Reproductive pattern of an exploited dusky grouper *Epinephelus marginatus* (Lowe 1834) (Pisces: Serranidae) population in the western Mediterranean

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SUMMARY: The reproductive parameters of the dusky grouper, *Epinephelus marginatus*, in the Balearic Islands (western Mediterranean) were studied with a histological analysis of specimens caught by artisanal and recreational spear fisheries. The histological examination of the gonads showed that the dusky grouper spawning season extends from late spring to the end of summer with clear spawning peaks in July and August. The monthly variation of the hepatosomatic index (HSI) and the Le Cren condition index suggests that reserves for gonad development were obtained from the energy stored in the liver before spawning. Females reach maturation at 49 cm total length (TL) and 6 years, and transitional individuals start to occur before all females are matured. Males were the largest and oldest specimens but females were also present in the largest and oldest classes and there was a large sex, size and age overlap in the population. The fecundity data showed that the dusky grouper is a determinate spawner with asynchronous oocyte development. Potential fecundity ranged between 65 thousand and 8 million oocytes in females from 39 to 92 cm TL and from 6 to 42 years of age, with a mean relative fecundity of 334 x 10^3 oocytes kg^{-1}. Females spawn an average of 10 batches during the spawning season, with a relative batch fecundity of 75 x 10^3 oocytes kg^{-1}. Fecundity increased significantly with length, weight, age and HSI, while relative fecundity was only related to HSI. Based on the results obtained in the present study (long reproductive lifespan of females, influence of maternal attributes on fecundity and size at maturity) management measures for the species were evaluated.

Keywords: *Epinephelus marginatus*, maturity, sex change, spawning, fecundity, Mediterranean.

RESUMEN: PATRÓN REPRODUCTOR DE UNA POBLACIÓN EXPLOTADA DE MERO *EPINEPHELUS MARGINATUS* (LOWE 1834) (PISCES: SERRANIDAE) DEL MEDITERRÁNEO OCCIDENTAL. – Los parámetros reproductores del mero, *Epinephelus marginatus*, en aguas de las Islas Baleares (Mediterráneo Occidental) se han estudiado mediante análisis histológico en ejemplares capturados por la pesca artesanal y recreativa. El análisis histológico evidencia que la reproducción tiene lugar desde finales de primavera a finales de verano, observándose una máxima actividad reproductora en los meses de julio y agosto. La variación mensual de los índices hepatosomático (HSI) y de condición, sugieren que la movilización de reservas para el desarrollo gonadal se obtiene de la energía almacenada en el hígado, antes de la puesta. Las hembras alcanzan la madurez sexual a los 49 cm de longitud total (TL) y los 6 años de edad. Si bien los machos son los ejemplares de mayor talla y edad, existe un amplio solapamiento entre ambos sexos, pudiendo alcanzar las hembras las máximas tallas y edades observadas en la población. La mayor parte de los criterios utilizados para identificar el tipo de fecundidad indican que el mero presenta una fecundidad determinada y un desarrollo ovocitario asincrónico. La fecundidad potencial de hembras entre 39 y 92 cm TL y 6 y 42 años de edad, está comprendida entre 65 mil y 8 millones con una fecundidad relativa media de 334 x 10^3 oocitos kg^{-1}. La fecundidad potencial aumenta significativamente con la talla, peso, edad y HSI, mientras que la fecundidad relativa esta únicamente relacionada con el HSI. Durante la estación reproductora las hembras realizan como media unas 10 puestas, con una fecundidad relativa por batch de 75 x 10^3 ovocitos kg^{-1}. En base a los resultados obtenidos en este estudio (longevidad de las hembras, influencia de las características maternas en la fecundidad y talla de primera madurez) se evalúan y proponen distintas medidas de gestión para la especie.

Palabras clave: *Epinephelus marginatus*, madurez, cambio de sexo, puesta, fecundidad, Mediterráneo.

INTRODUCTION

Identifying the reproductive pattern of a fish species is essential for understanding its population dynamics and establishing suitable management measures. The timing and duration of spawning, size/age at maturation and fecundity can change in response to environmental factors and to growth and mortality rates of the population (Stearns and Crandall, 1984; Wootton, 1990; Sanchez-Lizaso *et al*., 2000; Gust, 2004; Hamilton *et al.*, 2007). In hermaphrodite species, the mating system and sex inversion are also considered to be adaptable and are probably the result of social interaction rather than genetic cues (Warner and Swearer, 1991; Mackie, 2003; Muñoz and Warner, 2003a; Munday *et al*., 2006). Age and size at maturation, fecundity and the mechanisms underlying sex change are fundamental variables that determine the reproductive potential of a population and its capacity to withstand exploitation (Trippel, 1999; Coleman *et al*., 1996).

The dusky grouper, *Epinephelus marginatus* (Lowe 1834), is a slow growing species that can reach 60 years of age (Reñones *et al.*, 2007) and more than 1 m in length. It has a wide latitudinal and depth distribution range, occurring from the British Isles to South Africa and from the Bermuda Islands to Argentina, and is widespread in the Mediterranean (Heemstra and Randall, 1993; Irigoyen *et al*., 2005). It inhabits shelter-rich, hard substrates from the surface to 250 m (Bruslé, 1985) and reaches maximum densities above 50 m depth (Harmelin and Harmelin-Vivien, 1999). Like other grouper species, the dusky grouper is considered to be overexploited in its entire distribution range and is included in the IUCN red list of threatened species (www.iucnredlist.org). Its biological characteristics and trusting behaviour, together with strong pressure exerted by tourism and technological advances in fishing techniques, have contributed to its overexploitation. As a consequence, in the last decade there has been growing interest in its reproductive ecology in order to i) establish suitable management measures and ii) reproduce groupers in captivity as a means of restocking depleted populations (Marino *et al.*, 2003; La Mesa *et al*., 2008).

The reproductive behaviour and mating system of the dusky grouper have been studied in protected populations in the north Mediterranean (Zabala *et al*., 1997a,b; Hereu *et al.*, 2006), while systematic studies of sexual patterns and sexual maturation of exploited populations have been carried out in the south Mediterranean (Bouain, 1980; Bouain and Siau, 1983; Bruslé, 1985; Kara and Derbal, 1999; Marino *et al.*, 2001), Atlantic (Bertoncini *et al.*, 2003) and Indian Ocean (Fennessy, 2006). These studies report that the dusky grouper is a monandric protogynous hermaphrodite species which reproduces at sunset during summer and spawns in pairs (one male with one female). Female size at maturity varies among populations, ranging between 47 and 62 cm total length (TL). Males start to occur from 68 to 80 cm TL, and are generally the largest individuals, although females are also present in the largest size classes in all the studied populations. Currently the main gaps in the knowledge of the reproductive parameters are the age at maturation, age at sexual transition, and fecundity data. It has been suggested that the dusky grouper attains maturity at an age of around 5 to 6 years (Chauvet, 1988; Fennessy, 2006). Fecundity has been estimated in only two individuals from the wild population (Bouain and Siau, 1983) and in induced spawning females in culture conditions (Marino *et al*., 2003). There is no information on the reproductive parameters for the populations in the western Mediterranean, an area that seems especially suitable for the species considering its recuperation rate in the Marine Protected Areas (MPA) (Coll *et al.,* 1999, 2007; Reñones *et al*., 1999; García Charton *et al*., 2004; Harmelin-Vivien *et al*., 2007).

This study examined the reproductive parameters of the exploited dusky grouper population in the western Mediterranean and analyzed several poorly studied aspects of its reproduction. The specific objectives were to study the maturation process of the dusky grouper, including the reproductive cycle, fecundity, and female size and age at maturation and at sex transition.

MATERIALS AND METHODS

Sampling

A total of 399 specimens of dusky groupers captured along the coasts of Mallorca and Menorca (Balearic Islands, western Mediterranean) from 1998 to 2004 were analyzed. The specimens came from two sources: 1) artisanal bottom longline and trammel net commercial fishery catches (n = 92), and 2) spear-fished groupers captured in experimental fishing and in the spear-fishing championships (n = 307). Experimental fishing was carried out in order to obtain specimens under the minimum legal size established for the species in the area (45 cm TL for the commercial fishery and 2 kg weight in the spear fishing championships). The commercial and spear recreational fisheries are seasonal, with most catches occurring in summer and autumn, corresponding to the maximum trophic activity of the species. These capture methods exploit different depths, although their limits overlap: the recreational spear fishery targets groupers from 0 to 40 m depth (Coll *et al.*, 2004), while the artisanal fishery targets groupers from 20 to 65 m depth (Mallol and Goñi, 2008).

For each specimen, the TL was measured to the nearest mm, the total weight (W) and somatic weight (Ws) to the nearest gram and the liver (Lw) and gonad (Gw) weight to the nearest mg. A portion of the gonads was taken, weighed and fixed in 10% buffered formalin for histological observations. Subsamples were taken from 12 specimens of the anterior, central and posterior region of the gonad to verify whether oocyte development was homogeneous throughout the gonad. As no significant differences were observed, only a portion of the central

part of the right lobe was taken for histological determinations. In individuals smaller than 15 cm TL, the gonads were small, thread-like structures attached to the swim-bladder wall and coated by the dorsal mesentery. As they were difficult to dissect, the entire swimbladder wall was removed, preserved and sectioned transversally in order to observe the gonad structure. Subsamples of ovaries with visible developing oocytes, i.e. with secondary growth oocytes, were stored in Gilson fluid for 12 months to be used later for fecundity estimations. Sagittal otoliths were removed, cleaned and stored dried or in distilled water for age estimation.

Histological samples of gonads were embedded in Paraplast Plus (Kendall), sectioned at 3-4 μm and stained with Mayer's haematoxylin and eosin for routine microscopic examination. We used the terminology employed by Grau *et al.* (1996) in the histological description of oocyte development. Gonads were histologically sexed (ovaries, testes and transitional gonads) according to a modification of the microscopic classification reported by Marino *et al.* (2001) and staged in 15 different developmental stages: 8 developmental ovarian stages, 3 intersexual stages and 4 developmental testicular stages (Table 1). Ovary maturity stages were determined based on the most advanced oocytes, the occurrence of postovulatory follicles (POF), atretic vitellogenic oocytes (AO) and the amount of lamellar stroma (Fig. 1d). The difference between stage F8 and F2, in the absence of clear evidence of prior spawning (presence of POFs), was established according to a combination of criteria that determine female spawning history in inactive ovaries, such as the thickness of the ovarian wall or tunica albuginea, the presence of melanomacrophage centres (MMC), the presence of muscle bundles (Fig. 1) and the abundance of stroma in the gonad. The presence of prominent intralamellar muscle and connective bundles around blood vessels has been widely employed as a diagnosis criterion of prior spawning in other grouper species (Shapiro *et al.*, 1993; Sadovy and Colin, 1995; Fennessy, 2006; Pears *et al.*, 2006). These structures, along with the thickness of the tunica albuginea, are indicative of the expansion and subsequent post-spawning collapse of the ovaries (Shapiro *et al.*, 1993). For transitional specimens, the gonad stages were based on the relative proportions of female and male tissues. Male gonad stages were based on male germ cell development and the presence of spermatozoa in the lumen of lobules or in sperm sinuses (Fig. 1e).

