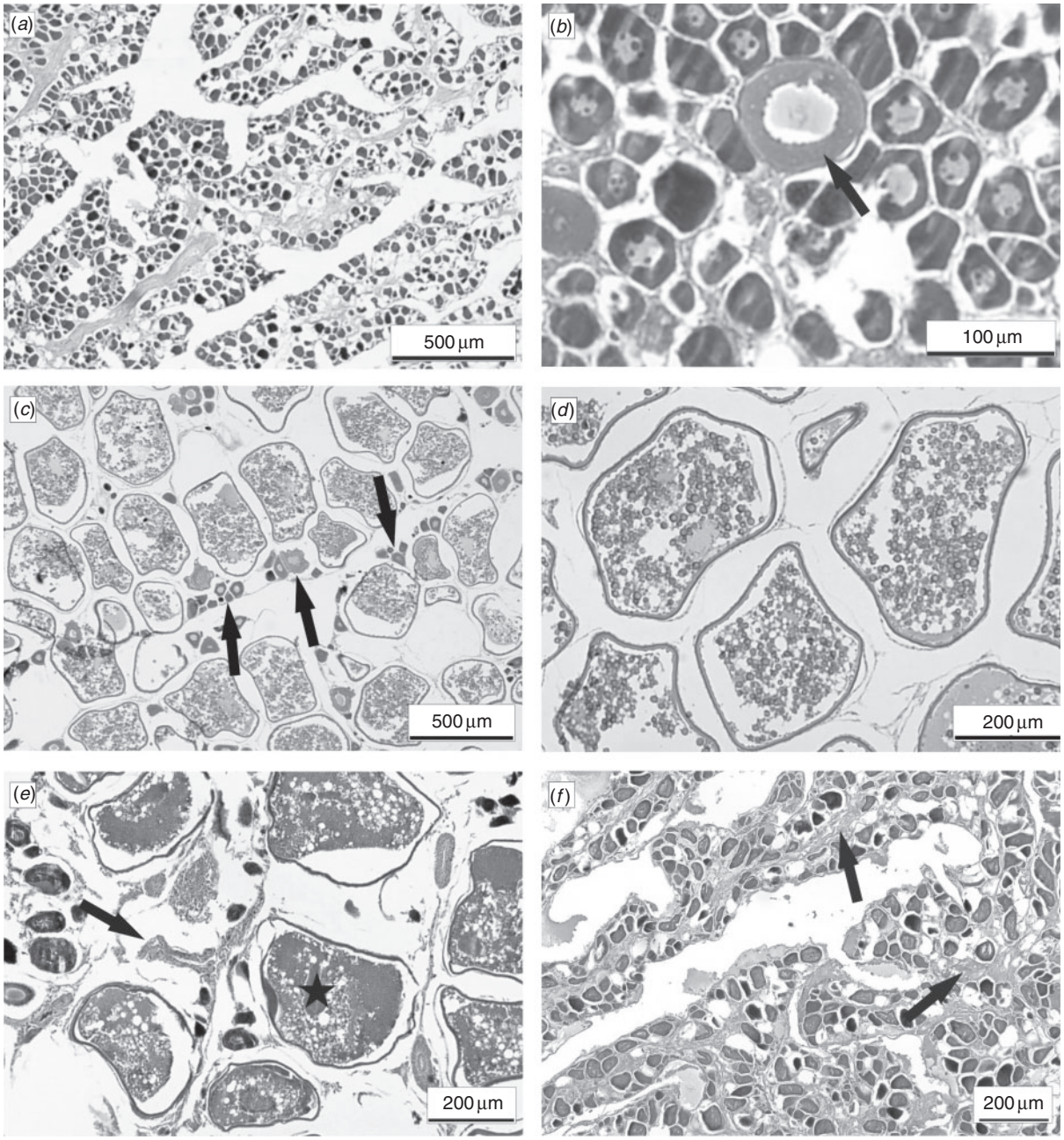

Fig. 3. Histological sections of hematoxylin-eosin (HE) stained ovaries of the dusky grouper (*Epinephelus marginatus*). (*a*) – Immature ovary containing ovigerous lamellae filled with previtellogenic ovarian follicles. (*b*) – Developing ovary, characterised by the presence of vitellogenic ovarian follicles (arrow) along with previtellogenic ovaries. (*c*) and (*d*) – Mature ovaries, with a predominance of ovarian follicles filled with yolk grains associated with the previtellogenic follicles (arrows). (*e*) – Partially spawned ovary, characterised by the presence of previtellogenic and vitellogenic follicles as well as an empty follicle (arrow). The star indicates a follicle undergoing hyalinisation. (*f*) – Recovered ovary, with previtellogenic ovarian follicles surrounded by a large quantity of connective tissue associated with muscle bundles (arrow) in the interior of the ovigerous lamellae.

