Egg masses of *Sepietta oweniana* (Cephalopoda: Sepiolidae) collected in the Catalan Sea *

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SUMMARY: Examination of 89 egg masses of *Sepietta oweniana* collected in the Catalan Sea (western Mediterranean) showed that 15.8% of them were composed of eggs at different developmental stages laid in more than one spawning bout; 6.7% of them were polyspecific and contained eggs of other sepioline species in addition to *S. oweniana*. The egg mass size, or number of eggs per egg mass, does not necessarily correspond to the spawning batch size, *i.e.* the number of eggs laid by a female in one spawning event. Furthermore the egg mass size appears to vary depending on the season.

Keywords: *Sepietta oweniana*, egg mass, reproduction, batch fecundity, Mediterranean.

RESUMEN: PUESTAS DE *SEPIETTA OWENIANA* (CEPHALOPODA: SEPIOLIDAE) COLECTADAS EN EL MAR CATALAN. – El examen de 89 puestas de *Sepietta oweniana* recogidas en el mar Catalán (Mediterráneo occidental) mostraron que el 15.8% estaba compuesto de huevos en diferentes estadios de madurez, procedentes de mas de un episodio de desove; el 6.7% era poliespecifico y contenía huevos de otra especie de sepiolido además de los de *S. oweniana*. El tamaño de las puestas, o número de huevos por puesta, no es una mediada apropiada del tamaño de un desove, es decir, el número de huevos desovados por una hembra en un episodio de puesta. Además el tamaño de las puestas parece variar con la estación del año.

Palabras clave: *Sepietta oweniana*, puestas, reproducción, fecundidad, Mediterráneo.

INTRODUCTION

The discovery of multiple spawning reproductive strategies in cephalopods (Boletzky, 1987; see review by Rocha, Guerra and González, 2001), made it necessary to reassess the estimations of the total fecundity of multiple spawners (Laptikhovsky and Nigmatullin, 1993). It also raised the need to estimate their spawning batch size, *i.e.* the number of mature oocytes released at each spawning act (Bello and Deickert, 2003). The most direct method to achieve the second aim is to count the eggs in the spawned egg masses, *e.g.* Gabel-Deickert (1995), for captive *Sepiola* spp. and *Sepietta* spp. (Sepiolida: Sepiolidae), and Guerra *et al*. (2002), for *Thysanoteuthis rhombus* Troschel, 1857 (Teuthida: Thysanoteuthidae). Indirect methods are based on counts of mature ovarian eggs that are ready to be spawned, *e.g.* Laptikhovsky and Nigmatullin (1993), in their study on *Illex* spp. (Teuthida: Ommastrephidae). In a few cases the indirect estimate of the spawning batch size was complemented by direct observations of captive females spawning, *e.g.* Bello and Deickert (2003), for *Sepietta oweniana* (d'Orbigny, 1841) (Sepiolida: Sepiolidae).

The purpose of this note is to assess the size of the *Sepietta oweniana* egg clutches collected from the sea and to verify whether this size can provide a

*Received June 16, 2004. Accepted October 8, 2004.

satisfactory estimate of the individual batch size. (In order to avoid any confusion, we term the egg mass actually laid by a female during a spawning act as an ‘egg batch’, and the egg mass collected in the field with an unknown history as an ‘egg clutch’.)

Sepietta oweniana is distributed in the eastern Atlantic Ocean from Mauritania to Norway, and also in the Mediterranean Sea, from a depth of 8 to 750 m (Mangold and Boletzky, 1987). The relatively large eggs (see Boletzky, 1974, for relative egg size) are fixed to hard substrates of a wide range of objects and living organisms, both in the wild and in captivity (Bergstrom and Summers, 1983; Gabel-Deickert, 1996). This behaviour is phylogenetically determined in Sepiolidae (Nesis, 1995). According to Mangold-Wirz (1963), *S. oweniana* prefers to spawn on sea bottoms inhabited by tunicate *Microcosmus sulcatus* (= *M. sabatieri* Roule, 1885). Collections of *S. oweniana* egg clutches from the sea have been reported briefly by Naef (1923), in the Gulf of Naples; by Jecklin (1934) and Mangold-Wirz (1963), in the Catalan Sea; by Thorson (1946), in the Kattegatt; and by Bergstrom and Summers (1983), in the Skagerrak. However, these papers give little information on the size of clutches in terms of egg numbers. Cephalopod eggs and egg masses collected in the wild are often disregarded because of identification difficulties (Boletzky, 1998). Very few statistical studies on cephalopod wild-collected egg masses have been carried out (Steer *et al*., 2002).

