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Reproductive biology of *Diplodus vulgaris* (Teleostei, Sparidae) in the southern Tunisian waters (Central Mediterranean)

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The reproduction of the common-two banded seabream (N=916), Diplodus vulgaris*, was studied in the Gulf of Gabes (Tunisia). The length at the first maturity averages 13.64 ± 0.18 cm for males and 13.84 ± 0.17 cm for females. Macroscopic examination of gonads and gonad-somatic index indicated that spawning occurs once a year between November and February with peak activity in November and December. Fecundity estimates ranged from 8 400 to 30 800 oocytes.*

Key words: *Diplodus vulgaris,* reproductive cycle, maturity, fecundity, central Mediterranean

INTRODUCTION

The common two-banded seabream, *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817) is a demersal species distributed in the Mediterranean and Black Seas, along the eastern Atlantic coast from France to Senegal (including the Madeira, Azores and Canaries Archipelago), and from Angola to South Africa (BAUCHOT & HUREAU, 1986, 1990). It can be found close to rocky and sandy bottoms to a maximum depth of 160 m. Juveniles often live in coastal lagoons and estuaries (MONTEIRO, 1989) and it is considered a resident species in artificial reefs (SANTOS, 1997).

Reproduction aspects have been studied in the Mediterranean by D'ANCONA (1949), LISSIA FRAU & PALA (1968), EL-MAGHRABY *et al.* (1981), KENTOURI & DIVANACH (1982), QUÉRO (1984), MAN-WAI (1985), CETINIĆ *et al.* (2002), BELTRANO *et al.* (2003), ZAKI *et al.* (2004), TSIKLIRAS *et al.* (2010) and the eastern Atlantic (LOZANO *et al.*, 1990; GONÇALVES & ERZINI, 2000; GONÇALVES *et al.*, 2003; PAJUELO *et al.*, 2006).

No studies have targeted the reproduction aspects of the species from southern Tunisian waters. Accordingly the study *D. vulgaris* seems to be important, since the species is one of the most important components of demersal fauna in Tunisia. It is therefore essential to determine the reproductive style of each sparid species in order to obtain a better understanding of its biology, a suitable evaluation of its population dynamics and a good management of its fisheries.

Thus, the aim of this paper was to study the reproductive cycle, maturity and fecundity of *D. vulgaris*.

MATERIAL AND METHODS

The Gulf of Gabes is a large neckline situated on the southern coastline of Tunisia (AZOUZ, 1971; BURROLET *et al.*, 1979), spreading over about 750 km from Cape Kapoudia (35th parallel) to the Tunisian-Libyan borders (Fig. 1). Fish samples were collected monthly from June 2006 to May 2007. They originated from commercial catches made along the southern Tunisian coasts

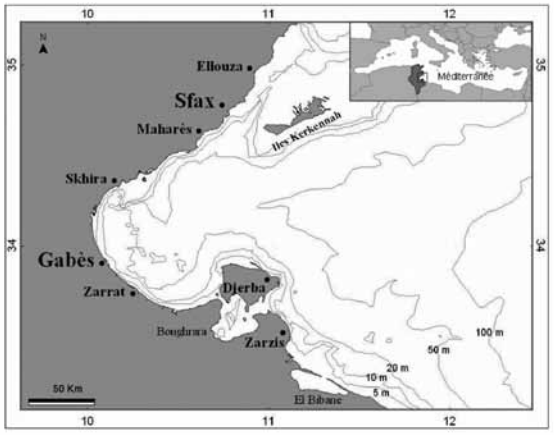

Fig. 1. Geographical position of the Gulf of Gabes (Tunisia)

and by the different types of artisanal fishing gears (seine nets, gill nets, fyke nets). Most fish were examined fresh, shortly after landing. A total of 916 specimens were collected, ranging in size from 10.9 to 25.8 cm of the total length (TL).

Each specimen's TL was measured to the nearest 1 mm and a total weight was measured using a top-loading digital balance (precision of 0.01 g). Gonad weight was recorded to the nearest 0.001 g. The macroscopic stage of gonad development in the fishes was determined using the classification of maturity stages: immature (I); resting (II); ripe (III); ripe and running (IV) and spent (V) (HOLDEN & RAITT, 1975).

