Pluri-annual study of the reproduction of two Mediterranean Oscarella species (Porifera, Homoscleromorpha): cycle, sex-ratio, reproductive effort and phenology

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ORIGINAL PAPER

Pluri-annual study of the reproduction of two Mediterranean *Oscarella* species (Porifera, Homoscleromorpha): cycle, sex-ratio, reproductive effort and phenology

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Abstract This study presents the phenology of two common Mediterranean sponges belonging to the genus *Oscarella* (Porifera, Homoscleromorpha). *Oscarella tuberculata* and *Oscarella lobularis* are two sibling species, dwellers of shallow benthic communities which tend to have distinct ecological behavior, respectively, euryecious and rather stenoecious. The comparative study of their reproductive cycle showed that both *Oscarella* species have a seasonal reproductive cycle with a successive phase duration differing from one species to another. In both species, there is a continuous oogenesis, with new oocytes appearing in spring, whereas the spermatogenesis generally starts later with the early warming of the sea. The embryonic development and the larval release are restricted to the warmest months of the year. We also observed a shift in the period of gametogenesis and larval emission depending on species and differences in their sensitivity to changes in thermal regime. It appears that an increase in seawater temperature can affect sex determination, with mainly a shift toward males in both species. Their reproductive efforts are variable in time, and can be in some cases influenced by the temperature regime. This is especially the case of *O. lobularis* which seems to be the most thermosensitive, its phenology responding significantly to changes in thermal regime, whereas *O. tuberculata* seems to be less sensitive and/or reactive. By detecting phenological changes among sponges, this study demonstrated the relevance of such monitoring to assess the possible biological response to climate change.

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Introduction

Reproductive biology studies, including mode of sexuality and sex-ratio (Szmant 1986), gamete production (Beiring and Lasker 2000), reproductive effort (Ereskovsky 2000), recruitment (Edmunds et al. 2001) and sexual phenotypic plasticity (Bates 2005) are fundamental to the understanding of marine population dynamics. Reproductive, physiological and life history patterns of marine invertebrates are influenced by several environmental factors, which include seasonal fluctuations of temperature and salinity, food availability and hydrodynamics (Morrissey and Sumich 2009; Lejeusne et al. 2010).

Sponges (phylum Porifera) are organisms with high morphological plasticity. Their life and reproductive cycles are also highly variable [for a review see (Fell 1993; Sarà

1993)], water temperature being frequently assumed as a major environmental factor regulating their reproduction (Storr 1964; Wells et al. 1964; Simpson 1968; Elvin 1976; Fell 1976; Fell and Jacob 1979; Kaye and Reiswig 1991; Witte 1996; Ereskovsky 2000; Ettinger-Epstein et al. 2007; Gerasimova and Ereskovsky 2007; Riesgo and Maldonado 2008; Whalan et al. 2007, 2008).

Until the end of the 1970s, data available on sponge reproduction were primarily based on sporadic field observations (see for instance (Simpson 1984; Ereskovsky 2010). Recently, some researches focused on the investigation of reproductive cycles of given sponge populations, and sometimes of labeled individuals monitored throughout their life cycle. In the Mediterranean Sea, some information on sponge reproductive cycles are already available for a number of Demospongiae (Siribelli 1962; Scalera Liaci et al. 1973; Corriero et al. 1996, 1998; Lepore et al. 2000; Meroz-Fine et al. 2005; Baldacconi et al. 2007; Mercurio et al. 2007; Riesgo and Maldonado 2008; Pérez-Porro et al. 2012; Di Camillo et al. 2012), but there is less knowledge related to the other sponge clades.

The Homoscleromorpha is a distinct monophyletic group with an unclear phylogenetic position within the Porifera (Gazave et al. 2010, 2012; Ivanišević et al. 2011). This group includes now 23 valid Mediterranean species. Most of them dwell in submarine caves or in the coralligenous community (Ereskovsky et al. 2009a, b). Among Homoscleromorpha, species belonging to the genus *Oscarella* are difficult to identify because of the lack of skeleton which is the fundamental character used in sponge systematics. Attempts to characterize *Oscarella* species by their morphological features led to a confusing reduction in the number of species, *Oscarella lobularis* having been long considered cosmopolitan (Ereskovsky et al. 2009a). Its sister species, *Oscarella tuberculata* which also occurs in the Mediterranean Sea, was only recognized in 1992 with several morphological and cytological characters (Boury-Esnault et al. 1992). The divergences between these two sister species has been recently supported by the combination of genetics and metabolomics (Ivanišević et al. 2011), but there is a high probability that the two species were inextricably confused in all previous biological and ecological researches. In some places, homoscleromorphs can be predominant and constitute distinct facies. This is particularly the case of the *Oscarella* species which seem to be strong competitors for space, overgrowing massive sponges, sea fans and erected bryozoans (Ereskovsky et al. 2009b; Pérez et al. 2011). Nevertheless, among these species only descriptions of the *Oscarella* cf. *lobularis, Corticium candelabrum* and *Oscarella balibaloi* reproductive cycles are available (Tuzet and Paris 1964; Riesgo et al. 2007; Pérez et al. 2011). Before these recent researches, little was known about Homoscleromorph sponge biology and ecology. Their gametogenesis and embryology were investigated in only a few species, including *O. lobularis* (Meewis 1938; Baccetti et al. 1986; Gaino et al. 1986a, b; Ereskovsky and Boury-Esnault 2002; Boury-Esnault et al. 2003; Ereskovsky 2010).

The aim of the present study is to provide a detailed description of the reproduction patterns of two sibling homoscleromorph species, *O. tuberculata* and *O. lobularis,* and to compare their reproductive cycles. According to our knowledge, these two species have distinct ecological behavior, one being euryecious and the other rather stenoecious. Indeed, *O. tuberculata* tends to be widely distributed in depth (from very shallow waters to the deep) and in a large variety of habitats, whereas *O. lobularis* is generally restricted to a given depth range (5–35 m) and habitat. We accurately measured their reproductive effort throughout several years of investigation and assessed the putative influence of seasonal temperature variations. Responses of the two species to a change in temperature conditions were compared.

Materials and methods

Sampling

This work was performed on two distinct collections of *Oscarella tuberculata* (Schmidt, 1868) and *O. lobularis* (Schmidt, 1862), sampled by scuba diving. A first collection was built up with individuals sampled between June 1987 and September 2005 in different locations of the NW Mediterranean around the Marseilles area. The second collection was obtained by sampling about 6 individuals per month in a given population located between 14 and 16 m depth, at the "Grotte à Corail" station (Maire Island, Marseilles 43.209592° N; 5.335312° E). Both collections of samples were used to describe the morphology, the gametogenesis, the embryonic development and the overall sex-ratio for *O. lobularis* and *O. tuberculata*. However, only the second collection was used for the characterization of the reproductive cycles and calculations of reproductive efforts. For *O. tuberculata* (Fig. 1a), the sampling was performed from October 2001 to August 2003 and from June 2007 to November 2009 (Table 1). For *O. lobularis* (Fig. 1b), the sampling was performed between June 2006 and November 2009 (Table 1). Once the period of reproduction was detected, the sampling frequency was increased to one sample per week. During the whole period of investigation, we studied 291 individuals of *O. tuberculata* and 303 individuals of *O. lobularis*, which are sufficient numbers to obtain indications for a better comprehension of the population dynamics. The sampling station was equipped with a permanent temperature recorder (HOBO Tidbit Data Logger).

