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Spawning behaviour of the Mediterranean dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Pisces, Serranidae) in the Medes Islands Marine Reserve (NW Mediterranean, Spain)*

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ABSTRACT: Spawning of the Mediterranean dusky grouper *Epinephelus marginatus* (Lowe, 1834) was observed for the first time in summer 1996 during scuba diving surveys in the Medes Islands Marine Reserve (NW Mediterranean). Ten spawning events were observed, which occurred at sunset, in the evenings around the new moon of August. A mature female (usually between 50 and 75 cm TL), in the standard mottled colour pattern, and a larger (more than 100 cm long) dominant male, in a conspicuous silver streaked colour-pattern, ascended up to 12 m side by side, spiralling in an anti-clockwise direction two or three times, from the bottom to shallow warmer waters, where the ova and sperm are released. Some traits of variability over this common pattern of spawning are described. Predation by saddled bream *Oblada melanura*, which concentrate in great numbers after each spawning suggests a very low survival of the spawned eggs.

Key words: Spawning, reproduction, behaviour, grouper, *Epinephelus*, Mediterranean Sea, marine reserves.

RESUMEN: LA FREZA DEL MERO MEDITERRÁNEO *EPINEPHELUS MARGINATUS* (LOWE, 1834) (PICES, SERRANIDAE) EN LA RESERVA MARINA DE LAS ISLAS MEDES (MEDITERRÁNEO NOROCCIDENTAL, ESPAÑA). – La freza del mero mediterraneo *Epinephelus marginatus* (Lowe, 1834) fue observada por primera vez el verano de 1996 en el curso de trabajos de seguimiento con escafandra autónoma en la Reserva Marina de las Islas Medes (Mediterráneo noroccidental). Los 10 episodios de freza que se observaron tuvieron lugar a la puesta del sol, durante los atardeceres próximos a la luna nueva de Agosto. Una hembra madura (generalmente entre 50 y 75 cm de largo TL), en la librea críptica estandard, y un macho de talla mucho mayor (mas de 100 cm TL), exhibiendo la conspicua librea plateada de los machos dominantes, ascienden verticalmente hasta 12 m, uno junto a otro, girando dos o tres veces en espiral en sentido antihorario, desde cerca del fondo hasta aguas superficiales más cálidas, donde liberan los huevos y esperma. Se describen algunas variaciones sobre esta pauta común de freza. Una intensa depredación por parte de obladas *Oblada melanura*, que se concentran en gran número tras cada episodio de freza sugiere una muy baja supervivencia de las puestas.

Palabras clave: Freza, reproducción, comportamiento, mero, *Epinephelus*, mar Mediterráneo, reservas marinas.

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INTRODUCTION

The dusky grouper *Epinephelus marginatus* (Lowe, 1834) is one of the largest and unquestionably the most popular of littoral fishes along the Mediterranean coasts. Although it has been watched by spearfishers and scuba divers for a long time, we paradoxally do not know much about its reproductive biology.

Spawning behaviour has been previously observed in the field in various tropical grouper species, as *Epinephelus guttatus* (Colin, 1992), *Mycteroperca phenax* (Gilmore and Jones,1992), *M.tigris* (Sadovy *et al.*, 1994) or *Plectropomus leopardus* (Samoilys and Squire, 1994). But, no direct observation of the spawning of the Mediterranean dusky grouper *E. marginatus* has come yet to our notice. This is not surprising, at least along the European coasts, were groupers are rather uncommon, and very "shy". Actually, Marine Reserves are today the only places in the north-western Mediterranean were these fishes can be encountered in reasonably high densities. The Medes Islands Marine Reserve (NE Spain) is probably one of the most interesting among these protected zones, since it shelters one of the highest numbers of *E. marginatus* in the area. Furthermore, in this place heavily frequented by divers, groupers get accustomed to human observers, and can be approached quite close without looking uneasy.