Ages were estimated from whole otoliths in specimens smaller than 20 cm TL and from sectioned otoliths in larger specimens according to the methodology described by Reñones *et al.* (2007).

Reproductive cycle, maturity and sex change

Histological determinations were used to establish sex, reproductive cycle, maturity and sex inversion. Specimens from different years and fishing methods were pooled by month for the different analyses due to the low sample size in relation to the age and size range attained for the species.

Females were considered mature from stage F3 to F8 and males from stage M1 to M4. The reproductive cycle was determined according to the maturity stages found throughout the year, and by the evolution of the gonadosomatic index (GSI = $100 \times Gw / (W - Gw)$), Le Cren condition index ($K_R = Ws / Wst$, where Wst is the somatic weight predicted from the length somatic-weight relationship) and hepatosomatic index (HSI = $100 \times Lw / Ws$). Only specimens over the size of the first mature female observed were considered. Analysis of variance (ANOVA) was used to test differences in K_R and HSI throughout the reproductive cycle and among gonad stages. Homogeneity of variance was tested using the Cochran test. The post-hoc comparison was tested using the Tukey test.

The relationship between spawning season and sea surface temperature (SST) was evaluated by linear regression of the percentage of spawning females (gonad stages F5 and F6) on mean monthly temperature during the study period. Data of females with ovaries containing hydrated oocytes and young POF, which indicate imminent or recent spawning, were taken as an indication of the timing of spawning events in relation to the lunar cycle.

Female length and age at 50% maturity (L_{50} and A_{50}) was estimated from the maturity ogive in the pre-spawning month (May) and during the spawning season. The proportion of mature females grouped into 5-cm length intervals or one-year increments were fitted to a logistic equation as described by Sparre and Venema (1992). A total of 196 females, ranging from 8.4 to 100.3 cm TL and from 0 to 52 years old, were included in the analysis. The sex change pattern was estimated by the length and age overlap between sexes in relation to the maximum size and age (Shapiro, 1987). To estimate length and age at sexual transition (Lsi_{50} and Asi_{50}), the proportion of females in each length or age class was fitted to a logistic equation as described above. The timing of sex change was analyzed by plotting the occurrence of transitional stages against the month.

Fecundity

The type of fecundity of a species determines the methodology for estimating it (Hunter *et al.*, 1992; Murua and Saborido Rey, 2003). Fecundity can be determinate or indeterminate (the number of eggs released during the spawning season is fixed or not fixed, respectively, prior to the onset of spawning). In determinate species, potential annual fecundity can be estimated by the number of vitellogenic oocytes prior to spawning, while in indeterminate species, annual fecundity should be calculated from batch fecundity, the percentage of females spawning per day (spawning fraction) and the duration of the spawning season (Hunter *et al.*, 1985).

TABLE 1. – Microscopic description of developmental gonad stages for females, transitionals and males of *Epinephelus marginatus*. Mean diameter of gametogenic stages (μm) and gonadosomatic (GSI), hepatosomatic (HSI) and Le Cren (K_R) indexes for the different gonad stages (± standard error) are indicated.

| Stages	Histological description
Females	
Immature stages	
Incompletely differentiated (F1)	Large ovarian cavity. Primary germ cells (PG), numerous oogonia (O) (11.4 ± 1.0) (nest of 4-6 cells) and chromatin-nucleolus oocytes (CN) (17.5 ± 4.3), together with some perinucleolar oocytes (PN) (55.3 ± 2.7), are seen in cortical region lining ovarian cavity. Scattered spermatogenic cysts can also be observed. GSI =0.08 (0.02); K_R = 1.01 (0.01); HSI = 1.33 (0.06)
Differentiated immature (F2)	Smaller ovarian cavity. More developed and closely packed lamellae with PG, abundant O and primary oocytes (especially at PN stage). Only scarce stroma along the axis of the ovigerous fold. Some spermatogenic cysts can be seen. No lipid globule stage (LG) or melanomacrophage centres (MMC) present. Ovary generally smaller in diameter than those of resting females. GSI = 0.11 (0.01); K_R = 1.01 (0.01); HSI = 2.0 (0.07)
Mature stages	
Developing (F3)	Numerous oocytes at LG (99.4 ± 2.7) and at cortical alveolus (CA) stage (146.9 ± 4.2) can be seen. Very few O and CN oocytes. PN oocytes are abundant. Rare spermatogenic cysts scattered in female tissue. LG and CA atresia can occasionally be observed. GSI = 0.59 (0.23); K_R = 0.97 (0.02); HSI = 2.34 (0.15)
Maturing (F4)	Batches of numerous secondary oocytes at yolk granule stage I (176.8 ± 4.2), II (225.4 ± 5.8) and III (407.2 ± 6.6). Very few O (or none) and less abundant previtellogenic oocytes and LG and CA oocytes. Rare spermatogenic cysts detectable at the periphery of the ovigerous folds. Some occasional oocyte atresia can be observed. GSI = 2.69 (0.36); K_R = 0.99 (0.02); HSI = 2.32 (0.16)
Ripe (F5)	One batch of oocytes in final maturation or hydrated (H) (548.5 ± 23.8) can be distinguished between groups of vitellogenic oocytes at different developmental stages. Few atretic vitellogenic oocytes (AO) can be observed. A large population of oocytes remain in the PG phase. No postovulatory follicles (POF) can be seen. The presence of mature oocytes suggests ovulation would have occurred within a few hours, so these fish are considered to be in spawning condition. GSI = 4.76 (0.76); K_R = 1.0 (0.02); HSI = 2.16 (0.22)
Spawning (F6)	Presence of POF, together with new batches of vitellogenic oocytes. Few AO, increasing only at the very end of the spawning period. A large population of oocytes remain in the PG phase. Ovarian cavity increases in size as spawning proceeds. GSI = 4.07 (0.57); K_R = 0.97 (0.02); HSI = 1.86 (0.10)
Spent (F7)	Wide central ovarian cavity. Empty and irregular ovigerous lamellae with numerous AO and POF, together with residual healthy yolked oocytes. A large population of resting oocytes present. GSI = 0.83 (0.10); K_R = 1.00 (0.02); HSI = 1.34 (0.19)
Resting (F8)	PG, abundant O and primary oocytes, mainly located at the periphery of the ovigerous fold. Some nests of spermatogonia. Many large MMC often present within the abundant stroma, especially along the axis of the ovigerous lamellae. Lipid vesicle stage oocytes present. Evidence of prior spawning activity is apparent in the form of a thick ovarian wall, the presence of MMC, the abundance of lamellar stroma and, sometimes, the presence of POF near the end of spawning period and the presence of muscle bundles. GSI = 0.41 (0.04); K_R = 1.00 (0.02); HSI = 1.30 (0.12)
Transitionals	
Early transitional (T1)	Predominance of female germinal tissue and 10-20% of developing testicular tissue. Abundant stroma in ovigerous folds. GSI = 0.48 (0.14); K_R = 1.04 (0.10); HSI = 1.42 (0.34)
Bisexual (T2)	30-70% testicular tissue not yet organized into lobules. Many MMC typically present. GSI = 0.37; K_R = 1.00; HSI = 2.34
Late transitional (T3)	10-20% of residual ovarian tissue scattered in testicular tissue, which is almost completely rearranged into lobules. Muscle layers in the gonad wall form empty spermatic sinuses. Abundant MMC. GSI = 0.43 (0.01); K_R = 1.02 (0.04); HSI = 1.58 (0.21)
Males	
Maturing (M1)	Lobules containing spermatogenic cysts in all developmental stages. No sperm within the sperm sinuses. GSI = 0.20; K_R = 1.13; HSI = 2.65
Ripe (M2)	Lobules containing cysts of male germ cells in all developmental stages. Spermatozoa (SP) free in the lumen. Little or no sperm in sperm sinuses. GSI = 0.31 (0.03); K_R = 1.00 (0.03); HSI = 2.17 (0.19)
Spawning (M3)	Numerous free SP in the large lumen of lobules and in the spermatic sinuses. GSI = 0.37 (0.03); K_R = 0.99 (0.03); HSI = 1.91 (0.16)
Resting (M4)	Some free residual SP within lobule lumen and sperm sinuses. Little spermatogenic activity and abundant cysts of spermatogonia inside the lobules. GSI = 0.18 (0.03); K_R = 1.05 (0.03); HSI = 1.66 (0.13)
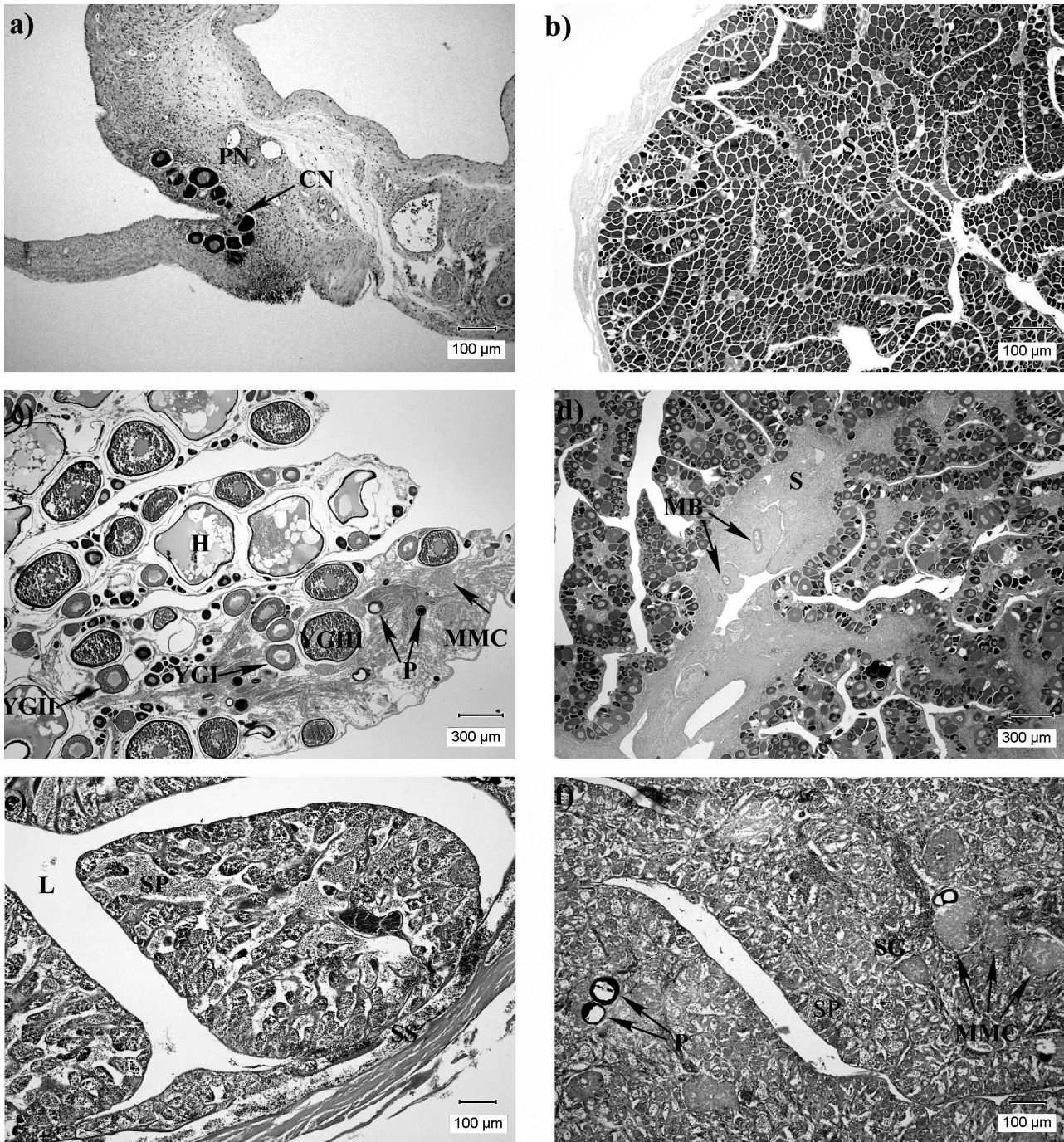
FIG. 1. – Sections of female and male *Epinephelus marginatus* gonads. a) Longitudinal section of an incompletely differentiated immature ovary (F1). Note the thread-like appearance, with previtellogenic oocytes (chromatin-nucleolus (CN) and perinucleolar (PN) stages) in the cortex and abundant stroma. b) Cross-section of a developing-immature ovary (F2), showing numerous previtellogenic oocytes inside closely packed lamellae and only scarce stroma (S) on the axis of the ovigerous fold. Ovarian lumen is very reduced. c) Cross-section of a ripe ovary (F5) showing a very advanced batch of hydrated oocytes (H) and new batches of vitellogenic oocytes (yolk granule (YG) stages I, II, and III). Melanomacrophage centres (MMC) and cysts of parasites (P) could also be seen. Note the abundance of stroma and the absence of female germ cells around the parasites. d) Cross-section of a resting ovary (F8), showing muscle bundles (MB), abundant stroma on the axis of the ovigerous fold, and postovulatory follicles (POF). Numerous previtellogenic oocytes can be seen. e) Cross-section of spawning testes (M3), showing numerous free spermatozoa (SP) in the lobule lumen (L) and inside spermatic sinuses (SS). f) Cross-section of resting testes (M4), showing free residual spermatozoa within the lobule lumen, many MMC , cysts of parasites and abundant cysts of spermatogonia (SG).