females, with smaller GSI values in autumn and winter (Fig. 6). The largest individual GSI values were 7.79% for females and 0.57% for males. Thus, based on the histological analysis, it was possible to infer the monthly frequencies of occurrence of gonads at different developmental stages and the average seasonal GSIs. Additionally, it was possible to infer that the spawning period of *E. marginatus* occurs in late spring and summer, periods in which the frequency of individuals with mature and partially spawned gonads is highest.

In the present study, the estimated length at first maturation (L50) for females was 496 mm TL (Fig. 7). It was not possible to estimate the average length at sexual transition for the dusky grouper because of the low number of males in the sample.

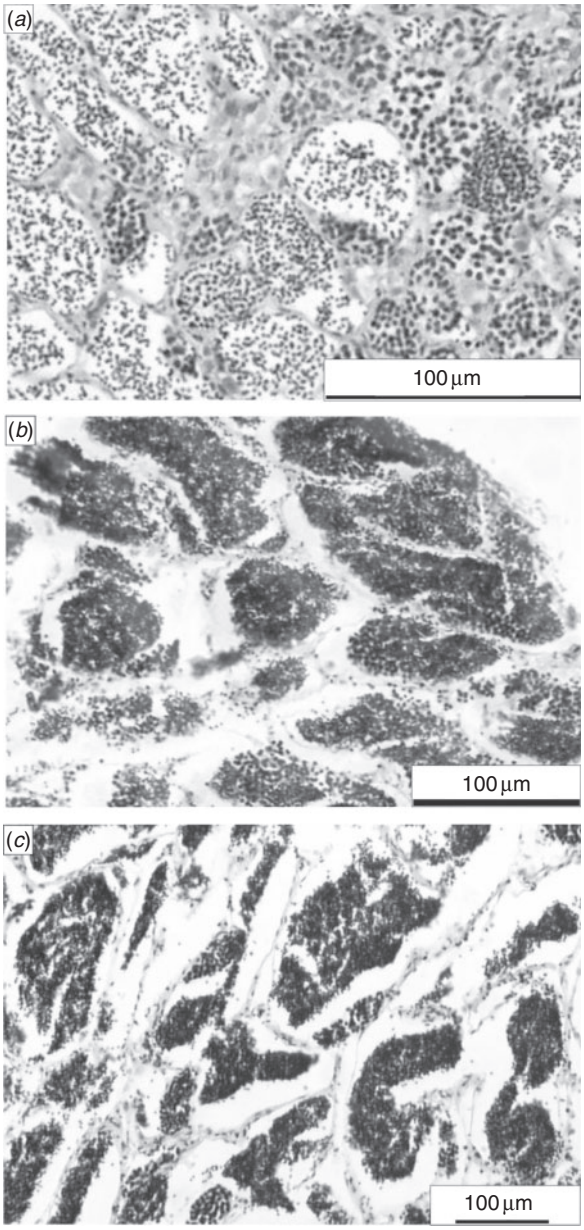

Fig. 4. Histological sections of hematoxylin-eosin (HE) stained testes of the dusky grouper (*Epinephelus marginatus*). (*a*) – Developing testis, characterised by the presence of different types of male germline cells. (*b*) – Mature testicle with the seminiferous tubules filled mainly by spermatozoids. (*c*) – Partially spawned testicle characterised by half-empty seminiferous tubules, showing a decrease in spermatozoids.

Discussion

Sexual pattern

The sex ratio (male : female) observed in the present study for the dusky grouper (1 : 6.6) was similar to that observed by Zabala *et al.* (1997*b*) and Reñones *et al.* (2010) (1 : 7.0 and 1 : 7.4, respectively) for two populations examined in Spain, and it was also similar to that reported by Fennessy (2006) in the African south-east (1 : 5.5). According to Coleman *et al.* (1996), species that use spawning aggregations are more susceptible to fishing activities and show an increase in the number of females compared with the number of males because fishing tends to capture larger individuals; therefore, males are removed first. However, both the studies performed by Zabala *et al.* (1997*a*, 1997*b*), which were conducted in a marine protected area (MPA), and the study by Reñones *et al.* (2010), who evaluated a population in an area that was open to fishing, showed similar sex ratios, which disagrees at first with the hypothesis of Coleman *et al.* (1996). However, the MPA studied by Zabala *et al.* (1997*a*, 1997*b*) was in existence for only 13 years at the time of the study. Considering that the dusky grouper population in this area was overexploited before the establishment of the MPA and that this is a long-lived species with slow growth, the sex ratio might not have re-stabilised at the time of the study and thus was similar to the ratios of Reñones *et al.* (2010).

As noted by Condini (unpubl. data), the discovery of only one individual undergoing sexual transition in the study area may be explained by the fact that not all of the females present in the population undergo sex reversal (Shapiro 1987; Coleman *et al.* 1996; Mackie 2003) as well as by the fast degeneration of the ovarian tissue and the fast development of the testicular tissue (Brulé *et al.* 2003*b*;). The number of individuals undergoing sexual transition was also small in the studies undertaken by Brulé *et al.* (2003*a*, 2003*b*), Fennessy (2006), Bustos *et al.* (2010) and Reñones *et al.* (2010). Dusky grouper individuals in the Atlantic Ocean have not been previously characterised as undergoing sexual transition (see Andrade *et al.* 2003; Gerhardinger *et al.* 2006; Seyboth *et al.* 2011).

Spawning season

Histological analysis of the gonads also indicated that the dusky grouper at the southern limit of its distribution in the south-western Atlantic is a partial spawner. This conclusion is corroborated by the presence of partially spawned ovaries and partially spawned testes, which has also been found in Mediterranean populations (Marino *et al.* 2001; Reñones *et al.* 2010).

The histological analysis of the ovaries and testes, along with the trends displayed by the mean monthly GSIs and the frequencies of occurrence of ovaries and testes at different stages of gonadal development, demonstrate that the spawning period of *E. marginatus* spans between late spring and summer, indicating that this population has a long spawning period similar to that of populations in the Mediterranean Sea (Marino *et al.* 2001; Reñones *et al.* 2010), the south-western Indian Ocean (Fennessy 2006) and the south-western Atlantic (Andrade *et al.* 2003). According to Garvey *et al.* (2002), a long spawning period ensures the recruitment of this species to areas with variable environmental factors because it increases the survival and growth of larvae and young-of-the-year.

In the northern hemisphere, the reproductive peak occurred in the summer months of July and August, which is slightly later than the early to mid-summer (i.e. December and January) spawning peak observed during the present study in the southern hemisphere.

Spawning aggregation behaviour of the dusky grouper in the southern region of the south-western Atlantic, as defined by Domeier and Colin (1997), was not observed in the present study. However, records from the Mediterranean Sea show that

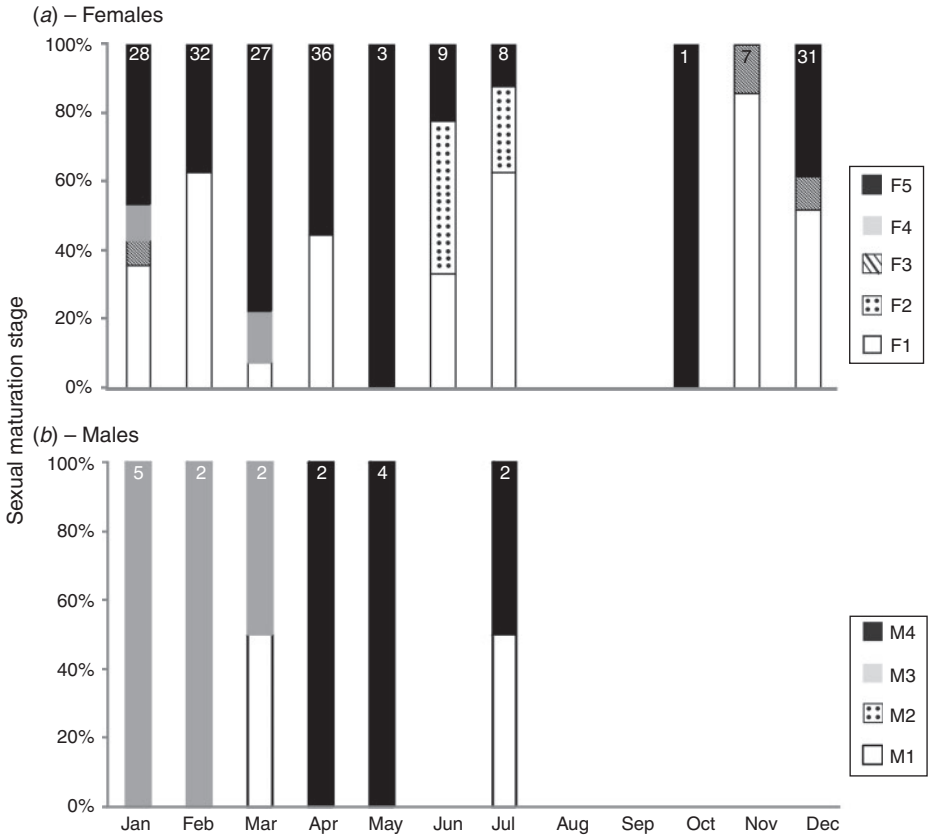

Fig. 5. Monthly frequencies of occurrence of sequential gonadal development stages in females (*a*) ($n = 182$) and males (*b*) ($n = 16$) of the dusky grouper (*Epinephelus marginatus*) at Carpinteiro Bank (CB) in southern Brazil. The ovarian stages are as follows: F1, immature; F2, developing; F3, mature; F4, partially spawned; F5, resting. The testicular stages of the males are as follows: M1, developing; M2, mature; M3, partially spawned; M4, spent.

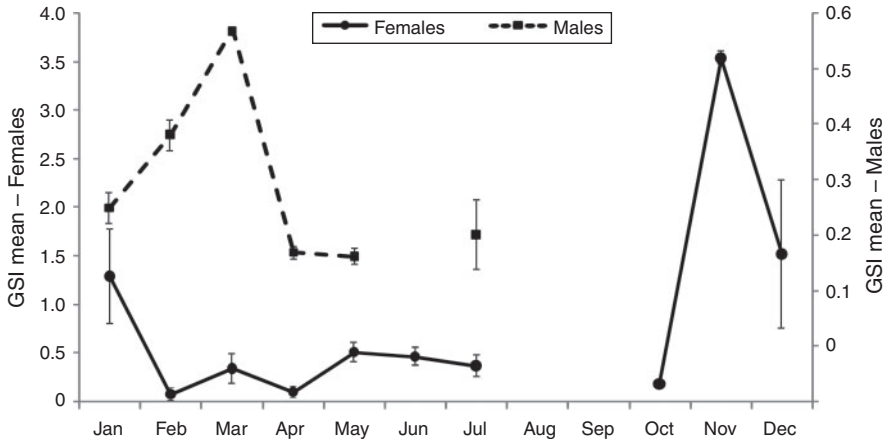

Fig. 6. Monthly average (±1 standard error) of the gonadosomatic indices (GSI) of dusky grouper (*Epinephelus marginatus*) females (*a*) and males (*b*) in the southern region of the south-western Atlantic.

this species forms spawning aggregations at depths of 20–30 m in June, July (Marino *et al.* 2001) and August (Zabala *et al.* 1997*a*, *b*). In the Mediterranean Sea, *E. marginatus* form harems, in which a single male is associated with many females (Zabala *et al.* 1997*a*, *b*). Although we did not obtain concrete evidence of spawning aggregations in the present study area, it is likely that these aggregations occur in CB because the histological analysis of nine individuals collected from the same fish landing on 13 January 2009, showed one mature (F3) female and three partially spawned (F4) females in addition to

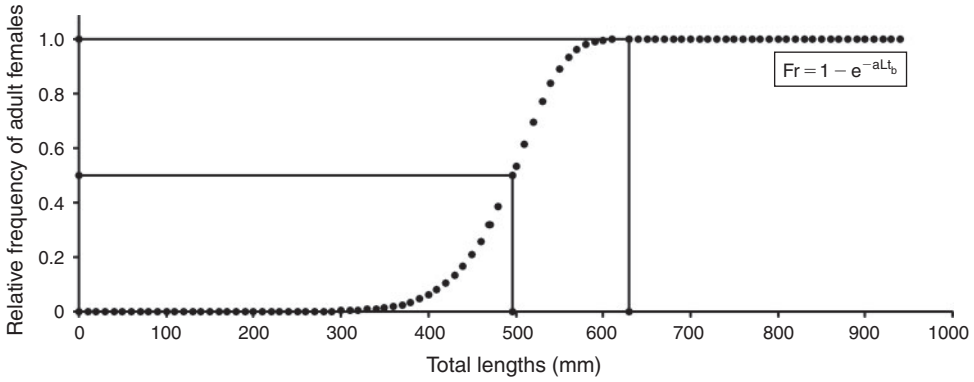

Fig. 7. The percentage of mature dusky grouper (*Epinephelus marginatus*) females in the different length classes in the southern region of the south-western Atlantic ($n = 182$). The sex ratio of mature females in each class is plotted as a logistic regression, with the line indicating the total length at which 50% of the females are mature (L50).

Table 2. Minimum size (Lmin) and mean (L50) of first maturation females; number of females, males and transitional individuals; sex ratio; and TL range of dusky grouper (*Epinephelus marginatus*) females and males sampled in different studies

Study	Local	Size at first maturation of females (cm)		Females (*n*)	Males (*n*)	Transitionals (*n*)	Sex ratio (M : F)	Range TL females (cm)	Range TL males (cm)
		Lmin	L50						
Kara and Derbal (1999)	Algerian coasts – Mediterranean	–	57.0	–	–	–	–	XX–72.0	79.5–XX
Marino *et al.* (2001)	Southern Mediterranean	36.7*	43.8*	321	59	25	1 : 3.5	24.5–97.0*	68.5–105.0*
Andrade *et al.* (2003)	South-western Atlantic	35.0	47.0	104	5	0	1 : 20.8**	19.7–79.5	80.0–100.2
Fennessy (2006)	South-western Indian	54.5	62.2	352	42	1	1 : 5.5	24.5–95.0	80.0–110.0
Reñones *et al.* (2010)	Western Mediterranean	38.6	49.1	346	43	10	1 : 7.4	6.6–100.3	58.4–105.6
Seyboth *et al.* (2011)	South-western Atlantic	34.3	45.1	111	0	0		26.0–80.0	
Present study	South-western Atlantic	39.1	49.6	184	16	1	1 : 6.6	27.8–93.3	51.5–116.0

*Marino *et al.* used standard length (SL) in their study.
**Sex ratio between number of males and females, disregarding the fact of the females are mature or not.

five partially spawned (M3) males. Additional large individuals that could not be sampled were also present on the ship (personal observation, MVC). Although the present study was not designed to determine the existence or location of these spawning aggregations, we understand that determining the occurrence, location and period of this event has great importance for the conservation of this species, which is highly vulnerable to fishing.

First maturity and sex reversal

In the present study, the female dusky grouper reached sexual maturity at 496 mm TL, which is approximately half of the maximum recorded length of 1200 mm (Heemstra and Randall 1993) that is characteristic of confamilial species. For example, females of *Mycteroperca microlepsis* and *Mycteroperca bonaci* both have L50s of 721 mm and attain maximum TLs of 1450 and 1500 mm, respectively (Brulé *et al.* 2003*a*, 2003*b*). However, the L50 of *Mycteroperca fusca* females (335 mm) is approximately half the maximum TL attained by this species (800 mm) (Bustos *et al.* 2010), suggesting that the sexual maturity of females is directly related to individual size in these species. The L50 recorded for the females in the current study was similar to the values observed in the Mediterranean Sea (Marino *et al.* 2001; Reñones *et al.* 2010), the south-western Atlantic along the coast of Santa Catarina (Andrade *et al.* 2003) and in a coastal area in close proximity to the region examined in the present study (Seyboth *et al.* 2011). However, distinct values were observed by Kara and Derbal (1999) in Algeria and by Fennessy (2006) in the south-western Indian Ocean (Table 2). According to Hunter *et al.* (1992), the method used to determine sex and

gonadal stage (microscopic or macroscopic identification), variation in sample number and variation in sample period may all influence the estimated length at first maturity. Therefore, the fact that Kara and Derbal (1999) used macroscopic analysis for sexual determination may explain the difference observed between their work and other studies.