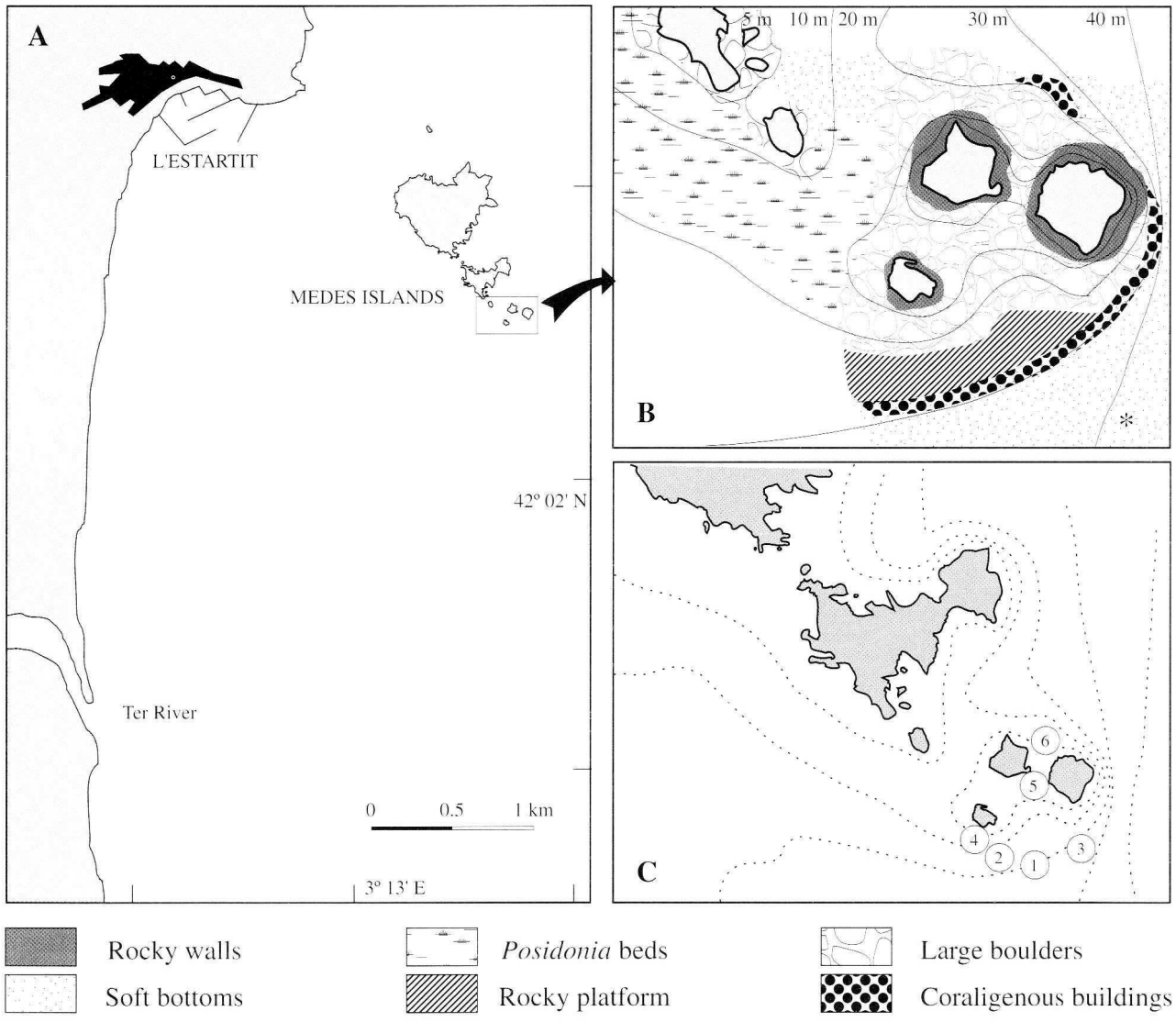


FIG. 1. – A. Map of the Medes Islands Marine Reserve with the area of study (rectangle); note de vicinity of the Ter River mouth (bottom left); B. bathymetric and community map of the studied area, also showing the location of the oceanographic station (asterisk); C. Male Grouper territories.

In July 1990, and each summer since then, strange behaviours and colour patterns were observed in some large males during routine censuses of fish densities in the Reserve (Garcia-Rubies and Zabala, 1994), suggesting they were dominant and reproductively active males. These and other colour patterns were described by one of us (Louisy, 1996) in the Cerbère-Banyuls Marine Reserve (France) in 1995, together with observations of the behavioural trends leading to spawning. Following these preliminary observations, we started in summers 1995 and especially 1996 an underwater survey of reproduction-related activities of the dusky grouper in the Medes Islands. We could thus observe for the first time the spawning of this species, and also the general socio-behavioural context which proved to be of major importance for the success of reproduction (these results are published separately: Zabala *et al.*, 1997).

MATERIAL AND METHODS

The site

Observations were carried out in the Medes Islands Marine Reserve (NE Spain, 3° 13' E, 42° 3' N). In a relatively small area (about 90 Ha strictly protected), this little Reserve encompasses 7 islands and islets grouped about 2 miles from the closest shore (Fig. 1a). Despite its size, it now shelters a dense population of *E. marginatus* (about 120 individuals in summer: Garcia-Rubies and Zabala, 1994) whereas groupers were very sparse in 1983, when the Reserve was created. Dusky groupers can be found in the whole protected area, but they are unevenly distributed. There is a special concentration in the south-eastern end of the Reserve, where up to 65 individuals can be seen in single census (Fig 1b).

Bottom topography in this small zone is a mixing of different environments: cliffs ranging from the surface to 5-15 m (35 m in the outer points), big boulders piling up between 5 and 20-25 m, and flat rocky platforms and "coraligenous" concretions extending from 20 to 32 m. Sandy bottoms and sparse *Posidonia* meadows are scarce, and mainly present in the shallow north-western part of the area (Fig. 1b).

Moderate to strong currents are common in the studied area, sometimes reaching speeds of 50 cm s^{-1}. Both direction and strengh can change repeatedly within a day, leading to sharp variations in water transparency, temperature and salinity, due to the vicinity of the Ter river effluent (lower left corner at Fig. 1a). The islets being roughly laid along a W-E axis and only separated by shallow passes, topography imposes two dominant directions to the flow : from North to South, and from South to North, the latter usually associated with clear waters. Water transparency is relatively low compared with other mediterranean regions, and ranges from 8.5 m in November and March (mean visibility measured with the Secchi disc) to 26.5 m in August. Seasonal and interannual changes in water temperature have been documented for more than 20 years (Pascual *et al.*, 1995). The shallow waters reach the highest temperature (up to 25°C) in August, and the lowest (down to 12°C) in February-March during the Tramontana storms (dry and cold winds blowing from the north). In summer, temperatures can change dramatically in the depths mostly frequented by groupers (12-25 m), following shifts of the upper limit of the thermocline.

The species

The dusky grouper *Epinephelus marginatus* (Lowe, 1834), commonly cited in the past under the nomen dubium *E. guaza* (L.) or *E. gigas* (Brünnich) (Heemstra, 1991), is a proterogynous hermaphrodite in which sex reversal seems to occur mainly when individuals are 14-17 years old and about 80-90 cm long (total lenght) (Bruslé and Bruslé, 1976; Bruslé, 1985; Chauvet, 1991; Gracia, 1996). Larger individuals, which are unquestionably males, can be up to 50 years old, and reach a maximum size up to 120 cm, and 40 kg in weight. The first sexual maturity is reached when females are 5 years old and 40 to 50 cm long (Chauvet, 1988).

The eggs of *E. marginatus* have been described (Spartà, 1935; Barnabé, 1974, 1976) but we lack informations on larval development and movements of planktonic stages, and we know very little about the sites of settlement (Lelong, 1993).