Following West (1990) and Murua and Saborido Rey (2003), five lines of evidence were used to identify the type of fecundity: i) development of a hiatus separating the stock of vitellogenic oocytes from the previtellogenic oocytes in the oocyte size-frequency distribution; ii) variation in the proportion of vitello-

genic oocytes in the oocyte size-frequency distribution during the spawning season; iii) variation in the mean diameter of advanced vitellogenic oocytes in spawning females during the spawning season; iv) incidence of atresia of mature oocytes at the end of spawning; and v) the presence of spent ovaries in the population. Oocyte size-frequency distributions were determined from histological sections, as this technique allows developmental characteristics to be associated with any given oocyte size class (West, 1990). Oocyte measurements were performed on maturing and spawning ovaries from 6 females (63 to 70 cm TL) captured in 1998. In order to obtain a true representative oocyte count with the minimum bias towards a particular oocyte size, counts were made of all oocytes present in 5 microscopic fields using a 4x objective. Oocyte size, obtained by taking the mean and maximum diameter, was only recorded for those oocytes that had been sectioned through the nucleus (Foucher and Beamish, 1980). Measurements ranged from 328 to 761 oocytes per gonad (mean 525 ± 150 SD). The nucleus increases relatively little in size with growth, and the bias towards larger cells is minimized (Foucher and Beamish, 1980); therefore, a correction factor was not applied to the observed oocyte size frequency measured in histological sections, because the purpose of our analysis was only to compare oocyte size-frequency distributions between individuals and not to quantify oocyte abundances.

As fecundity in the dusky grouper is determinate (see results), the potential annual fecundity (Fp), defined as the number of mature oocytes per female in a reproductive season uncorrected for atretic losses (Hunter *et al*., 1992), was estimated by a gravimetric method in females in stages F4 and F5. In order to eliminate primary growth oocytes and tissue debris, the ovaries preserved in Gilson fluid were filtered through a 100-µm mesh and weighed. Only cortical alveolus and vitellogenic oocytes were counted. Six random subsamples of about 0.5% of total filtered ovarian weight were taken and the oocytes were counted under a dissecting microscope. The average of the six counts was used to estimate Fp and relative fecundity (number of vitellogenic oocytes per kg of somatic weight). Analysis of covariance (ANCOVA) was carried out to determine whether the capture method and parasitic infestation of the gonads by the nematode *Phylometra jordanoi* have any influence on fecundity. It has been indicated that infection of the ovaries by *Phylometra* species can have a detrimental effect on reproduction (Hesp *et al*., 2002; Moravec and Justine, 2005). The relationship between fecundity and somatic (TL, Ws, age) and physiological (HSI and K_R) variables was analysed by simple regression analysis. Multiple stepwise regression analysis was used to identify which predictor variables influence fecundity. The variables entered in the model were significant at p<0.05 and removed if p>0.1,due to the high correlation between them. The predictors and dependent variables, except

the HSI and K_R, were log-transformed to achieve normality and homogeneity of variances.

Batch fecundity (BF) was estimated in four ovaries that showed hydrated oocytes but no POF or other evidence of extended spawning activity (such as collapsed ovarian wall, general organization of lamella, high number of primary growth oocytes and oocyte atresia). Batch fecundity was estimated using the hydrated oocyte method (Hunter *et al*., 1985), which consists in extrapolating the number of hydrated oocytes in a subsample to the total ovary weight. In these samples the standing stock of secondary growth oocytes (i.e. the yolked oocytes still remaining in the ovary) was also estimated. Hydrated oocytes were counted manually in 4 subsamples (150 mg). For these individuals the number of developing oocytes was also estimated.

RESULTS

Length and age structure of sampled population

The sampled *E. marginatus* ranged from 6.6 to 105.6 cm TL, 4 g to 22.3 kg total weight and 0 to 60 years of age. Females were collected all year round, while transitional specimens and males were present only in the samples from April to October. The absence of males and transitional specimens in late autumn and winter is due to the seasonality of the fisheries; thus, specimens sampled during these months came from experimental spear fishing with a maximum size of 55 cm TL.

Of the 399 individuals, 346 were females, 10 were transitionals and 43 were males. Females ranged from 6.6 to 100.3 cm TL (mean 44.0 cm $\pm$ 17.2 SD), 4 g to 19.8 kg total weight (2.1 kg $\pm$ 2.3) and 0 to 52 years of age (6.8 years $\pm$ 7.3). Transitional specimens ranged from 52.1 to 76.9 cm TL (63.0 cm $\pm$ 8.0), 2.2 to 6.5 kg weight (4.5 kg $\pm$ 1.6) and 7 to 17 years old (11.0 years $\pm$ 3.5), and males from 58.4 to 105.6 cm TL (83.2 cm $\pm$ 14.7), 3.1 to 22.3 kg (11.3 kg $\pm$ 5.7) and 7 to 60 years old (32.3 $\pm$ 17.6). The length and age frequency distribution indicated that males were the largest and oldest individuals in the sampled population (Fig. 2); however, females were also present in the largest size and age groups. Females were on average smaller and younger than transitionals (length: $t_{0.05\ (1),\ 11.8} = -7.1$, p<0.001; Age: $t_{0.05\ (1),\ 9.2} = -3.0$, p=0.01) and males (length: $t_{0.05\ (1),\ 57.1} = 15.9$, p<0.001; Age: $t_{0.05\ (1),\ 33.6} = 8.0$, p<0.001).The length and age frequency distribution of transitionals and males also showed significant differences, and transitionals were generally smaller and younger (length: $t_{0.05\ (1),\ 25.6} = 5.8$, p<0.001; Age: $t_{0.05\ (1),\ 38.6} = 6.3$, p<0.001).

Considering only specimens over the size of the first mature female observed, the sex ratio (M:F) of the population sampled from the spear recreational fishery was 1:7.4. The sex ratio of samples from the artisanal fishery was not estimated as the sampling size structure was not representative of the landings.
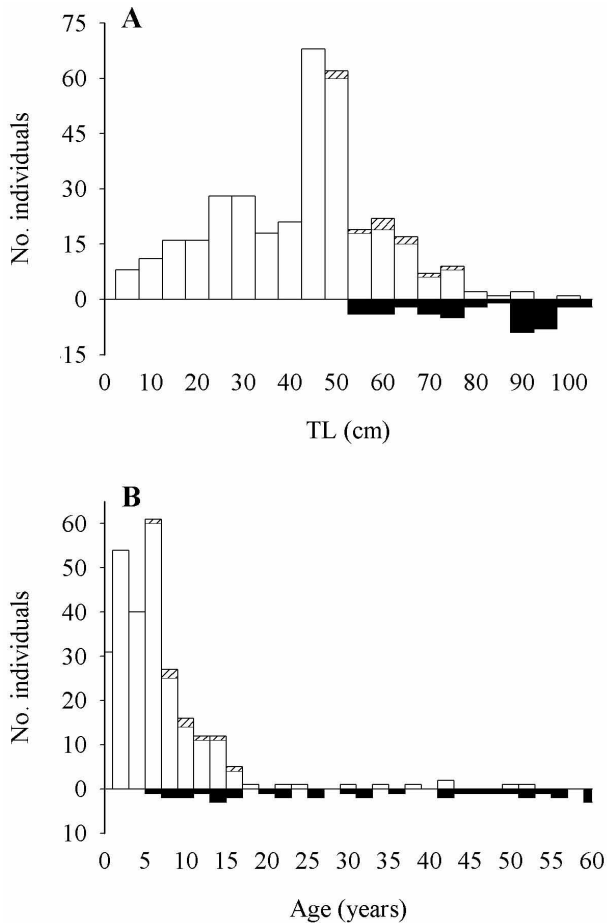
FIG. 2. – Length (A) and age (B) frequency distributions for females (white bars), transitionals (striped bars) and males (black bars) of *Epinephelus marginatus*. Data grouped by 5 cm TL and 2 years.
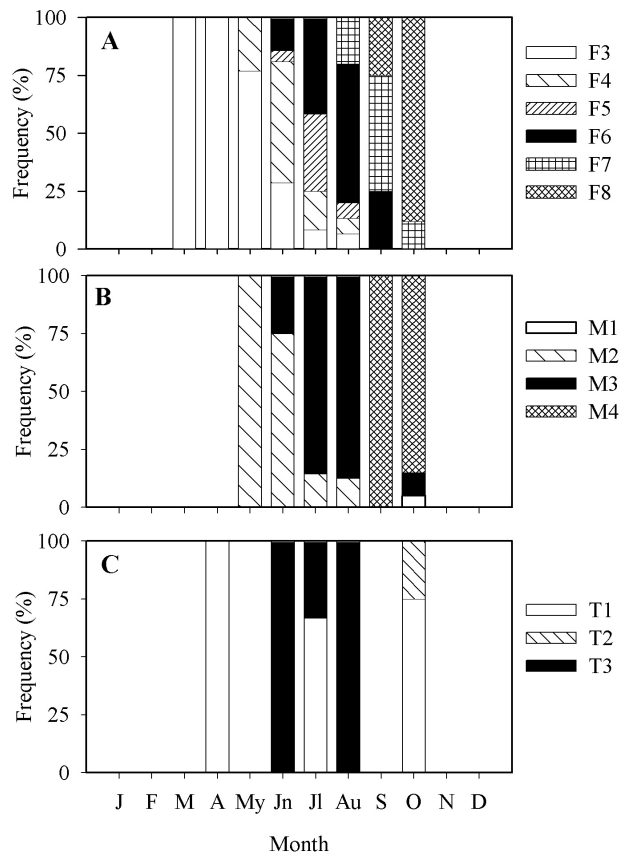

