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Sexual reproduction in demosponges: ecological and evolutive implications

Reproducción sexual en demosponjas: implicaciones ecológicas y evolutivas

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Barcelona 2007

Chapter 1:

Introduction

In nature, aquatic invertebrates are subjected to a variety of fluctuations in environmental factors including light, salinity, pressure and temperature. Temperature is probably the factor controlling most of the biological processes that occur in an individual, grouped under the category of metabolism and activity (Kinne 1970). Unanimously, reproduction falls into the most affected processes by temperature (Sastry 1966; Kinne 1970; Levin and Creed 1986; Bates 2005). Many marine invertebrates begin to reproduce when a certain temperature level is reached after a period of either increasing or decreasing temperature, or in response to sudden temperature changes (Kinne 1970), often confining their reproductive period to relatively narrow thermal ranges. The effect of temperature on the reproductive cycle is expected to be more important in lower than higher invertebrates, particularly in those lacking defined gonads and tissue systems for regulatory control of the reproductive activity. Among the lower invertebrates, sponges lack not only gonads but also a distinct germ cell line, with somatic cells transdifferentiating into oogonia and spermatogonia when required (e.g., Harrison and de Vos 1991). The environmental stimuli inducing such cell transformation remain little understood (see Fell 1974 and Simpson 1984 for reviews), although temperature is thought to be the key factor (e.g., Sarà and Vacelet 1973; Fell 1974; Simpson 1984). It has frequently been proposed that a certain temperature threshold must be attained for gametogenesis to initiate in some sponges (e.g., Fell 1983; Simpson 1984), and that such temperature values must hold to ensure its completion (Kaye and Reiswig 1991). A strong correlation between seawater

temperature and gametogenesis has also been reported in many studies, with raising temperatures being suggested to induce gamete production in most cases (e.g., Hartman 1958; Storr 1964; Fell 1974, 1976b; Scalera-Liaci and Sciscioli 1975; Johnson 1978; Tanaka-Ichihara and Watanabe 1990; Kaye and Reiswig 1991; Fromont 1994, 1999; Fromont and Bergquist 1994; Witte et al. 1994; Ereskovsky 2000; Mercurio et al. 2007). Unfortunately, such a straightforward relationship between temperature and gametogenesis does not always emerge clearly, not even for temperate demosponges, which are subjected to severe annual temperature fluctuations. In habitats where water temperature experiences minor variations over the year, such as at very high or very low latitudes and in deep sea, the role of temperature in triggering reproduction may not be relevant, if any (Kaye and Reiswig 1991). In these situations, the photoperiod (Elvin 1976) or peaks in vertical fluxes of particles (Witte 1996) may become more important clues. Sometimes, minimum rather than maximum temperature values appear to be the stimulus required for the onset of gametogenesis, as reported for some cold-water sponges (Ereskovsky 2000). In other cases, gametogenesis appeared to be unrelated to the temperature cycle, such as in *Sycon ciliatum* (Sarà and Relini-Orsi 1975), *Haliclona permollis* (Elvin 1976) and 2 species of *Tethya* (Corriero et al. 1998).

The many recent findings are leading to a more realistic view of the dynamics of sponge gametogenesis, pointing out that the relationship between seawater temperature and gametogenesis does not always follow a simple, easily generalizable pattern, in contrast to the view offered by earlier reviews on this issue. Therefore, a clear understanding of the relationship between reproductive activity and temperature may be of major importance if we are to evaluate and predict the effects of climate change on marine invertebrates (Walther et al. 2002). This study investigates the dynamics of the gametogenic cycle in 4 common Mediterranean demosponge species that share habitat in a sublittoral rocky-bottom community. By assessing between-species differences in gametogenic dynamics and exploring the relationship between gamete production and temperature, we expect to improve our ability to assess the potential ecological effects of climate change on the reproductive cycle of these key organisms.

Material and Methods

Species and study sites This study dealt with 4 demosponge species, representing 4 different taxonomic orders: *Axinella damicornis* (Halichondrida), *Corticium candelabrum* (Homosclerophorida), *Raspaciona aculeata* (Poecilosclerida), and *Chondrosia reniformis* (Chondrosida). All 4 sponges are fairly common, sharing habitat at a typical Mediterranean rocky-bottom community (e.g., Ros et al. 1985) located between Blanes (2º48.12'N, 41º40.33'E) and Tossa de Mar (2º54'55.77''N, 41º42'33.25''E), in the northeastern coast of Spain.

Sample processing and analyses For a long-term monitoring of the reproductive activity in the studied populations, we tagged 5 presumably mature (according to size) individuals of each species. These individuals were sampled monthly during 2 consecutive years (from November 2003 to November 2005) using scuba diving (8 to 15 m depth). A small tissue piece (approx. 1 x 0.5 x 0.5 cm) of each sponge was collected using surgical scissors at each sampling time. In no case tissue collection caused death of the sampled sponges or perceivable functional damage. When samples revealed that gametogenesis activity was about to peak in the populations, we increased sampled individuals to a number of 25.

Tissue samples for optical microscopy were maintained in ambient seawater for transportation to the laboratory (1 to 2 h) and fixed in 4% formaldehyde in seawater for 24 h. Then, samples of *Corticium candelabrum* were desilicified with 5% hydrofluoric acid for 1.5 h; 5 h were required for desilicification of *Raspaciona aculeata* and *Axinella damicornis*; samples of *Chondrosia reniformis* were not acid-treated, since they lacked spicules. Tissue samples were subsequently rinsed in distilled water, dehydrated through a graded ethanol series (70%, 96%, 100%), cleared in toluene, and embedded in paraffin before cutting them into 5 µm-thick sections using an Autocut Reichert-Jung microtome 2040. After deparaffining with xylene, sections were stained with Hematoxylin-PAS and examined through a Zeiss Axioplan II compound microscopy connected to a Spot Cooled Color digital camera. To obtain oocyte number per tissue area unit and document the occurrence of spermatic cysts, we took 3 pictures (x100) of each of 2 non-serial sections per individual, which rendered a total surveyed area of 7 mm^2 per individual. Pictures were taken at least 240 µm from each other to

avoid the overlapping of oocytes leading to overestimation. We counted number of oocytes on the digital histological images. Then, we estimated average density of oocytes (mean number per unit area ± SD) for each species. Regarding sperm, we recorded only the occurrence of spermatic cysts in the tissue over the 2 years, since sperm investment can not accurately be inferred from cyst area and density. It would rather require counts of spermatozoa per cyst, which were impossible to separate visually in the histological sections.

To investigate the potential relationship between temperature and reproductive activity, we measured seawater temperature (± 0.5 ºC) at the sampling sites monthly, placing an electronic underwater thermometer (Suunto) on the rocky walls where the sponges grew. Then, monthly temperature values were plotted versus estimated density (number per mm^2) of oocytes and presence/absence of spermatic cysts.

Results

Axinella damicornis It was a gonochoristic and oviparous sponge (Fig. 1A-B) with a gametogenesis that extended for 7-8 months at the population level (Fig. 2). All tagged individuals were reproductively active during the entire study, and the sex-ratio was about 1:1. Gametes in both females and males were homogeneously spread in the sponge tissue. Young oocytes were amoeboid, emitting evident pseudopodia. Mature oocytes, measuring approximately 150 µm, became rounded, showing a nucleolate nucleus (Fig. 1A). Scarce nurse cells occurred around the oocytes during their development. Over the 2 years of study, oogenesis was confined to the coldest period of each year. