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Multiple spawning and spawning batch size in *Sepietta oweniana* (Cephalopoda: Sepiolidae)

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Abstract: In order to gather information on the reproductive strategy of the sepiolid squid *Sepietta oweniana*, direct observations of egg-laying in the aquarium by three wild collected mated females were combined with the examination of the ovaries of 59 specimens. The record of multiple spawning events in two aquarium-kept females (two and eight events, respectively) and the co-existence in the ovaries of oocytes at various stages of maturation, including mature oocytes, showed that *S. oweniana* is a multiple-spawner. An estimate of the spawning batch size, *i.e.* the mean number of mature oocytes released at each spawning act, was 54.4 eggs; the overall fecundity was estimated to be on the order of 10^3 eggs. The multiple spawning reproductive strategy of this sepiolid squid, which continues to grow during its long reproductive phase, was found to differ largely from that of some teuthid squids.

Résumé : *Pontes multiples et taille des pontes chez* Sepietta oweniana *(Cephalopoda: Sepiolidae).* L'objectif du présent travail était d'obtenir des informations sur la stratégie de reproduction de la sépiole *Sepietta oweniana* par des observations en aquarium sur la ponte de trois femelles capturées en mer après leur accouplement, ainsi que par l'examen des ovaires de 59 exemplaires. Les pontes multiples chez deux femelles captives (deux et huit pontes, respectivement) et la présence simultanée dans les ovaires d'ovocytes à différents stades de développement, y compris des ovocytes mûrs, montrent que *S. oweniana* est un céphalopode à ponte multiple. La taille moyenne de la ponte, c'est-à-dire le nombre moyen d'ovocytes mûrs à chaque ponte, a été estimée à 54,4 œufs ; la fécondité totale a été évaluée à environ 10^3 œufs. La stratégie de reproduction de cette sépiole, caractérisée par des pontes multiples et par la croissance somatique pendant sa longue phase de reproduction, est très différente des modes de reproduction connus chez certains teuthidés.

Keywords: Reproductive strategy, oocyte, fecundity, Mediterranean, Cephalopoda, Sepiolidae.

Introduction

In contrast to the once widely held opinion that coleoid cephalopods are strictly semelparous 'big-bang' spawners, it has become clear in recent years that the females of many species are indeed multiple or 'long-continued' spawners, *e.g.* Sepiida (Boletzky, 1987a), Sepiolida (Gabel-Deickert, 1995), Teuthida Myopsida (Maxwell et al., 1998), Teuthida Oegopsida (Laptikhovsky & Nigmatullin, 1993), Octopoda (Villanueva, 1992), see also the review by Rocha et al. (2001). Multiple spawning may be defined as the co-occurrence of two processes: at least two bouts of egg-laying and the maturation of new oocytes between these

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bouts (Harman et al., 1989; Maxwell et al., 1998; Rocha et al., 2001).

The evolutionary significance of multiple spawning in cephalopods as well as in other marine animals is fairly clear. Multiple spawning favours the increase of the overall number of spawned eggs in one reproductive season and, in addition, spreads eggs and hatchlings over a long period of time thus counteracting temporary unfavourable environmental conditions; at the same time, also the energetic investment for reproduction is spread over a long period of time. The reproductive advantage of multiple spawning is particularly evident in Sepiolids and other small-size cephalopods laying large eggs (Boletzky, 2002).

Very little is known about the real overall fecundity of Atlanto-Mediterranean sepiolids, as well as about their spawning batch size, *i.e.* the number of mature oocytes released at each spawning act. The only sources of information are the paper by Bergström & Summers (1983) on *Sepietta oweniana* (d'Orbigny, 1841) and those by Gabel-Deickert (1995, 1996) who maintained eight sepiolid species in the aquarium, to observe their spawning.

The purpose of this paper is to investigate the reproductive strategy of *Sepietta oweniana*, which is the largest sepioline species in the Atlanto-Mediterranean area, the one with the widest geographical distribution (from Mauritania to Norway), the widest depth distribution (8 750 m), and the highest density in the Mediterranean Sea (Mangold-Wirz, 1963; Mangold & Boletzky, 1987).