FIG. 3. – Monthly evolution of percentage of mature gonad stages for females (A), males (B) and transitionals (C) of *Epinephelus marginatus*. Female gonad stages are: F3: developing, F4: maturing, F5: ripe, F6: spawning, F7: spent, F8: resting; male stages are: M1: maturing, M2: ripe, M3: spawning, M4: resting; and transitional stages are: T1: early transitional, T2: bisexual, T3: late transitional.

Reproductive cycle, maturity and sex change

The monthly evolution of different gonad stages indicates that the reproductive cycle is asynchronous at population level (i.e. not all the individuals are at the same gonad development stage at the same time). Females have vitellogenic oocytes from March to October (Fig. 3). Females with developing and maturing ovaries were initially observed in March and May, respectively. The percentages of females in these initial ripening stages decreased progressively after May, but they were present in the population until the beginning of August, when together they represented around 13% of mature females. The spawning season, defined by the presence of females with hydrated oocytes and/or POF (F5 and F6), extends from early June to the middle of September with a peak in July (75%) and August (67%). Females with spent gonads were first observed at the end of August (20%), while resting individuals started to appear in September. In October the number of resting individuals rose sharply to become 90% of mature females. The absence of resting females from November to February could be mainly due to the size range sampled during these months and the difficulty

outside the reproductive period of distinguishing between differentiated immature (F2) and resting (F8) ovaries of small females previously involved in a small number of spawning events. Thus, during these months only 22% of females analyzed were over the size at first maturity (see below) with a maximum size of 55 cm TL. The presence of prominent intralamellar muscle and connective bundles around blood vessels was only observed in a reduced number of resting (36%), developing (23%) and maturing (11%) ovaries.

Males showed slightly wider spawning activity, which extended from early June to October (Fig. 3b). The highest percentage of spawning males was found in July (85%) and August (87%).

There were few females undergoing sexual transition in the sampled population (10 individuals). Transitional specimens were captured from spring to autumn (Fig. 3c), but the absence of transitionals during winter could be attributed to the size range sampled during these months, as indicated above. The 6 individuals with initial transition gonads (T1) were observed in April, July and October, and late transitional and bisexual individuals were observed during and at the end of the spawning season respectively.
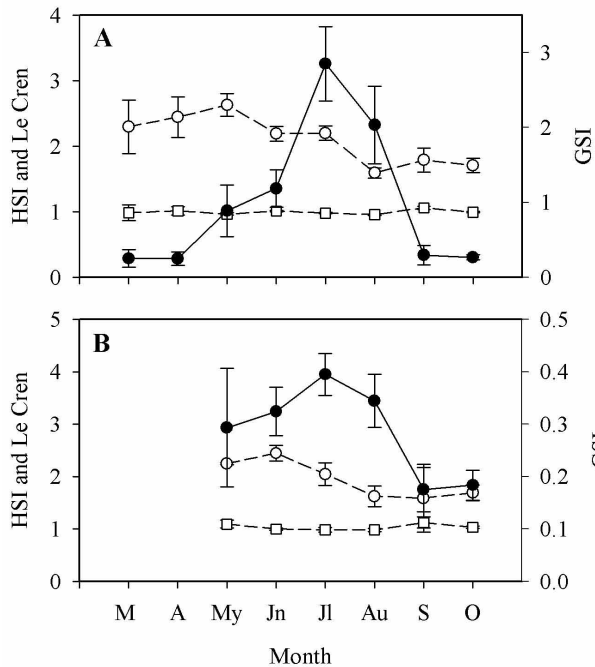
FIG. 4. – Monthly mean (± SE) gonadosomatic index (GSI) (●), hepatosomatic index (HSI) (○) and Le Cren index (□) for females (A) and males (B) of *Epinephelus marginatus*.

The spawning pattern identified histologically in females and males seems to be corroborated by the seasonal variability in the GSI. In Females, the mean GSI remains low (<0.5%) from March to April, increases in May and reaches the maximum values in July and August (>2%), which are the months with the highest proportions of ripe and spawning females (Fig. 4a). In males, seasonality was not so pronounced as in females. The GSI showed maximum values in July and August, which declined sharply afterwards (Fig. 4b).

The HSI fluctuated over the year almost in parallel with the GSI but following the opposite trend (Fig. 4). In females, the HSI increased from March to May (>2.5%), prior to the onset of spawning activity, decreased over the spawning season and reached minimum values from August to October. In contrast, the K_R annual change was low and only a slight increase was observed in September. A similar trend in both indexes was observed in males (Fig. 4b). Monthly differences in the HSI were significant for females (HSI: $F_{7, 115} = 9.80$, $p<0.001$), but not for males ($p>0.05$). Females had a significantly higher HSI in May than from August to October. Neither males nor females had seasonal differences in K_R. The HSI differed significantly among gonad stages for females ($F_{7, 338} = 6.58$, $p<0.001$). The highest values were observed in developing, maturing and ripe females (>2%), with a decreasing trend from spawning to resting individuals (Table 1). A similar trend was observed in males but it was not significant ($p=0.1$), with maturing and ripe males showing the highest HSI values (Table 1). K_R did
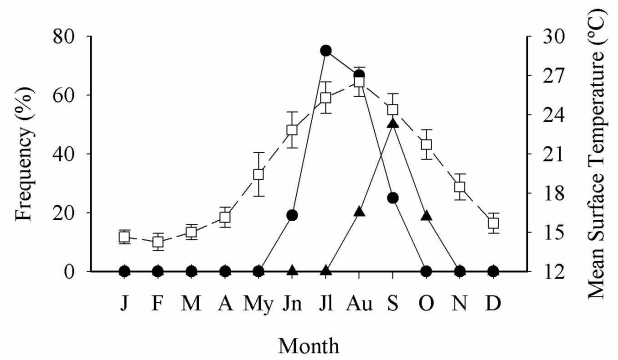

FIG. 5. – Monthly percentage of ripe and spawning (●) and spent (▲) vitellogenic gonads of *Epinephelus marginatus* females and monthly mean sea surface temperature (□) (± SD) during the sampling period in the study area.

not differ significantly between development stages in either of the two sexes ($p>0.05$) (Table 1).

Spawning activity, as indicated by the maturity stages, was related to sea surface temperature ($F_{1, 10} = 18.45$, $p=0.002$, $r^2 = 0.65$). Spawning took place in the months when the mean SST was over 23ºC (Fig. 5). Females with gonads containing hydrated oocytes occurred throughout the lunar month (37% in the full moon, 13% in the new moon and 50% in the half moon).

The smallest and youngest mature female was 38.6 cm TL and 5 years old while the largest and oldest immature female was 54 cm TL and 7 years old. Size and age at 50% maturity were estimated as 49.1 cm TL (lo-
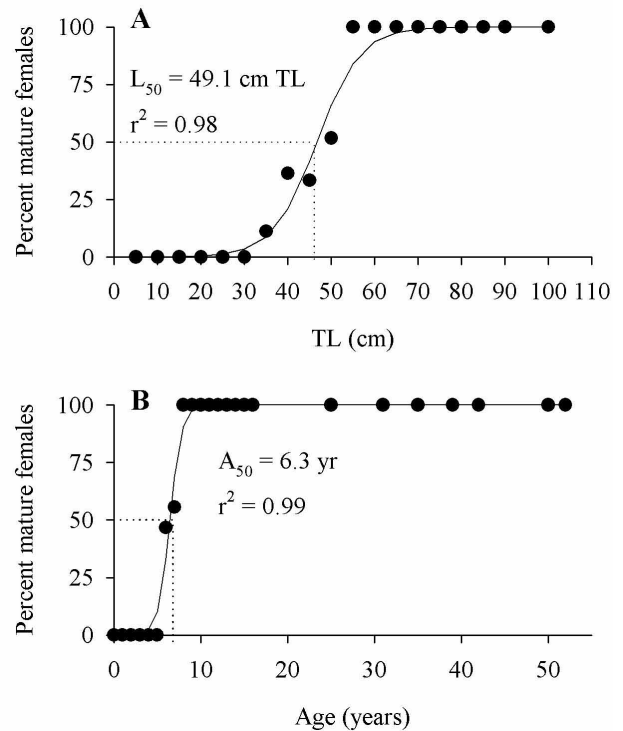

FIG. 6. – Percentage of mature females per length (A) and age (B) classes of *Epinephelus marginatus*. Dashed lines indicate estimates of size and age at 50% maturity.
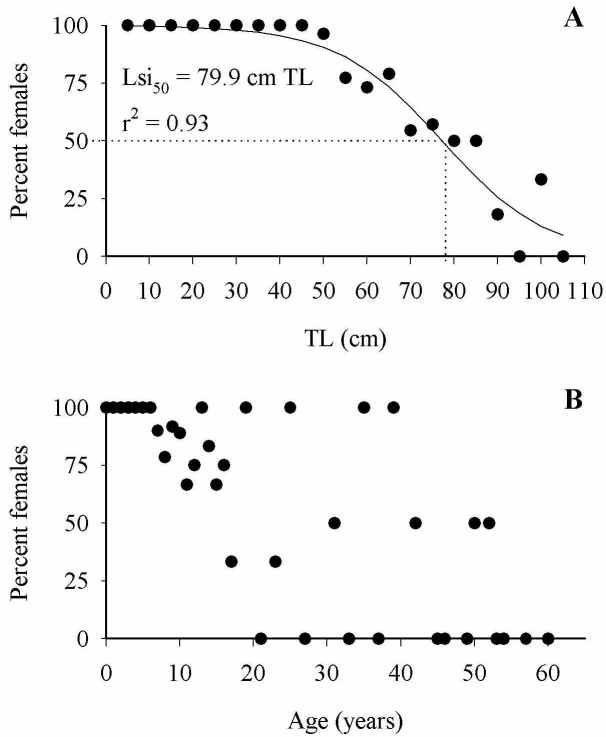
FIG. 7. – Percentage of *Epinephelus marginatus* females per length (A) and age (B) class. Dashed lines indicate 50% of females by length increments of 5 cm TL.

gistic model 95% CI = 47.3 – 51.0 cm) and 6.3 years of age (logistic model 95% CI = 6.1 – 6.4 years) (Fig 6).

Transitionals and males started to occur in the samples from 52.1 cm TL and 58.5 cm TL respectively and from 7 years of age for both sexes. The transitional range was 25% for length and 19% for age of the maximum female size and age. The overlap between females and males extended from 58.5 to 100.3 cm TL (Fig. 2), which corresponds to 40% of the maximum size. The age overlap is wider, and extends from 7 to 52 years, which represents 75% of the maximum age observed for the species. Fitting the logistic model to the proportion of females yielded an Lsi_{50} of 80 cm TL (logistic model 95% CI = 76.2 - 83.5 cm) (Fig. 7). The evolution of the proportion of females per age class did not significantly fit a logistic curve and Asi_{50} could not be estimated (Fig. 7).

Fecundity

The oocyte size-frequency distribution of the dusky grouper showed an asynchronous pattern before spawning (Fig. 8a), i.e. when no hydrated oocytes or oocytes in the migratory nucleus stage were present. However, a bimodal ovarian organization was observed during the spawning season (Fig 8b, c) with a relatively synchronous population of "large" vitellogenic oocytes and a more heterogeneous population of smaller oocytes from which the clutches were recruited. Therefore, there was a hiatus between previtellogenic and vitellogenic

oocytes, although it was only evident at the end of the spawning season (Fig. 8c). The proportion of vitellogenic oocytes (oocyte diameter >170 mm) decreased significantly ($F_{1, 3}$ = 54.1, p=0.005) as the spawning season progressed. However, mean vitellogenic oocyte diameter did not change over the spawning season ($F_{1, 3}$ = 0.3, p=0.6). A generalized prevalence of atresia at the end of the spawning season was never observed in the histological sections. Moreover, the presence of flaccid, empty and bloodshot appearance spent ovaries, with atretic and residual healthy vitellogenic oocytes, was observed in August, which marks the beginning of the post-spawning period. These evidences indicate that oocyte development is asynchronous, but fecundity is determinate.

Fecundity was estimated in a total of 39 females with sizes ranging from 38.6 to 91.5 cm TL and ages between 6 and 42 years. The Fp ranged between 65424 (in a females of 40.5 cm TL) and 7984835 oocytes (in
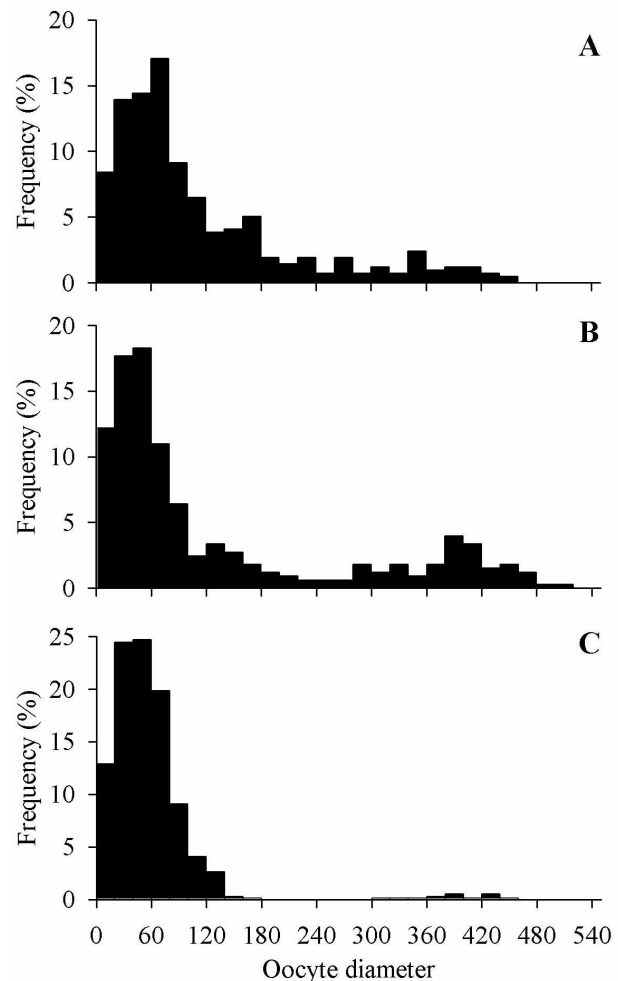

FIG. 8. – Size frequency distribution of oocyte diameter grouped per 20 μm from histological sections of 3 *Epinephelus marginatus* females. A: a 69.5 cm TL female captured in the middle of June, maturity stage 4, B: a 64.7 cm TL female captured in the middle of July, maturity stage 6; C: a 63.5 cm TL female captured at the end of August, maturity stage 6. Number of oocytes measured A: 416, B: 328, C: 593.

TABLE 2. – Results of the significant regression analysis for the effects of total length (TL), somatic weight (Ws), age, Le Cren index (K_R) and hepatosomatic Index (HSI) on potential (Fp) and relative (Fr) fecundity of *Epinephelus marginatus*.

Fecundity	F	p	r^2
$Fp = 0.14 \, LT^{3.82}$	47.47	<0.001	0.57
$Ln \, Fp = 3.71 + 1.23 \, Ln \, Ws$	50.67	<0.001	0.58
$Ln \, Fp = 10.43 + 1.30 \, Ln \, Age$	17.83	<0.001	0.37
$Ln \, Fp = 12.08 + 0.83 \, HSI$	13.00	0.001	0.26
$Ln \, Fr = 11.70 + 0.40 \, HSI$	5.72	0.02	0.13

a female of 87 cm TL), although the number of vitellogenic oocytes in females of similar length, weight, age and conditions indexes varied greatly, as shown by the relatively low r^2 (Table 2, Fig. 9). We investigated the potential sources of this variability and found that females collected from spear-fishing showed significant highest Fp (ANCOVA, $F_{1,33} = 7.15$, p<0.01) than those collected from the artisanal fishery (Fig. 9). Parasitic infestation rate has apparently no relevance on this variability (ANCOVA, $F_{1,33} = 2.38$, p=0.11); however, within the samples collected from the spear-fishing, infestation rate showed a significant impact on fecundity (ANCOVA, $F_{1,11} = 5.0$, p<0.05), i.e. infested females showed lower Fp. The number of developing oocytes was significantly and positively related to all morphometric characteristics and to the HSI (Table 2). The TL and Ws explained the highest part of the variance, 57 and 58% respectively. K_R did not have a significant effect on fecundity (Table 2). The relationship between

Fp and TL was best described by a power equation, while the relationship with the other variables was linear. Stepwise regression analysis indicated that only TL and the HSI significantly determined fecundity ($F_{2,36} = 28.53$, p<0.001), and they explained 61% of the total variance:

$$Ln \, FP = -0.69 + 3.31 \, LnTL \, (cm) + 0.40 \, HSI \, (r^2 = 0.61)$$

Relative fecundity ranged between $42.9 * 10^3$ and $913.1 * 10^3$ (mean $334.1 \times 10^3 \pm 196.4 \times 10^3$ SD) and were recorded from females of quite similar sizes (67 and 71 cm TL). Relative fecundity increased significantly with the HSI, although the variance explained was low (13%). The TL, Ws, age and K_R were not significantly related to relative fecundity (p>0.05) (Fig. 9b).

Batch fecundity ranged between 306 and 475 thousand hydrated oocytes (mean 400806 ± 78.3 SD). BF was positively and highly related to female length and somatic weight:

$$BF = -203566.4 + 8554.5 \, TL \, (cm) \, (r^2 = 0.75)$$

$$BF = 6193.7 \, Ws^{0.4841} \, (g) \, (r^2 = 0.77)$$

However, in spite of the high r^2, these relationships were not statistically significant (p=0.14 and 0.13 respectively) due to the low sample size (only four specimens).

Relative batch fecundity yielded a mean of $75.3 \times 10^3 \pm 16.3 \times 10^3$ (SD) hydrated oocytes per kg of gutted female. For these four females, the number of developing oocytes ranged from 2.7 to 5.1×10^6 for females between 63.7 and 80.2 cm. These values are similar to the Fp estimated as above. Therefore, the number of batches to be spawned (i.e. the number of developing oocytes divided by batch fecundity) was estimated to be between 8.9 and 11.3. The number of batches was positively correlated with size ($r^2 = 0.66$).

DISCUSSION

Epinephelus marginatus has been fished for more than 10000 years in the western Mediterranean (Desse and Desse-Berset, 1999). In the study area the decreasing numbers of this species caught in the recreational spear-fishing championships (Coll *et al.*, 2004) and differences in abundance and biomass between the MPA and fished areas (Coll *et al.*, 1999, Reñones *et al.*, 1999; García-Charton *et al.*, 2004; Harmelin-Vivien *et al.*, 2007) were evidences of the overfished status of the *E. marginatus* population in the shallow range of its distribution. The reported landings of the commercial fishery have remained around 5 Tm from 1996 to 2007 (Local fishery statistics) but with an increasing contribution over time of captures from areas open exclusively to the artisanal fishery (Reñones, 2007). No information is available on the catches of the recreational spear fishery.
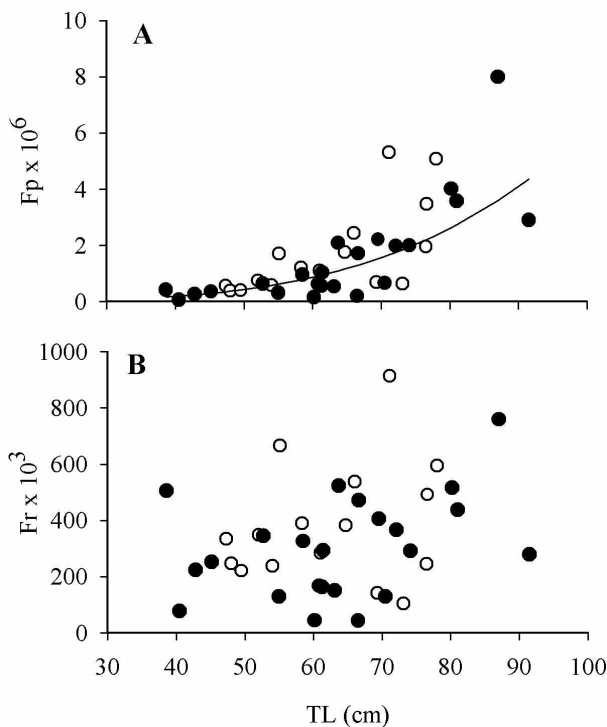

FIG. 9. – Relationship of potential (A) and relative (B) fecundity with TL (cm) of *Epinephelus marginatus* specimens from artisanal (●) and spear recreational (○) fisheries. The values of the relationship are given in Table 2.

