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Temporal patterns of spawning of the dusky grouper *Epinephelus marginatus* in relation to environmental factors

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ABSTRACT: The dusky grouper *Epinephelus marginatus* pair spawns in summer in the NW Mediterranean. Only a few spawning sites have been identified, mostly in marine reserves. Because the dusky grouper is vulnerable to fishing, there is a need to identify and predict the occurrence of spawning for conservation purposes. To gain insight into the temporal patterns of dusky grouper spawning, we monitored a reproductive population in the Medes Islands Marine Reserve, Catalonia, from 1996 to 1999. We observed 44 successful spawning events. At a monthly scale, the highest reproductive activity occurred in August, when surface temperatures were at a maximum (monthly average of 24 to 25°C), and during days with 14 daylight hours, 2 mo after the summer solstice (21 to 22 June). At a daily scale, the greatest number of spawns occurred when the surface temperature was highest (25 to 26°C) and the temperature at 20 m was 17 to 19°C, during anticyclonic conditions, and when tidal amplitude and wave height were largest. Spawning occurred during all moon phases, although it was more frequent during the new moon and the first quarter. Reproductive males did not show sustained spawning activity during consecutive days and had an average spawning rate of 1.5 spawns d^{-1}. All spawning occurred at dusk, between 1 h before and 30 min after sunset. There was no population-wide synchronicity of spawning, since we never observed all monitored males spawning within the same day. These results should allow researchers to predict the temporal occurrence of dusky grouper spawning elsewhere in the Mediterranean.

KEY WORDS: *Epinephelus marginatus* · Spawning · Reproductive patterns · Environmental factors · Marine reserves

INTRODUCTION

Many species of reef fishes aggregate to spawn at specific locations and in specific seasons, often in relation to phases of the moon (e.g. Johannes 1978, Carter & Perrine 1994, Domeier & Colin 1997). For most species, relationships between spawning and environmental variables have been derived more from fishers' observations than from scientific inquiry (Johannes 1981, Colin 1992, Johannes et al. 1999). Because many reef-fish spawning aggregations have been overfished (Carter et al. 1994, Sadovy 1997, Sala et al. 2001), an understanding of the reproductive biology of fishes is essential for fisheries management and conservation purposes. Here we present an analysis of the relationship between spawning behavior of the Mediterranean dusky grouper (*Epinephelus marginatus,* Lowe 1837) and associated environmental conditions.

In the Mediterranean, observations of dusky grouper spawning were first reported only recently, from the Medes Islands Marine Reserve in Catalonia (Zabala et al. 1997a,b). We monitored a spawning aggregation of dusky groupers in the Medes Islands and collected data on spawning frequency from 1996 to 1999. Simul-

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taneously, we collected data on environmental variables including moon phases, temperature from the surface to 35 m, currents, water transparency, tides, waves, rain, cloud cover, and atmospheric pressure. The objective of this study was to determine the temporal predictability of spawning of the Mediterranean dusky grouper in relation to atmospheric and oceanographic factors.

MATERIALS AND METHODS

Study site and species. The study site was located in the Medes Islands Marine Reserve in Catalonia, northeast Spain (Fig. 1). This small marine reserve (94 ha of complete protection from fishing) harbors a spawning aggregation of more than 100 dusky groupers (Zabala et al. 1997a). Dusky groupers occupy benthic habitats containing fields of large boulders. Large males (>100 cm total length) establish territories in summer and pair spawn with females that enter their territories (Zabala et al. 1997a). Spawning occurs in the water column at depths of 5 to 25 m. Although spawning occurs throughout the reserve, we focused our sampling efforts on a small area (about 4 ha) in the southeast part of the archipelago (Fig. 1) where density of reproductive adults was the highest (Zabala et al. 1997a).

Grouper spawning behavior. We monitored reproductive activity of groupers using SCUBA diving. We conducted observations of grouper behavior throughout the year. During winter and early spring (November to March), because grouper activity and density are very low (Zabala et al. 1997b), we conducted a census every 2 wk. During spring (May to July), when grouper densities increase and the fish are more active, we conducted controls twice a week, in order to find the first signals of reproductive activity. Daily surveys were performed (weather permitting) between late June and late September, when most of the reproductive activity occurs (Zabala et al. 1997b). Territories of 5 to 12 reproductive males were visited daily at dusk (between 17:00 and 19:00 h) simultaneously by several divers, in June to September from 1996 to 1999.