Up to recent years, reproduction was supposed to occur only south to 41.5° N in the western Mediterranean, water temperature being adduced as the main reason for this pattern (Chauvet, 1991; G.E.M., 1996). But the finding of young groupers (less than 10 cm long) along the northen mediterranean coasts of Spain and France (Harmelin and Robert, 1992; Lelong, 1993) brought the evidence that reproduction now occurs in the northwestern

basin. The spawning season of *E. marginatus* is approximatively known by dissection of mature individuals speared or caught by fishermen: around July in the straits of Messina (Spartà, 1935), from June to August along the coasts of Tunisia (Bruslé and Bruslé, 1976; Bouain, 1980; Bouain and Siau, 1983; Bruslé, 1985; Chauvet, 1988), in July and August in Croatia (Skaramuca *et al.*,1989), the south of Spain (Barnabé, 1974) and the NE Mediterranean coast of Spain (Gracia, 1996). The success of each reproductive event can be indirectly assessed by the extent of the recruitment of youngs the next year; good recruitments were reported in 1984 in Spain, in 1986 in Corsica and Sardinia, in 1998 in Tunisia, Sardinia and Spain, and in 1990 around most of the occidental basin (G.E.M., 1996), and some juveniles were caught in Catania in 1981 and 1982 (Battiato, 1985) and in France in 1991 and 1993 (Harmelin and Robert, 1992; Lelong, 1993).

Louisy (1996) described the main colour patterns of *E. marginatus* during the reproductive activities. Accordintg to his work, and following the terminology we used in the course of this study (see Zabala *et al.*, 1997), the most frequent colour patterns (C.P.) are the standard mottled C.P. 1, the dark with 3 blotches C.P. 2, the uniformly dark C.P. 3 (the 3 of them being displayed at any time of the year), the light C.P. 4, the dark streaked C.P. 5 (both being displayed during the reproductive season, mainly by small to medium sized groupers) and the silver C.P. 6, typically displayed by large dominant males (Fig. 2).

The groupers of Medes Islands

Used to underwater visitors (about 65,000 dives a year), the groupers of the Medes Islands Marine Reserve have lost their natural shyness. They even are strongly attracted by divers, who are allowed to feed them. In a hand, this helps the observer to approach the fishes during reproductive activities, but also prompts him to be very cautious about any possible undesirable interaction.

In summer at least, most of the groupers of the Medes Islands use to wander widely, often swimming in open water (i.e. usually 1 to 5 m above the bottom). If disturbed, they look for a temporary shelter, but rarely remain hidden in a fixed hole.

From late June to early september at least, large males (> 95 cm TL) display the silver C.P. 6 (Fig. 2) and are territorial (Zabala *et al.*, 1997). Territories are rather wide (reaching 100 m in their largest dimension), although variable in size. They are preferably localized below 20 m (range: 10 to 30 m), thus remaining most of the time under the upper

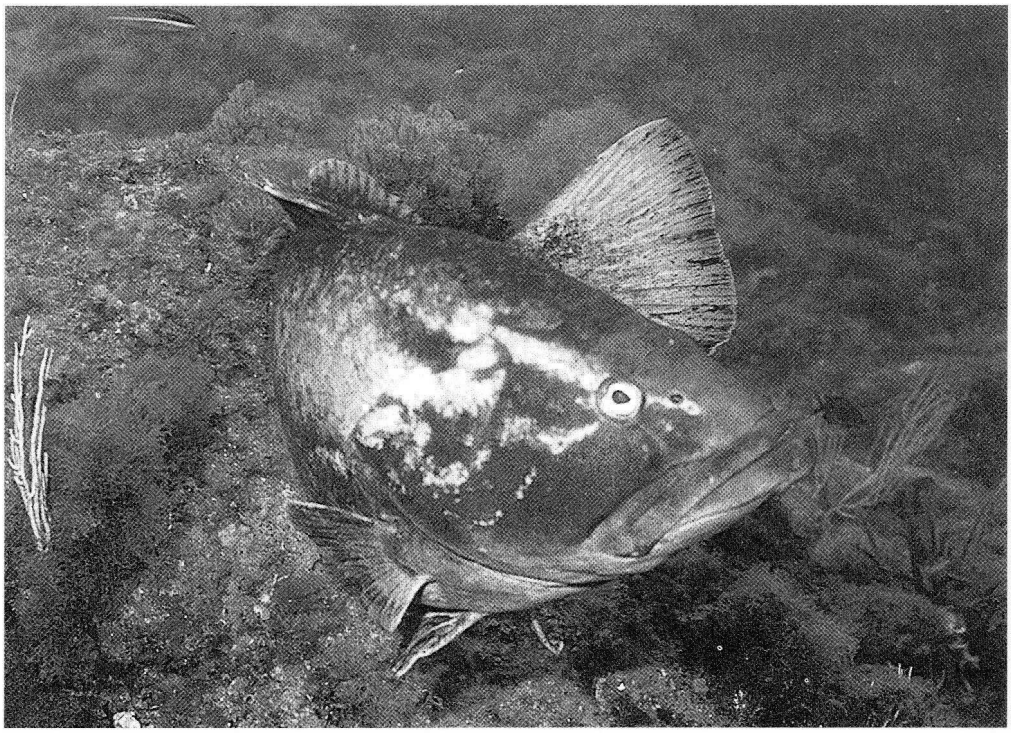


FIG. 2. – At the beginning of its full territorial activity (19th of July, 1995), this dominant male (about 100 cm TL) was photographed when shortly patrolling in shallow waters, in bright silver colour pattern 6, before getting back to its deeper neighbouring territory (Photo A. Garcia-Rubies).

limit of thermocline. The bottom is rather flat, but includes some outstanding boulders serving as landmarks. In summer 1996, six males at least owned a territory in the study area (Fig. 1c; Zabala *et al.*, 1997); they patrolled their territory tirelessly all the day long, and would drive away any encountered grouper, neighbouring males being especially attacked and chased. From time to time, they made incursions into shallower zones were females used to stay. In these situations, males could display in different ways : frontal, lateral, with tail flapping, sometimes tilting the body (Louisy and Zabala, unpublished data). But most displays and chases where so fast that it was difficult to tell whether they would prefigurate courtship display.