This study was accomplished by gathering evidence for multiple spawning through both direct observations on the reproductive behaviour of captive females and the examination of ovary conditions in wild-collected squids. The approach of combining anatomical information with observations of actual egg-laying was adopted by Maxwell et al. (1998) to investigate multiple spawning in *Loligo pealei* Lesueur, 1821. In addition to establishing that females of *S. oweniana* spawn repeatedly, the spawning batch size was deduced from direct observations of spawning and especially by counting the mature oocytes of wild-collected females. A similar method has been used with clupeiform fishes to estimate their 'batch fecundity' (*e.g.* Hunter et al., 1985) (since the definition of the term 'batch fecundity' as used in the procedure to determine the total fecundity of a fish stock is not univocal [*cf.* Parker, 1985], we prefer to use instead the term 'spawning batch size' or simply 'batch size').

Materials and methods

This study was carried out on two samples of *Sepietta oweniana* collected in the Catalan Sea (western Mediterranean) and the Adriatic Sea (eastern Mediterranean) respectively.

The sample collected in the Catalan Sea consisted of seven females of *S. oweniana* caught by bottom trawl on sandy and muddy grounds, between 10 and 120 m of depth, during daytime, in January 1988 (one mature female) and in March-July 1989 (one immature and five mature females) (maturity established according to Mangold, 1989). The presence of discharged spermatophores in the *bursa copulatrix* showed that all mature females had mated before being collected. These bobtail squids were transferred to the aquarium soon after capture and were kept separately, without any males, in 50 litre plastic tanks (base: 42 x 60 cm). All tanks were supplied with running sea water filtered through a gravel bed. Water temperatures in the aquarium varied from 9 °C in February to 23 °C in July. The sepiolids were fed on live mysids, palaemonid and crangonid shrimps *ad libitum*. Daily routine controls permitted the recording of egg numbers for each spawned clutch, the individual spawning duration (time from first to last egg laying) and the total number of eggs laid by each female. Daily controls on females were carried on until their death, that is up to 32 days following their capture. The specimens found dead were weighed to the nearest 0.1 g after removing the excess of water with paper tissue, the mantle being cut open (fresh weight being a more reliable measure than dorsal mantle length *ML* in sepiolid squids [Bello, 1991]). Their ovaries were then removed, fixed with Bouin's solution and preserved in 70% ethanol; afterwards they were dissected to count and measure all the oocytes larger than 0.1 mm.

The other sample was collected by bottom trawl in three stations similar in depth (93 to 98 m) and temperature (13 to 14 °C) off the Apulian coast, south-western Adriatic Sea, from the 27th of October to the 3rd of November 1985. A total of 92 specimens, 40 males and 52 females, were caught and used for batch size estimation by the same method as used for bony fishes, *viz.* the count of ripe oocytes, ready to be spawned, found in the ovary (Hunter et al., 1985). The squids were fixed in 10% formalin and then preserved in 70% ethanol. Measurements and counts were taken on the preserved animals. *ML* was measured to the nearest 0.5 mm. All specimens were dissected to determine their sex and maturity condition. Mated females were recognized by discharged spermatophores remaining in their *bursa copulatrix*. The ovary of each mated female was removed from the mantle cavity and the mature oocytes were counted and measured. Fully mature oocytes are easily recognized due to their smooth surface (because of this they are also called 'smooth eggs', Mangold-Wirz, 1963) and their gold-brownish colour, that is darker than the vitelline oocytes. The number of mature oocytes in each female is abbreviated as *MO*. Since the frequency distribution of *MO* values was similar in all three sampling stations, the sub-samples were pooled and used as one single sample. The lunar periodicity

was taken into account in the Adriatic specimens to evaluate the distribution of *MO* values, since the lunar phase was shown to affect some aspects of the reproductive cycle in sepiolid squids (*viz.* the development of spawned eggs in *Rossia pacifica* Berry, 1911 in Summers, 1985).

Results

Observations on aquarium-kept females of Sepietta oweniana *from the Catalan Sea*

Three out of six wild-caught mature females of *Sepietta oweniana* died the day of collection (without any spawning).