We focused our observations on males, because they occupy specific territories and have distinct marks that allowed us to distinguish them. Females were much more abundant and moved between male territories, which made it impractical to follow individual females. Grouper territories had variable sizes, up to 100 m in length and 20 m along the depth gradient. Most territories were contiguous, allowing divers to observe each male for 5 to 15 min during each dive. Longer observation times and videotape recording were carried out whenever possible. At each site, divers noted the number of courtships, the number of false rises, and the number of spawns. Courtship occurs when a male approaches a female with a lateral display, shaking the rear part of the body in a ritualized caudal flapping. A false rise occurs when both male and female ascend vertically side by side for a few meters (6 to 8 m) but then swim apart without spawning (Zabala et al. 1997a,b). Spawning occurs when a rise is finished by a short (2 to 3 m) acceleration ending up with the emission of gametes and the immediate separation of the pair.

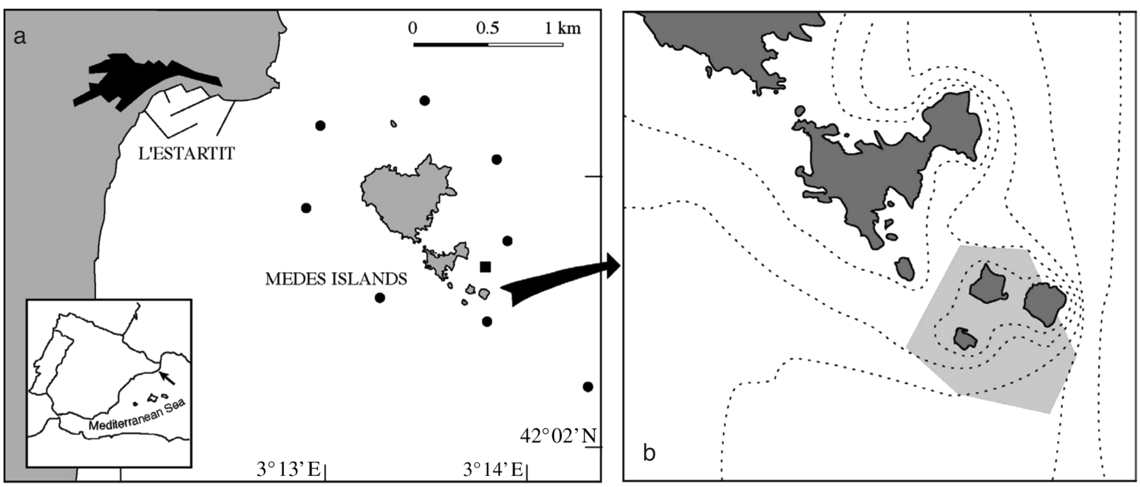

Fig. 1. (a) Medes Islands Marine Reserve showing the study area and the situation of the oceanographic stations (CTD: circles, acoustic Doppler current profiler [ADCP]: square), (b) monitored territories of male dusky grouper *Epinephelus marginatus*. Dotted lines: 10 m depth contours. Shaded area: area where most of the observations were conducted

Environmental variables. We collected information about phases of the moon, atmospheric and oceanographic conditions, and tidal stages. In order to evaluate the relationship between the moon and spawning activity, each moon cycle (28 d) was divided into 4 phases–first quarter, full moon, last quarter, and new moon–each period lasting 7 d. Two weather stations provided continuous records for standard atmospheric variables (atmospheric pressure, temperature, strength and direction of wind, rain, humidity, photoperiod, irradiance, and cloud cover). Regional barometric maps were acquired and recorded twice daily (morning and evening). Tidal amplitude was continuously recorded by a tide gauge installed at the harbour of L'Estartit, 1 km from the Medes Islands. Water transparency was measured by a Secchi disk at the beginning and the end of each dive. Transparency data were pooled into the following categories: bad (<11 m), moderate (11 to 16 m), good (16 to 21 m) and excellent (>21 m). Wave height was estimated daily using a visual scale situated on the shore at L'Estartit.

A description of the thermohaline structure of the waters surrounding the Reserve was recorded from fixed stations routinely sampled from July to October by a conductivity–temperature–depth recorder (CTD). Eight CTD stations were visited weekly in 1996; one station placed 1.6 km off the Medes Islands (at 80 m depth) was sampled weekly between 1997 and 1999 (Fig. 1). Water temperature was recorded at 35 m ($T35$); at 20 m ($T20$), which is the mean depth of the spawning territories; and at 5 m ($T5$), where most spawning activity occurs (Zabala et al,1997a). TD_{5-35}, the difference between water temperature at 5 and 35 m depth, was selected to describe the strength of the thermocline gradient.

From 1996 to 1998 we estimated the direction and the strength of the current using a visual method developed by Zabala et al. (1997a). Current strength was estimated at 3 levels using a weighted rope hanging from the boat: (1) undetectable; (2) moderate, when weighted ropes tilted underwater; and (3) strong, when weighted ropes were pulled by the current more than 30° away from the vertical axis. In 1999, an acoustic Doppler current profiler (ADCP) provided continuous current records, with which we evaluated our visual estimations.

Statistical analyses. We conducted 2 different analyses to determine the relationship between environmental factors and the spawning of the dusky grouper. First, we investigated patterns of variation in reproductive activity at a monthly scale using monthly averages of environmental variables. Second, we used the data collected over a period of 87 d each year, from the first to the last spawning event observed, to search for daily patterns of variation. In both analyses we modeled spawning success as a function of environmental factors with generalized additive models (GAMs; Hastie & Tibshirani 1990).

GAMs are non-parametric generalizations of multiple linear regression that are not restricted to specific functional relationships (i.e. linearity) or underlying statistical distributions (i.e. normality) of the data (Hastie & Tibshirani 1990, Swartzman et al. 1992). Hence, GAMs can be useful for examining environmental and biological relationships that are unlikely to be monotonic, linear or parametric (Maravelias & Reid 1997). In GAMs the dependent or response variable is modeled as the additive sum of unspecified covariate or predictor variables, whereby scatterplot smooths replace the least-squares estimates used in multiple linear regression (Hastie & Tibshirani 1990). The general form of a GAM is:

$$g(E[Y|x]) = g(\mu) = \alpha + \sum_{i=1}^{p} f_i(x_i)$$

where $E(Y)$ is the expected response (spawning) and x_i are the values of the covariates (environmental variables), α is a constant parameter to be estimated, f_i is a nonparametric smoothing function for the ith covariate, μ is the mean response of the predictor variables, and g is the link function, which depends on the error distribution (see below, this section).

We used smoothing splines with varying degrees of freedom (df = 2, 4, and 6) in a flexible model selection, to allow for the most adequate degree of smoothing (a function with 1 df is equivalent to a linear response; an increasing number of degrees of freedom allows for more non-linearity).

Categorical covariates included in the analyses were moon phase, water transparency, wind direction and strength, and current direction and strength. Continuous covariates, included as linear or nonlinear functions, were atmospheric pressure, water temperature, photoperiod, tidal amplitude, and wave height. We selected the covariates that best explained the timing of spawning given the observed data in a stepwise model selection. In this process, each covariate was excluded, retained as linear or included as nonlinear from an initial linear model including all covariates. The best combination of most explanatory covariates was selected by the lowest Akaike information criterion (AIC; Burnham & Anderson 1998).

Two different GAMs were used to model the timing of spawning at different time scales:

(1) Presence–absence. To analyze the role of the environmental variables in triggering spawning at a monthly scale, we used absence or occurrence of courtship or spawning. Zabala et al. (1997a,b) showed that courtship was closely associated to spawning.

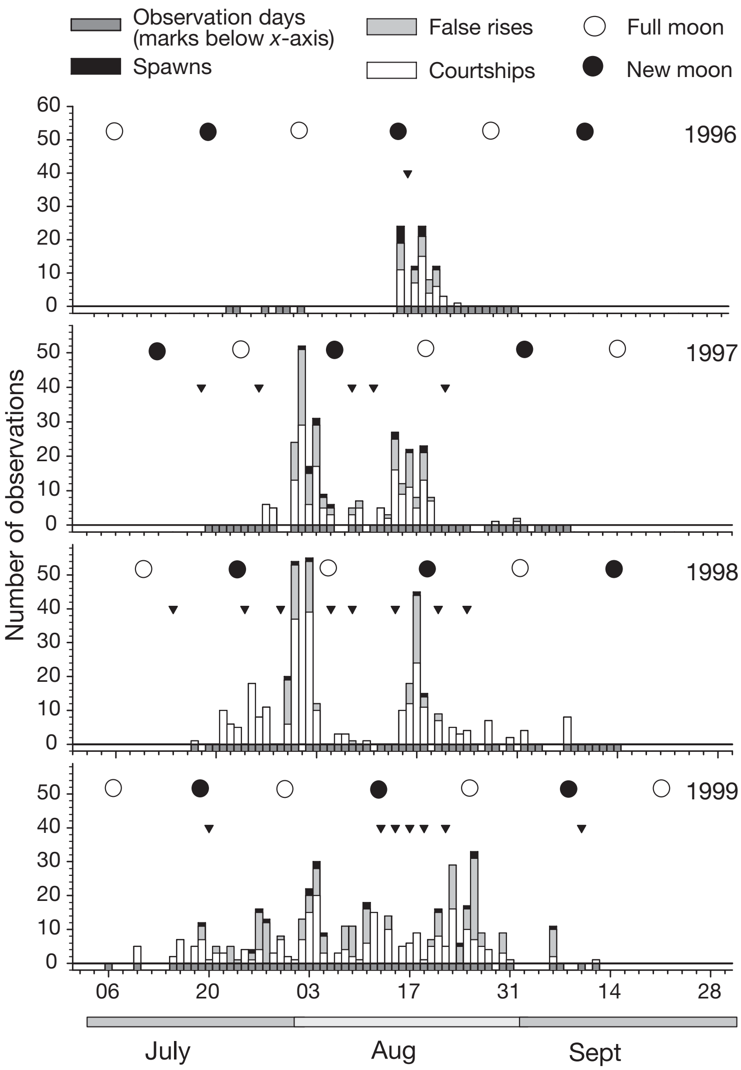

Fig. 2. *Epinephelus marginatus.* Number of observed courtships, false rises and spawns of pairs during the reproductive season in 1996 to 1999. Triangles: days with strong current

Presence/absence of spawning was modeled as a binomial response with a logit function, $\log(\mu/(1-\mu))$, to link the response to the covariates.

(2) Reproductive rate. To analyze trends in spawning at the daily scale, we used number of spawns divided by the time of observation. Spawning rates were assumed to follow a Poisson distribution, as observed in the histogram of the observed data. Because of some degree of sparseness in the observations over time, we used a robust Poisson distribution where the link function is $\log(\mu)$, and a relation between μ and variance proportional to an estimated over-dispersion factor (Chambers & Hastie 1992). To take into account the variation in the number of groupers observed during each dive, we incorporated the number of groupers observed per dive as an offset variable.

RESULTS

Spawning of the dusky grouper was rarely observed. Despite an intensive effort in which a team of divers conducted more than 130 surveys (>500 dives), systematically repeated during the reproductive season (July to September) over 4 consecutive summers (1996 to 1999), only 44 spawns were observed, distributed over 30 different days (Fig. 2).

Spawning: monthly scale

Although there was some interannual variability in peak time of spawning from 1996 to 1999, we observed most spawning events in August (Fig. 2). The selected model for the monthly trends of spawning include the following covariate selected with smoothers *s* of df = 2: $s(T0)$. Comparison of the AICs during the stepwise selection showed that surface temperature was the most significant covariate, followed by *T*35 and photoperiod. The spawning peak occurred during maximum surface temperatures (monthly mean = 24 to 25°C), and during days with 14 daylight hours, 2 mo after the summer solstice (21 to 22 June; Fig. 3).

All grouper spawning in the Medes Islands occurred between the last week of July and the first week of September. The temporal distribution of spawning was significantly different from random ($\chi^2 = 25.6$, $p < 0.001$). In 1996 to 1998, 5 distinct spawning periods were observed, each lasting 2 to 8 d (16–21 August 1996, 1–7 August 1997, 14–21 August 1997, 31 July–3 August 1998 and 18–19 August 1998), which seemed to suggest the existence of 2 short periods of spawning activity each summer, during the first and the third weeks of August respectively, and separated by a period of inactivity during the second week of August (Fig. 2). In 1999 we observed a different pattern, with an almost continuous spawning period from 18 July to 6 September 1999.

Spawning: daily scale

Spawning frequency was less predictable at a daily scale within the reproductive season. There was large variation in the intensity of spawning activity between days, not only for spawning but also for all pre-

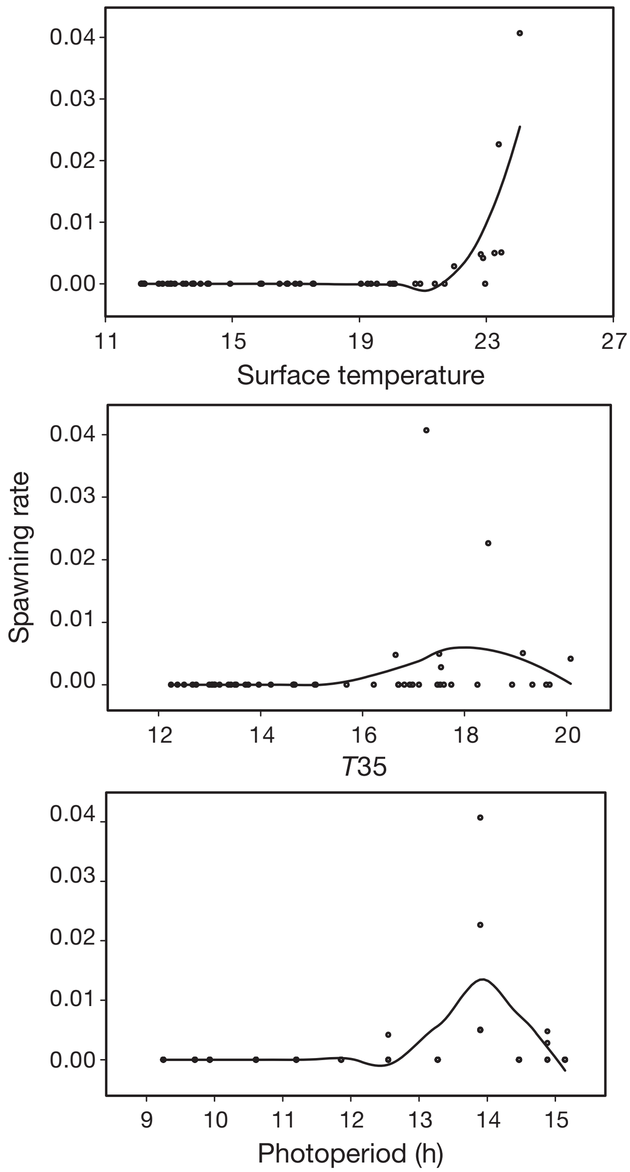

Fig. 3. *Epinephelus marginatus.* Spawning rate (successful spawns per minute of observation) as a function of surface temperature, temperature at 35 m depth (*T*35), and photoperiod

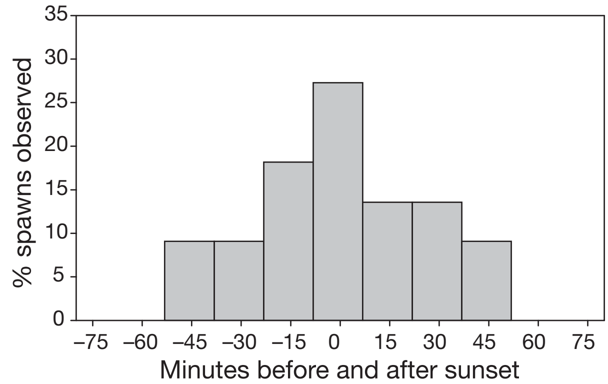

Fig. 4. *Epinephelus marginatus.* Frequency and timing of spawning during the study period

spawning behavior and courtship. Males generally did not exhibit sustained spawning activity during consecutive days. In 38% of the observations of spawning activity, a male exhibiting false rises in one day was able to repeat them the next day. However, after a male actually spawned, it was only able to spawn again on consecutive days 30% of the time. During the spawning period, the average frequency of observation of males spawning was only 1.46 spawns d^{-1}. The maximum observed activity was 5 spawns d^{-1}, which were conducted by 2 different males, but on 75% of the spawning days we observed only 1 spawning by 1 male. On 6 occasions we observed 2 spawnings by the same male. Spawning showed a clear circadian pattern, all spawning occurring at dusk in less than 1.5 h around sunset (the 44 spawning events were observed between 45 min before and 45 min after sunset; Fig. 4).

The selected model for the temporal trends of spawning included the covariates *s*(*T*5) and *s*(*T*35) (selected with smoothers of df = 2) and *s*(pressure) (selected with smoothers of df = 4), together with untransformed *T*20, moon phase, tidal amplitude, and wave height (Fig. 4). The comparison of the AICs during stepwise selection showed that temperature and atmospheric pressure were the most significant covariates, followed by moon phases.

The highest frequency of spawning occurred when the thermocline was shallowest. This occurred when temperature at the surface (5 m) was highest (25 to 26°C) and at the same time temperature at 20 and 35 m was 17 to 19°C (Fig. 5). The maximum difference in temperature between grouper territories at 20 m and the spawning depth was about 7°C.

Spawning occurred during prevailing anticyclonic conditions, and highest spawning frequency occurred during largest tidal amplitude and wave height (Fig. 5e,f). Spawning occurred during all moon phases, but it was not related to any specific phase (median test, $\chi^2 = 2.76$ p = 0.43). In 1997 and 1998, we observed 2 main spawning events separated by 14 d, half a lunar cycle. Despite the average highest frequency of spawning occurring under the above environmental conditions, there was significant variability in spawning between consecutive days. We never observed fully synchronized spawning activity with all the monitored males spawning simultaneously.

Extreme events may reduce spawning activity. Because of the rarity of these events, we could not conduct statistical analyses, although we observed that extremely strong currents (>50 cm s^{-1}) or low temperatures (<15°C) inhibited the reproductive activity of

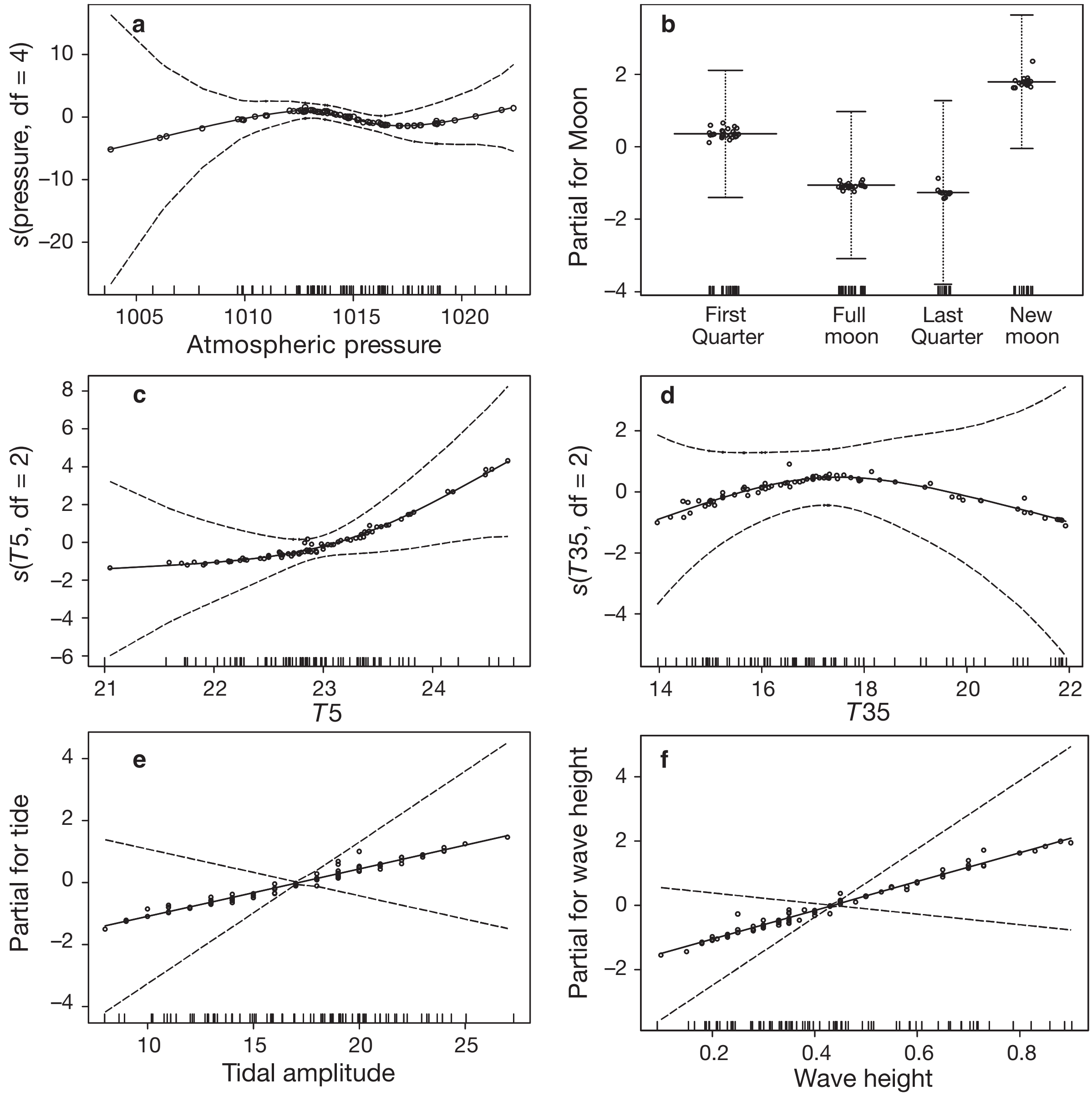

Fig. 5. *Epinephelus marginatus*. Partial fits for the general additive model (GAM) of grouper spawning with (a) atmospheric pressure, (b) phase of the moon, (c) surface temperature (*T*5), (d) temperature at 35 m (*T*35), (e) tidal amplitude and (f) wave height as covariates, showing the relationship estimated by a smoothing spline (solid line). 95% confidence intervals are shown (dotted lines). Rugplot on *x*-axis: number of observations; *s* on *y*-axis: a non-linear function; df: degrees of freedom of the smoothing spline

males. On days with strong currents, both northerly and southerly, males ceased patrolling their territories and withdrew to shelters, where they remained immobile lying on the bottom. Males experiencing cool temperatures near the thermocline generally left their territories for warmer-water sites just above the thermocline, where they hovered over the territory. During these days, any signs of patrol activity, aggression or courtship coloration were absent. Additionally, spawning was never observed on days with turbid waters (Secchi-disk visibility < 8 m), although evidence of turbidity inhibiting spawning is limited by our inability to differentiate no spawning from no observations of spawning because of poor visibility.

DISCUSSION

Dusky grouper spawning in the Medes Islands appears to be a predictable event at a monthly scale. With only a few exceptions, all observed spawning events during 1996 to 1999 occurred in August. It has been reported that photoperiod is the principal environmental determinant of the timing of seasonal breeding in other fishes (e.g. salmonids: Bromage et al. 1993; cod: Norberg et al.1999). Since the first reproductive activity of dusky groupers occurred about 30 d after the summer solstice, the reversal of the photoperiodic length trend could provide an excellent trigger for synchronising reproduction.

The main weakness of our study is the relatively low number of observed spawnings (44 in total). Observation of spawning of the Mediterranean dusky grouper is extremely difficult. Moreover, despite the large number of dives conducted throughout the Mediterranean over the last 50 yr, grouper spawning was not observed until 1996 (Zabala et al. 1997a). We conducted a very intense effort to obtain as much data as possible. Additional studies involving large teams of divers at several locations would provide more detailed data on the relationship between spawning and environmental factors. However, our results show some clear patterns that we believe will be the basis for understanding the environmental triggers of the spawning of the Mediterranean dusky groupers.

Temperature was the environmental parameter which was most significantly related with *Epinephelus marginatus* reproductive activity at a monthly scale. All spawning was detected after the sea-surface temperature reached 21.8°C. The annual cycle of activity of dusky groupers in the Medes Islands was also correlated with the annual cycle of water temperature, and the first signs of aggregation and territorial activity start in late May to early June (Zabala et al. 1997b). High water temperatures may enhance female gonad maturation, as only females permanently inhabit shallow, warmer waters (around 22 to 24°C). Males, in contrast, whose territories are located deeper (commonly below 20 m) and spawn above the thermocline, experience a wider temperature range (15–17 to 24°C). Marine reserves could thus enhance reproduction of the dusky grouper, because groupers are more abundant and distributed in shallow waters within reserves, generally above the thermocline (Garcia-Rubies et al. 2003). In unprotected areas, in contrast, and because of intense spearfishing pressure, reproductive adults are distributed in deeper waters generally below the thermocline. The waters below the thermocline during the spawning season ranged between 15 and 17°C, and this could inhibit spawning.

We would thus expect the reproductive season to start earlier in warmer years, and perhaps to last longer along warmer coasts (e.g. Balearic Islands, North African coasts). For instance, there have been observations of large individuals exhibiting spawning colorations in late October and November in the Cabrera National Park, Balearic Islands, and Lavezzi Marine Reserve, Corsica, although spawning was not observed during those months (authors' pers. obs., P. Quignard & J. M. Culioli pers. comm.). Colin (1992) described a similar pattern of latitudinal variation in spawning time in *Epinephelus striatus*, where spawning occurred in different months in different areas, but always when surface temperature was about 25 to 26°C. The apparent recent warming of seawater in the NW Mediterranean (Francour et al. 1994, Pascual et al. 1995) could also lengthen the reproductive period of the dusky grouper.

Spawning of the dusky grouper appears to be variable and less predictable at a smaller temporal scale (days), although there was a significant relationship between some environmental variables and spawning. Because there were >60 reproductive females in the Medes Islands (Garcia-Rubies et al. 2003), our observations covered only between 10 and 20% of the potential spawning in the marine reserve. In addition, we were not able to monitor all pairs simultaneously. Nevertheless, our imperfect estimation of reproductive activity sufficed to show that high variability between consecutive days was the rule. We never observed days of fully synchronized activity with all the monitored males spawning simultaneously, and, at the individual level, a day of successful mating was commonly followed by a mostly unsuccessful day.

At a circadian scale during the spawning period, dusky groupers exhibited a clear pattern in reproductive activity. As described for other groupers (e.g. Colin 1992, Samoilys & Squire 1994, Sala et al. 2001) all dusky grouper spawns were observed near sunset. Zabala et al. (1997a) suggested this timing would reduce egg and larval mortality because spawning occurs at a time when diurnal egg predation would be relatively satiated (Colin & Clavijo 1988), and also because darkness would reduce predator efficiency, as suggested by Samoilys & Squire (1994). However, predation on eggs by sparid fishes, mainly *Oblada melanura*, was frequent (Zabala et al. 1997a).

Epinephelus marginatus spawned during all moon phases, in contrast with the more restricted lunar synchronicity showed by some tropical groupers (e.g. Domeier & Colin 1997). This absence of synchronicity with lunar phases may be due to the relatively long reproduction period of *E. marginatus* (2 to 4 wk). Some tropical species that reproduce in synchrony with moon phases can have spawning periods that last only a few days (e.g. 7 d for *E. striatus*; Sala et al. 2001) and the moon period may then be a precise signal for spawning. Johannes (1978) suggested that synchronized spawning with moon phases allows gametes to be released during strong tidal flows which could facilitate the offshore transport of eggs, thereby reducing potential predation. However, there are no empirical data to support this hypothesis. Although spawning of *E. marginatus* was more frequent during the largest tidal amplitude, the tidal range in the NW Mediterranean is small (<50 cm; Hopkins 1985) and does not produce significant increases in water flow. Relating spawning events of dusky groupers to egg and early larval transport will be difficult, given the nonsignificant relationship between spawning and strength and direction of currents in the spawning area.

Reproductive behavior of dusky groupers in the NW Mediterranean has only recently been described (Zabala et al. 1997a,b). Although much is known about spawning behavior, much more needs to be learned about the underlying mechanisms of spawning and recruitment in order to ensure proper management of this valuable species.

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