

## Sexual reproduction, larval development and release in *Spongia officinalis* L. (Porifera, Demospongiae) from the Apulian coast

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**Abstract** The reproductive cycle of the bath sponge *Spongia officinalis* L. has been studied over 1 year on 11 tagged specimens of different sizes (from 82 to 886 ml, in volume) from Ionian coasts of Apulia (SE Italy). According to literature data, the sponge is viviparous. All the monitored specimens showed sexual reproduction, even if the process usually involved small portions of the sponge tissue. Ten specimens were gonochoric (sex ratio 1:1), one specimen showed successive hermaphroditism, with alternate production of oocytes and spermatocysts in the same reproductive season. Young oocytes occur almost all year round, whereas large mature eggs show a peak in October–November, concomitantly with the appearance of spermatocysts. No relationships were observed between the sponge size and the presence of sexual elements within the range of the sponge size considered in this research. Embryo development occurs in patches of choanosomal tissue, which contain four or more elements. Cleavage is total and equal; it starts in November and in May leads to a solid stereoblastula, which develops into a parenchymella larva, from May to July. The stereoblastula lacks flagella and its surface is delimited by elongated cells well segregated from the internal ones. Parenchymella larvae are released from June to July, asynchronously, either at the individual or population

level, with a few days of de-phasing. Up to 523 larval elements/48 h for a sponge specimen were counted. The free-swimming larvae are ovoid and uniformly flagellated. Flagella are longer at the posterior region, than on the rest of the larval body. Flagellated cells form a pseudo-stratified epithelial layer, delimiting the outermost larval surface, and are filled with electron-lucent vesicles showing a homogeneous content. No choanocyte chambers, pinacocytes or skeletal elements are present in the newly released larvae.

### Introduction

*Spongia officinalis* L. is a sponge known worldwide and much utilized for cosmetic purposes. Prolonged over-fishing and some recent epidemics have caused its disappearance from wide areas of the West Mediterranean Basin (Gaino et al. 1992; Pronzato et al. 1996; Cerrano et al. 2000), thus determining the inclusion of this bath sponge among the marine endangered species requiring specific management measures (Annex 3 of the Bern Convention on the Protection of Wildlife). In this context, knowledge on the reproductive pattern and larval behavior are essential for understanding the biological and ecological needs of this species, and may yield information useful for proper management, here including conservation plans (Kaye and Reischwig 1991).

Data on the reproductive biology of *S. officinalis* are due to Gaino et al. (1984), who investigated sperm cyto-differentiation at the ultra-structural level. Some aspects of the sexual cycle are reported in a former work in which about 400 specimens were examined, collected over 3 years at different sites from the Adriatic and Ionian Seas (Scalera Liaci et al. 1971). Reproductive processes of some West Indian species of *Spongia* have also been investigated (Kaye 1990; Kaye

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and Reiswig 1991). On the whole, the studies were based on specimens randomly collected within different populations. This sampling technique does not allow the determination of the ratio between gonochoric versus hermaphroditic specimens or whether reproduction is an age/size dependent event or not. By contrast, Ayling (1980), Wapstra and van Soest (1987), Witte et al. (1994), Usher et al. (2004) and Sidri et al. (2005) report valuable information on the sexual cycle of some demosponges obtained from investigating tagged individuals over time. This method, which requires large sponges and experienced SCUBA divers, has several advantages with respect to the traditional technique, such as a stricter definition of the sex ratio and of the reproductive periodicity of a population. In addition, such a technique makes it possible to ascertain the possible relationships between the biological features of a sponge (age, size, morphology) and its reproductive process.

The present work deals with the reproductive cycle of 11 specimens of *S. officinalis* investigated in the same area for 1 year. The investigation was carried out by utilizing the technique of tagging each individual with a PVC stick and cutting off tissue samples from each of them every month for histological and ultra-structural analyses. Larval release was also investigated.

## Materials and methods

### Study site

The study was carried out at a depth of 6–7 m, on the rocky bottom of La Pierta, a large embayment off the coast of Porto Cesareo (SE Italy, Ionian Sea-N 40°16'312" E 17°51'593"). The rocky bottom consists of a horizontally exposed wide carbonatic platform. The rocky surface is affected by high water movement and exposed to high solar radiation. The benthic community is characterized by a very rich sponge population with the dominance of *Sarcotragus spinosulus*, *Chondrilla nucula* and *Spongia officinalis*, with a few frondose algae (Corriero et al. 2004b).

### Species studied

*Spongia officinalis* (Demospongiae, Dictyoceratida, Spongiidae) displays a wide variety of massive forms (Pronzato et al. 2003). The specimens studied in the present work show a recurrent shape, characterized by a very large base and a limited thickness, as a result of high mechanical stress due to water movement conditions. The consistency of the specimens is elastic, the surface smooth and finely conulose, with a few oscules irregularly scattered. The external color is black, owing to high light exposure, and the internal color is brownish-yellow.

### Sampling protocol

Within an area of about 400 m<sup>2</sup>, eleven specimens of *S. officinalis* of different volume were selected and tagged with numbered plastic labels. The volume of these specimens was estimated at the beginning of the study by measuring in situ, with a caliper, the height of each sponge (five replicas for each measurement) and estimating the area of each sponge upper surface by analyzing underwater photographs (Nikonos V system) using a KURTA IS/ADB digitizer connected to a Macintosh PC.

From April 2004 to March 2005, with monthly frequency, small sponge fragments (about 3 cm<sup>3</sup> on the whole) were carefully collected from each specimen by scuba divers, to minimize damage. Samples were fixed in a solution of formaldehyde in sea water (4%) and preserved in ethanol 70%. To detect the presence of sexual reproductive elements, the samples were dehydrated in ethanol and embedded in Paraplast for routine histological examination. Sections 7 µm-thick were then stained with toluidine blue.

Sections were observed under a light microscope to determine the monthly percentage of specimens with oocytes, spermatid cysts, embryos and larvae, the mean diameter (considering them spherical) and the density (number/mm<sup>3</sup>) of reproductive elements. In evaluating the phases of the embryonic development of *S. officinalis*, the elements in which a clear segregation between the superficial layer and the internal cells was evident were counted as larvae and all the previous developmental phases as embryo.

The quantitative evaluation of the reproductive elements was carried out using the Abercrombie (1941) formula as suggested by Elvin (1976).

Univariate statistical analysis was carried out considering only reproductive specimens. The relationship between reproductive parameters (frequency of reproductive specimens, number of sexual elements, percentage of reproductive tissue) and the size of the sponge specimens were calculated using the Spearman ( $r_s$ ) coefficient of correlation.

For transmission electron microscopy (TEM), samples fixed in a solution of glutaraldehyde in sea water (4%, pH 7.4) for 24 h were rinsed in artificial sea water, used as a buffer, and post-fixed for 30 min in osmium tetroxide (1% in sea water). Afterwards they were repeatedly rinsed in the same buffer, dehydrated in a graded ethanol series and embedded in an epon-araldite mixture. Ultra-thin sections were obtained with an LKB ultramicrotome, contrasted with uranyl acetate and lead citrate, and examined with a Philips EM 208 transmission electron microscope. For scanning electron microscopy (SEM), fixed material was dehydrated and critical point dried in a Pabish CPD 750 apparatus and coated with gold palladium (20 nm). Some paraffin embedded samples, after the cutting of a face view of the tissue including embryos and/or larvae, were treated

to remove the embedding medium and then processed using the above reported technique for SEM analysis. Specimens were observed under a Philips XL 30 scanning electron microscope at an accelerating voltage of 18 kV.

In the month of June, concomitantly with the occurrence of larvae in the sponge tissue, traps were employed to collect the larvae released in the field. The trap consisted of a conical net (base diameter = 20 cm; height = 30 cm), the base of which was provided with a closure system, made of a plastic clamp. The cloth used in the net was high capacity nylon monofilament screen with a mesh of 250  $\mu\text{m}$ . The sponges which had been previously found to be reproductively active (specimens no. 2, 4, 10) were enclosed in the collecting traps, which, in turn, were closed at the base of the sponge by the plastic clamp. The traps were placed four times at 48 h intervals, with weekly frequency. After collection, larvae were placed at 20°C in filtered seawater and immediately preserved for histological and ultra-structural investigations.

## Results

### Sponge size

Sponge thickness never exceeded the value of 6 cm in any of the studied specimens. The area of the sponge upper surface varied between 81.7 and 393.9  $\text{cm}^2$ . The estimated volumes varied between 81.7 and 886.2 ml, with a highest frequency around 200–400 ml. These data were coherent with unpublished observations referring to about 100 specimens studied from the study site.

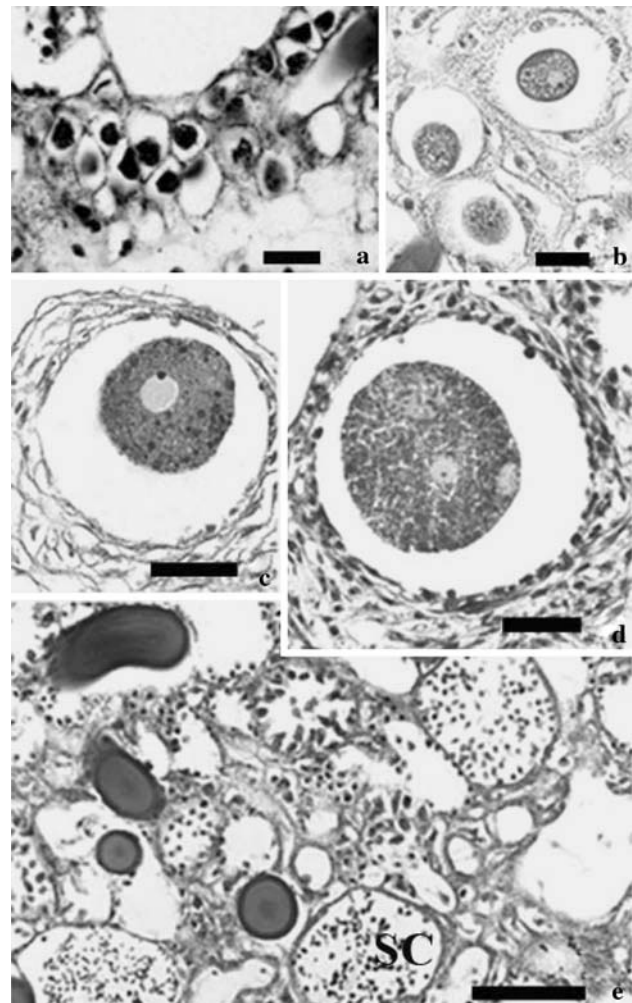
### Description of sexual elements

#### Female lineage

In the early phase of differentiation, the oocytes of *S. officinalis*, measuring about 15  $\mu\text{m}$  in diameter, were fairly triangular shaped (Fig. 1a). Growing oocytes acquired a spherical shape and showed an eccentric nucleolated nucleus and cytoplasm with scarce inclusions (Fig. 1b). They were found in low densities throughout the choanosomal region, separated from one another by mesohyl elements and collagen. In the successive steps of development, the cytoplasm was gradually enriched with yolk stored material (Fig. 1c) up to its complete filling (Fig. 1d). In the last phase of development, they reached their maximum diameter (up to 200  $\mu\text{m}$ ).

#### Male lineage

Spermatic cysts (Fig. 1e) occurred in the choanosomal region. Germinal cell maturation occurred synchronously in



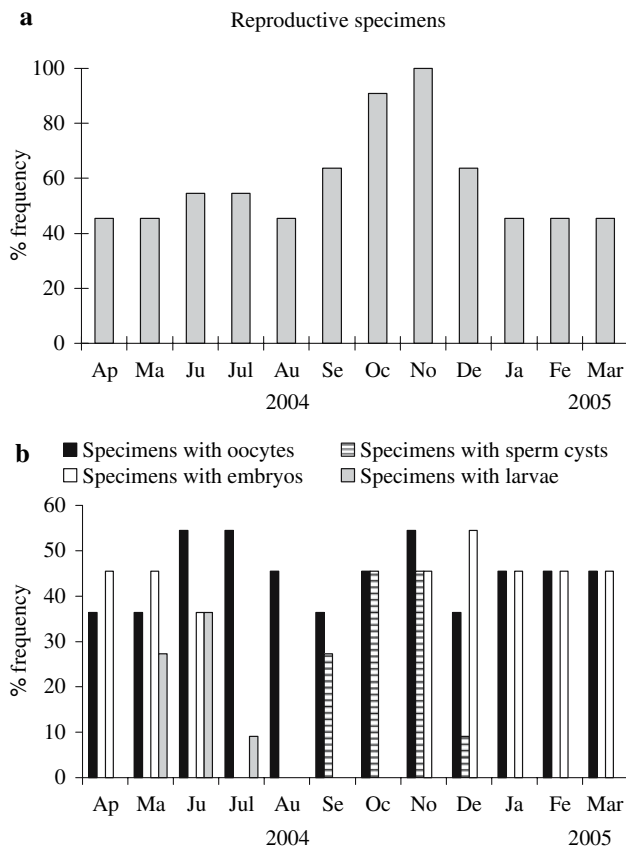
**Fig. 1** **a** Oocytes of *S. officinalis* in the early phase of differentiation (bar = 20  $\mu\text{m}$ ). **b** Growing oocytes showing a spherical shape and eccentric nucleolated nucleus and cytoplasm in its initial phase of yolk accumulation (bar = 20  $\mu\text{m}$ ). **c** Oocyte with nucleolated nucleus and cytoplasm in its initial phase of yolk accumulation (bar = 50  $\mu\text{m}$ ). **d** An oocyte whose cytoplasm is filled with yolk stored material (bar = 50  $\mu\text{m}$ ). **e** Spermatic cyst (SC) in the choanosomal region. Different cysts contain sperm in various phases of differentiation (bar = 50  $\mu\text{m}$ )

the same cysts, whereas it was asynchronous in different cysts of the same specimen.

#### Sexual reproductive cycle

Sexual elements (oocytes, spermatic cysts, embryos, larvae) were found throughout the year, with a monthly frequency of reproducing sponges gradually increasing from September (63.6%) to November (100%) (mean annual value  $58.3 \pm 5.4\%$ ) (Fig. 2a).

Specimens with oocytes occurred almost all year round (mean frequency  $44.7 \pm 2.1\%$ ) (Fig. 2b). The density trend showed some fluctuations during the study year, with the maximum value recorded in June ( $6.0 \pm 2.8$  oocytes/ $\text{mm}^3$ )



**Fig. 2** Sexual cycle trend of *S. officinalis* in the study site. **a** Monthly frequency of reproducing specimens. **b** Monthly frequency of specimens with oocytes, sperm cysts, embryos and larvae

(Fig. 3a). Oocytes grew in size from July ( $50.9 \pm 6.6 \mu\text{m}$  in diameter) to October ( $84.8 \pm 22.2 \mu\text{m}$ ) (Fig. 3a), then they fell. Concomitantly with the maximum size of the oocytes, spermatocysts became evident. They were found in autumn and early winter (mean frequency =  $10.6 \pm 5.2\%$ ) (Fig. 2b) with density values about 100 times higher than those estimated for female elements. In contrast with oocytes, the size of the spermatocyst showed moderate oscillations (from 50 to 60  $\mu\text{m}$ ) (Fig. 3b).

Fertilized eggs showed total and equal cleavage. The process started in November, giving rise to a solid morula stage measuring  $361.0 \pm 12.4 \mu\text{m}$  in means, which consisted of few cells (4–16 cells). In December embryos consisted of numerous cells (Fig. 4a). The ensuing development took place up to April and led to embryos measuring  $429.8 \pm 21.2 \mu\text{m}$  as a maximum (Fig. 3c). In May, the cleavage resulted in a solid white-translucent stereoblastula and parenchymellae measuring  $402.4 \pm 16.9 \mu\text{m}$ . Larvae grew up to a final size of 480  $\mu\text{m}$  in July (Fig. 3d).

In electron microscopy, the cells of the embryo possessed a cytoplasm packed with both the large electron-dense and small electron-translucent inclusions (Fig. 4b).

The former contained dark granular bodies variously dispersed. The nucleolate nucleus (Fig. 4c) tended to be slightly eccentric. Just outside the nucleus, Golgi-derived vesicles with electron-dense granules were evident (Fig. 4d). Although embryonic cells tend to maintain a certain distance from one another, on occasions a cell establishes contact by means of membrane bridges (Fig. 4e) that interact with the membrane of an adjacent cell (Fig. 4f).

Cleavage led to an early solid stereoblastula (Fig. 5a) presenting a large amount of inclusions, mainly accumulated in the central region of the embryo. Under TEM they revealed their heterogeneity, being the result of electron-dense granules, varying in size and content (Fig. 5b). The largest granules consisted of different types of material (Fig. 5c), among which even bacteria (Fig. 5c, inset). Small cells with micro-granular electron-dense inclusions and short microvilli, protruding from the outer cell surface, were evidently dispersed among the inclusions (Fig. 5d).

The peripheral region of the early stereoblastula showed numerous cells whose morphology was consistent with a migration activity. They were elongated in shape with electron-dense inclusions, an eccentric nucleolated nucleus and pseudopodia surrounding the bacteria dispersed in the mesohyl matrix (Fig. 5e). Some granules were minute and encased in large vacuoles.

Images of sectioned specimens appropriately processed for SEM analysis, after having removed the embedding paraffin (see “Materials and Methods” section), revealed that the developing embryos and the ensuing larvae tended to gather together to form clumps (Fig. 6a). Such brood chambers (Leys and Degnan 2002) were scattered in the mesohyl of the sponge. The development of the embryos was dephased in such a way that in some clumps late stereoblastulae coexisted with mature parenchymellae (Fig. 6a). Embryos and larvae were individually included in a cavity (Fig. 6b), the wall of which were delimited by a monolayer of flattened cells (Fig. 6c).

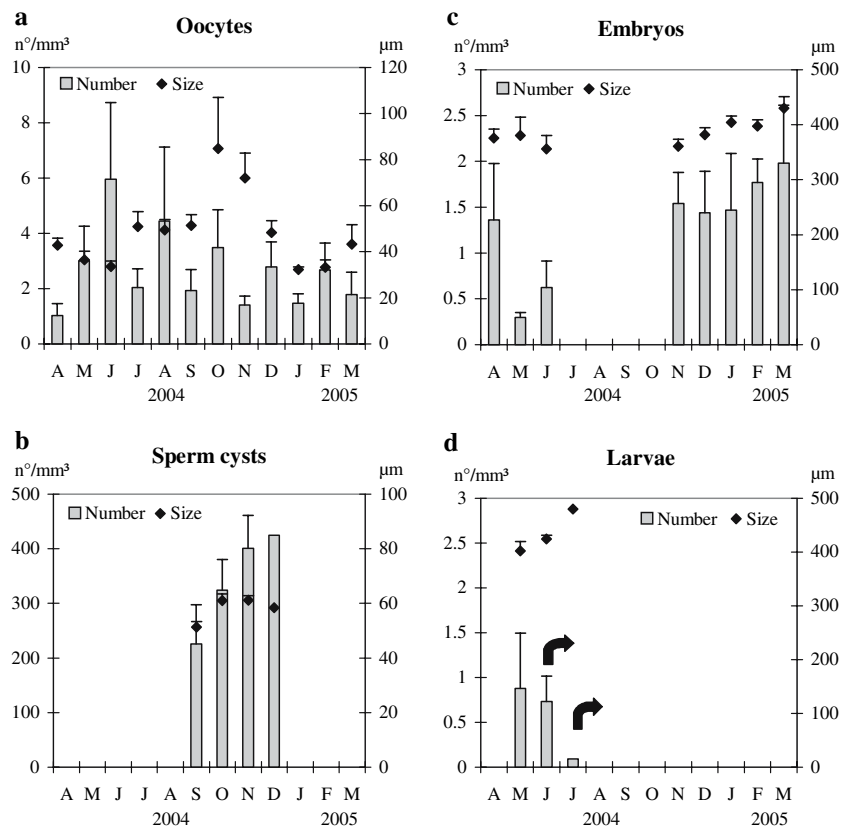
The solid late stereoblastula showed a clear segregation between peripheral non-flagellated cells, showing an elongated shape and the more-rounded internal cells (Fig. 6d).

Mature parenchymellae (up to 600  $\mu\text{m}$  in size along the main axis), were totally flagellated and their outermost surface protruded in numerous folds (Fig. 7a). The flagella extended towards the cavity walls in such a way that they crossed the space in between (Fig. 7b). The cavities were separated from one another by a narrow collagen region (Fig. 7c). The parenchymellae maintained the solid organisation of the stereoblastula, but their superficial layer showed a flagellated epithelium (Fig. 7d).

The body of released larvae can be approximated to an oval (Fig. 8a), whose posterior pole had a slightly depressed region encircled by an uplifting ring (Fig. 8b),



**Fig. 3** Sexual cycle trend of *S. officinalis* in the study site: monthly mean size and number of oocytes (a), sperm cysts (b), embryos (c) and larvae (d) per mm<sup>3</sup> of sponge tissue ( $\pm$ standard error). Arrows indicate the larval release



made of cells whose flagella were longer than those of the cells delimiting the rest of the larval surface.

TEM images gave evidence of the arrangement of the flagellated cells. They formed a pseudo-stratified continuous layer, were elongated in shape and possessed cytoplasmic extensions surrounding the single flagellum (Fig. 8c). Most of the cell body was filled with electron-lucent inclusions showing a homogeneous content, whereas mitochondria and electron-dense granules tended to accumulate in the apical region (Fig. 8d).

Numerous bacteria were dispersed in the mesohyl matrix and several TEM images proved that they are taken up along the peripheral border of the cells with electron-dense microgranules. These cells showed an elongated shape, an eccentric nucleolated nucleus, scarce cytoplasm and numerous protrusions (Fig. 8e), which tended to branch and come back to the original surface to surround bacteria. On occasions, bacteria were seen within blind ended pockets formed by lobose pseudopodia wrapped around themselves (Fig. 8f). No skeletal elements, choanocyte chambers or pinacocytes were observed.

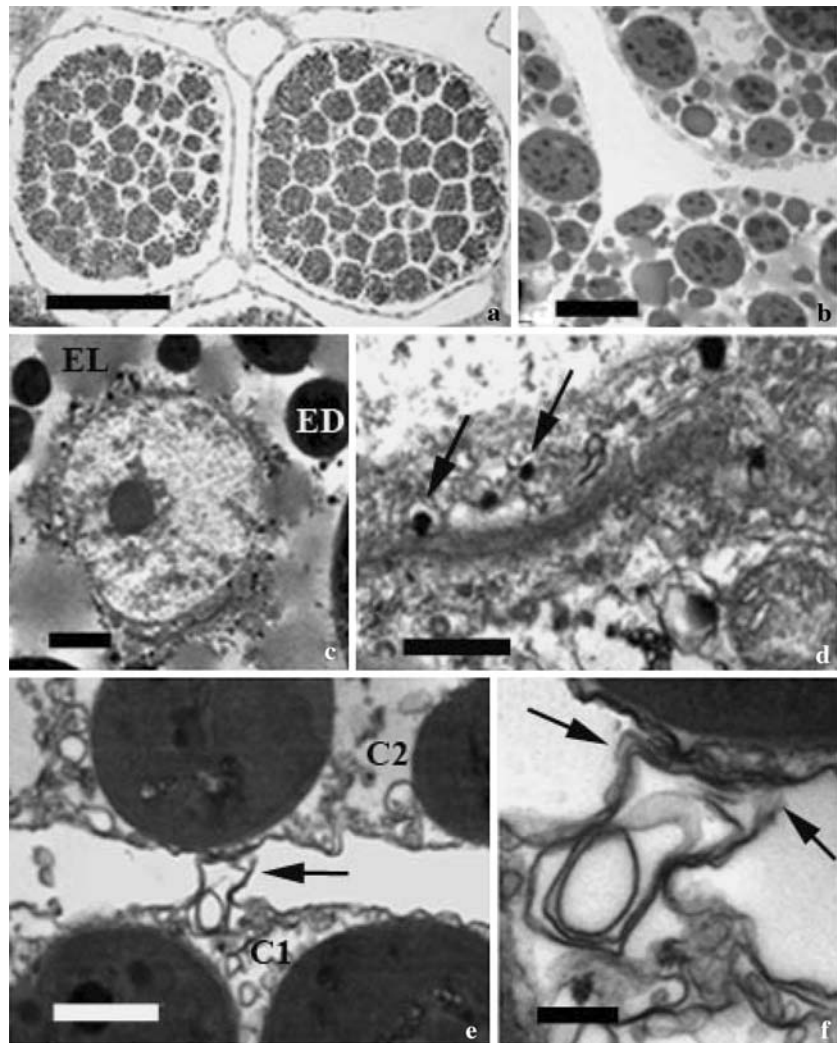
The embryo annual mean density in the mother sponge was  $1.3 \pm 0.6$  no./mm<sup>3</sup> (Fig. 3c). The progressive larval development took place from May (mean annual density:  $0.6 \pm 0.4$  no./mm<sup>3</sup>) (Fig. 3d); the parenchymella was ready to be released from June to July. The mean monthly fre-

quency of specimens with embryos and larvae was  $30.3 \pm 6.6\%$  and  $6.1 \pm 3.6\%$ , respectively (Fig. 2b). From August to October neither embryos nor larvae were detected (Fig. 3c, d).

The reproductive activity of each of the eleven monitored specimens is shown in Fig. 9. During the study period, ten specimens were found to be gonochoric (sex ratio 1:1) and one hermaphrodite (specimen no. 6). In the six sponges producing oocytes (five plus the hermaphrodite), the reproductive effort and periodicity were different: specimens no. 1, 2, 4 and 10 sustained oogenesis nearly all year round (11 months), while no. 5 and 6 showed female gametes for 10 and 5 months, respectively. Among the six sponges producing spermatic cysts, the longest period of spermatogenesis was detected in specimen no. 7 (from September to December), in which the highest values of density were also observed, while no. 3 and 6 produced spermatic cysts for 1 month (November and October, respectively). Sponge no. 9 showed the lowest values of cyst density. As far as embryos and larvae are concerned, the general behavior observed was coherent with the above trend described for oocytes: specimens no. 1, 2, 4 and 10 were the most involved in embryo and larval production, a process that took place in 8/9 months.

No relationships were observed between sponge size and density of sexual elements ( $r_s = 0.028$ ;  $P > 0.05$ ).

**Fig. 4** Embryogenesis of *Spongia officinalis*. **a** Morula stage (bar = 150  $\mu$ m). **b** Electron micrograph showing the variety of inclusions packed in the morula cells (bar = 10  $\mu$ m). **c** Detail of a nucleolated nucleus of a morula cell showing the surrounding electron-dense (ED) and electron-lucent (EL) inclusions (bar = 2.5  $\mu$ m). **d** A zoomed view of the region squared in (c). Note some Golgi vesicles with membrane-bound electron-dense granules (arrows) (bar = 250 nm). **e** Two adjacent cells ( $C_1$ – $C_2$ ) showing a contact by a membrane bridge protruding from  $C_1$  (arrow) (bar = 1  $\mu$ m). **f** Detail of the contact region (arrows) (bar = 250 nm)



### Larval release

Larvae were released from June to July (Fig. 3d), asynchronously, either at the individual or at the population level, with few days of de-phasing. All the three monitored specimens of *S. officinalis* released larvae during the third week of June with values varying from 50 (specimen no. 4) to 211 (no. 10) and 523 (no. 2) elements/48 h of monitoring. A severe and unexpected storm in the last week of June removed the trap and hampered further observations.

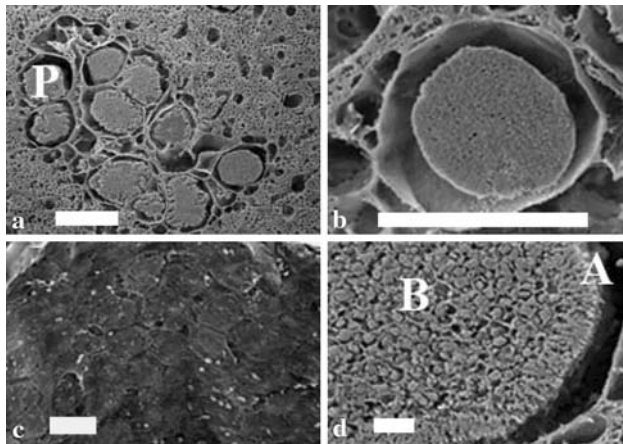
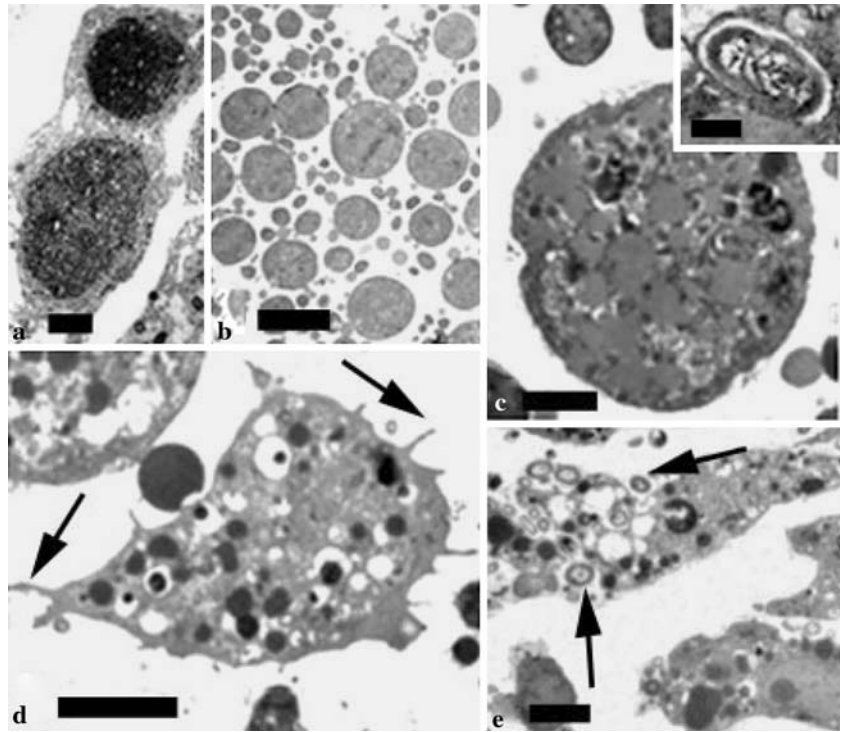
### Discussion

The technique used in the present investigation, based on tagged individual (Mercurio et al. 2006), allowed us to confirm a prevalent gonochoric condition of *S. officinalis*. In addition, a low percentage of specimens capable of sexual reverse, showing a successive hermaphroditism. Sexual reverse leads to successive hermaphroditism. Successive

hermaphroditism has been suggested for several sponge species (Sarà 1961; Diaz 1973) even if, so far, changes of sex have only been demonstrated in tagged specimens of freshwater demosponges consecutively sampled (Van de Vyver and Willenz 1975; Gilbert and Simpson 1976). Moreover, the occurrence of five males out of ten gonochoric specimens refutes a sex ratio in favour of the females, a feature that seems to be fairly common among Porifera (Scalera Liaci et al. 1971; Wapstra and van Soest 1987; Corriero et al. 1996, 1998; Mercurio et al. 2000; Meroz-Fine et al. 2005).

The general pattern of oogenesis and embryo development in *S. officinalis* is similar to that reported for other viviparous demosponges (Saller and Weissenfels 1985; Saller 1988; Leys and Ereskovsky 2006; Maldonado 2006) and particularly for the Caribbean species of *Spongia* (Kaye 1990; Kaye and Reiswig 1991). Oogenesis begins with the differentiation of primary oocytes, about 15  $\mu$ m in diameter, probably differentiated from archaeocytes. They grow up to 200  $\mu$ m in diameter and acquire a round shape. Their cytoplasm becomes gradually filled with yolk granules.

**Fig. 5** Embryonic development of *Spongia officinalis*. **a** Two solid stereoblastulae (bar = 100  $\mu$ m). **b** Electron micrograph showing the variety of inclusions internally positioned in the stereoblastula (bar = 10  $\mu$ m). **c** Detail of a large inclusion showing the different components and an encased bacterium (inset) (bar = 1  $\mu$ m; bar of inset = 100 nm). **d** A microgranular cell with evident microvilli-like marginal extensions (arrows) (bar = 1.5  $\mu$ m). **e** Microgranular cells gathered along the outermost surface of the solid stereoblastula. Note the bacteria amidst the cell cytoplasmic extensions (arrows) (bar = 1.5  $\mu$ m)



**Fig. 6** Scanning electron microscopy view of stereoblastula and parenchymella stages of *Spongia officinalis*. **a** Steroblastula and parenchymella (P) grouped to form a clump within brood chambers scattered in the mesohyl of the sponge (bar = 500  $\mu$ m). **b** A stereoblastula included in its chamber (bar = 500  $\mu$ m). **c** Zoomed view of the epithelium (E) of the chamber wall in which the stereoblastula develops (bar = 25  $\mu$ m). **d** A stereoblastula showing a clear segregation between the non flagellated peripheral (A) and the internal (B) cells. Note the solid mass of the internal cells (bar = 25  $\mu$ m)

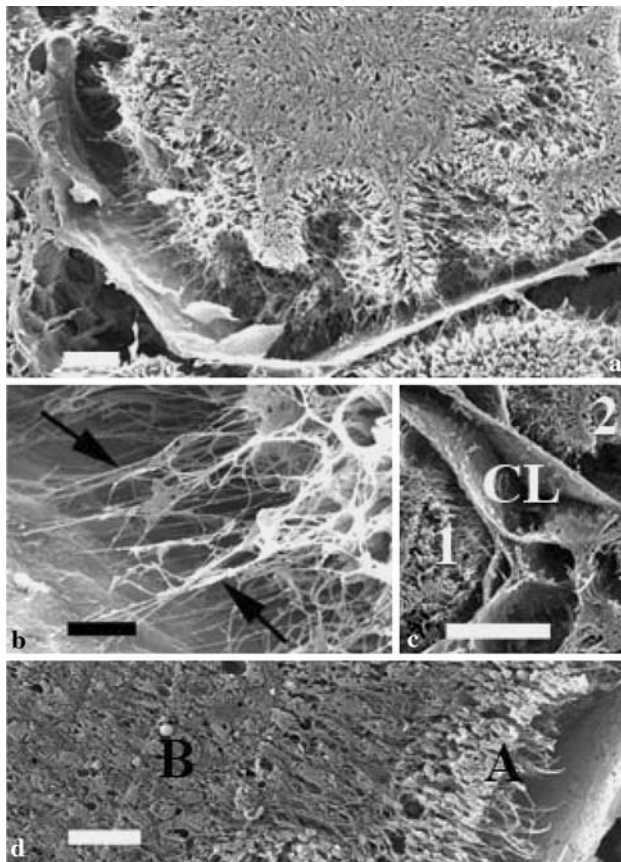
Spermatogenesis inside spermatic cysts, deriving from choanocyte chambers (Scalera Liaci and Sciscioli 1969; Gaino et al. 1984), does not occur synchronously either in individuals or in a population level and it is limited to 4 months as a maximum. This temporal lag overlaps the presence of the largest oocytes, thereby corroborating the

hypothesis of Kaye (1990) that these elements can be zygotes, even if a fertilization process has never been detected.

The development of the parenchymella of *S. officinalis* follows the typical stages of the formation of a solid, non-flagellated stereoblastula followed by the differentiation of a flagellated epithelium (see review in Simpson 1984). A general monitoring of the parenchymella architecture shows that the cell component is similar to that described by Kaye and Reiswig (1991) for caribbean *Spongia* species, where amoeboid cells prevailed, whereas pinacocytes, skeletal elements, choanocytes and other components of the aquiferous system were lacking. This feature differs from other observations on parenchymellae of haplosclerid sponges (Saller and Weissenfels 1985; Saller 1988; Ereskovsky 1999; Leys and Degnan 2002; Leys and Ereskovsky 2006), thus emphasising the striking difference in the sponges belonging to this order, in which choanocyte chambers, a pinacocyte lined cavity and siliceous spicules differentiate precociously. According to Maldonado et al. (2003), the cytoplasmic extensions protruding from the apical region of the flagellated layer could be involved in functional beating.

The occurrence of bacteria inside the large electron-dense inclusions of the early stereoblastula as well as along the peripheral border of its electron-dense microgranular cells, which come to surround bacteria by cytoplasmic extensions and store them within intracellular vacuoles, proves that the uptake of such micro-organisms already takes place in the

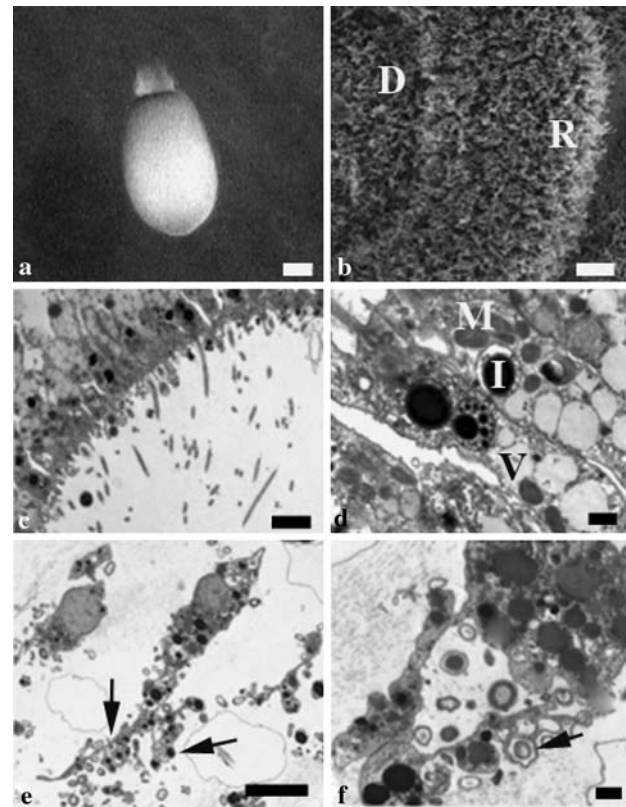




**Fig. 7** Scanning electron micrographs of parenchymella of *Spongia officinalis*. **a** Section of a mature larva showing the folds of its outermost flagellated surface (bar = 25  $\mu\text{m}$ ). **b** Detail of the flagellated epithelium. Note the flagella (arrows) crossing the space interposed between the larva and the chamber wall (bar = 10  $\mu\text{m}$ ). **c** The chamber walls of two adjacent larvae (1, 2) are separated by a narrow collagen-containing region (CL) (bar = 100  $\mu\text{m}$ ). **d** Portion of the flagellated surface (A) and internal region (B) of the larva (bar = 25  $\mu\text{m}$ )

initial phase of larval development. This process is particularly evident in the newly released parenchymella. We can suppose that the bacteria, derived from the mother sponge, constitute the main food source for the free-swimming larva owing to the lack of an aquiferous system able to support its filter-feeding activity. Likewise, the accumulation of the large inclusions in the central region of the stereoblastula, as a result of cell disintegration, could be a way of providing nutrients, a phenomenon already reported for the larval development of other sponge species (Lévi 1956; Reiswig 1976). The bulk of data are in agreement with the fact that most sponge larvae are lecithotrophic (Maldonado 2006) and therefore self-sustaining by utilizing stored material. This mechanism has also been observed in asexually produced bodies described in *Tethya* species where an aquiferous system was lacking (Gaino et al. 2006a).

Bacteria of the parenchymella escaping phagocytosis can pass to the new generation, a feature supporting a verti-



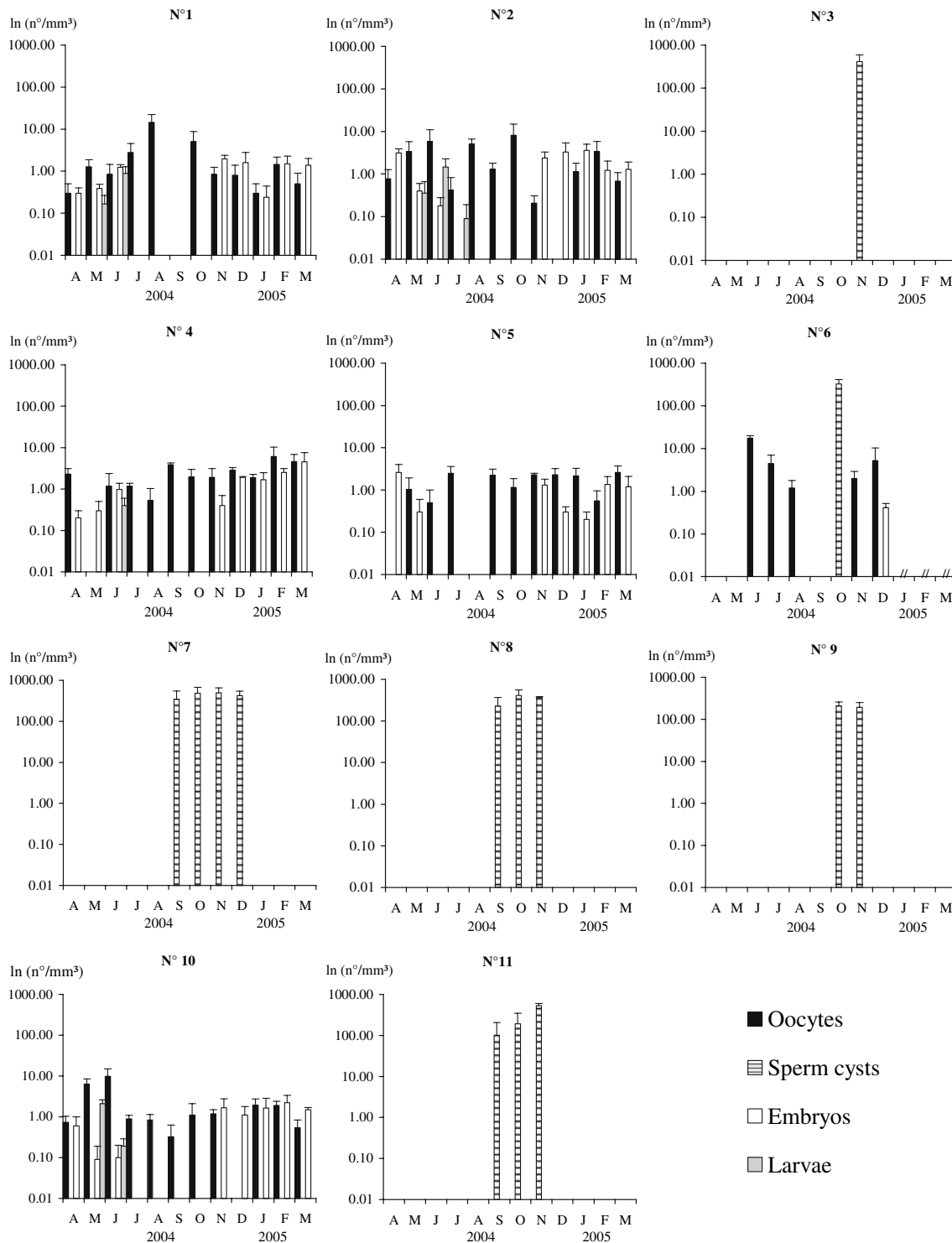
**Fig. 8** A newly released parenchymella of *Spongia officinalis*. **a** Scanning electron microscopy of a whole larva (bar = 50  $\mu\text{m}$ ). **b** Detail of the posterior larval pole showing the slight depression (D) delimited by an uplifted ring (R) (bar = 25  $\mu\text{m}$ ). **c** Transmission electron microscopy of the posterior larval ring showing the arrangement of the flagellated cells elongated in shape (bar = 5  $\mu\text{m}$ ). **d** Detail of the flagellated cells showing their cytoplasmic organelles (mitochondria M; electron-dense inclusions I) in the apical cell region, and the numerous electron-lucent vesicles (V) with homogeneous content (bar = 1  $\mu\text{m}$ ). **e** Microgranular cells with long branching cytoplasmic extensions surrounding bacteria (arrows) (bar = 10  $\mu\text{m}$ ). **f** Detail of a microgranular cell whose pseudopods converge to form a pocket that includes bacteria. Note the presence of bacteria within membrane-bounded vacuoles located along the cell margin (arrow) (bar = 1  $\mu\text{m}$ )

cal transmission of symbiotic bacteria throughout sexually produced elements (Kaye 1990). A similar mechanism has also been observed in the buds of *Tethya orphei* where filamentous cyanobacteria located in the mother sponge enter these asexually produced elements (Gaino et al. 2006b).

The homogenous material included in the vacuoles of the parenchymella flagellated epithelial cells could be extruded from the larva at settlement, thus acting as a matrix substance able to assure the adhesion to the substratum at metamorphosis. Indeed, matrix substances are doubtlessly involved in determining to a large extent the successful adhesion allowing cell movement, repositioning and growth processes, which take place in newly formed sponges.

Current literature reports that the annual reproductive index in sponges is usually low, with only a limited number





**Fig. 9** Sexual abundance trend (mean monthly number of oocytes, sperm cysts, embryos and larvae per mm<sup>3</sup> of sponge tissue, ±standard error) for each monitored individual of *S. officinalis*

of specimens, generally not exceeding 50%, able to differentiate gametes (Scalera Liaci et al. 1973; Corriero et al. 1996, 1998). Scalera et al. (1971) reported for *S. officinalis* a mean reproductive index of 49.4% with a peak of reproductive specimens in December (87.5%). It is quite surpris-

ing that all the individuals of *S. officinalis*, monitored in the present study reproduce all year round. Likewise, the prolonged presence of embryos within the same specimen corroborates previous observations carried out on some tropical and subtropical sponges, which may brood within

large chambers scattered in the mesohyl of the sponge (Leys and Degnan 2002), of variable amounts of embryos at any time during the year, as reported by Whalan et al. (2005) and Maldonado (2006). It should be emphasized that our technique of tagging specimens proved the storage of embryos in the same individual, thus giving a better opportunity to investigate the reproductive strategies in sponges.

In *S. officinalis* the density of spermatocysts is higher than that of the oocytes ( $343.9 \pm 89.7$  no./mm<sup>3</sup> and  $2.7 \pm 1.4$  no./mm<sup>3</sup>, respectively), a pattern previously observed in other viviparous species, such as *Tedania anhelans* (Nonnis-Marzano et al. 2000) and *Pellina semitubulosa* (Mercurio et al. 2000). Moreover, if we compare viviparous and oviparous demosponges, the percentage of volume occupied by female gametes in the latter is much higher, likewise observed in *Tethya aurantium* (up to 1%) and *T. citrina* (up to 3.5%) (Corriero et al. 1996).

The mechanisms controlling the onset of reproduction, its duration and the growth of gametes are so far scarcely documented. It is well known that in sponges gamete differentiation varies from individual to individual, probably according to age and size (see Simpson 1980 for a review). Indeed, it has been frequently observed that the relative amount of resources put into reproduction increases with age (Graham 1985). According to Storr (1964) in the bath sponge *Hippospongia lachne* the minimum reproductive size corresponds to about 14 cm in maximum diameter. Our data prove full sexual activity even in specimens of *S. officinalis* with a volume of 82 ml, corresponding to about 10 cm in maximum diameter. On the other hand, despite their phylogenetic affinity, these two species exhibit remarkable differences in other aspects of their life strategy, more rapid growth in the tropical species *H. lachne* (Storr 1964) and slower in the temperate *S. officinalis* (Corriero et al. 2004a). Nevertheless, among demosponges, the pattern of sexual activity is strongly variable, in *Suberites ficus*, for instance, oogenesis usually occurs only in specimens no larger than about 5 cm in size (Lévi 1951).

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