The remaining three females survived in the aquarium tanks for durations ranging from 7 to 32 days. The shortest-lived of them spawned only once; the remaining two females achieved multiple-spawning. Table 1 summarizes the observations on the number of spawning events and the size of spawned egg batches produced by these three females. The average spawned egg batch contained 48.54 eggs (= overall number of spawned eggs / overall number of spawning events = 534 / 11).

Only one specimen (no. 2) continued spawning at roughly regular intervals (1 to 4 days) during its whole stay in the aquarium. This female spawned 8 times. The daily and cumulative distributions of the number of eggs laid by this individual is shown Fig. 1. According to the regression equation of the line fitting the cumulative frequency distribution of eggs laid over time ($eggs = 83.70 + 16.74 \cdot days$; $r = 0.968$; $n = 8$; $P < 0.001$), the average number of eggs laid per day is 16.74 ± 1.78 ($= b \pm s_b$, where b is the slope and s_b the slope standard error).

Oocyte counts

The size frequency distributions of oocytes in the seven females (six mature and one immature) collected in the Catalan Sea are reported in Fig. 2. In all females, oocytes smaller than 0.5 mm make up the bulk of oocytes (Fig. 2; Table 2); their percentage ranges from 60.7 to 78.7%. The

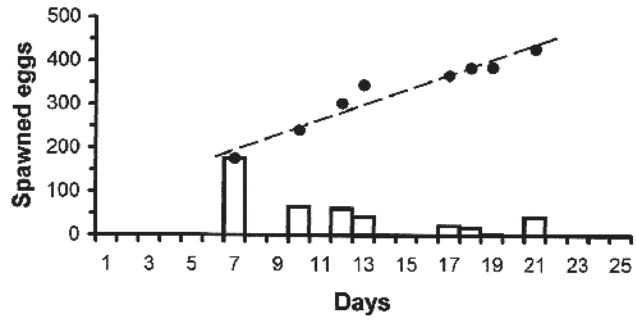

Figure 1. Daily and cumulative distributions of number of eggs laid in aquarium by female n° 2 of *Sepietta oweniana* during 21 days. Broken line: regression line for cumulative distribution.

Figure 1. Distribution journalière et cumulée du nombre d'oeufs pondus dans l'aquarium par la femelle n° 2 de *Sepietta oweniana* pendant 21 jours. Droite en pointillés: régression linéaire calculée sur la distribution cumulée.

total number of oocytes plus eggs laid in the aquarium (Table 2) is here considered cautiously to represent the overall production of oocytes; indeed it is not known whether the six mature wild-collected females had spawned before being caught or whether they would have laid more eggs if they had not been caught.

No significant correlation was found between the overall number of produced oocytes and body weight in mature females or between numbers of oocytes and body weight, *i.e.* the regression line slopes are not significantly different from 0 (Table 3). Therefore the numbers of oocytes and eggs are independent of the size of the animal.

Mature oocyte counts

The sample collected in the Adriatic Sea contained 8 virgin females, all of them immature (*ML* range: 16.5 - 27.0 mm), and 44 mated females (*ML* range: 24.5 - 38.5 mm); all females larger than 27.0 mm *ML* were mature and mated. The number of mature oocytes (*MO*) per mated female ranged from 0 to 64, except in one specimen, which had an ovary containing 133 mature oocytes. The mean value of

Table 1. Spawning duration, batch size and total number of spawned eggs of aquarium-kept females of *Sepietta oweniana* from the Catalan Sea; the day of first spawn, exact duration of spawning, and individual batch sizes of the third female are unknown.

Tableau 1. Durée de la ponte, taille des pontes et nombre total d'œufs déposés en aquarium par trois femelles de *Sepietta oweniana* de la Mer Catalane; le jour de la première ponte, la durée exacte de la ponte et le nombre d'œufs de chaque ponte de la troisième femelle ne sont pas connus.