MATERIAL AND METHODS

The egg clutches were collected in the Catalan Sea (western Mediterranean) at Rech Lacaze-Duthiers, 12 nautical miles off Banyuls-sur-Mer (Pyrénées-Orientales, France), by means of bottom trawling while fishing for sepioline squid (Gabel-Deickert, 1996). Trawling was carried out on sandy and muddy sea bottoms, in depths of between 90 and 120 m, in March-July 1989. All pieces of hard substrate caught in the net (stones, shells, sessile animals, and man-made objects) were examined for sepiolid egg clutches. The eggs were recognized as the species *S. oweniana* thanks to their general shape and by comparing them with eggs laid in aquarium tanks by captive females of the same species (see Boletzky, 1998, for the identification difficulties due to egg changes during embryogenesis). Within hours after collection the clutches were transferred to the laboratory and the eggs were examined and counted. Then the eggs were transferred to aquarium tanks to be kept until hatching. The hatchlings were examined to confirm the tentative egg identification (Gabel-Deickert, 1996).

RESULTS AND DISCUSSION

Altogether 103 pieces of hard substrate bearing sepiolid egg clutches were collected. Eighty-nine egg clutches belonged to *Sepietta oweniana*. Most of them (59.6%) were fixed to sea squirt individuals *Microcosmus* spp. (Tunicata: Pyuridae); 21 clutches (23.6%) were fixed to empty shells of the pen shell *Atrina pectinata* (Linnaeus, 1767) (Bivalvia: Pinnidae); the remaining clutches were attached to other empty mollusc shells (4.5%), colonies of bryozoans (4.5%), sponges (4.5%), stones and man-made objects, namely a life belt, a car tyre and a plastic sheet (3.4%). The size of the collected egg clutches ranged from 3 to 62 eggs. A total of 2128 eggs were collected. Table 1 gives details of the clutches gathered in the three collecting campaigns. The May survey provided a comparatively large number of clutches; the mean number of clutches per haul was about three times that of the March and July surveys (Table 1). The mean clutch size of July (= 18.48) was significantly smaller than that of both March and May, pooled (= 27.11) (Student’s *t*-test: $t = 2.84$; $df = 87$; $P < 0.01$). Furthermore, the clutch size frequency distribution in the three surveys (Fig. 1) reveals that there are no July-collected clutches larger than 30 eggs and that the only clutches larger than 55 eggs are from the May survey. The 89 egg clutches consisted of closely to loosely packed eggs mainly placed in a single layer (Figs. 2 and 3). Most

TABLE 1. – Egg clutches of *Sepietta oweniana* collected in the field.

Date of collection and number of hauls (*n*)	Number of collected clutches	Mean number of collected clutches/haul	Range of eggs/clutch	Mean clutch size ± standard deviation
10.3.89 (5)	20	4.0	6-53	23.65 ± 14.69
23.5.89 (3)	36	12.0	4-62	29.03 ± 16.70
15/16.7.89 (9)	33	3.7	3-30	18.48 ± 7.67

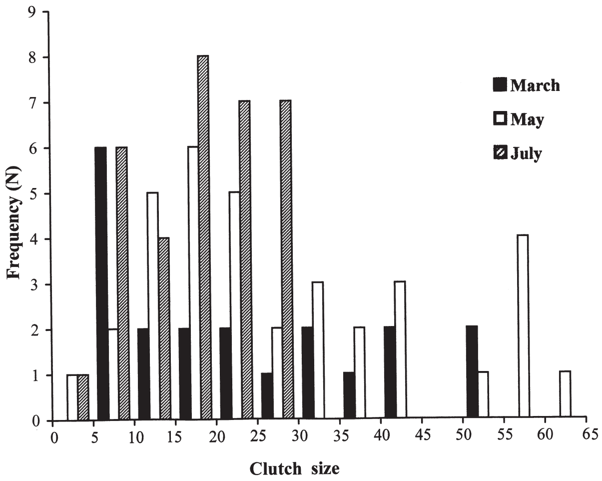

FIG. 1. – Frequency distribution of *Sepietta oweniana* clutch sizes (number of eggs) in three surveys.

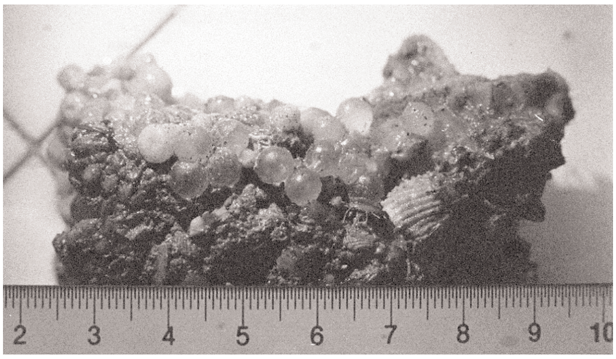

FIG. 2. – A 21 egg clutch of *Sepietta oweniana* attached to *Microcosmus* sp., collected in the Catalan Sea.