Reproductive cycle

The histological analysis showed that *E. marginatus* has asynchronous ovarian development, i.e. oocytes of all developmental stages are present without dominant cohorts (Murua and Saborido-Rey, 2003). This finding contradicts the findings in the study by Marino *et al*. (2001), who found this species to be group-synchronous. However, this seems to be a different interpretation of the ovarian organization as their study shows the coexistence of several cohorts of developing oocytes (Marino *et al*., 2003). Developing oocytes are recruited and ovulated in several batches over a relatively protracted spawning season that, in the study area, extends from the end of spring to the end of summer with clear spawning peaks in July and August. Batch spawning and a protracted spawning season at population level, also observed in previous studies (Bouain and Siau, 1983; Marino *et al*., 2001; Bertoncini *et al*., 2003; Fennessy, 2006), seems to be related to the constancy of the environmental factors (Allain, 2001). In contrast, Garvey *et al*. (2002) suggest that protracted reproduction at the population level may increase expected recruitment in variable environments, and influence growth and survival over multiple life stages.

The spawning peak is also shown by the GSI, which has often been used to delimit the spawning season (Wilk *et al*., 1990). However, simply observing the GSI in the dusky grouper suggests a shorter spawning season (from July to September) than that found with histological data. Therefore, although the GSI provided a good approximation of the spawning season, the duration of the season could only be determined accurately from histological data (Alonso-Fernandez *et al*., 2008; Grau *et al*., 2009). In addition, the presence of a GSI peak and the sharp decrease in the index indicate quick egg loss without replacement, which corresponds to a batch spawning determinate species (see below). In contrast, in indeterminate species, the GSI curve is either dome-shaped or has no trend at all because egg release occurs at the same time as oocyte recruitment, and therefore the ovary weight only changes slightly over the spawning season (Wilk *et al*., 1990; Domínguez-Petit, 2007; Alonso-Fernandez *et al*., 2008).

The spawning activity seems to be clearly synchronized with temperature at a population level, as it occurs when the SST is above 23ºC. This suggests that a SST threshold is probably a critical environmental cue for spawning, which was also indicated by Hereu *et al*., (2006), and could explain the differences in timing and duration of spawning over the geographic range of the species. In areas where the SST is over 23ºC for more than 3 months, the spawning season is similar to that observed in this study (Bouain and Siau, 1983; Marino *et al*., 2001; Bertoncini *et al*., 2003). However, spawning in northerly waters is more than one month later and only half as long (Pelaprat, 1999; Hereu *et al*., 2006). In tropical areas, ripe females are present in the population for about 8 months (Fennessy, 2006).

For tropical grouper species the relationship between spawning and lunar phases has been related to the reproductive pattern (Sadovy *et al*., 1994). Thus, species that aggregate to spawn show a short spawning season related to lunar phases, while non-aggregating species, such as the dusky grouper, show longer spawning with no apparent lunar component. As expected, in this study moon phases did not influence spawning activity, which is also in accordance with previous studies (Hereu *et al*., 2006). The duration of the spawning period can thus be explained by temperature stability and the reproductive pattern.

In many fish species, the HSI and condition index are inversely associated with the GSI (Alonso-Fernandez *et al*., 2008). In this study, the HSI, but not the condition index, had the inverse seasonal pattern to the GSI in both sexes, and the HSI showed a decreasing trend from developing to spent gonads. This pattern, together with the influence of the HSI on fecundity (see below), suggests that mobilization of reserves for gonad development and thus for egg production would be dependent on energy stored in the liver prior to spawning. Temporal differences in feeding intensity, behaviour and metabolic rate may be responsible for the stability in condition over the year. The spawning season of the dusky grouper coincides with the maximum water temperatures, and the increase in feeding intensity during the reproductive season could compensate for the high energetic cost of both the higher metabolic rates and the reproductive effort (temporal dimorphism, polygamy, male territorial defence and elaborate courtship; Zabala *et al*., 1997a).

Maturity and sex inversion

Age and size at 50% maturity of *E. marginatus* females were estimated as 6 years old and 49 cm TL, respectively. The result for L_{50} was similar to that estimated with histological analyses for the south of Sicily (43.8 cm standard length [SL], corresponding to 51 cm TL; Marino *et al*., 2001) and south-east Brazil (47 cm TL; Bertoncini *et al*., 2003), but lower than that reported for Argelia (57 cm TL; Kara and Derbal, 1999) and south-east Africa (58-62 cm TL; Fennessy, 2006), which used macroscopic and histological criteria, respectively. The dusky grouper is a long-lived, slow growing species with a highly variable growth pattern (Reñones *et al*., 2007). As the onset of maturity is sensitive to condition and growth during the juvenile stage (Trippel, 1995), spatial differences in growth related to food supply, environmental condition and population density result in females maturing at different sizes. Moreover, estimates of size and age at 50% maturity depend on the methodology used (macroscopic vs. histological criteria), number and size range of the sample, and the sampling period considered in the estimations (Hunter *et al.,* 1992).

While maturation takes place within a relatively narrow size and age range (39-54 cm TL and 5-7

years), sex change occurred over a broad size and age range (52-77 cm TL and 7-17 years). Females showed a long reproductive lifespan, and were found in the largest and oldest size and age classes recorded in this study. Transitional specimens started to occur slightly above the size and age of females at 50% maturity and before 100% of females were mature. However, no evidence of primary males was observed in the histological analysis. Functional males were also present at younger ages (7 years) and at quite small sizes (58 cm TL) and represented 50% of the population above 80 cm TL. In other populations sex change also occurs over an extended length range (Marino *et al.*, 2001), and populations often differ in lengths and ages at which sexual transition occurs. These differences between populations, together with the extended overlap between sexes within each population suggest that sex change is socially mediated rather than genetic as shown in other grouper species (e.g. Coleman *et al.*, 1996; Harris *et al.*, 2002; Mackie, 2003). The relative constancy of the sex-ratio in shallow habitats between the exploited and protected populations (1:7.4 in the present study and 1:7, Zabala *et al.*, 1997b) also supports the hypothesis that sex change is socially mediated for this species.

Interestingly, the size at which sexual transition occurs was considerably lower in this study than in other Mediterranean areas, such as the south of Sicily (Marino *et al.*, 2001), where sex change was recorded from 69 to 93 cm SL (corresponding to 80 to 107 cm TL). This may indicate that large males are overfished in the study area, which would favour a sex change at smaller sizes. The sex allocation theory predicts that individuals should change sex when their reproductive value increases (Warner, 1975). Based on the size-advantage hypothesis, sex change should occur early and/or at smaller sizes in social groups with slower growth rates or higher mortality rates (Warner, 1988). The smaller and younger transitional and male specimens observed in our samples came from recreational spear fishing, which has the highest impact on the species, although spear-fished groupers showed higher growth rates than those captured by the artisanal fishery (Reñones *et al.*, 2007). These results, together with the presence of transitionals and males with sizes and ages close to those of females at first maturity, suggest a population response in the size/age at sex change to high fishing mortality. A shift towards smaller sizes and earlier ages for sex change has been reported for hermaphrodite species both as a result of elevated natural predation rates in the absence of fishing (Gust, 2004) and due to increased fishing mortality (Hawkins and Roberts, 2003; Hamilton *et al.*, 2007).Females in the largest and oldest classes have been observed in all the dusky grouper populations studied. Their presence has been attributed to a genetic inability to undergo sex change or lack of environmental cues (Marino *et al.*, 2001). However, recent manipulative and theoretical studies provide evidence that the sex change process may be more complex than predicted by the size-advantage model (Mackie, 2003; Muñoz and Warner, 2003a,b). Muñoz and Warner (2003a,b), working with *Sparisoma radians*, show that the largest female in a group will not increase its reproductive value by changing sex if the combined fecundity of the other females in the group is less than her current fecundity and/or if sperm competition is high. In these situations large females may leave sex change to smaller females. Specific studies are necessary to identify the pattern and factors that affect the sex change process in this species.

The timing of the sex change was difficult to assess as there were few transitional specimens in the population. When the sex change is socially mediated it occurs in relation to the social system and the aggregation spawning behaviour of the species (Shapiro *et al.*, 1993; Coleman *et al.*, 1996). Zabala *et al.*, (1997b) followed a complete reproductive cycle of a protected *E. marginatus* population and found that the two sexes co-occur at least from April to December. Therefore, the sex change could potentially occur at any time during that period. Transitional specimens in our samples were found from April to October, which is consistent with the time of "coexistence" indicated by these authors.

Fecundity

Among the evidence for assessing the type of fecundity of a species, the development of a hiatus in the oocyte size frequency distribution during spawning could be considered as the most conclusive evidence of determinate fecundity (Hunter *et al.*, 1992; Murua and Saborido-Rey, 2003). In *E. marginatus* the oocyte size frequency distribution did not have a well developed hiatus between previtellogenic and vitellogenic oocytes, except at the end of spawning. It is not rare, however, to find species with determinate fecundity and asynchronous oocyte development, which would prevent the occurrence of the aforementioned hiatus (Greer-Walker *et al.*, 1994; Murua and Saborido-Rey, 2003; Alonso-Fernández *et al.*, 2008). In this study, the decreasing relative standing stock of vitellogenic oocytes in spawning females due to new oocytes not being recruited after every batch episode, together with the absence of a generalized prevalence of atresia towards the end of spawning and the presence of post spawning spent ovaries, provided further evidence of determinate fecundity. However, the diameter of vitellogenic oocytes did not increase over the spawning period, as would be expected in a species with determinate fecundity (Hunter *et al.*, 1992). This feature has normally been reported for typical determinate species, i.e. species with group-synchronous oocyte development, while asynchronous oocyte development species may show no trend in oocyte size over the spawning season (Alonso-Fernandez *et al.,* 2008).

The relative batch fecundity of the dusky grouper in this study was $75.3 * 10^3$ eggs kg^{-1}. The ratio between

the number of developing oocytes and batch fecundity had an average value of 9.7. Assuming that the dusky grouper is a determinate spawner, a female would spawn an average of 10 batches during the spawning season. This is a very similar value to the maximum (9) obtained in captive-reared experiments (Marino *et al*., 2003), which also indicates that fecundity in the dusky grouper is determinate.