Female # weight (g)	Body length (days)	Survival of spawning	First day duration (days)	Spawning of spawning events	Number size (number of eggs)	Batch of spawned eggs	Total number batch size	Average
1	10.3	7	3rd	1	1	30	30	30
2	10.3	21	7th	15	8	2-176	428	53.5
3	11.0	32	?	?	2	?	76	38

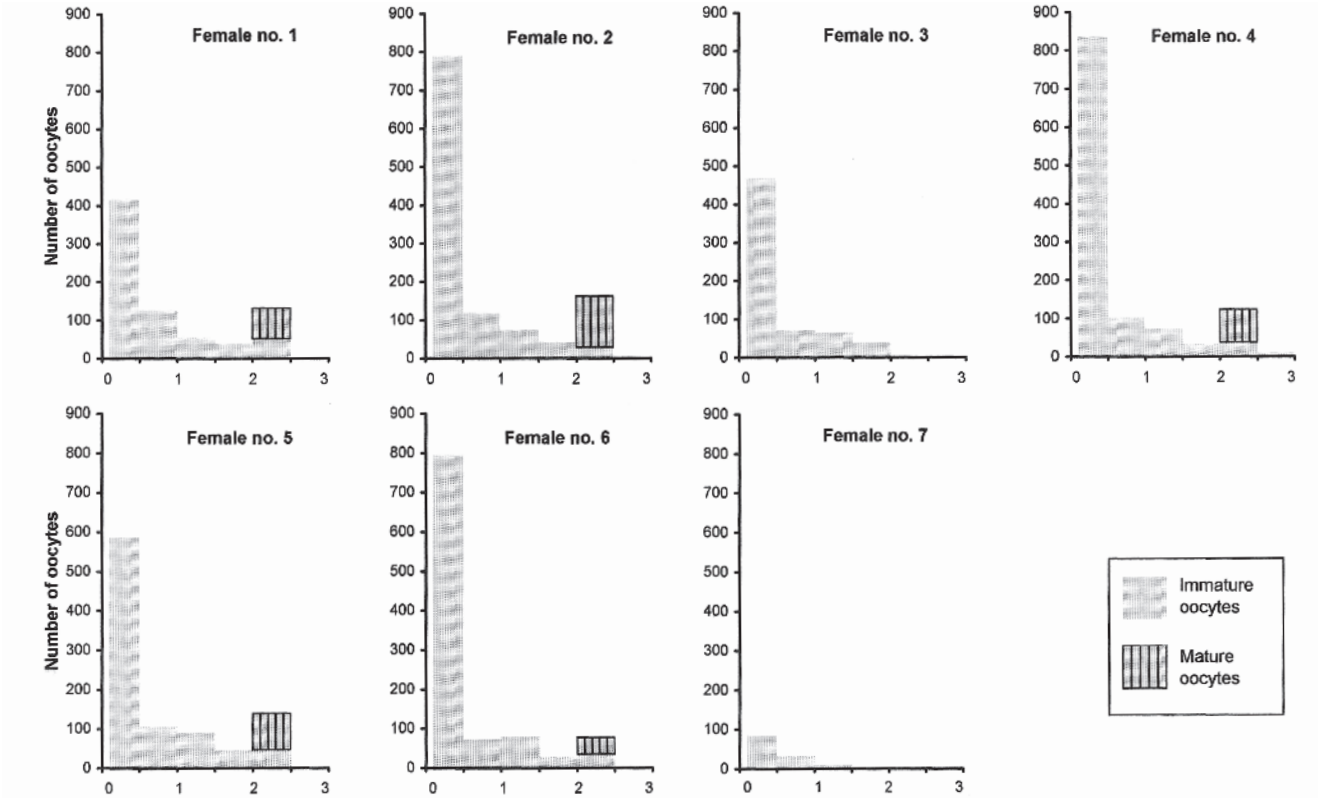

Figure 2. Size frequency distribution of the oocytes of the seven females of *Sepietta oweniana* from the Catalan Sea. The striped part of bars indicates the number of mature oocytes. Oocyte size in mm.

Figure 2. Distribution des fréquences de taille des ovocytes de sept femelles de *Sepietta oweniana* de la Mer Catalane. La partie hachurée de l'histogramme indique le nombre d'ovocytes matures. La taille des ovocytes est en mm.

MO is 27.2 mature oocytes per female. In addition, the ovary of all mature females contained a large number of immature oocytes (not counted) at various stages of maturity.