We calculated the sex proportion and the results were tested using the χ^2-test (SCHERRER, 1984).

To quantify the changes in gonad weight during the annual sexual cycle and to determine the spawning season, we calculated the gonad-somatic index (GSI) for 783 specimens (399 females and 384 males) by the following formula:

$$GSI = GW*100/EW \quad (2)$$

GW: gonad weight (g) and EW: eviscerated weight (g).

Accumulation and depletion of reserves of two-banded seabream in the Gulf of Gabes were studied through the analysis of monthly changes of hepatic-somatic index (HSI) and the condition coefficient (K). These indexes were calculated as follows:

$$HSI = LW*100/EW \quad (3)$$

LW: liver weight (g) and EW: eviscerated weight (g);

$$K = EW*100/TL^3 \quad (4)$$

EW: eviscerated weight (g) and TL: total length (cm).

Analysis of variance, followed by Tukey's post hoc test (ZAR, 1996), was used to confirm critical differences in the indexes (GSI, HSI, and K) per month. The results are presented as the mean (± confidence interval) and the significance level used for the tests was $P = 0.05$.

The total length at first maturity was estimated during the spawning season by the proportion of mature specimens (i.e. in stages III-V). It is defined as the total length at which 50% of fish are mature and was estimated by means of a logistic function fitted to the proportion of the mature specimens pooled in 1 cm length class (TL).

To estimate the size at first sexual maturity (TL_{50}), L_{25} and L_{75} corresponding to 25 and 75% of mature individual, we calculated the proportion (Pi) of mature individuals by sex and by size class:

$$Pi = Mi*100/Ni \quad (5)$$

where Mi is number of mature individuals in the size class;and i, Ni is the number of examined individuals in the same size class i.

The obtained data were fitted to a logistical function by using the software 'FSAS' (SAUL *et al.*, 1988).

The used equation was:

$$P = 1/(1+e^{-a(TL-TL50)}) \quad (6)$$

P - proportion of mature individuals; TL - total length corresponding to the proportion (P); a - constant and TL_{50} - total length of 50% mature fish. This function (6) has the advantage of estimating with precision the lengths TL_{25}, TL_{50} and TL_{75} that are often required by most fisheries science's software to carry out fish stock assessments (e.g. PAULY, 1980; GHORBEL *et al.*, 1996).

Ovaries used for fecundity estimates were from ripe stage; small pieces from each pair of ovaries were weighed to the nearest 0.1 mg and preserved in a 7% formalin solution. After approximately 3 months, the ovaries were care-

fully washed under running water, which helped in separating the oocytes from the tissue. For fecundity estimates the volumetric method was employed (DULČIĆ *et al*., 1998). The ovaries were placed in a beaker with a known volume of water and mixed with a magnetic stirrer. Five subsamples were obtained from the ovaries of each fish, using a 2-ml Stempel pipette and subjected to an analysis of variance to test their homogeneity. Fecundity, defined as the number of ripening eggs in females prior to spawning, was determined in 67 specimens sampled in 2006 and 2007. The number of maturing oocytes for both ovaries was calculated from the sampled ovary.

To establish the relationship between fecundity (F) and TL, TW and GW, the multiplicative ($y = ax^b$) regression model was used.

RESULTS

Sex-ratio

Out of 916 specimens examined, 107 were undifferentiated (< 10 cm TL). Macroscopic study of gonads showed that the sample consisted of 399 females, 384 males and 26 intersexual individuals. These data showed that the number of females is almost equal to that of males. Overall sex-ratio can be considered 1:1; anyway males dominated the length intervals between 10 and 16 cm, with the exception of lengths 13 and 15 cm (a small number of specimens), females were the most abundant in classes > 18 cm and intersexual individuals were most frequent between 14 and 18 cm (Table 1).

The χ^2-test did not clearly confirm these findings since the difference was not significant, as the χ^2 calculated (15.375) was less than χ^2theoritical (19.675). The difference was significant only for the last length class (≥21 cm; χ^2 calculated = 4.637 vs. χ^2 theoretical = 3.84).