On the basis of the studies performed by Marino *et al.* (2001) and Reñones *et al.* (2010), it is clear that dusky grouper in the Mediterranean Sea have shown a decrease in the magnitude of sex reversal over the past two decades. This decrease is most likely related to intensive fishing and overexploitation of the large males in the area, leading to a variation in the reproductive strategy, with sex reversal now occurring earlier in life. Previous studies have shown that some hermaphroditic species have a tendency to anticipate their sex reversal because of increased natural mortality rates (Gust 2004) or in response to increased mortality from fishing (Hawkins and Roberts 2004). In the present study, we observed only one male with a TL (515 mm) below the size reported in the literature (e.g. between 680 and 1100 mm TL in Bruslé and Bruslé (1975) and between 690 and 930 mm TL in Marino *et al.* (2001)). The absence of other males with similar lower-than-expected sizes precluded us from associating this finding with a possible sex reversal-dependent size reduction in the south-western Atlantic species.

Although one male was collected with a length that was similar to the average size of females at first maturity, no evidence of primary males was found in the histological analyses. That is, all the observed males originated from females and are therefore secondary males. These observations suggest that sex change is related to the social behaviour of this population, as observed for other grouper species (Shapiro 1987; Coleman *et al.* 1996; Mackie 2003).

Comparison between deep and coastal habitats in the southern region of the south-western Atlantic

Dusky grouper inhabiting rocky jetties in the littoral zone (<5 m deep) located ~16 nautical miles from the Carpinteiro Bank area (15–25 m deep), which were studied by Seyboth *et al.* (2011), showed different reproductive characteristics from those observed in the present study. Seyboth *et al.* (2011) noted that 52% of the individuals observed were immature females and 48% were females in the resting stage. No reproductive activity was detected in the littoral zone, and no males or individuals undergoing sexual transition were found (Table 2). In contrast, males, individuals undergoing sexual transition and females in different stages of sexual maturity were observed during the present study at Carpinteiro Bank. Therefore, these results confirm the hypothesis proposed by Seyboth *et al.* (2011), which states that the dusky grouper occurring in the far southern region of Brazil spawns over offshore rocky reefs. In this context, it appears reasonable to hypothesise that deeper rocky reefs (15–25 m) constitute better quality habitats than coastal rocky reefs (<5 m) for the dusky grouper in the extreme southern region of the south-western Atlantic. According to the source-sink metapopulation model, a lower quality habitat does not possess all the environmental prerequisites that the species needs to reproduce and complete its life cycle (i.e. it is a sink), and the habitat depends on the dispersal of individuals (larvae and/or juveniles) from higher quality habitats (sources) (Pulliam 2000). According to this model, the studied coastal region could be considered as a lower quality habitat when compared with deeper rocky reefs such as the Carpinteiro Bank.

Future studies examining the dispersal and movement of individuals between these two regions using techniques such as telemetry and otolith microchemistry should be performed to evaluate the hypothesis regarding the connectivity between the two areas. Moreover, future conservation plans to protect this endangered grouper should take into account the need to protect this important reproductive site from increasing and unregulated fishing pressure.

Acknowledgements

We thank Beatrice Padovani Ferreira, Luís Romano and Gonzalo Velasco for comments on the manuscript; Jorge Castello for assisting with infrastructure and sample processing; and Lauro Madureira for the 3D bathymetric map of the Carpinteiro Bank. The study received financial support from the Fundação O Boticário de Proteção à Natureza (0753–20072) and a fellowship from the 'Coordenação de Aperfeiçoamento de Pessoal de Nível Superior' (CAPES).

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