The frequency distribution of *MO* is polymodal (Fig. 3). The averages of the three modal groups of the *MO* frequency distribution are 9.0, 33.0, and 58.6, respectively. No significant correlation was found between *MO* and *ML*: $r = 0.185$, $df = 42$, $P = 0.229$ (n.s.) (Fig. 4) (note that the outlying point in the graph has a high standardized residual, but a rather low leverage, which does not influence the regression line too much [Sokal & Rohlf, 1981]). The lack of statistical significance indicates that the number of mature oocytes in the ovary is independent of the size of the animal.

No correlation was found between the *MO* values of the three Adriatic squid sub-samples and the lunar periodicity.

Discussion

The sepiolid squid *Sepietta oweniana* is a multiple spawner. This was revealed both by direct observations on three aquarium-kept females and by the examination of the ovary of 50 mature females. Lewis & Choat (1993) and Maxwell et al. (1998) likewise linked the condition of the ovaries to the actual reproductive histories in females of the squids *Idiosepius pygmaeus* Steenstrup, 1881 and *Loligo pealei*, respectively, to establish their capability of multiple spawning.

The method of direct observations on aquarium-kept cephalopods (*e.g.* Boletzky, 1987a, 1988) showed that two females of *S. oweniana* spawned 2 and 8 times respectively (Table 1). Such observations on a small number of females are strongly corroborated by the examination of the ovary in many specimens. The coexistence of oocytes at various stages of maturation, including mature oocytes, is generally deemed evidence of multiple spawning in different animal taxa, *e.g.* fishes (Hunter et al., 1985), decapod crustaceans (García-Flórez & Fernández-Rueda, 2000), gastropods (Henninger & Hodgson, 2001). In cephalopods, multiple spawning was postulated in the tropical oegopsid squid *Stenoteuthis oualaniensis* (Lesson, 1830) (Harman et al., 1989), the cirrate octopods *Opisthoteuthis* spp. (Villanueva, 1992) and the myopsid squids *Loligo vulgaris* Lamarck,

Table 2. Number of oocytes and eggs of six mature and one immature (marked *) females of *Sepietta oweniana* collected in the Catalan Sea. Means, standard deviations (*s*), and coefficient of variation corrected for bias, $V = [1 + (1 / 4n)](s \cdot 100 / mean)$, were computed for the six mature females only.

Tableau 2. Nombres d'ovocytes et d'œufs de six femelles mûres et d'une femelle immature (signée avec *) de *Sepietta oweniana* de la Mer Catalane. Les moyennes, les deviations standard (*s*) et les coefficients de variation ajustés, $V = [1 + (1 / 4n)](s \cdot 100 / moyenne)$, ont été calculés seulement pour les six femelles mûres.

Female #	Body weight (g)	Number (and percentage) of oocytes		total number of oocytes [size (mm)]	Number of mature oocytes	Number of eggs laid [size (mm)]	overall number of oocytes produced
		small (0.1 < Ø < 0.5 mm)	large (Ø > 0.5 mm)				
1	10.3	415 (60.67%)	269 (39.33%)	684	80 [2.4]	30 [2.4]	794
2	10.3	790 (75.17%)	261 (24.83%)	1051	134 [2.1]	428 [2.1]	1613
3	11.0	467 (72.63%)	176 (27.37%)	643	0	76 [2.4]	719
4	8.9	835 (77.03%)	249 (22.97%)	1084	87 [2.5]	0	1171
5	10.7	586 (67.32%)	285 (32.68%)	872	93 [2.4]	0	965
6	11.7	795 (78.71%)	215 (21.29%)	1010	43 [2.4]	0	1053
7*	7.4	84 (67.74%)	40 (32.26%)	124	-	-	-
	Means ± *s*	648.2 ± 183.0 (71.92%)	242.5 ± 40.2 (28.08%)	890.7 ± 190.6	72.8 ± 46.0		1052.5± 320.6
	V	29.41%	17.27%	22.29%	65.82%		31.73%

Table 3. Relationships between overall number of produced oocytes, *i.e.* sum of oocytes and spawned eggs (*O* + *E*), total oocytes (*TO*), small oocytes (*SO*), large oocytes (*LO*) and body fresh weight (*W*).

Tableau 3. Relation entre le nombre total d'ovocytes produits, c'est-à-dire la somme des ovocytes et des œufs pondus (*O* + *E*), tous les ovocytes (*TO*), les petits ovocytes (*SO*), les grands ovocytes (*LO*) et le poids somatique frais (*W*).