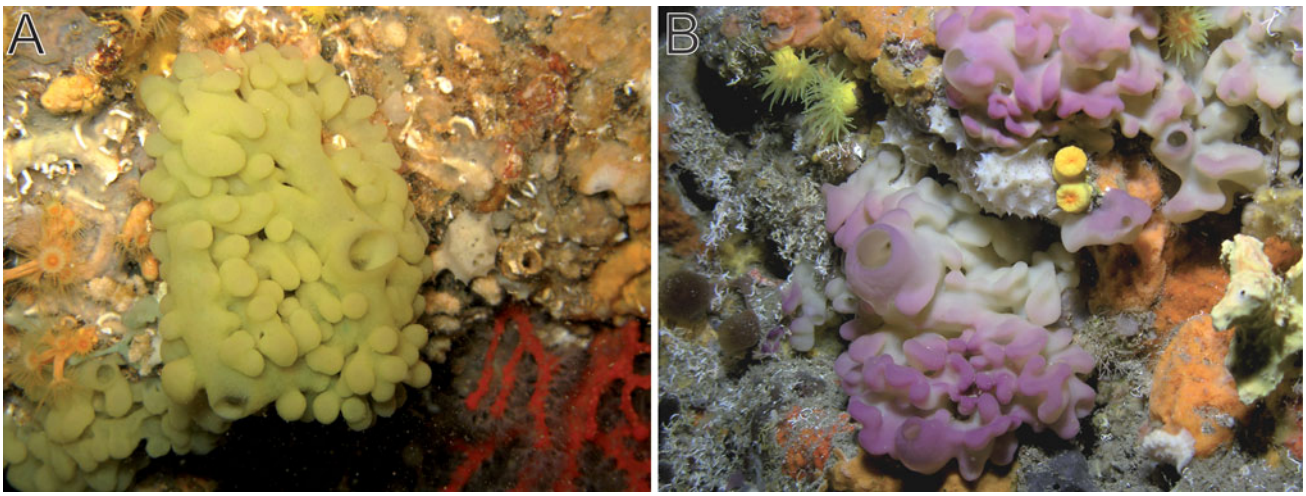


Fig. 1 Living *Oscarella* in situ. **a** *Yellow–green* color morph of *Oscarella tuberculata;* **b** *violet* color morph of *Oscarella lobularis* (Color figure online)

Morphological and ultrastructural analysis

To characterize the life cycle and assess the reproductive effort, sub-samples were fixed in Bouin's fixative. Then tissue fragments were dehydrated through an ethanol series and embedded in paraffin. Serial sections of 6 µm in thickness were mounted on glass slides and stained with Trichrome of Masson and Goldner hematoxylin and then observed under a light microscope WILD M20.

To describe gametogenesis and embryonic development, sub-samples were fixed with a standard fixation method using glutaraldehyde 2.5 % in a mixture of 0.4 M cacodylate buffer and seawater (1 vol: 4 vol: 5 vol; 1,120 mOsm). After fixation, samples were washed in 0.1 M phosphate buffer and post-fixed in 2 % OsO4 in sea water at room temperature, dehydrated through a graded ethanol series and embedded in araldite resin for semi-thin and ultra-thin sections. Semi-thin sections were stained with toluidine blue and then observed under a light microscope WILD M20. Digital photographs were taken on the microscope Leica DMLB with the system of photo capture Evolution LC color. Ultra-thin sections were stained with uranyl acetate, contrasted with lead citrate and observed in a LEO 910 and LEO 912 transmission electron microscopes (TEM). For scanning electron microscopy (SEM), the specimens were fractured in liquid nitrogen, critical-point-dried, sputter-coated with gold–palladium and observed under a Hitachi S 570 SEM.

Data analysis

The calculations of reproductive effort (RE) were carried out on serial histological sections. For each specimen of the second collection, we analyzed digital photographs of 16 sections for every sponge. Thus, four photographs (a total surveyed area of 5 mm^2 per individual), from serial sections were taken in order to avoid the overlapping of reproductive products that would lead to overestimation. We determined the number of reproductively active sponges over time and estimated number and area (μm^2) of each reproductive product (spermatic cyst, oocyte, embryo and larva) within the tissue sample using ImageJ Software (http://rsb.info.nih.gov/ij/index.html).

Reproductive efforts were expressed as percentage of reproductive tissue (mean ± SD) for each type of reproductive product in the reproductive individuals. The difference between RE in the two species and for each sex was assessed with a Kruskal–Wallis nonparametric rank-sum tests (H) and a Tukey's (honestly significant differenced or HSD) test.

Temperature data were provided by members of the MEDCHANGE program. Raw data were used to calculate daily means, and then annual means for male and female reproductive periods. Therefore, annual mean values are different for males and females. We also took into account maximal values and the coefficient of variations for each reproductive period. All those indicators of the temperature regime during the reproduction period were related to the percentage of each sex in the population and to the mean values of each sex reproductive efforts, in order to assess the putative influence of a change in temperature on the sponges' reproductive strategies.

Results

General characteristics

Oscarella tuberculata (Fig. 1a) is thinly encrusting to lobate, but its color is highly variable (yellow, green, blue

Table 1 The number of individuals of *O. tuberculata* and *O. lobularis*, monthly sampled

Years	Months	*O. tuberclata* N	*O. lobularis* N
2001	10	6	–
	12	6	–
Total		***12***	–
2002	1	6	–
	2	6	–
	4	6	
	6	6	–
	7	6	–
	8	16	–
	9	6	
	10	6	
	12	6	
Total		***64***	–
2003	1	6	–
	2	6	–
	4	6	–
	7	6	–
	8	6	–
Total		***30***	
2006	6	–	9
	7	–	35
	8	–	24
	9	–	6
	10	–	8
	11	–	2
	12	–	6
Total			***90***
2007	2	–	3
	3	–	6
	6	11	4
	7	14	17
	8	–	23
	9	8	–
	10	5	3
	11	9	6
	12	7	8
Total		***54***	***70***
2008	1	9	6
	2	7	3
	3	6	8
	4	6	7
	5	6	6
	6	11	25
	7	12	15
	8	6	11
	9	7	11
Total		***70***	***92***
2009	1	6	2
	3	7	1
	4	6	6
	5	6	6
	6	12	12
	7	6	6
	9	12	12
	11	6	6
Total		***61***	***51***

Bold italic values indicate total for each collected year

Fig. 2 Histology and cell composition of *Oscarella tuberculata* and *Oscarella lobularis*. Semi-thin micrographs of *O. tuberculata* **a** and *O. lobularis* **b** tissues. **c** SEM micrograph of flagellated exopinacoderm (*exp*) and ostium (*o*) of *O. lobularis*; **d** SEM micrograph of flagellated endopinacoderm (*enp*) of *O. lobularis*; **e** TEM micrograph of vacuolar cell of *O. tuberculata*; **f** TEM micrograph of vacuolar cell type 1 (*vt1*) and vacuolar cell type 2 (*vt2*) of *O. lobularis*. *cc* choanocyte chambers, *exc* exhalant canal, *f* flagella, *inc* inhalant canal, *n* nucleus, *o* ostia, *sb* symbiotic bacteria, *v* vacuole

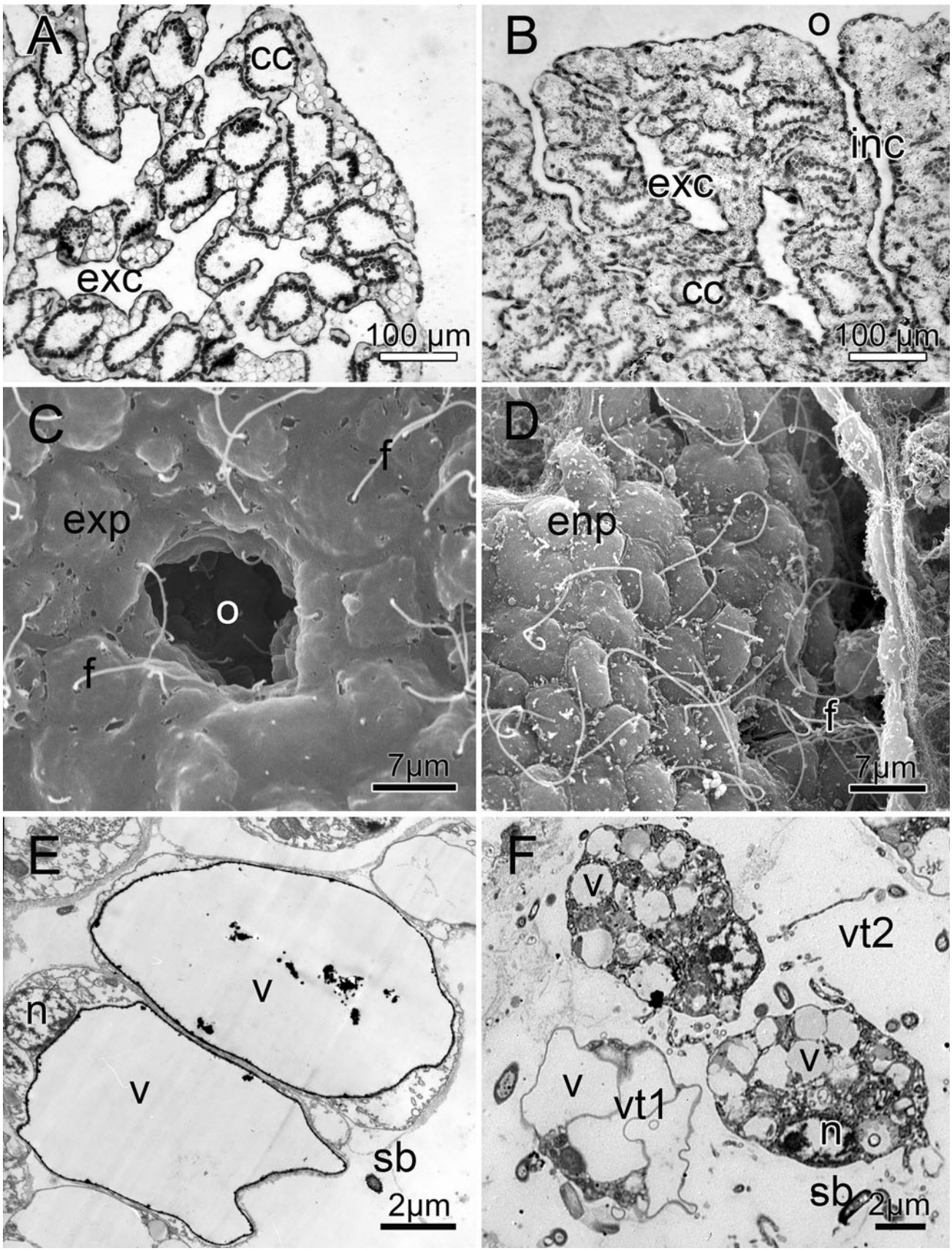
and sometimes pink). *Oscarella lobularis* is also thinly encrusting to lobate, from white to deep purple and sometimes blue (Fig. 1b). Both species can cover surfaces of up to 400 cm^2 and have a thickness of 3–8 mm. Their consistency is different, rather cartilaginous for *O. tuberculata*, and soft for *O. lobularis*. The two species can live in sympatry in the coralligenous community (Ereskovsky et al. 2009b).

The anatomy of the two species is similar, with inhalant ostia regularly scattered over the surface (Fig. 2b), oscules at the top of lobes (Fig. 1), a sylleibid-like aquiferous system (radial arrangement of chambers around exhalant canals) and eurypylous choanocyte chambers (Fig. 2a, b). The thin unspecialized ectosome and the canals are lined by a regular layer of pinacocytes which are relatively flat uniflagellated cells (Fig. 2c, d). A thin mesohyl layer located beneath the epithelium contains regularly distributed choanocyte chambers, symbiotic bacteria and scattered cells, including vacuolar cells which are specific for the two *Oscarella* species. *Oscarella tuberculata* harbors a high density of one distinct type of large vacuolar cell (Fig. 2e), whereas *O. lobularis* has two other types of vacuolar cells (Fig. 2f) (Boury-Esnault et al. 1992). The two species also possess specific symbiotic bacteria belonging to two and three morphotypes, respectively, each type being easily distinguishable using electron microscope (Vishnyakov and Ereskovsky 2009) (Figs. 3, 4, 5).

Sex-ratios

Both species are ovoviviparous. During the reproductive season, many individuals did not have any reproductive elements. Among our large collection of samples of the two series, most of the reproductive individuals belonged to only one sex. However, very few hermaphrodite individuals were found among the *O. lobularis* populations from Maire Island (3 individuals in August 2007 and only one in September 2008). In those cases, spermatic cysts were distributed in the choanosome, whereas some embryos were located at basal part of the sponges. Both spermatic cysts and embryos did not exhibit any evidence of degeneration.

Overall, the monitored population of *O. tuberculata* from Maire Island included 12.9 % of males, 24.4 % of females



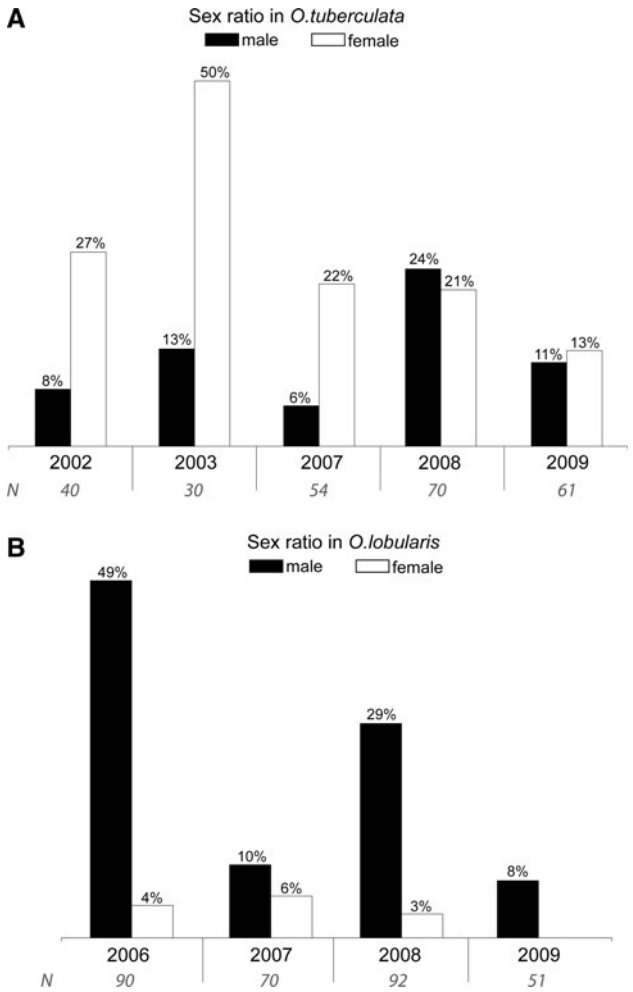


Fig. 3 Sex-ratios: Proportions of females, males and hermaphrodites (when present) in Maire Island populations in NW Mediterranean Sea. *Oscarella tuberculata* **a** and *Oscarella lobularis* **b**

and 63.1 % of non-reproductive sponges throughout the monitoring period. Considering all color morphs sampled, the sex-ratio in *O. tuberculata* is thus about 1/3 (male/female). Nevertheless, there was a little variation throughout years: 1/3.6 in 2002, 1/3.7 in 2003, 1/4 in 2007, 1.1/1 in 2008 and 1/1.14 in 2009 (Fig. 3a). The monitored population of *O. lobularis* included 27.1 % males, 3.6 % females, 1.3 % hermaphrodites and 68 % sponges without reproductive elements. In this species, the sex-ratio is thus about 10/1 (male/female) (Fig. 3b). Compared to *O. tuberculata,* a greater variation over time was recorded: 11/1 in 2006, 1.75/1 in 2007, 9/1 in 2008 and 4/0 in 2009 (Fig. 3a).

Gametogenesis

The spermatogenesis is the same in both *Oscarella* species. It occurs in spermatic cysts, which originate from the differentiation of the choanocyte chambers and are located in the sponge choanosome (Figs. 4a, b, 6a, c, e, f). They are ovoid, 15 to 60 μm in size (Fig. 4a) and surrounded by a thick layer of collagen. The spermatogenesis is asynchronous. Cysts generally contain several generations of male germ cells (Fig. 4a, b). Mature spermatozoa have a terminal long flagella and a slightly elongated head containing the acrosome and a large mitochondrion.

Young oocytes are spherical to amoeboid cells, approximately 15–40 μm in diameter, randomly distributed throughout the mesohyl (Fig. 4c). During vitellogenesis, they increase rapidly up to 130–150 μm in diameter. Mature eggs are isolecithal and polylecithal, with a cytoplasm full of yolk granules and some lipid droplets surrounding the nucleolate nucleus (Fig. 4d). Eggs are located in the basal part of the choanosome (Fig. 6b, d). Mature eggs are completely surrounded by a follicle made of endopinacocytes (Fig. 4d).

Embryonic development

Both *Oscarella* species have a similar pattern of embryonic development. It occurs at the basal part of the choanosome (Fig. 6b, d). The cleavage is holoblastic, equal, and asynchronous (Fig. 5a), and results in a solid apolar stereoblastula (morula), 130–170 μm in diameter, with undifferentiated yolk-rich blastomeres (Fig. 5b). The coeloblastula is the intermediate stage of development, coming from a morula after multipolar egression which is a migration of internal cells toward the periphery of the embryo (Fig. 5c). The cell differentiation results in a cinctoblastula larva, measuring from 130 to 200 μm in diameter (Fig. 5d–f). Larvae are then released from the maternal sponge by exhalant canals and the osculum. They remain free in the water column for a few days before settling on a substrate, where they metamorphose into a young sponge. The main difference between cinctoblastulae of the two species is the presence of the two maternal vacuolar cell types inside the larval cavity of *O. lobularis* (Fig. 5e), absent in the larval cavity of *O. tuberculata* (Fig. 5d) (Boury-Esnault et al. 2003).

Reproductive cycle and reproductive effort

Reproductive efforts (RE) of *O. tuberculata* and *O. lobularis* were calculated separately for males and females and related to natural fluctuations of the seawater temperature from January 2002 to September 2003 for *O. tuberculata* only, and January 2006 to October 2009 for both species. The reproductive efforts were highly variable in time.

In *O. tuberculata*, the spermatogenesis occurred between May and July (Fig. 7a). The male RE was almost undetectable until June, and it reached its maximum in June or July depending on the year. The highest male RE was recorded in June 2002, with spermatic cysts

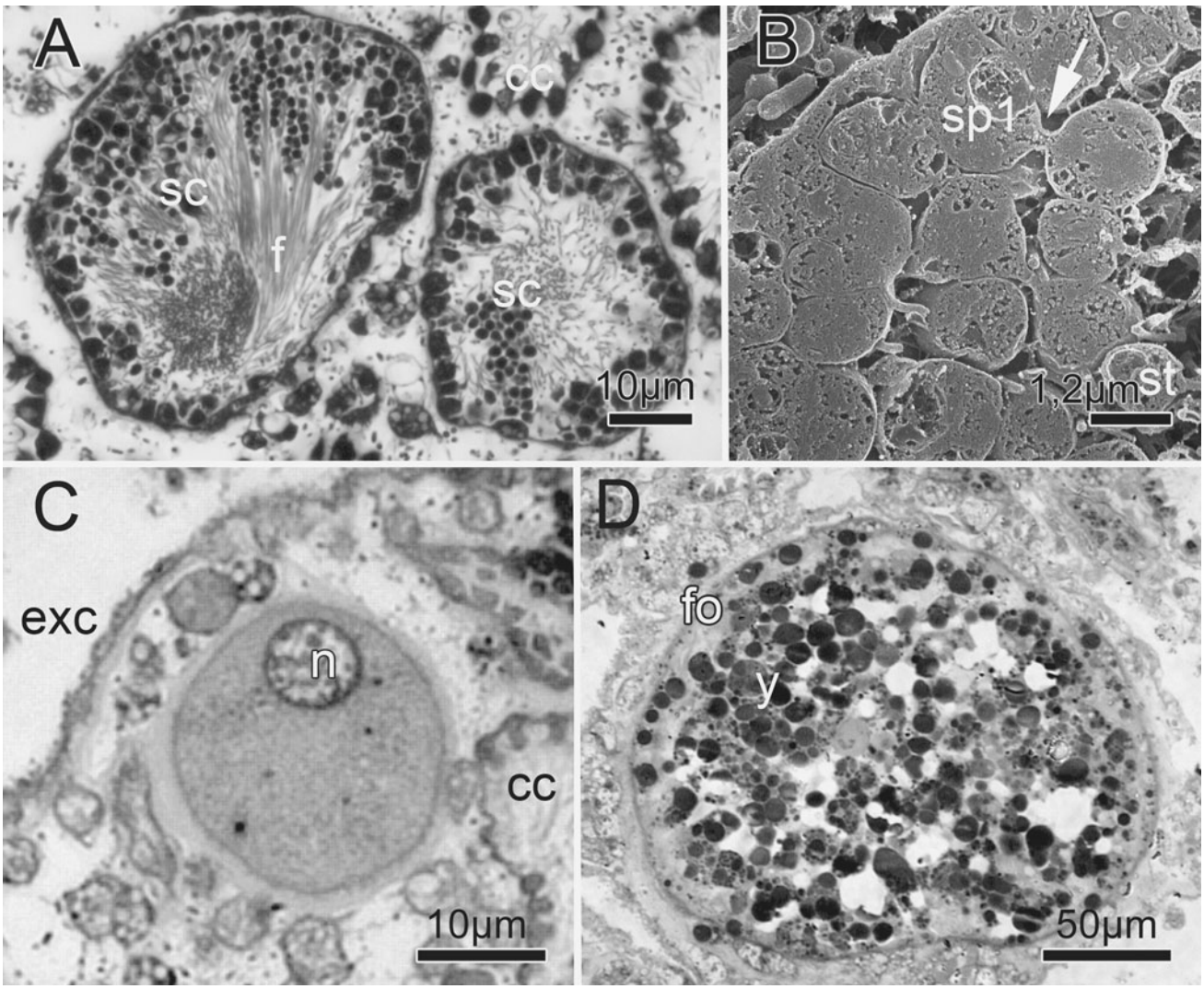


Fig. 4 Spermatogenesis (**a, b**), oogenesis (**c, d**) in *Oscarella tuberculata* and *O. lobularis*. **a** Semi-thin micrographs of two spermatic cysts (*sc*) with the different generation and composition of male germ cells. **b** SEM image of peripheral part of spermatic cyst. Intercellular bridge (*arrow*) is seen between two primary spermatocytes (*sp1*), inherited after the spermatogonia division. **c** Semi-thin micrographs of young oocyte in the mesohyl; **d** Semi-thin micrographs of mature egg surrounding by follicle (*fo*). *cc* choanocyte chambers, *exc* exhalant canal, *f* sperm flagella, *n* nucleus, *sc* spermatic cyst, *spI* primary spermatocytes, *st* spermatids, *y* yolk granules

representing 9 % in average of the sponge volume (Fig. 7a). However, considering the two studied periods, we did not find any statistical differences in male RE between 2002 and 2003, and between 2007, 2008 and 2009 (Table 2). The differentiation of young oocytes started between January and April, the vitellogenesis occurring from May to July (Fig. 7a). The first embryos were observed in mid-June, and the release of larvae occurred between the end of July and early September (Fig. 7a). For the female, the maximal RE usually occurred in July, with the highest female RE recorded in July 2008 with 15.6 % in average of the sponge volume represented by few mature oocytes and a great number of embryos (Fig. 8b). The female RE differed significantly between 2002 and 2003 (Table 2; Fig. 8a). During the second period studied, female RE differed between 2007, 2008 and 2009, mean values being, respectively, 3.6, 7.7 and 2.6 %, but this difference was not supported statistically (Table 2; Fig. 8b).

In *O. lobularis*, there was no reproductive event in winter (Fig. 7b). Both sexes exhibited their maximal RE in late June or July (Fig. 8c). The spermatogenesis was generally achieved within 10 weeks—between June and August (Fig. 7b). The male RE varied significantly between years (Table 2). The highest effort was found at the end of June 2006, with spermatic cysts representing 4.4 % of the sponge volume, against 0.21 % in July 2007, 2.89 % in July 2008 and 2.34 % in June 2009 (Fig. 8c).

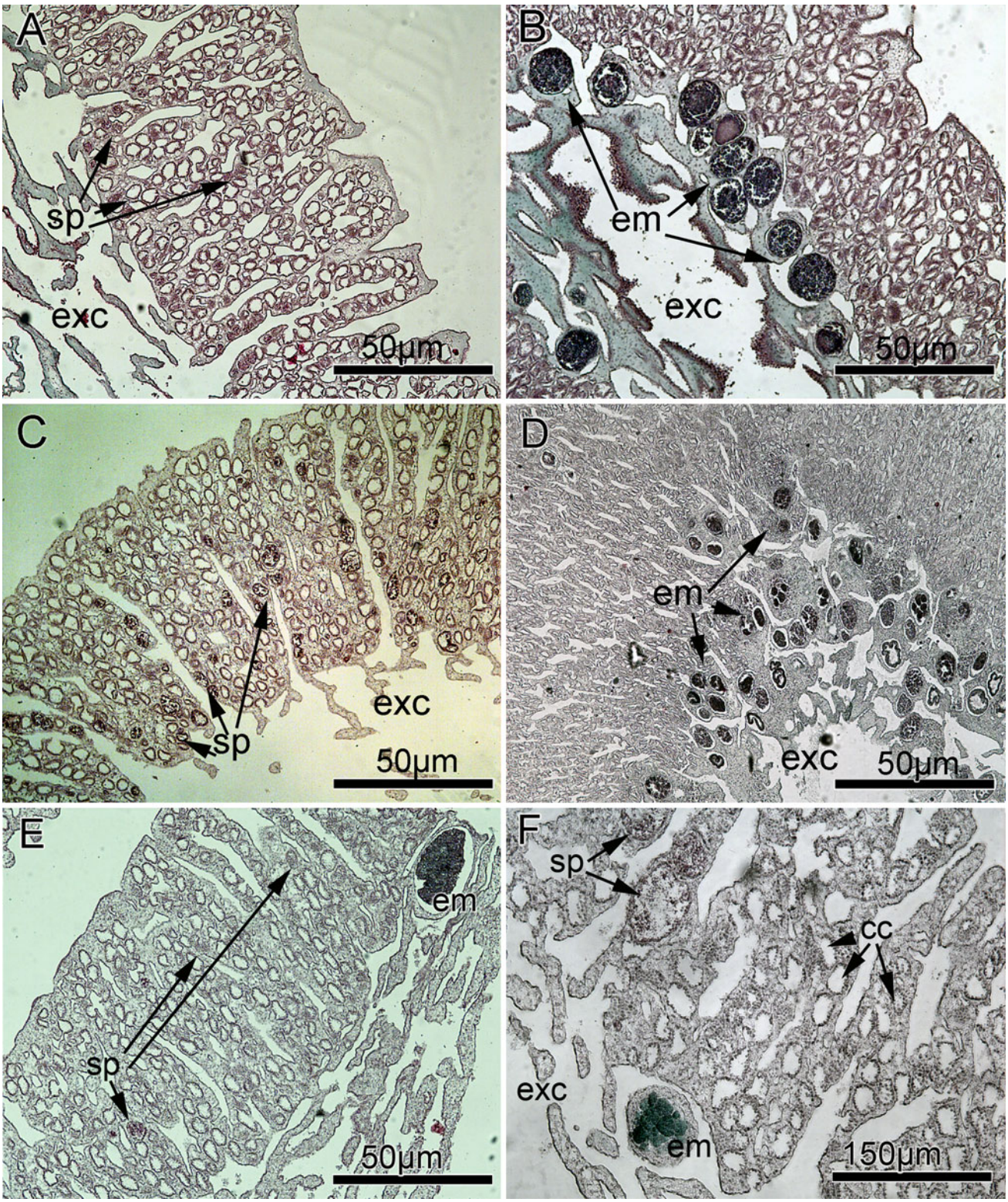


Fig. 5 Embryogenesis (**a–c**) and larvae (**d–f**) in *Oscarella tuberculata* and *O. lobularis*. **a** SEM image of asynchronous cleavage of *O. lobularis*. **b** SEM image of morula of *O. tuberculata*. **c** Semi-thin micrographs of embryo during multipolar egression and cell differentiation of *O. tuberculata*. **d** SEM image of larva inside of maternal mesohyl of *O. tuberculata*. **e** SEM image of larva inside of maternal mesohyl of *O. lobularis*. **f** SEM image of cinctoblastula larva of *O. tuberculata*. *bl* blastomeres, *cc* ciliated cells of larva, *ecm* extracellular matrix, *fo* follicle, *mc* maternal cells

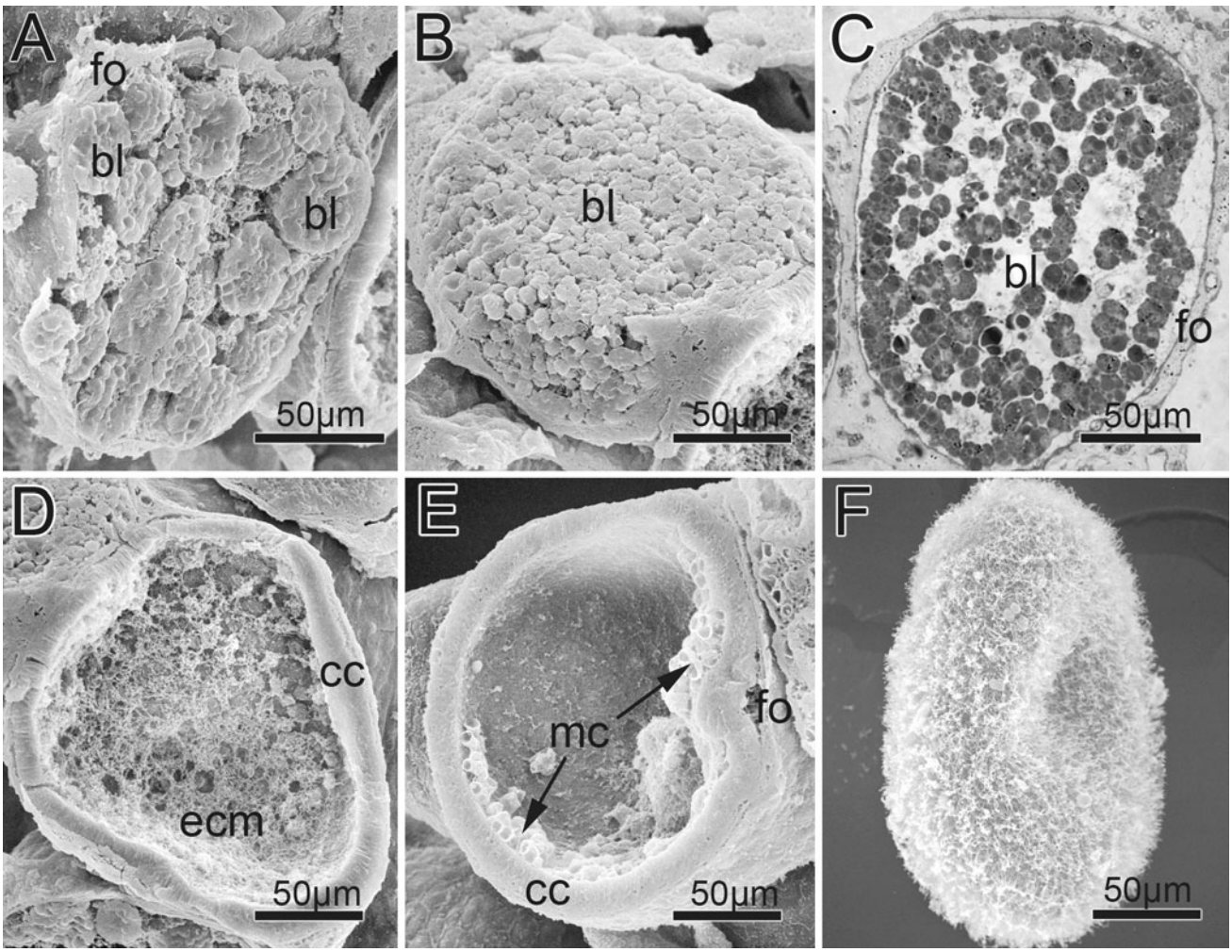


Fig. 6 Histology images of *Oscarella tuberculata* (**a**, **b**) and *O. lobularis* (**c**–**f**) reproductive sponges. **a** Male sponge (July 2007) with the spermatic cysts (*sp*) in the choanosome. **b** Female sponge (June 2006) with the embryos (*em*) on different stages of development. **c** Male sponge (July 2007) with the spermatic cysts (*sp*) in the choanosome. **d** Female sponge (June 2006) with the embryos (*em*) on different stages of development. **e, f** Hermaphrodite sponges August 2007 **e** and September 2008 **f**. *cc* choanocyte chambers, *exc* exhalant canal

The difference of male effort was significant between pairs of years 2006–2007 and 2007–2008, but was not supported statistically between 2008 and 2009 (Table 2). The differentiation of young oocytes in *O. lobularis* started in May. In this species, the vitellogenesis is slightly longer than in *O. tuberculata*. It generally started in late May and occurred until mid-August (Fig. 7b). The synchronization of the two sexes is mainly observed in July (Fig. 7b). The embryogenesis occurred from mid-July to the beginning of September, and the larvae release took place from the end of August to September. The female RE varied significantly between years (Table 2). The maximal female RE was recorded in July 2006, with 6.46 % of the sponge volume represented by oocytes or embryos, whereas it was only 1.86 % in July 2007 and 0.95 % in September 2008 (Fig. 8).

Reproduction versus seawater temperature

Temperature records allowed characterizing the main features of the thermal conditions in our monitored site at 15 m depth. Overall, the thermal regime is typical of the NW Mediterranean, with stable and rather cold temperature in winter and early spring, minima ranging from 12 to 13.5 °C, followed by a gradual increase in the temperature which usually reaches its maximal values at the end of the summer (maxima from 22.0 to 25.5 °C in late August or September during the study period) (Fig. 8). In spring and summer, the seawater is usually highly fluctuating at this depth (variations extending up to 6 °C in few hours), but whatever the studied year considered, the annual mean temperature is always about 16 °C and the coefficient of variation about 15–20 %.

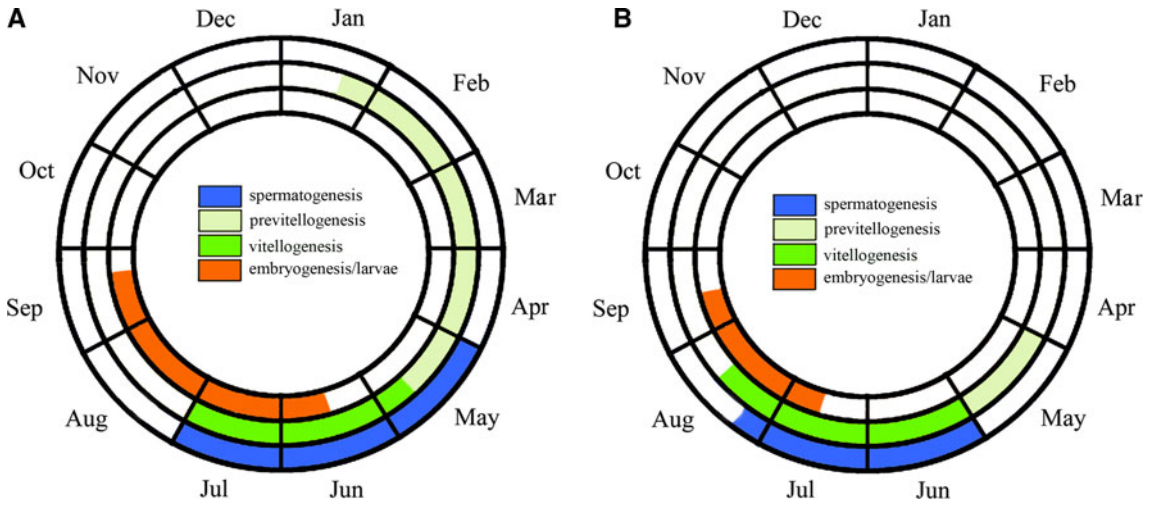


Fig. 7 Generalized diagram of reproductive cycle of *Oscarella tuberculata* **a** and *Oscarella lobularis* **b** for all investigated period

Table 2 Results of the Kruskal–Wallis nonparametric tests performed in order to assess differences in reproductive efforts between years

Data obtained for both species and sex were processed separately

Significant differences are indicated in bold

Species	Male/Female	Years	N	dl	H	*p* Value ($\alpha = 0.05$)
O. tuberculata	Male	2002/2003	63	1	3.14535	0.076
		2007/2008	15	1	3.5208	0.0606
		2008/2009	19	1	0.2571	0.6121
	Female	2002/2003	167	1	6.33144	**0.011**
		2007/2008	27	1	0.6881	0.4068
		2008/2009	23	1	1.5042	0.2200
O. lobularis	Male	2006/2007	51	1	17.0837	**0.00004**
		2007/2008	34	1	9.2739	**0.0023**
		2008/2009	31	1	0.0312	0.8597
	Female	2006/2007	8	1	4.0833	**0.0433**
		2007/2008	7	1	1.1250	0.2888
		2008/2009	–	–	–	–

In order to study the putative influence of the thermal regime on the reproduction of the two *Oscarella*, we calculated the coefficient of variation, the mean and maximal temperature of each reproductive period (distinct between species and distinct between males and females). Then, we related the percentage of each sex in their population and their annual mean RE to these proxies of the temperature regime during the reproductive season. The two species' response to a change in the temperature regime is rather different, and it even varies in relation to the indicator of the temperature regime considered.

In *O. lobularis*, both the proportion of male individuals and their RE were positively influenced by an increase in the seawater temperature (both annual mean and maximal value) and its variation over the year. On the other hand, the proportion of females was not affected by a change in the temperature regime during the reproductive season (Fig. 9). When considering the female reproductive efforts, the tendency varies between the indicators of the temperature regime: higher when the temperature variability increases and no special trend with an increase in the mean or maximal temperature during the reproductive season.

In *O. tuberculata*, the response seems to be more heterogeneous. As it was observed for *O. lobularis*, the proportion of males in the population seems to be positively influenced by an increase in the maximal temperature and of the temperature variability during the reproductive season, but no trend is observed with a change in the mean temperature. The proportion of females is also slightly positively influenced by changes in the maximal values and coefficient of variation, but there is a strong negative effect of an increase in the mean temperature during the reproductive season. There is also a strong negative effect of an increase in the mean temperature on the reproductive efforts for both sexes. This tendency is reduced when considering the temperature variability, and there is no

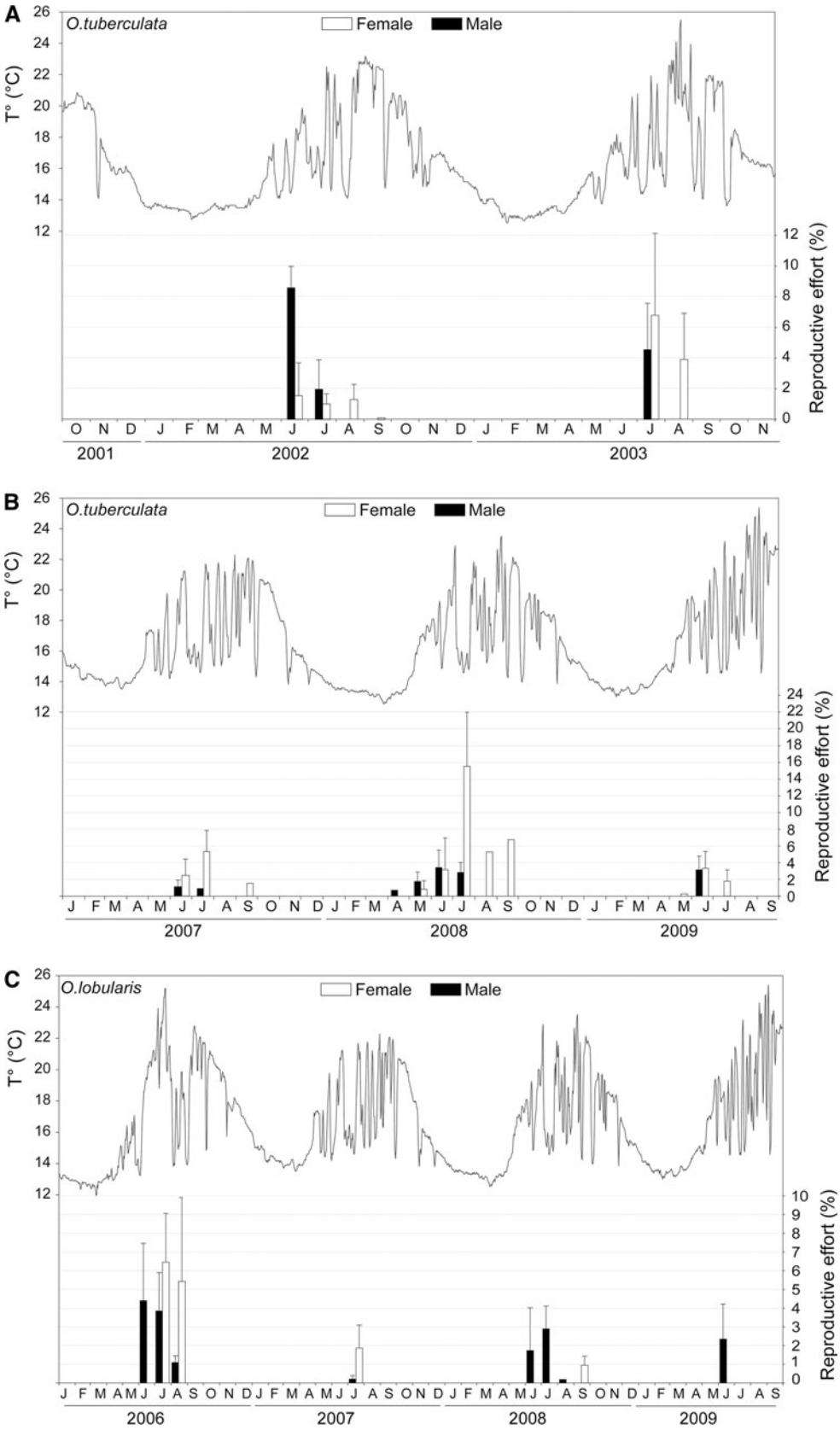


Fig. 8 Diagram of the correlation of water temperature and the monthly reproductive efforts (%) of males and females in *Oscarella tuberculata* in 2002–2003 **a** and in 2007–2009 **b**, and *Oscarella lobularis* in 2006–2009 (**c**)

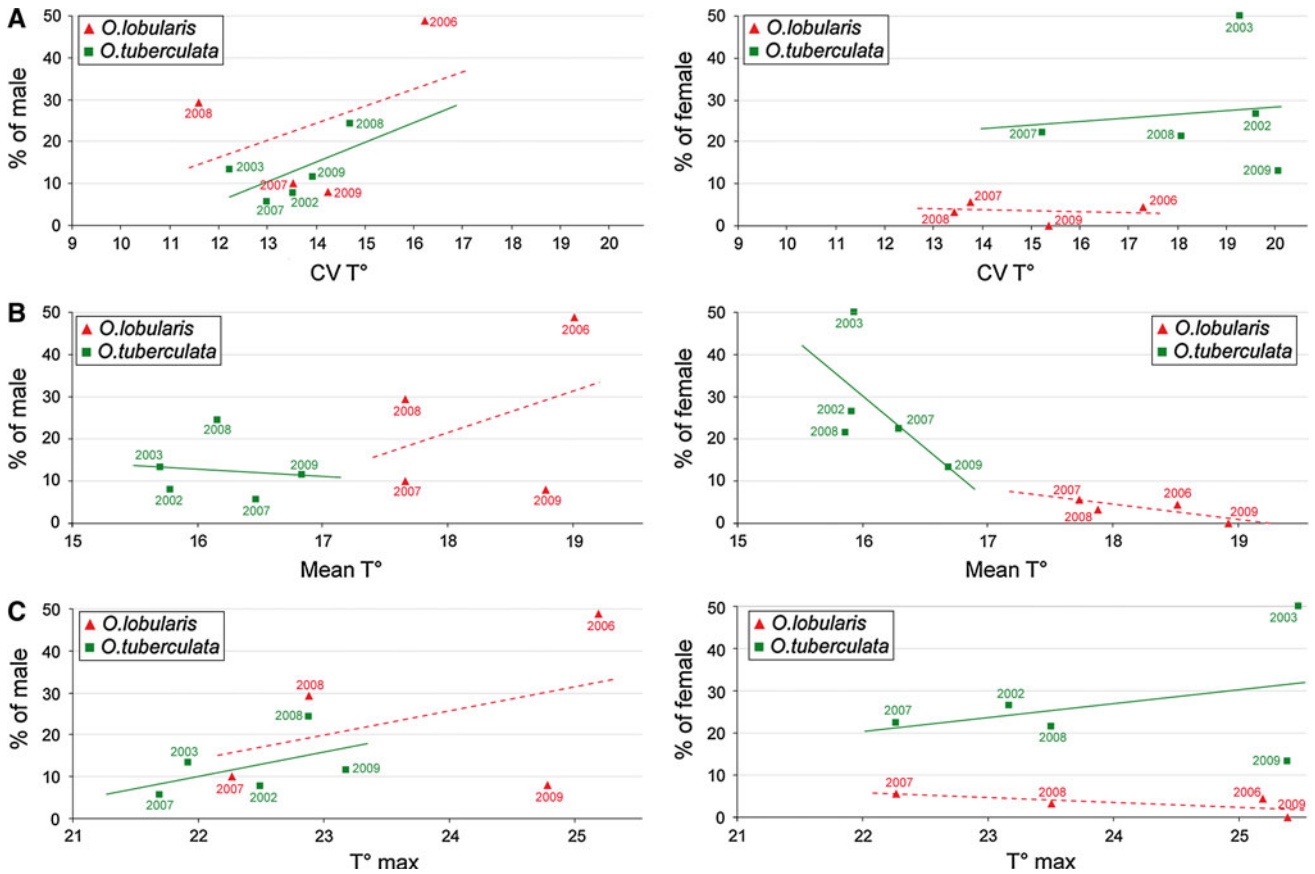


Fig. 9 Diagrams of the annual sex-ratio (%) of males and females in *Oscarella tuberculata* in 2002–2003 and in 2007–2009, and *Oscarella lobularis* in 2006–2009 versus variation coefficient (**a**), mean (**b**) and maximal (**c**) temperature

visible effect of a change in the maximal temperature during the reproductive season (Fig. 10).

Discussion

In Demospongiae and Homoscleromorpha, spermatogenesis occurs inside temporary cytological structures, the spermatic cysts, which differentiate from choanocyte chambers (Boury-Esnault and Jamieson 1999; Ereskovsky 2010). The absence of synchronization of the spermatogenesis, with different development stages (from spermatogonia to spermatozoa) within one spermatic cyst, is a characteristic feature of all investigated Homoscleromorpha which contrasts with Demospongiae (Gaino et al. 1986b; Riesgo et al. 2007; Ereskovsky 2010). Our study confirms for two *Oscarella* species the early observations by Schulze (1877) that female sexual cells can develop asynchronously inside one sponge. The cleavage pattern in both *Oscarella* is holoblastic, equal, asynchronous, and gives rise to a regular solid morula, as in all previously studied homoscleromorph species (Schulze 1877, 1880, 1881; Meewis 1938; Tuzet and Paris 1964; Ereskovsky and Boury-Esnault 2002; de Caralt et al. 2007; Maldonado and Riesgo 2008). Then, the stereoblastula develops into a coeloblastula by multipolar egression (Ereskovsky and Boury-Esnault 2002) and the subsequent development results in a cinctoblastula larva (Boury-Esnault et al. 2003).

Different patterns of sexual differentiation exist in sponges (Fell 1993). Many sponges are contemporaneous hermaphrodites, whereas many others are gonochoric. Different types of hermaphroditism and gonochorism occur among sponges. In some apparently gonochoric species, a successive hermaphroditism has been demonstrated, after a detailed study of their reproductive cycle (Sarà 1993). However, the successive or temporal hermaphroditism is difficult to distinguish from gonochorism unless the periods of male and female gamete production overlap. Only a survey of labeled individuals would allow to confirm a prevalent gonochorism, an ability of sex reversal or successive hermaphrodism (Baldacconi et al. 2007; Riesgo et al. 2007), but this monitoring strategy is hardly feasible throughout several years with tiny sponge species such as *Oscarella*. Our results from the pluri-annual monitoring showed that only rare hermaphrodite individuals, containing both spermatic cysts and embryos, can be found among

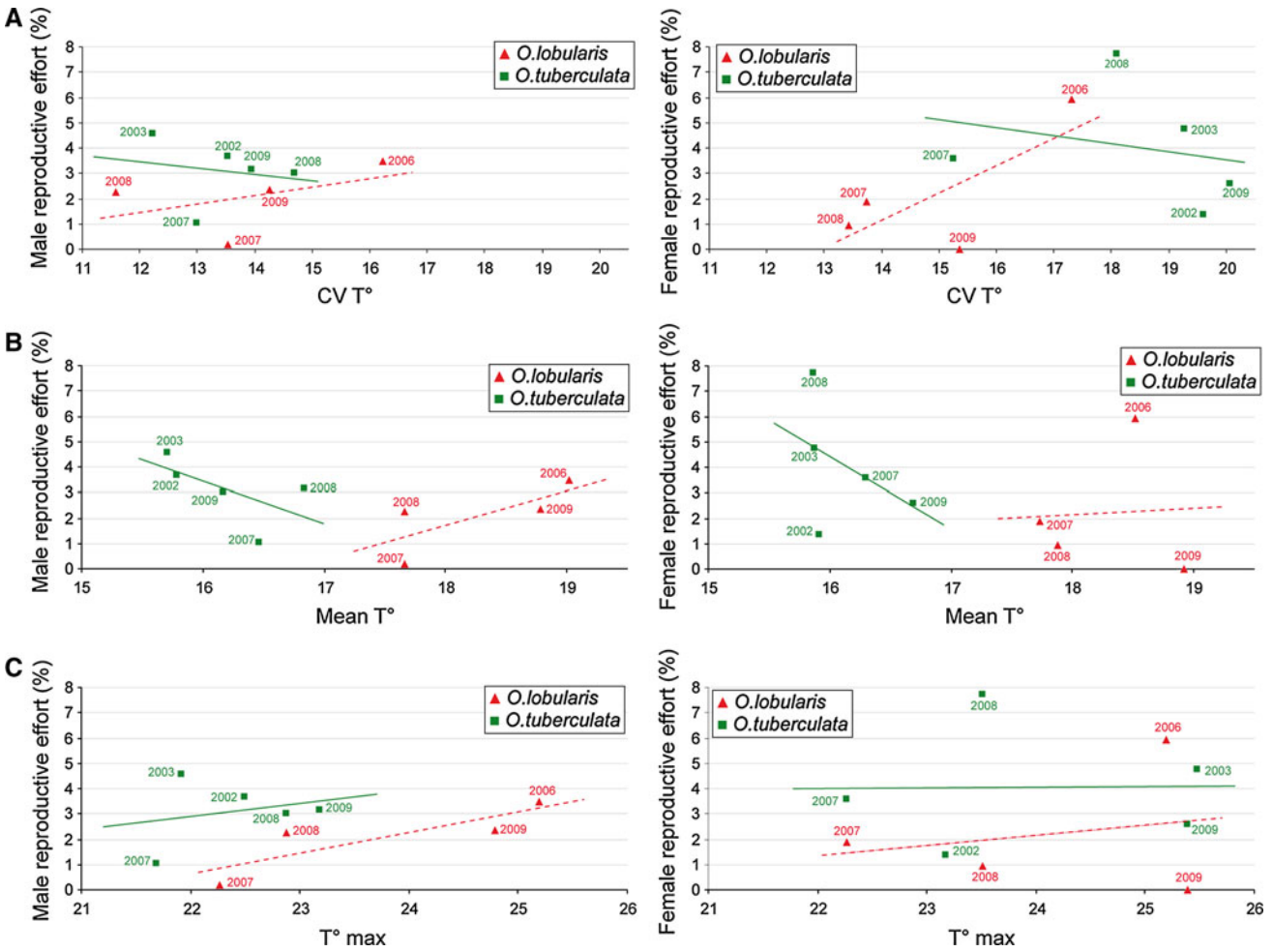


Fig. 10 Diagrams of the annual reproductive efforts (%) of males and females in *Oscarella tuberculata* in 2002–2003 and in 2007–2009, and *Oscarella lobularis* in 2006–2009 versus variation coefficient (**a**), mean (**b**) and maximal (**c**) temperature

an *O. lobularis* population thoroughly sampled. Our observation contrasts with the study performed by Meewis (1938) who found that *Oscarella* cf. *rubra* from Roscoff (Atlantic coast of France) was hermaphrodite, female gametes developing more deeply within the sponge body than spermatic cysts. On the other hand, our work confirms the previous findings of Tuzet and Paris (1964) who considered that representatives of *Oscarella* cf. *tuberculata* (which was wrongly called *Octavella galangaui*) from Banyuls-sur-Mer (NW Mediterranean) were mainly gonochoric, but with very rare hermaphrodites. Finally, we may suppose that sex determination in some individuals of *O. lobularis* may be labile in the population, this hypothesis being supported by similar observations in other marine ovoviviparous sponge species (Diaz 1973, 1979; Elvin 1976; Sarà 1993).

The sex-ratio of *O. tuberculata* is generally weighted in favor of females (male/female 1:3), this feature being apparently rather common among Demospongiae (Wapstra and van Soest 1987; Tanaka-Ichihara and Watanabe 1990; Witte et al. 1994; Corriero et al. 1996, 1998; Witte 1996; Meroz-Fine et al. 2005; Mercurio et al. 2007; Riesgo and Maldonado 2008). A comparison of the sex-ratios over the studied years showed that it remains almost constant in *O. tuberculata*. In comparison, the sex-ratio of *O. lobularis* is more variable and always weighted in favor of males (from about 2:1 to 11:1). This predominance of males is a very rare feature among sponges (Simpson 1984; Sarà 1993).

Maturation and spawning of male and female gametes between individuals of marine invertebrate populations must be highly synchronized to ensure reproductive success (Lawrence and Soame 2004). This synchronization is moderated by external cues, and for many species; temperature is the principal factor triggering the biology of the organism.

Sex determination and/or sex-ratio in *Oscarella* seems to be influenced by seawater temperature variation. In general, the proportion of males seems to be more influenced by the thermal regime than the proportion of

females. In both species, the proportion of male individuals was higher when the temperature maxima and the temperature variation increase. The same trend is observed in *O. lobularis* under the influence of a change in seawater mean temperature during the male reproductive season. By contrast, the proportion of females in both species declines when the mean annual temperature increases, the most puzzling trend being for *O. tuberculata*, whereas there is no special trend under the influence of a change in maxima or temperature variations over the female reproductive season.

A modification of the sex-ratio has been recorded in some marine invertebrates (cnidarians, crustaceans and protochordates) as a differential response of both sexes to external disturbances (mostly thermal stress) (Lawrence and Soame 2004; Bates 2005; Cerrano et al. 2005; Ribes et al. 2007). For example, in the ascidian *Botryllus schlosseri*, a greater effect on male reproduction was observed under cold and food-limited conditions (Newlon et al. 2003). These results are consistent with the sex allocation theory (Charnov 1982), which predicts that a less favorable environment will result in a relative increase in energy allocation to sperm production, because sperm are less costly to produce than eggs. Labile sex determination in these cases would allow the adjustment of the actual sex-ratio according to the local need.

A better knowledge of reproductive cycle must provide clues to those intrinsic and environmental factors, which initiate gametogenesis, control sexual differentiation and influence the rates of gamete production. Several researches already searched for a correlation between environmental fluctuations and the reproductive activity of marine invertebrates (Lawrence and Soame 2004). The influence of the environment is particularly critical when there is a marked seasonality of the reproductive events.

Sponges exhibit a wide variety of reproductive strategies. Sexual reproduction may occur throughout the year, the level of reproductive activity being relatively constant, or at the opposite, may change seasonally (Fell 1993). In temperate regions, most sponges are reproductively inactive during the coldest part of the year, the initiation of the sexual reproduction being often triggered by an increase in the water temperature (Simpson 1984; Fell 1993). *O. tuberculata* and *O. lobularis* are iteroparous sponges that produce offspring in successive (annual) cycles. Their period of embryonic development is related to the warmest period of year. In these species, the onset of gametogenesis is not a continuous process, but it occurs only once a year, concomitantly with the increase in the water temperature in the case of *O. lobularis*. In *O. lobularis*, the gametogenesis and embryogenesis occur during a shorter period than in *O. tuberculata*. This sexual pattern probably represents the most common means of sexual reproduction among sponges in temperate seas (Tanaka-Ichihara and Watanabe 1990; Fell 1993; Ereskovsky 2000; Baldacconi et al. 2007; Gerasimova and Ereskovsky 2007; Mercurio et al. 2007). However, in another homoscleromorph species, *Corticium candelabrum*, the spermatogenesis starts during the coldest months and is extended over a period characterized by both cold and warm seawater. In this species, the temperature is not a direct factor triggering oogenesis, as new oocytes can be produced all year round (de Caralt et al. 2007; Riesgo et al. 2007).

Organisms obtain resources from their environment and allocate energy to different cellular compartments. The allocation of resources to somatic or reproductive compartments varies considerably according to the reproductive strategy. One of the integrative indicators of this allocation is the reproductive effort (RE) defined as the proportion of an organism's total energy which is directed toward reproduction. Marine organisms can exhibit highly variable correlations between cyclic RE and natural variation of the sea water temperature (Adiyodi and Adiyodi 1993; Olive 1995). A positive correlation between RE and the thermal regime has been shown in several researches on sponge reproductive cycles. In these previous works performed in different seas (polar, temperate and tropical), the maximal RE occurs during hydrological summer, so when the seawater temperature reaches its maxima (Fell 1976; Fell and Jacob 1979; Ereskovsky 2000; Meroz-Fine et al. 2005; Ettinger-Epstein et al. 2007; Mercurio et al. 2007; Riesgo et al. 2007; Riesgo and Maldonado 2008; Whalan et al. 2008).

Our pluri-annual monitoring showed that reproductive efforts of *O. tuberculata* and *O. lobularis* varied in time under the influence of a change in environmental conditions. Although the two *Oscarella* species can share the same habitat, they seem to respond differently in terms of RE to a change in temperature regime. In *O. lobularis,* RE of both sexes increased with an increase in the temperature variability, whereas there is almost no effect on *O. tuberculata* RE. In *O. lobularis*, there is also a positive influence of the seawater warming (both in terms of annual mean and in terms of maxima) on the REs, although it is less pronounced in females than in males. On the other hand, a change in the temperature maxima has no effect on *O. tuberculata*, and it seems that an increase in the annual mean reduces the both sexes RE. Thus, *O. lobularis* and *O. tuberculata* exhibit opposite responses to a change in the temperature regime. The contrasted responses of these species may reflect distinct adaptive strategies and/or sensitivities to environmental changes. Because it responds in a more homogeneous and significant way to changes in the thermal regime, we hypothesize that *O. lobularis* is more sensitive than *O. tuberculata*. This may be partly explained by the fact that its periods of gametogenesis initiation are

more subject to temperature fluctuations (May–June) than in *O. tuberculata* (January–April). The most pronounced responses of males RE could also be explained by the sex allocation theory (Charnov 1982).

Phenology is the timing of seasonal activities of animals and plants, including biological phenomena such as growth and reproduction. These phenological changes are sensitive and easily observable indicators of changes in the ecology of species in response to climate change. Today, there is substantial evidence from continental systems of species responding to climate change through changes in their phenology. In marine environments, there is much less evidence, because of a lack of long-term biological series, and also because it is not possible to observe in situ biological events at a high frequency. Our pluri-annual investigation of the *Oscarella* reproduction showed the relevance of such strategy to demonstrate the putative biological effect of change in temperature regime. The next step of our research will be to test experimentally the effect of a change in temperature in order to support our hypothesis of distinct adaptive strategies and explain their habit in a continual changing environment.

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