Methods

Physical parameters

Water transparency (horizontal distance of disappearance of the Secchi disc) was measured at the beginning and at the end of each dive, together with the evaluation of direction and strengh of the current (with 3 levels of strengh: 0) undetectable; 1) moderate (about 3-30 cm s^{-1}), when unloaded ropes tilt underwater; 2) strong (more then 30 cm s^{-1}), when even ropes loaded with a diving tank tilt, and divers must struggle to swim against the current). A precise description of the thermic structure of the waters surrounding the Reserve was provided by CTD profiles from 8 stations routinely sampled every week. In addition, the depth of the upper limit of the thermocline was noted at each dive.

Behavioural observations

Although the Medes Islands groupers are usually attracted by stationnary divers, it appeared that a single observer behaving properly (i.e. looking "unconcerned") would not interfer with the fishes, especially when they were engaged in agonistic or reproductive behaviours. Thus, the very special context of the Reserve does not alter the descriptive value of the observations.

Eggs collection

When spawning events eventually happened, eggs were collected using a large zooplankton net (diameter 55 cm, 450 micron mesh) handled by SCUBA divers. The net was lifted to strain the water from below the point were the cloud of sperm was released, less than 30 seconds after spawning. Within 3 minuts, the net with captured eggs was taken to the boat and emptied in a container filled with 50 l of clean sea water and provided with an air pump. Eggs were brought to the laboratory in less than 30 minuts, and maintained in an aquarium at a constant temperature of 20 °C.

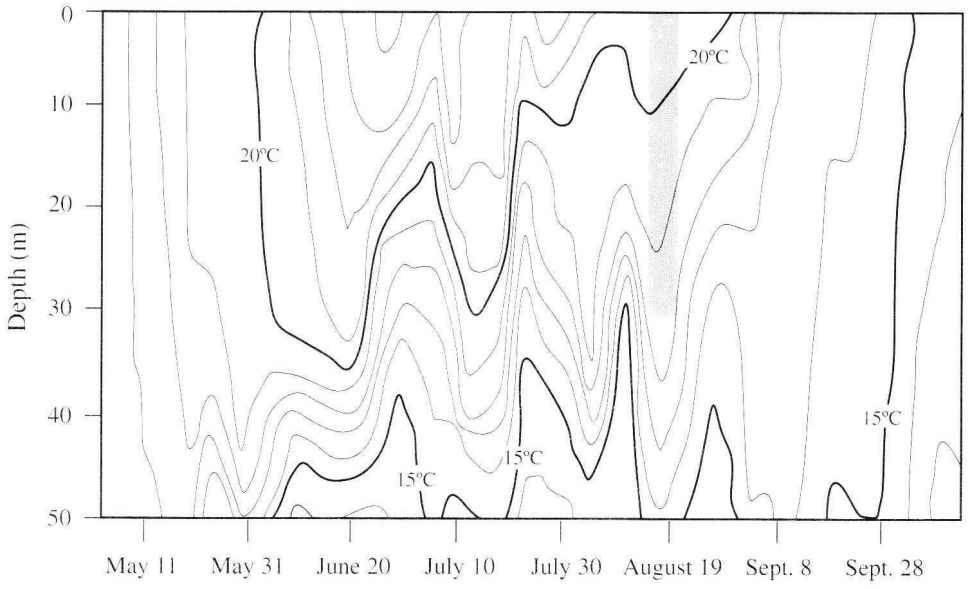


FIG. 3. – Distribution of temperature (T°C) at the oceanographic station neighbour of the zone of spawning throughout the summer 1996 (based on the CTD data) The rectangle shows the days when spawning was observed.

RESULTS

The present study allowed us to describe the spawning behaviour of the dusky grouper. It also showed that spawning is only the final event of a long term process engaged at least one and a half month earlier. This important social and behavioural context, is dealt with in an other article (Zabala *et al.*, 1997).

Physical conditions

From June to August, shallow waters undergo a process of warming and thermo-haline stratification (see Zabala *et al.*, 1997). Nevertheless, both temperature and transparency can change suddenly due to vertical shifts of the upper limit of the thermocline (Fig. 3). Thus, groupers moving about at medium depths (15-20 m) may be exposed, sometimes within an hour, to temperature changes as high as 5 °C while visibility may double or lower by half.

In August, when spawning occured, the surface temperature reached its maximum of 25 °C, and the water transparency also was at its highest levels. Table 1 summarizes the values of some physical parameters at the time of observed spawnings.

Occurence of spawnings

Ten effective spawnings were observed during four different evenings between the 16th and the 21st of August (despite an intense deployement of watchers, no spawning could be seen the 17th and 20th, and after the 21st). Thus, we can almost be sure that spawnings ended the 21st of August, while we do not know exactly when they began (due to planning constraints, observations were interrupted from the 4th to the 15th of August).

All the observed spawnings occured betwen an hour before and half an hour after sunset (see tables 1 and 2). The moon was in a new lunar phase (new moon on the 15th).

Spawning behaviour

During a spawning event, a dominant male (> 95 cm TL) performs courtship activities within only a small area of its usual territory (30-50 × 7-10 m) generally using definite boulders as landmarks, thus indicating where females should wait for mating. It patrols this reduced area by following repeatedly, in a ritualized manner, a more or less elliptical path. The male displays a bright silver colour pattern 6, so that, with clear water (Secchi disc visibility >20 m), a whole lap can fully be observed from 10 m above. In this way, the male continuously drives away smaller groupers entering the area, and also is especially aggressive towards neighbouring dominant males.