Sex-ratio was calculated by season (Table 2). This parameter significantly varied with season

Table 2. Seasonal variation of females as percentages of Diplodus vulgaris *in the Gulf of Gabes.*

Season	Total	% of females	χ^2 calculated
Summer	184	55.98	1.86
Autumn	202	54.46	0.99
Winter	212	42.45	6.14
Spring	185	51.89	0.06
Annual	783	50.96	9.05

Table 1. Percentage and number of males, females and intersexual individuals as a function of length in Diplodus vulgaris *in Gulf of Gabes. N, number of fish*

Length (cm)	*Females* %	*Males* %	*M+F* N	*Intersexual* N	χ^2 *calculated*
£10	33.33	66.67	9		1.119
11	45	55	40		0.568
12	47.11	52.89	121	1	0.718
13	51.13	48.87	133		0.002
14	48.82	51.18	127	4	0.233
15	55.17	44.83	87	11	0.618
16	42.71	57.29	96	5	2.614
17	50.91	49.09	55	1	5.10^{-5}
18	57.14	42.86	35	1	0.536
19	67.86	32.14	28		3.200
20	63.16	36.84	19	2	1.132
³21	69.7	30.3	33	1	4.637
Total	50.96	49.04	783	26	15.375

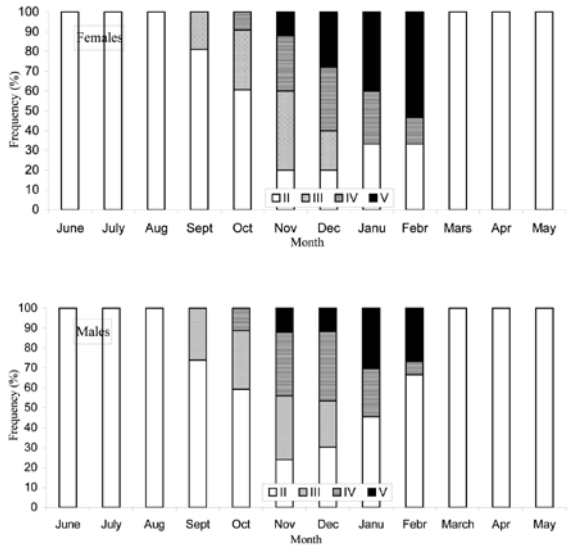

Fig. 2. Monthly evolution of the maturity stages for females and males of Diplodus vulgaris *in the Gulf of Gabes*

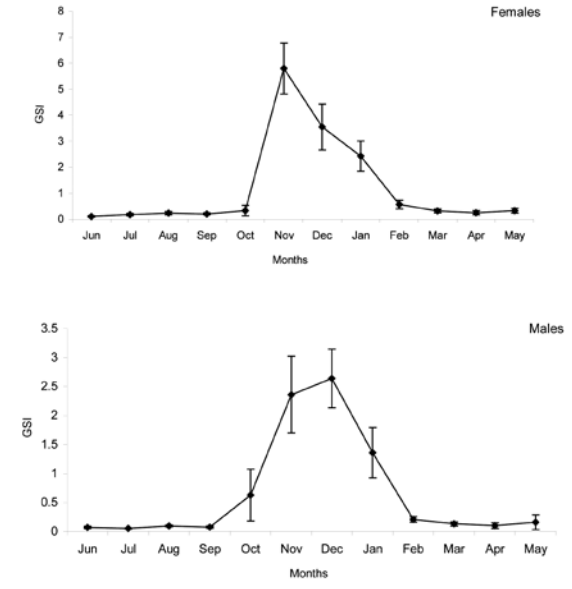

Fig. 3. Monthly variation of the gonad-somatic index (GSI) for Diplodus vulgaris *in Gulf of Gabes (mean ± confidence interval)*

(χ^2 calculated = 9.05 > χ^2 theoretical =7.81) and males were only significantly abundant in winter (χ^2 cal = 6.14).

Spawning period

Monthly mean values of gonad-somatic index for females and males with the 95% confidence limits are shown in Figure 2. The highest mean value was found in stage II, in which the weight of the gonads represented 7.5% of the total weight of females. Peaks of gonad-somatic indexes, both of males and females, coincided with the spawning period as determined by maturity stage changes (Fig. 2).

For females, the monthly evolution of gonad-somatic index (GSI) showed that it increased slowly during the period between June and September (2006) from 0.107% to 0.206% (Fig. 3). Then, the slight increase was shown in October (0.33%) followed by a rapid increase of GSI in November (5.793%) when it reached a maximum value. From November to February, there was a sharp drop of the GSI to 0.572% which reflected the phenomenon of spawning. Indeed, while analyzing the evolution of the individual GSI, we saw that a few individuals began to emit their gametes from November.