FIG. 3. – An empty pen shell, *Atrina pectinata*, with a 10 egg clutch of *Sepietta oweniana* (top left) and a 7 egg clutch plus an empty egg case of *Sepiola* sp. (bottom left), collected in the Catalan Sea.

clutches were composed of eggs at one development stage. In the remaining clutches, eggs at two or more than two development stages co-occurred. Six clutches were polyspecific, that is they contained eggs of *S. oweniana* and other sepiolid species namely *Sepiola robusta, Sepiola* spp., and *Rossia macrosoma* (Table 2). All egg clutches developed to hatching in the laboratory. However, 18 clutches (20.2%) were found to contain a total of 73 abnormal eggs, *viz.* 3.4% of the overall egg number. Most of these eggs were milky-looking and did not develop at all, and the others contained either dead or malformed embryos.

According to the present results and the data from the literature, the overall size of *Sepietta oweniana* egg clutches collected in the field ranges from 3 to 130 eggs (Table 3). Clutches from the North Sea seem to be larger than clutches from the Mediterranean (Table 3). However, the North Sea data are too limited to allow a sound statistical comparison. Moreover, seasonal and/or habitat differences may be the cause of the different clutch sizes, as suggested by the present results. The larger number of clutches collected in May and the larger absolute and mean clutch sizes of the March and May samples (largest sizes in May) (Table 1; Fig. 1) are probably due to a higher spawning activity in spring.

TABLE 2. – Composition of *Sepietta oweniana* wild-collected egg clutches.

Developmental stages co-occurring in one clutch	Clutches N	Clutches %	Range of eggs in the clutches	Mean clutch size (eggs / clutch) ± standard deviation
1	69	77.5	3 -59	21.90 ± 12.63
2	12	13.5	13 - 60	33.83 ± 6.72
> 2	2	2.3	53 -62	57.50
polyspecific	6	6.7	7 - 30	16.00 ± 9.29

TABLE 3. – Size range of egg clutches of *Sepietta oweniana* collected from the sea.

Reference	Geographical location	Number of examined egg clutches	Size range of egg clutches (number of eggs)	Mean clutch size (number of eggs / clutch) ± standard deviation
Thorson (1946)	Kattegatt	1	130	
Mangold-Wirz (1963)	Catalan Sea	not reported	30 - 60	-
Bergstrom and Summers (1983)	Skagerrak	4	32 - 103	53.25
present results	Catalan Sea	89	3 - 62	23.91 ± 14.39

According to Salman (1998), *S. oweniana* exhibits two spawning peaks, in April-May and October-November respectively.

The overall size of clutches collected from the sea (3 to 130 eggs) lies within the range of the batch size of eggs spawned in aquariums, which is 2 to 176 eggs (Bello and Deickert, 2003). This fact suggests that there is a relationship between clutch size and batch size. But in fact the comparison between the mean clutch size and the mean batch size for the Mediterranean population shows that the average size of egg clutches observed in the present study (23.91 eggs) is half the average batch size observed in aquariums (48.5 eggs) and the average batch size estimated by mature oocyte counts (54.4 eggs) (Bello and Deickert, 2003). Hence, the examination of egg clutches collected from the sea in order to deduce the batch size may be misleading. Indeed two opposite sources of bias may be involved. The possibility that each egg mass found in the wild may be the result of more than one spawning event by the same female or by several different females cannot be excluded, as suggested by the observation of eggs at different development stages within one clutch (present results). This phenomenon was observed directly in another sepioline squid, *Sepiola affinis,* kept in aquariums (Gabel-Deickert, 1996). Mangold-Wirz (1963), inferred the same type of bias about the 130 egg clutch reported by Thorson (1946). Furthermore, spreading one large batch laid during one single spawning event into several small clutches was also directly observed in Sepiolinae. For instance, a female *Sepiola intermedia* was observed spawning a batch of 100 eggs in the aquarium, between egg laying the animal moved around exploring the substrate with its tentacles. It eventually arranged its eggs in smaller clutches ranging from 2 to 49 eggs (mean clutch size = 12) (Gabel-Deickert, 1996). In the field, *S. oweniana* might also spread one batch of eggs between two or three objects if there is enough suitable substratum for egg laying that is not too far apart.

ACKNOWLEDGEMENTS

We are grateful to Dr. Sigurd von Boletzky (Laboratoire Arago, Banyuls-sur-Mer) who provided the facilities for collecting and keeping sepiolid eggs, guided A.D. during her doctoral thesis work, and gave advice on the present paper. We also acknowledge the useful suggestions given by two anonymous referees.

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