Eventually, a female slips into the territory and waits laying on the bottom or close to it, showing the standar colour pattern 1. When the male approaches, the "non-escape" reaction of the female turns the aggressive display into courtship.

Then follow the different phases of the spawning event :

- *Approach* (Fig. 4 , phase a)

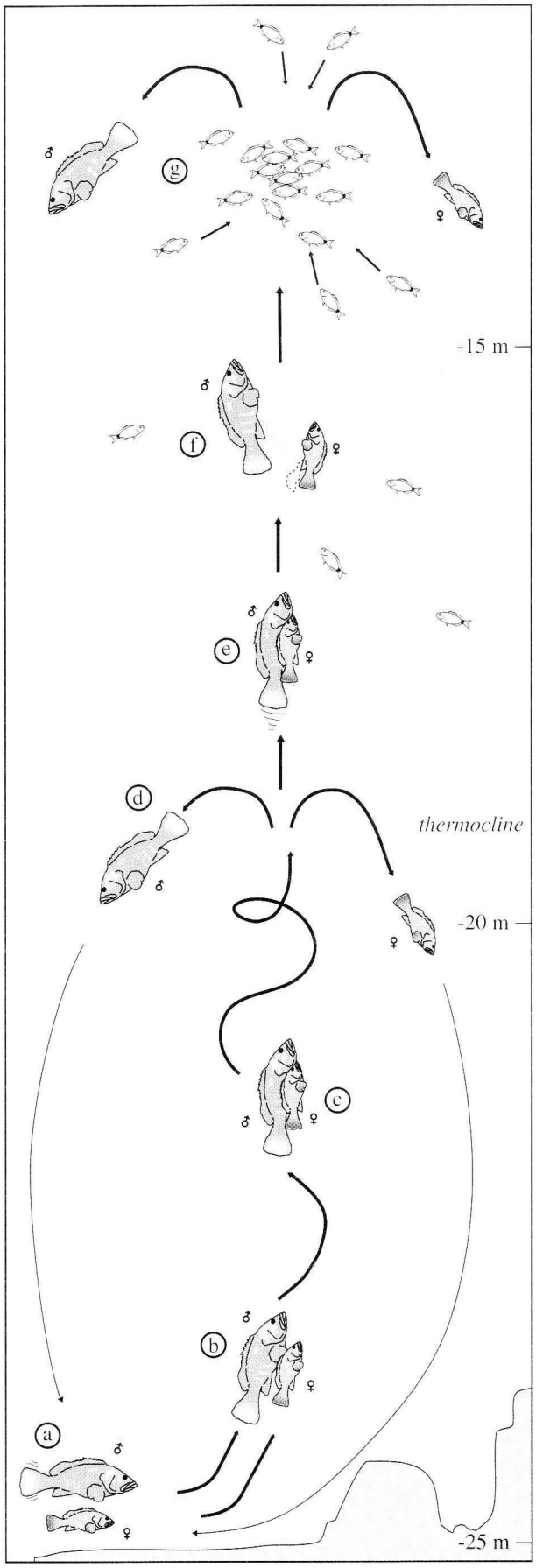
First approaching with a lateral display, the male then comes from behind the female until it is placed above its mate. During this movement, it tilts sideways in an almost horizontal position, shaking the rear part of the body in a ritualised caudal flapping.

- *Ascent and "false rise"* (Fig. 4 , phases b to d)

The courtship of the male causes the female to ascend, first at an angle from the bottom, then vertically. The male joins the female just after the start, and they rise in parallel, side by side, less than 20 cm apart. Usually much bigger than the female, the male

TABLE 1. – Dates, range of daytime(Greenwich meridian time) and environmental conditions when the spawning of *Epinephelus marginatus* was observed. Currents direction (dir) and speed (spe) (see text for codes). The upper boundary of the thermocline (thermocl. upper) as detected by divers, and the distance of loss of vision of the Secchi disc, above (up) and below (down) the thermocline; and the number of false rises and successful spawnings.

Date	Time (GMT)	Sunset (GMT)	Currents dir	Currents spe	Thermocline upper L (m)	Secchi disc up	Secchi disc down	False rises	Succes spawns
16 Aug. 96	1743-1907	1849	S	1	>27	26.5	?	8	5
17 Aug. 96	1730-1910	1848	NW	3	18	17.5	13	0	0
18 Aug. 96	1740-1808	1847	S	1	18-20-25	>25	21	4	1
19 Aug. 96	1730-1828	1845	-	0	15	19	19	6	3
20 Aug. 96	1740-1815	1844	N	1	15	25.5	17	4	0
21 Aug. 96	1801-1837	1842	N	2	19	19.5	17	5	1



is always more or less on top of it. During the ascent, the head of the male touches that of the female, inducing two to three slow gyrations (always counterclockwise in our observations).

Often, after 6-8 m of ascent - seemingly when the pair reaches the upper level of the thermocline -, the female gives up and gets back to the bottom. The male may flap its tail in a display towards the female, trying to resume the rise, but would finally also get down to the bottom and start again the courtship of this female or another. These false rises usually reoccur two to three times in a few minuts before the actual spawning (but there can be none to 7).

- *Actual spawning* (Fig. 4 , phases e to g)

Eventually, when the pair reaches the warmer waters above the thermocline, the rising process is continued by a short (2 - 3 m) but frenetic acceleration of swimming, ending up with the emission of gametes and the immediate separation of the pair, each mate going back to the bottom.

The release of sperm could be seen as an ephemerous whitish cloud (about 30 cm long), while the ova could not be detected (although they were collected; see later). The whole process leading to actual spawning could last 20 seconds to some minuts, depending on the number of false rises. No changes were observed in the colour patterns during courtship, ascent or descent, the male keeping the bright silver C.P. 6 while the female remained in standart C.P. 1.

Variability of spawnings

The few observed spawning events showed that spawning behaviour is somewhat variable for most of the quantifiable parameters: zone and depth of courtship territory, male(s) involved, number of false rises and frequence of spawnings, height of ascent and depth of spawning, females availability, size of spawning females and male's activity. This is illustrated by the data summarized in Table 2.

During the first witnessed spawning event (August, 16th), the male (# 1 in Fig. 1c) was able

FIG. 4. – Simplified representation drawing of a spawning event. a. a male (above) approaching from behind incites a receptive female to begin the ascent by flapping his tail; b. the male joins the female in the ascent; c. the male displays by approaching his head to touch the head of the female and, forces the pair to describe some counterclockwise gyres; d. after a false rise, the pair separate and come back to the bottom to resume the procedure; e. a vertical rush preceeds the spawn; f. the gametes are then released (only the cloud of sperm can be seen); g. a school of saddle breams predates on the eggs without any defensive reponse of the spawners.

TABLE 2. – Variability of spawning patterns on *Epinephelus marginatus* of Medes Islands Marine Reserve during summer 1996. N (number or capitals) identifies different mating individuals (see Fig. 1c for the position of the males).

Date	Time (GMT)	Territory Zone	Territory Depth	Male N	Male TL (cm)	Female N	Female TL (cm)	False Rises	Ascent Height(m)	Depth Spawn(m)
16 Aug. 96	1817	h	27	1		A	50	7	12	15
	1822	h	27	1	100	A		0	9	18
	1823	h	27	1		A		0	8	19
	1825	h	27	1		B	50	0	10.5	16.5
	1907	h	27	1		C	60	?	?	circa 14
18 Aug. 96	1808	j	27	2	110	D	60	4	10	17
19 Aug. 96	1744	e	19	3	100	E	50	2	7	12
	1754	e	19	3		F	60	0	7.5	11.5
	1828	e	19	3		G	65	3	12	7
21 Aug. 96	1837	g	10	4	105	H	75	1	8.5	1.5

to achieve four successful spawnings in only 8 minuts; three different females at least were involved. That was the observed maximum of reproductive activity: females were very receptive and the mere approach of the male from behind was enough to trigger off the rise. In the meantime, two more dominant males with silver C.P. 6 (# 2 and 3 in Fig. 1c) were active in the vicinity, displaying towards other groups of small females. In two instances, two males were seen courting a female at the same time, only 20 m apart. Once, male # 1 left an ascent in the process, to drive away a neighbouring male. After this high reproductive activity, male # 1 was not seen to reproduce successfully for the next seven days.

The second spawning event was observed two days later (August, 18th), in the territory of male # 3. Only one spawning was observed, following two false rises; this male was inactive for the next six days. In the third observed spawning day, male # 4 was found active, but the females were few, and less responsive than the previous days. As a result, the male had to display vigorously on top of the female (ritualized caudal flapping) before it would begin to rise. This male also was unable to mate successfully the next days, despite an intense courting activity. The last observation of spawning (August, 21st) occured at a very shallow depth (10 m), when divers were coming back after the unsuccessful watching of four other dominant males. The involved male (# 5) mated with a large female (75 cm TL), and they spawned at the second attempt, only 1.5 m below the surface.

In most instances, males did not seem to compete directly for females, but courting males spent most of their time -and energy- driving away other groupers, and especially chasing neighbouring big males. Although courtship and spawning usually involved a single pair, a second female was seen in one occasion closely following the mating pair ; when the first female gave up during the ascent (false rise), the second one finished the rise with a successful spawning.

Despite the different depths where courtship started (10-31 m), the height of the ascent was somewhat constant (7-12 m), and the depth of spawning could notably vary between 19 and 1.5 m.

Mating groupers did not seem to be disturbed by observers (spawning could occur only 2 m from the divers), although some males reacted aggressively to white patches in the divers' equipment (plastic sheet, flippers...).

Eggs and predation on eggs

When sampled about 20 seconds after release, hundreds of eggs were collected for a single spawning (Fig. 5). Fresh eggs were extremely translucent, about 0.7 mm in diameter (avg. = 0.67 ; s.d. = 0.04 ; n = 50), with a yellowish oil globule 0.37 mm in diameter. Most of the collected eggs seemed unfertilised; this can explain (no hydratation) why their size was smaller than that given by Barnabé (1974, 1976), i.e. 0.75 mm after fixation in Bouin solution, and by Skaramuca *et al.*, (1989). The first author also took the only known photograph of newly hatched larva of the dusky grouper (Barnabé, 1976), but it unfortunately never was published in a scientific journal; this is why we chose to show it here (Fig. 6), with the kind authorization of the author.

In all the observed cases, the emission of gametes was immediatly followed by intense predation by smaller fishes (essentially saddled breams *Oblada melanura*, and a few white breams *Diplodus sargus*). Gathered by hundreds, saddled breams

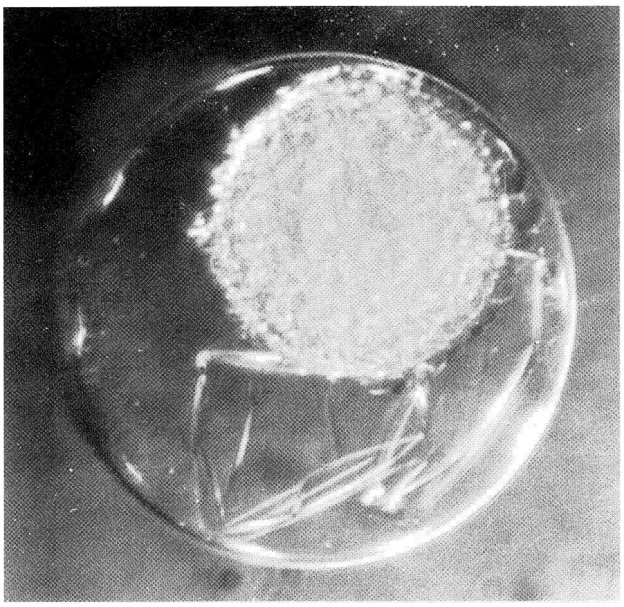


FIG. 5. – Photograph of an unfertilized egg spawned the 21th August 1996 (Photo A. Sabatés).

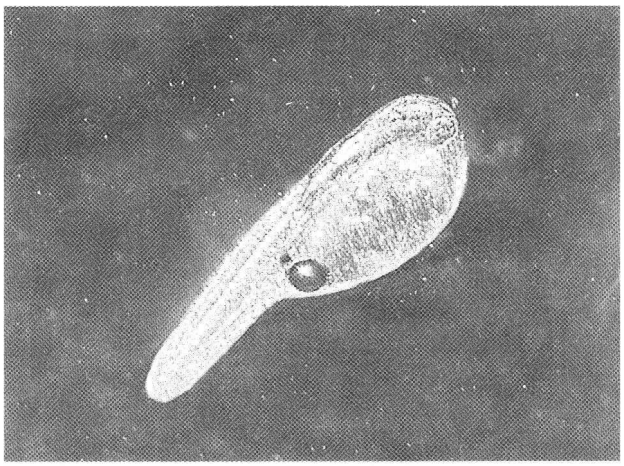


FIG. 6. Just hatched larva of *Epinephelus marginatus*, about 40 hours after fertilization (after Barnabé, 1976) (Photo G.Barnabé).

immediately swam wards the cloud of gametes and picked at what we assumed to be the eggs for a few minutes; they infallibly discriminated true spawning ascents from false rises, and some of them even rushed towards the spawning pair before the release of gametes. The groupers made no effort to protect their eggs.

DISCUSSION

During the observed period, the general scheme of spawning behaviour (Fig. 4) seems fairly clear, with four main phases:

1) *Approach* of the male from behind and above the female; 2) *Initial ascent* with usually a spiralling movement, which may end before spawning in a false rise; 3) the *final spawning rush*, with release of the gametes; and 4) *separation and descent* of the mates.

Spawning behaviour had been previously observed in the field in various tropical grouper species, as *Epinephelus guttatus* (Colin, 1992), *Mycteroperca phenax* (Gilmore and Jones, 1992), *M.tigris* (Sadovy *et al.*, 1994) or *Plectropomus leopardus* (Samoilys and Squire, 1994). Involved behaviours were described in detail only for *E. fasciatus* and *P. leopardus*.

Unlike *Epinephelus marginatus*, *E. fasciatus* is not a strictly proterogynous species: males are more numerous, and not bigger than females. In this species, Colin (1992) described group spawning involving at least various males, and possibly also some females, following a bigger leading female. Despite this fundamental diference, *E. fasciatus* displays some of the behaviours we could observe in the spawnings of the *E. marginatus*, like vertical spirals or short vertical rushes followed by outward radiating of the group.

In *P. leopardus*, Samoilys and Squire (1994) reported various phases in the courtship, including an initial approach of the female by the male, displaying and guivering in a tilted position. Spawning occurred as single female/male pairs, and gamete release followed an ascent termed spawning "rush", although no false rises like those we observed repeatedly in *E. marginatus* (as in other reef fishes: Colin and Clavijo, 1988; Colin, 1989) were mentioned (nor for any of the above cited grouper species). These overall similarities are especially interesting since the general socio-behavioural context of reproduction also appears some what similar to what we observed in *E. marginatus*: dominant males display a specific colour pattern, and also show a patrolling behaviour along the reef wall, deeper than the area where females stay during the day.

Remaining uncertainties

The above examples open interesting fields of comparison, but our observations on *E. marginatus* showed that some parameters could be highly variable (see Results, Variability of spawnings), and some uncertainties remain on various points, some of which are discussed below.

Beginning of the spawning period

When we first observed spawnings, in the 16th of August (after a 11 days gap in our dives), sexual activity was at its maximum, and it decreased the following days. Thus, we obviously witnessed the end of the spawning period, which most likely started some days before. Did the first spawning evenings look like those we could study? We can reasonably suppose that the first days of spawning activity were more intense (higher activity of males, better readiness of females), which may imply a mating behaviour slightly different from what we could see.

Repeated spawnings

Our observations of spawnings are insufficient to tell how many times a given female may spawn. At least once, a female was seen to spawn twice with the same male in a six minutes interval, but most females disappeared after a single spawning.

The males are indeed able to mate repeatedly during the evening, and may display active courtship throughout several days. But a male successfully mating one day seems unable to find females receptive enough the day(s) after (or alternatively is unable to prompt them to spawning).

Within the whole reproductive season, males seem ready to spawn for several weeks at least, whereas females seem to mature only for a few days, even if this period differs from female to female. One should notice that "thin" females (i.e. with no clearly egg-swollen abdomen) were seen spawning, and therefore can be mature.

Multiple mating

Observed courtships and spawnings involved only single pairs. But since one female was seen to take over from a first one during a spawning rise (see Results, Variability of spawnings), the idea that a male could mate with various females at a time should not be discarded. In the other hand, the extreme aggressivity between males makes any possibility of cooperative fertilization highly improbable.