After spawning, females were in sexual rest stage, extending from March to June while the gonad-somatic index remained almost constant (Fig. 3).

The evolution of the GSI in males was almost similar to that observed in females (Fig. 3). The maturation phase of gametes was between September and October. The maximum value of the GSI was observed in December; afterwards the values of the GSI began to decrease.

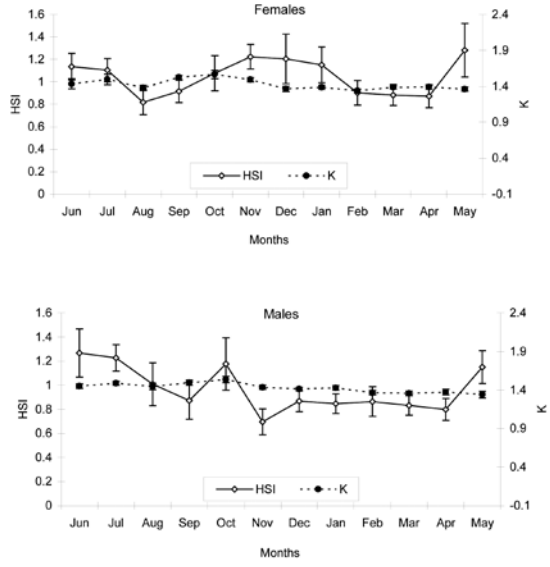

Fig. 4. Monthly variation of the hepatic-somatic index (HSI), and the condition factor (K) of Diplodus vulgaris *in the Gulf of Gabes (mean ± confidence interval)*

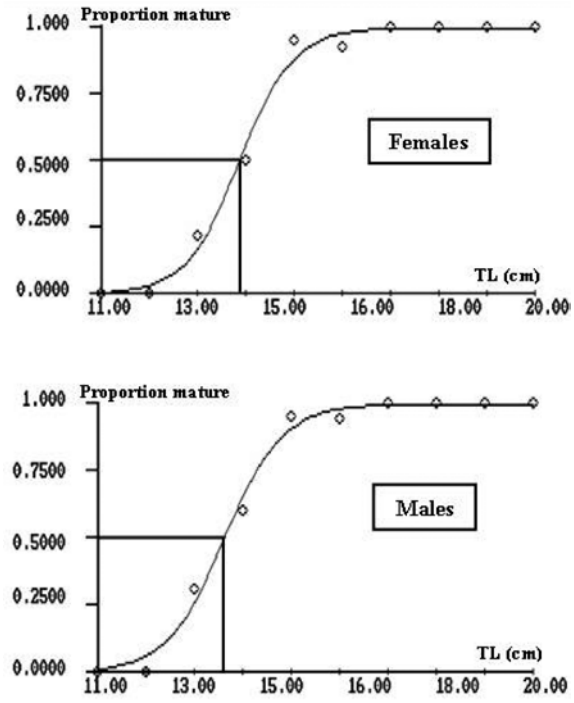

Fig. 5. Graphic representation of Diplodus vulgaris *maturity of males and females in the Gulf of Gabes*

For males, November and December mean GSI were not significantly different (ANOVA, F = 0.449, P > 0.05).

Sexual cycle in both sexes of *D. vulgaris* was synchronized, except that the peak of the GSI was observed in November for females (5.793) and in December for males (2.639).

The hepatic-somatic index (HSI) and the condition coefficient (K) for both sexes gradually increased from August to October. For males in November, during the spawning of this species, the two indexes showed a decrease. However, the HSI reached its maximum in May with 1.28 and 1.15 for females and males, respectively and attained its minimum in August (0.82) for females and in November (0.70) for males. The variations in the condition coefficient (K) were not very perceptible and its decrease had less importance for both sexes. Hence, it seems that the changes in the HSI are associated with the sexual cycle (Fig. 4).

Length at first maturity

An analysis of length at first maturity L_{50} showed that males were mature at an average

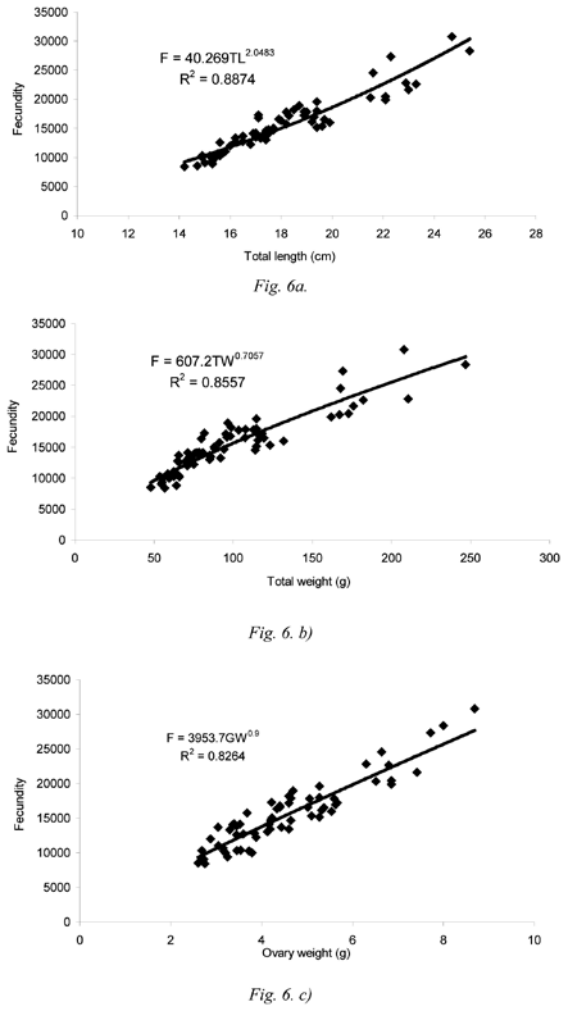

Fig. 6a.

Fig. 6. b)

Fig. 6. c)

Fig. 6. a) relationship of fecundity to length for Diplodus vulgaris *in the Gulf of Gabes b)relationship of fecundity to total weight for* Diplodus vulgaris *in the Gulf of Gabes, c) relationship of fecundity to ovary weight for* Diplodus vulgaris *in the Gulf of Gabes*

of 13.64 ± 0.18 cm in TL or 10.27 cm standard length whereas females at 13.88 ± 0.17 cm in TL or 10.45 cm standard length (Fig. 5). Thus, males mature at shorter size than females.

The application of the χ^2-test confirmed these findings: for males (χ^2 calculated = 0.523) and females (χ^2 calculated = 0.521) calculated values were lower than the theoretical value of 16.92 (ddl = 9 and risk $\alpha = 0.05$).

Calculated L_{25} of 12.98 cm for males and 13.27 cm for females, and L_{75} of 14.3 cm for males and 14.49 cm for females corresponded respectively to 25% and 75% of mature individuals.

Fecundity

The 67 ripe females examined ranged from 14.2 to 25.4 cm TL (ages 3 and 8 years, respectively), while the total mass ranged from 47.94 to 246.92 g. The absolute fecundity varied between 8400 and 30800 eggs per fish. Plots of fecundity versus length (Fig. 6a); fecundity versus total weight (Fig. 6b) and fecundity versus ovary weight (Fig. 6c) indicated a regression model.

The mean fecundity obtained for *D. vulgaris* through the direct summation procedure was 15437 ± 1126 eggs per fish. The diameter of oocytes contained in the ovaries ranged from 0.34 to 0.62 mm, with an average of 0.41 ± 0.03 mm.

DISCUSSION

Analysis of the GSI together with maturity stage data suggest that the spawning period took place in the winter season from November to February, with a resting period during the remainder of the year. Our data showed a similarity period spawning as in other Mediterranean areas (KENTOURI & DIVANACH, 1982; QUÉRO, 1984; MAN-WAI, 1985; BAUCHOT & HUREAU, 1986; RIEDL, 1986; FISCHER *et al.*, 1987; CETINIĆ *et al.*, 2002; ZAKI *et al.*, 2004; BELTRANO *et al.*, 2003; TSIKLIRAS *et al.*, 2010).

Previous results in Tunisian waters (Gulf of Tunis and Gulf of Gabes) confirmed that spawning occurs in the same period (EL-AREM, 1980; BRADAI, 2000) while in Algeria (DIEUZEIDE *et al.*, 1955), for fish > 25 cm spawning starts in December and ends in March and in Italy (TORTONESE, 1975), spawning starts in October and finishes in November.

Such an extensive spawning period could indicate that environmental conditions for hatching and larval development are favourable for a longer period (TSIKLIRAS *et al.*, 2010). An extensive spawning period has also been found for *P. erythrinus* (GHORBEL, 1996) and for *S. aurata* (HADJ TAIEB *et al.*, 2010) and an even extended period was found for *D. vulgaris* for the Atlantic Ocean (GONÇALVES & ERZINI, 2000; GONÇALVES *et al.*, 2003; PAJUELO *et al.*, 2006). The spawning season of *D. vulgaris* could change from year to year as a function of sea surface temperature (SST) and the number of hours of direct sun light, because the start and the duration of the spawning season are negatively correlated with these environmental parameters (GONÇALVES, 2000).

Diplodus vulgaris saves some lipid reserves in the liver as well as in the muscles. Hence, it seems that the changes in the HSI and K are associated with the sexual cycle. The annual variation in the HSI showed that energy storage decreased during the spawning season and increased afterwards.

The presence of individuals with well-formed ovaries and residues of degenerated testes and the predominance of males at smaller sizes confirms that it is a protandric hermaphrodite. This characteristic, which is common among sparids (BUXTON & GARRATT, 1990; MICALE & PERDICHIZZI, 1994; VITALE *et al*., 2011), has been documented for this species in the Mediterranean (D'ANCONA, 1949; LISSIA FRAU & PALA, 1968; EL-MAGHRABY *et al*., 1981; MAN-WAI, 1985; GORDOA & MOLI, 1997).

The differences observed in sexual maturity between the sexes may be explained adequately by protandry. Fish attain maturity between the second and the third year of life (HADJ TAIEB, 2012). DULČIĆ *et al.* (2010) pointed out that this species also attains sexual maturity at the age of 2-3 years in the eastern Adriatic Sea.

The two-banded seabream reached sexual maturity at a smaller size than reported by LOZANO *et al.* (1990) and GONÇALVES *et al.* (2003). They reported 19.7 and 17.24 cm as the lengths at first maturity, but they analysed only a few individuals smaller than 20 cm, and 50% of the individuals of the smallest length class were mature. As reported by other authors (MAN-WAI, 1985; GORDOA & MOLI, 1997) there was a slight increase in the proportion of males with size, which could be related to the protandrous hermaphroditism associated with *D. vulgaris* (GONÇALVES & ERZINI, 2000). However, sex change may not only be related to individual determinism but could depend on the environmental and social conditions (HAPPE & ZOHAR, 1988).

Fecundity estimates of *D. vulgaris* were smaller in the Gulf of Gabes than in the south-west coast of Portugal ranging between 31 523 to 250 608 (GONÇALVES & ERZINI, 2000). The reproductive efficiency of *D. vulgaris* appears to be different to that in the Atlantic. This difference can be explained with the size of the females, in the present study females were smaller than in the Atlantic study (>25cm).

There was a significant positive relationship between fecundity and fish length; this result is in agreement with the one found in other sparid in the same area by GHORBEL (1996) for *Pagellus erythrenus* and by HADJ TAIEB *et al.* (2012) for *Sparus aurata*.

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Reproduktivna biologija vrste *Diplodus vulgaris* (Teleostei, Sparidae) u vodama južnog Tunisa (središnji Mediteran)

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SAŽETAK

Razmnožavanje fratra (N=916) *Diplodus vulgaris*, istraživano je u zaljevu Gabes (Tunis). Dužina prve spolne zrelosti je u prosjeku između 13,64 ± 0,18 cm za mužjake i 13,84 ± 0,17 cm za ženke. Makroskopsko ispitivanje gonada i gonadsko-somatskog indeksa pokazuje da se mriješćenje odvija jednom godišnje, između siječnja i veljače sa vrhuncem u studenom i prosincu. Procjene fekunditeta su u rasponu od 8 400 do 30 800 oocita.

Ključne riječi: *Diplodus vulgaris,* reproduktivni ciklus, zrelost, fekunditet, središnji Mediteran