Regression equation	*r*	*df*	*P*
$(O+E) = 2093.161 - 99.271\ W$	-0.289	4	0.578 (n.s.)
$TO = 1573.263 - 65.113\ W$	-0.319	4	0.537 (n.s.)
$SO = 1145.968 - 47.501\ W$	-0.242	4	0.643 (n.s.)
$LO = 427.648 - 17.661\ W$	-0.411	4	0.418 (n.s.)

1798 and *Loligo forbesii* Steenstrup, 1856 (Rocha & Guerra, 1996) based on the co-occurrence of mature and immature oocytes in the ovary (see also Laptikhovsky & Nigmatullin, 1993; Nigmatullin et al., 1995).

The comparatively wide size-range of the mature females and the lack of any significant correlation between the number of oocytes (small and large, both separately and pooled) and the size of six mature females (Table 3) suggest that females keep on growing in size during their long spawning phase; this is further evidence for multiple egg-laying (Lewis & Choat, 1993; Harman et al., 1989).

Spawning batch size and overall fecundity

According to the direct observations in aquaria, the batch size of *S. oweniana*, *i.e.* the number of eggs laid at each spawning event, ranges from 2 to 176 eggs, mean value = 48.5 eggs. The female that spawned 8 times laid on the average 16.7 eggs per day. Such figures, of course, cannot be extrapolated to the whole Mediterranean population, because the animals observed in captivity are too few to be representative of the whole population and the behaviour of captive animals may differ greatly from that of wild animals.

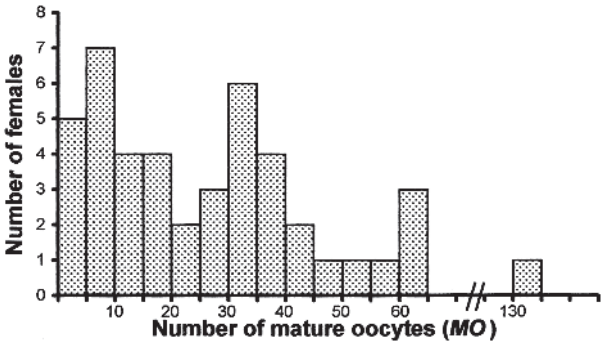

Figure 3. Frequency distribution of number of mature oocytes per female (*MO*) in *Sepietta oweniana* from the Adriatic Sea ($N = 44$).

Figure 3. Distribution de fréquence du nombre d'ovocytes matures par femelle (*MO*) chez *Sepietta oweniana* de la Mer Adriatique ($N = 44$).

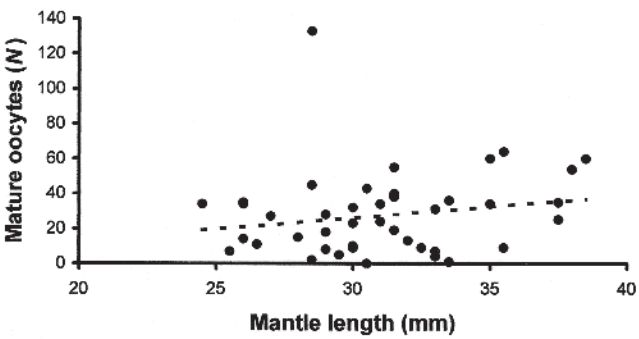

Figure 4. Relationship between number of mature oocytes per female (*MO*) and mantle length (*ML*) in *Sepietta oweniana* from the Adriatic Sea ($N = 44$).

Figure 4. Relation entre le nombre d'ovocytes matures par femelle (*MO*) et la longueur du manteau (*ML*) chez *Sepietta oweniana* de la Mer Adriatique ($N = 44$).

The counts of mature oocytes (*MO*) in wild-collected squids provided an unexpected polymodal distribution of the *MO* values (Fig. 3). To explain this phenomenon, once the heterogeneity of the sample being dismissed, we hypothesize that the batch of mature oocytes to be spawned at the first as well as subsequent spawning events is built up by the addition in the ovary of sub-batches of mature oocytes. Since the experiments in the aquarium showed that mated females of *S. oweniana* as well as those of other sepioline species did not spawn daily (present results; Gabel-Deickert, 1996), each sub-batch of mature oocytes might represent the daily production of mature oocytes. Therefore, the number of mature oocytes found in wild-collected squids may represent the mature egg production of one or several days. According to our hypothesis, the range of *MO* values is representative of various situations in females: on the average, large *MO*s (*i.e.* many mature oocytes) occur in females that have not laid eggs for a few days, whereas very small *MO*s (*i.e.* very few mature oocytes) might be indicative of an immediate post-spawning situation, *i.e.* females that have spawned only a few hours before being collected. Consequently, the mean *MO* value (= 27.2) represents an intermediate situation between two spawning events; hence, the average spawning batch size may be estimated as *mean MO* x 2 = 27.2 x 2 = 54.4. Such an indirect estimate of the batch size of *S. oweniana* (54.4 eggs) is in good agreement with that derived through direct observations in aquaria (48.5 eggs); furthermore, this similarity supports the hypothesis of multiple sub-batches.

The lack of statistical significance in the correlation between *MO* and *ML* (Fig. 4) indicates that the number of mature ovarian eggs and, hence, the batch size are most probably independent of the size of the animal. In other terms, the body growth during the spawning season does not cause any significant increase in the individual batch size.

The size range of mature females shows that they can grow more than 10 mm in *ML* during their spawning phase; according to their growth rate of 5 mm/month (Bergström & Summers, 1983), 10 mm growth is achieved in about two months (in Sepiolinae growth rates appear to be independent of temperature, *cf* Boletzky, 1975). Thus, putting the spawning phase duration at 60 days (*cf.* Gabel-Deickert (1996) about *Sepiola affinis* Naef, 1912) and the daily egg production at 16.7 eggs (see Results), the individual female fecundity is on the order of 10^3 eggs (16.7 eggs/day x 60 days = 1002 eggs), which is one order of magnitude larger than the value (120-140 eggs) reported in the review by Mangold (1989: Table XXVIII). This preliminary rough estimate is further corroborated by the number representing the 'overall number of oocytes produced' (range: 719-1613) (Table 2).

Reproductive strategy

Sepietta oweniana is a small-size multiple spawner whose main features are: continuous production of oocytes by asynchronous ovulation, continuous ripening of oocytes, internal fertilization and continuous laying of comparatively small batches of eggs over an extended period of time; the species is a multiple spawner that feeds and grows during its long spawning life phase. These features are distinctive of the reproductive strategy named "continuous spawning" in the classification proposed by Rocha et al. (2001), which reproductive pattern is typical of cirroctopods and some pelagic octopods. Rocha et al. (2001) consider the decabrachian *Idiosepius pygmaeus* also a continuous spawner because it lays egg batches continuously during most of its short adult life. Indeed the performance of *S. oweniana* multiple-spawning is similar in several respects to that of the pygmy squid *I. pygmaeus* (Lewis & Choat, 1993) (*cf.* discussion in Boletzky (2002) on the different adaptive solutions evolved in coleoids within the scope of

small adult size); on the contrary, it differs to a great extent from other coleoids such as *Illex* spp. (Laptikhovsky & Nigmatullin, 1993) in that the latter animals spawn a few very large batches of eggs in a burst-off way while the female body progressively deteriorates.

The peculiarity of *S. oweniana* within the Sepiolinae is a smaller relative egg size with respect to *Sepietta obscura* Naef, 1916 and four examined *Sepiola* species, namely *S. affinis, Sepiola intermedia, Sepiola robusta* Naef, 1912, and *Sepiola rondeletii* (Gabel-Deickert, 1996: Fig. 4.5). This feature is responsible for the higher values of fecundity (see Table 4.10 about the counts of oocytes in the above-mentioned sepiolines in Gabel-Deickert, 1996).

Sepietta oweniana is a most abundant and widely distributed species. This may be largely ascribed to its reproductive strategy: at the population level, an extended spawning season, throughout the year in the Mediterranean Sea (Salman, 1998; Sartor et al., 1998) and, at individual level, multiple spawning and production of eggs with a relative small size, which nevertheless are large enough to give birth to benthic hatchings (Boletzky, 1977, 1987b).

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