Diel, lunar and seasonal patterns of spawning

Further observations (in next summers) will be needed before the correlation between spawning and the moon lunar phase can be established. Even so, it seems fairly clear that Mediterranean dusky groupers (at least those from the Medes Islands) exhibited a strongly synchronized pattern of spawning at the three levels: diel, lunar and seasonal. All the events were observed between 1 hour before and 32 minutes after the sunset (Tables 1, 2); they occurred between 1 and 6 days after the new moon of August (but may be a few days before); and, most probably no more spawnings occurred througouth a year-round cycle. Tropical groupers use to aggregate in traditional spawning grounds (Smith, 1972; Thresher, 1984; Tucker *et al.*, 1993; Shapiro *et al.*, 1993) after long migrations of tens of kilometers (Colin *et al.*, 1987). Some of these species provide extreme examples of diel, lunar and seasonal synchronized spawning (Colin *et al.*, 1987; Shapiro *et al.*,1993). The association of lunar spawning cycles with (hipothetic) migration and aggregation patterns of the dusky groupers (Zabala *et al.*, 1997) may be related to adult biology constraints (Colin *et al.*, 1987). Temperature, which drives the maturation of gonads (Gracia, 1996), together with peaks of food availability needed to support gametes production can explain better than larval survival hypotheses the seasonal pattern of spawning (Ross, 1991). On the contrary, the diel pattern seems more related to larval survival constraints. Since tide and potential predators of the spawners are almost absent, the narrow diel spawning period of the Mediterranean dusky groupers, provides a good example to support of the egg-predation hypothesis (Ross, 1991) for the explanation of grouper's diel spawning pattern; this hypothesis assumes that groupers would reduce eggs and larval mortality by spawning at a time when diurnal egg-predators would be relatively satiated (Colin and Clavijo, 1988) and darkness would reduce the pick-up's efficiency.

Spawning and environmental parameters

Circumstancial evidences suggest that environmental parameters have, in the dusky grouper, a strong influence on reproductive activity, spawning place and spawning time.

Topography

The main reproductive area (see Fig. 1c) encompasses shallow zones (5-15 m) where numerous boulders provide shelter to the groupers, and after a steep rocky slope, deeper and rather flat zones with a few

"landmark rocks", where the big males set up their territories. Due to the steepness of the bottom, areas where males patrol and where females move about are usually less than 20 m apart, thus allowing continuous visual contact. The spawning sites themselves, although variable in depth, are settled close to steep rocky outgrowths in such a way that mates can rise in the water column without getting far from shelter.

Despite a different geological nature and shallower surrounding bottoms (18-25 m), a similar context was encountered in the Rédéris shelf reproductive area (Cerbère-Banyuls marine Reserve), where courtships took place along a cliff-like slope, close to shallower resting areas (Louisy, personnal observation).

Hydrology

Situated in a prominent part of the Reserve area, the main reproductive zone is exposed to currents: a favourable fact, both for food availability and for eggs dispersal. Maximum observed spawning activity took place during a period of moderate northern current promoting clear water conditions. In the contrary, reproductive activity was reduced by strong currents (because groupers were forced to shelter) and / or by poor visibility (this fact suggesting that visual relationship is important). Both factors can account for the failure of spawning in the 17th and the 20th of August.

Temperature has been proposed to be a key factor for the reproduction of Mediterranean groupers (G.E.M., 1996). In the one hand, it can be considered as one of the activating factors of spawning : observed spawnings occured when the surface water temperature reached its maximum (one should notice however that this factor affects mainly the females, since dominant males remain in the deeper and cooler waters under the thermocline, at least during the daytime activity). In the other hand, temperature may work as a limiting factor, either because low temperatures may inhibate gonadal maturation, or because sudden cooling of shallow waters (due to winds and currents, the depth of thermocline may change dramatically) may hinder, and possibly stop reproductive activity. Both these factors, together with a temporary inversion of dominant currents, may explain the unexpected interruption of reproductive activity observed by mid August 1996 in the Cerbère-Banyuls Marine Reserve (Louisy, personal observations).

Eggs predation and reproductive success

The quantitative impact of eggs predation by saddled bream could not be assessed. Nevertheless, the number, speed and feeding frenzy of these fish may cause an extreme decimation of the gametes, even before fertilization occurs. This predatory behaviour suggests that saddled breams have been accustomed for a long time to the spawning behaviour of groupers (then supporting the hypothesis that groupers used to spawn the years before), although one may alternatively suppose, when seeing their fast reaction to divers, that the fish-feeding habit simply "trained" them to react at once to any "strange-behaving" animal. Anyway, this predation was seen to occur systematically after any observed spawning, and this may partly explain why the recruitment of young groupers seems so poor in our area.

An additional explaination may be related to the planktonic larval development, although we do not know much about eggs, larvae, and newly settled benthic juveniles of the dusky grouper. Barnabé (1974, 1976) could obtain - in quite rough conditions - the hatching and the beginning of larval development (see his photograph in Fig. 6). The eggs hatched in about 40 hours, and yolk resorption occured about 10 days later; the last living larva, measured 14 days after fertilization, was 3.8 mm long. Lelong (1993) found a young grouper only 2 cm long in September 1991; kept in aquarium, this individual wheighed 2 g and measured 3.9 cm in November (Lelong's photograph of this juvenile was published in Harmelin and Robert, 1992, and in Louisy, 1996). The smallest grouper reported by this author, seen in November 1992, was between 1 and 2 cm long.

Thus, according to Barnabé's results, the duration of the planktonic phase would probably exceed one and a half to two months, before the benthic juvenile settles at a possible minimum size of about 1 cm. Then, sharp falls of surface water temperatures, which often occur in October-November, may hinder dramatically the survival of both planktonic larvae and newly settled juveniles. That would be an other explaination of possible failures in the recruitment following late reproductive events (i.e. August?) in the NW Mediterranean.

Future research

The spawning behaviour rarely could be described in such detail in a grouper species, but

some uncertainties remain, especially on the beginning of spawning activity. The problem of the duration and evolution of the actual spawning activity would deserve further study.

Almost every aspect of the planktonic larval life of the dusky grouper remain to be studied. The occurence of natural spawning may allow to work on early development and larval rearing for aquiculture purposes, a promising field according to its growth performances (Gracia, 1996). Such researchs would also be relevant to understand the dispersion and colonisation patterns of the species, and together with studies on climatic parameters and reproduction itself, would permit a sustainable management of this most renowned fish of the Mediterranean heritage.

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