Batch fecundity in wild dusky groupers was estimated for the first time. The resulting values were lower than the number of ovulated eggs obtained in captive-reared experiments (Marino *et al*., 2003), although hormone induction may have increased egg production in each batch event. The number of potential offspring that a dusky grouper female can produce in a spawning season was influenced by the maternal attributes and the HSI. Thus, potential fecundity increased exponentially with size and linearly with weight and age. However, females with similar body size and weight showed very different fecundity values. Part of this variability was due to the effect of the liver index on fecundity. Thus, for a given length/age the potential fecundity increased as the energy surplus stored in the liver before spawning increased. The reproductive effort (number of vitellogenic oocytes per body gram) did not change over the reproductive lifespan, and only the energy stored in the liver seems to have a significant effect on it. It has been reported for several species that reproductive success depends on female condition, which in turn depends on environmental factors. Differences in individual condition (liver index) have been related to differences in habitat quality, in terms of food availability, habitat type and temperature, among others (Wootton, 1990; Lloret and Planes, 2003). The relative fecundity estimated in the present study was in the same order as that found by Bouain and Siau (1983) for the Tunisian population (312 eggs g^{-1}) and lower than that reported for *E. polyphekadion* (1350 eggs g^{-1}) (Rhodes and Sadovy, 2002), which is the only other determinate *Epinephelus* species for which fecundity has been estimated. Species with asynchronous oocyte development normally show little dependence on energy storage because egg production depends more on food assimilation during the spawning season (Domínguez-Petit, 2007), as food and temperature are not limiting factors during this season. This strategy is therefore found more often in temperate and tropical waters than in cold waters, and is normally associated with indeterminate fecundity. While in the study area, the food and temperature should favour indeterminate fecundity in the dusky grouper, the energetically costly reproductive effort, as mentioned above, may support determinate fecundity and hence the relevance of energy storage, in this case in the liver.

However, the liver index alone did not explain the high variability observed in fecundity. Although potential fecundity was estimated in ovaries without postovulatory follicles, the possibility remains that some females had already initiated the spawning season and therefore some batches had already been released. Nevertheless, the highest potential fecundity, disregarding size effect, was observed among females caught by spear-fishing, and thus samples from the commercial fishery generally showed the lowest fecundity. This is important as it may show a spatial difference in biological performance due to either depth or differential fishing pressure that produces different stress. High exploitation rates alter fish density as well as social behaviour, and thus favour early maturation as well as modifying the sex change rate, as described above, which has a clear impact on reproductive potential, including fecundity (Trippel, 1999). More research in this direction is necessary.

Management

The effects of maternal attributes on reproductive potential (age, size and HSI), fecundity and the strategy of the species to keep females to the largest and oldest classes highlight the importance of maintaining a wide length/age distribution and relatively high number of spawners in the larger/older year-classes in order to protect the stock reproductive potential. In addition, bathymetric differences in growth and size/sex distribution (Zabala *et al*., 1997b; Reñones *et al*., 2007) and reproductive performance (this study), together with the lack of knowledge on recruitment dynamics, indicate that management measures should be taken to protect the species over its depth distribution range. In the present study we have only demonstrated that female size/age and condition (HSI) have an effect on the number of offspring produced, but it is becoming widely recognized that for long-lived species, such as the dusky grouper, maternal attributes also affect the quality of the progeny and subsequent recruitment (Heath, 1992; Marteinsdottir and Begg, 2002; Berkeley *et al*., 2004a,b; Birkeland and Dayton, 2005), which highlights the importance of having a significant proportion of older fish in the population.

A slot limit (minimum and maximum capture limits) is a management method that could help protect the largest/oldest individuals of the population. However, the effectiveness of this management measure will depend on the rate of survivorship of released individuals (this has to be estimated for the species), the total fishing mortality exerted and the level of compliance with the regulation. The management measures implemented in the area for the dusky grouper, which are based on size and bag limits, have done little to protect the species: first, the agreed minimum landing size of 45 cm TL is under the size at 50% maturity of females, as shown in this study; second, bag limits are ineffective in reducing fishing mortality if effort (number of fishers) is not controlled; and third, the degree of compliance with these management measures is very low, as indicated by differences in demographic structure between protected and fished populations (Reñones *et al*., 1999; Coll *et al*., 2007). The spear recreational

and artisanal fisheries that target the dusky grouper are scattered over a wide area, which makes it difficult to effectively apply measures based on size or bag limits. Therefore, technical measures that prevent growth and recruitment overfishing must be complemented with a reduction in fishing mortality. In accordance with the results obtained in this study, the minimum landing size should be increased to 50 cm TL and a maximum landing size should be established at 80 cm TL to protect the largest/oldest members of the population, as has been done for other populations of large serranids (Pears *et al.*, 2006). At the same time, fishing should be banned in designated protected areas (MPA), as this has been shown to be the best management strategy for protecting the *E. marginatus* population all around the Mediterranean (e.g. Sanchez-Lizaso *et al.*, 2000; Garcia-Charton *et al.*, 2004; Harmelin-Vivien *et al.*, 2007) as well as other long-lived hermaphrodite species.

ACKNOWLEDGEMENTS

REFERENCES

Allain, V. – 2001. Reproductive strategies of three deep-water benthopelagic fishes from the northeast Atlantic Ocean. *Fish. Res.*, 51: 165-176

Alonso-Fernández, A., R. Domínguez-Petit, M. Bao, C. Rivas and F. Saborido-Rey. – 2008. Spawning pattern and reproductive strategy of female pouting *Trisopterus luscus* (Gadidae) on the Galician shelf of north-western Spain. *Aquat. Living Resour.*, 21: 383-393.

Berkeley, S.A., M.A. Hixon, R.J. Larson and M.S. Love. – 2004a. Fisheries sustainability via protection of age structure and spatial distribution of fish population. *Fisheries*, 29: 23-32.

Berkeley, S.A., C. Chapman and S.M. Sogard. – 2004b. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. *Ecology,* 85(5): 1258-1264.

Bertoncini, A.A., L.F. Machado, M. Hostim-Silva and J.P. Barreiros. – 2003. Reproductive biology of the dusky grouper *Epinephelus marginatus* (Lowe, 1834). *Bra. Arch. Biol. Technol.,* 46: 373-381.

Birkeland, C. and P.K. Dayton. – 2005. The importance in fishery management of leaving the big ones. *Trends Ecol. Evol.,* 20(7): 356-358.

Bouain, A. – 1980. Sexualité et cycle sexuel des mérous (Poissons, Téléostéens, Serranidae) des côtes du sud tunisien. *Bull. Off. natn. Pêch. Tunisie,* 4(2): 215-229.

Bouain, A. and Y. Siau. – 1983. Observations of the female reproductive cycle and fecundity of three species of groupers (*Epinephelus*) from the southeast Tunisian seashores. *Mar. Biol.,* 73: 211-220.

Bruslé, J. – 1985. Expose synoptique des donnés biologiques sur les mérous *Epinephelus aeneus* (Geoffroy Saint Hilaire, 1809) et *Epinephelus guaza* (Linnaeus, 1758) de l'océan Atlantique et de le Méditerranée. *FAO Synopsis sur les pêches,* 129: 1-64.

Chauvet, C. – 1988. Etude de la croissance du mérou *Epinephelus guaza* (Linné, 1758) des côtes tunisiennes. *Aquat. Living Resour.*, 1: 277-288.

Coleman, F., C.C. Koenig and A. Collins. – 1996. Reproductive style of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. *Env. Biol. Fish.*, 47: 129-141.

Coll, J., A. Garcia-Rubies, J. Moranta, J. Stefanni and B. Morales-Nin. – 1999. Efectes de la prohibició de la pesca esportiva sobre l'estructura poblacional de l'anfós (*Epinephelus marginatus* Lowe 1834, Pisces: Serranidae) en el Parc Nacional de Cabrera. *Boll. Soc. Hist. Nat. Balears*, 42: 125-138.

Coll, J., M. Linde, A. Garcia-Rubies, F. Riera and A.M. Grau. – 2004. Spear fishing in the Balearic Islands (west central Mediterranean): species affected and catch evolution during the period 1975-2001. *Fish. Res.*, 70: 97-111.

Coll, J., A. Garcia-Rubies, G. Morey, O. Navarro, S. Martino, F. Riera and A.M. Grau. – 2007. The north of Minorca Marine Reserve (W-Mediterranean) as a tool for a suitable management of natural resources. The case of the dusky grouper *Epinephelus marginatus* (Lowe, 1834). In: P. Francour and J. Gradiot (eds.), *Second International Symposium on the Mediterranean Groupers*, May 10-13th 2007, pp. 55-58. Nice University Publication, Nice.

Desse, J. and N. Desse-Berset. – 1999. Préhistoire du mérou. *Mar. Life*, 9(1): 19-30.

Domínguez-Petit, R. – 2007. *Study of reproductive potential of* Merluccius merluccius *in the Galician shelf*. Ph.D. thesis, Univ. Vigo.

Fennessy, S.T. – 2006. Reproductive biology and growth of the yellowbelly rockcod *Epinephelus marginatus* (Serranidae) from South-East Africa. *Afr. J. mar. Sci.,* 28(1): 1-11

Foucher, R.P. and R.J. Beamish. – 1980. Production of nonviable oocytes by pacific hake (*Merluccius productus*). *Can. J. Fish. Aquat. Sci.*, 37: 41-48.

García-Charton, J.A., A. Perez-Ruzafa, P. Sánchez-Jerez, J.T. Bayle-Sempere, O. Reñones and D. Moreno-Lampreave. – 2004. Multi-scale spatial heterogeneity, habitat structure, and the effect of marine reserves on Western Mediterranean rocky reef fish assemblages. *Mar. Biol.,* 144: 161-182.

Garvey, J.E., T.P. Herra and W.C. Leggett. – 2002. Protracted reproduction in sunfish: the temporal dimension in fish recruitment revisited. *Ecol. Appl.*, 12(1): 194-205.

Grau, A., S. Crespo, F. Riera, S. Pou and M.C. Sarasquete. – 1996. Oogenesis in the amberjack *Seriola dumerili* Risso, 1810. An histological, histochemical and ultrastructural study of oocyte development. *Sci. Mar.,* 60(2-3): 391-406.

Grau, A., M. Linde and A.M. Grau. – 2009. Reproductive biology of the vulnerable species *Sciaena umbra* (Pisces, Sciaenidae) Linnaeus, 1758. *Sci. Mar.,* 73(1): 67-81.

Greer-Walker, M., P.R. Witthames and I. Bautista de los Santos. – 1994. Is the fecundity of the atlantic mackerel (*Scomber scombrus*: Scombridae) determinate? *Sarsia,* 79: 13-26.

Gust, N. – 2004. Variation in the population biology of protogynous coral reef fishes over tens of kilometres. *Can. J. Fish. Aquat. Sci.*, 61: 205-218.

Hamilton, S.L., J.E. Caselle, J.D. Standish, D.M. Schroeder, M.S. Love, J. Rosales-Casian and O. Sosa-Nishizaki. – 2007. Size-selective harvesting alters life histories of a temperate sex-changing fish. *Ecol. Appl.*, 17(8): 2268-2280.

Harmelin, J.G. and M. Harmelin-Vivien. – 1999. A review on habitat, diet and growth of the dusky grouper, *Epinephelus marginatus* (Lowe, 1834). *Mar. Life*, 9(2): 11-20.

Harmelin-Vivien, M., J. García-Charton, J. Bayle-Sempere, E. Charbonel, L. Le Diréach, D. Ody, A. Pérez-Ruzafa, O. Reñones, P. Sánchez-Jerez and C. Valle. – 2007. Importance of marine reserves for the population dynamics of groupers (Epinephelidae) in the Western Mediterranean. P. Francour and J. Gradiot (eds.), In: P. Francour and J. Gradiot (eds.), *Second International Symposium on the Mediterranean Groupers*, May 10-13th 2007, pp. 91-94, Nice University Publication, Nice.

Harris, P.J., D.M. Wyanski, D.B. White and J.L. Moore. – 2002. Age, growth, and reproduction of scamp, *Mycteroperca phenax*, in the southwestern North Atlantic, 1979-1997. *Bull. Mar. Sci.*, 70(1): 113-132.

Hawkins, J.P. and C.M. Roberts. – 2003. Effects of fishing on sex-changing Caribbean parrotfishes. *Biol. Conserv.,* 115: 213-226.

Heath, M.R. – 1992. Field investigations of the early life stages on

marine fish. *Adv. Mar. Biol.*, 28: 1-174.

Heemstra, P. C. and J. E Randall. – 1993. Groupers of the world (Family Serranidae, Subfamily Epinephelidae). An annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known. FAO Species catalogue. *FAO Fish. Synop.,* 16(125): 1-382.

Hereu, B., D. Diaz, J. Pascual, M. Zabala and E. Sala. – 2006. Temporal patterns of spawning of the dusky grouper *Epinephelus marginatus* in relation to environmental factors. *Mar. Ecol. Prog. Ser.*, 325: 187-194.

Hesp, A.A., R.P. Hobbs and I.C. Potter. – 2002. Infection of the gonads of *Glaucosoma hebraicum* by the nematode *Phylometra lateolabracis*: occurrence and host response. *J. Fish. Biol.,* 60: 663-673.

Hunter, J.R., B.J. Maciewicz, N.C.H. Lo and C.A. Kimbrell. – 1992. Fecundity, spawning and maturity of female Dover sole, *Microstomus pacificus*, with an evaluation of assumptions and precision. *Fish. Bull. U.S.*, 90: 101-128.

Hunter, J.R., N.C.H. Lo and H.J Leong. – 1985. Batch fecundity in multiple spawning fishes. In: R. Lasker, (ed.), *An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, Engraulis mordax*. *NOAA Tech. Rep. NMFS,* 36: 67-77.

Irigoyen, A.J., D.E. Galva and L.A. Venerus. – 2005. Occurrence of dusky grouper *Epinephelus marginatus* (Lowe, 1834) in gulfs of northern Patagonia, Argentina. *J. Fish. Biol.*, 67: 1741-1745.

Kara, M.H. and F. Derbal. – 1999. Données biologiques sur le mérou *Epinephelus marginatus* (Lowe, 1834) des côtes algériennes. *Mar. Life,* 9(2): 21-27.

La Mesa, G., A. Longobardi, F. Sacco and G. Marino. – 2008. First release of hatchery juveniles of the dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Serranidae: Teleostei) at artificial reefs in the Mediterranean: results from a pilot study. *Sci. Mar.*, 72(4): 743-756.

Lloret, J. and S. Planes. – 2003. Condition, feeding and reproductive potential of white seabream *Diplodus sargus* as indicators of habitat quality and the effect of reserve protection in the northwestern Mediterranean. *Mar. Ecol. Prog. Ser.*, 248: 197-208.

Mackie, M.C. – 2003. Social induced sex-change in the half-moon grouper, *Epinephelus rivulatus*, at Ningaloo reef, Western Australia. *Coral Reefs,* 22: 133-142.

Mallol, S. and R. Goñi. – 2008. Rotación estacional de la pesca artesanal en la reserva marina de Cala Rajada y Llevant de Mallorca: herramientas de protección sobre las especies y hábitats. In: G. X.Pons (edit). *V Jornades de Medi Ambient de les Illes Balears. Ponències i Resums*, pp. 292. Soc. Hist. Nat. Balears, Palma de Mallorca.

Marino, G., E. Azzurro, A. Massari, G. Finoia and A. Mandich. – 2001. Reproduction in the dusky grouper from the southern Mediterranean. *J. Fish. Biol.,* 58: 909-927.

Marino, G., E. Panini, A. Longobardi, A. Mandich, M.G. Finoia, Y. Zohar and C.C. Mylonas. – 2003. Induction of ovulation in captive – reared dusky grouper, *Epinephelus marginatus* (Lowe, 1834), with a sustained –release GnRHa implant. *Aquaculture,* 219: 841-858.

Marteinsdottir, G. and G.A. Begg. – 2002. Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. *Mar. Ecol. Prog. Ser.,* 235: 235-256Moravec, F. and J.L. Justine. – 2005. Two species of *Phylometra* (Nematoda, Phylometridae) from serranid fishes off New Caledonia. *Acta Parasitol.*, 50(4): 323-331.

Munday, P.L., P.M. Buston and R.R. Warner. – 2006. Diversity and flexibility of sex-change strategies in animals. *Trends Ecol. Evol.,* 21(1): 89-95.

Muñoz, R.C. and R.R. Warner. – 2003a. Alternative contexts of sex change with social control in the bucktooth parrotfish, *Sparisoma radians*. *Environ. Biol. Fish.,* 68: 307-319.

Muñoz, R.C. and R.R. Warner. – 2003b. A new version of the size-advantage hypothesis for the sex-change: Incorporating sperm competition and size fecundity skew. *Am. Nat.,* 161: 749-761.

Murua, H. and F. Saborido-Rey. – 2003. Females reproductive strategies of marine fish species of the North Atlantic. *J. Northw. Atl. Fish. Sci.,* 33: 23-31.

Pears, R.J., J.H. Choat, B.D. Mapstone and G.A. Begg. – 2006. Demography of a large grouper, *Epinephelus fuscoguttatus*, from Australia's Great Barrier Reef: implications for fishery manage-ment. *Mar. Ecol. Prog. Ser.*, 307: 259-272.

Pelaprat, C. – 1999. Observations on the spawning behaviour of the dusky grouper *Epinephelus marginatus* (Lowe, 1834) in the north of Corsica (France). *Mar. Life,* 9(1): 59-65.

Reñones, O., R. Goñi, M. Pozo, S. Deudero and J. Moranta. – 1999. Effects of protection on the demographic structure and abundance of *Epinephelus marginatus* (Lowe, 1834). Evidence from the Cabrera Archipelago National Park (West-Central Mediterranean). *Mar. Life,* 9(2): 45-53.

Reñones, O. – 2007. Monitoring of groupers in a MPA with fishery management. In: J. García (ed.), *Management of habitats and species in Mediterranean Marine Protected Areas, MedPAN Workshop nº7.* pp. 15-17, MMA, Palma de Mallorca.

Reñones, O., C. Piñeiro, X. Mas, and R. Goñi. – 2007. Age and growth of the dusky grouper *Epinephelus marginatus* (Lowe 1834) in an exploited population of the Western Mediterranean. *J. Fish. Biol.,* 71: 346-362.

Rhodes, K.L. and Y. Sadovy. – 2002. Temporal and spatial trends in spawning aggregations of camouflage grouper, *Epinephelus polyphekadion*, in Pohnpei, federated states of Micronesia. *Environ. Biol. Fish.,* 63: 27-39.

Sadovy, Y.A., Rosario and A. Román. – 1994. Reproduction in an aggregating grouper, the red hind *Epinephelus guttatus*. *Environ. Biol. Fish.*, 41: 269-286.

Sadovy, Y. and P.L. Colin. – 1995. Sexual development and sexuality in the Nassau grouper. *J. Fish. Biol.*, 46: 961-976.

Sanchez-Lizaso, J.L., R. Goñi, O. Reñones, J.A. García-Charton, R. Galzin, J.T. Bayle, P. Sanchez-Jerez, A. Rérez-Ruzafa and A. Ramos. – 2000. Density dependence in marine protected populations: a review. *Environ. Conserv.,* 27: 144-158.

Shapiro D.Y. – 1987. The reproduction in groupers. In: J.J. Polovina and S. Ralston (eds.), *Tropical snappers and groupers: Biology and management of snappers and groupers*, pp. 295-327. Westview Press Inc, Boulder, Colorado.

Shapiro, D., 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(3): 1151-1162.

Sparre, P. and S.C. Venema. – 1992. Introduction to tropical fish stock assessment. Part 1 Manual. *FAO Fish. Tech. Pap.,* 306.

Stearns, S.C. and R.E Crandall. – 1984. Plasticity for the age and size at sexual maturity: a life-history response to unavoidable stress. In: G.W. Potts and R.J. Wootton (eds.), *Fish Reproductive Strategies and Tactics*, pp. 13-30. Academic Press, New York.

Trippel, E.A. – 1995. Age at maturity as a stress indicator in fisheries. *BioScience,* 45: 759-771.

Trippel, E.A. – 1999. Estimation of stock reproductive potential: History and challenges for Canadian Atlantic gadoid stock assessments. *J. Northw. Atl. Fish. Sci.*, 25: 61-81.

Warner, R.R. – 1975. The adaptative significance of sequential hermaphroditism in animals. *Am. Nat.,* 109: 61-82.

Warner, R.R. – 1988. Sex change and the size advantage model. *Trends Ecol. Evol.*, 3: 133-136.

Warner, R.R. and S.E. Swearer. – 1991. Social control of sex change in the bluehead wrasse *Thalassoma bifasciatum* (Pisces: Labridae). *Biol. Bull.,* 181: 199-204.

West, G. 1990. Methods of assessing ovarian development in fishes: a review. *Aust. J. Mar. Freshw. Res.,* 41: 199-222.

Wilk, S.J., W.W. Morse and L.L. Stehlik. – 1990. Annual cycles of gonado-somatic indices as indicators of spawning activity for selected species of finfish collected from the New York Bight. *Fish. Bull.,* 88: 775-786.

Wootton, R.J. – 1990. *Ecology of Teleost Fishes*. Chapman and Hall, New York.

Zabala, M., A. García-Rubies, P. Louisy and E. Sala. – 1997a. Spawning behaviour of the Mediterranean dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Pisces: Serranidae) in the Medes Islands Marine Reserve (NW Mediterranean, Spain). *Sci. Mar.,* 61(1): 65-77.

Zabala, M., P. Louisy, A. García-Rubies and V. Gracia. – 1997b. Socio-behavioural context of reproduction in the Mediterranean dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Pisces: Serranidae) in the Medes Islands Marine Reserve (NW Mediterranean, Spain). *Sci. Mar.,* 61(1): 79-89.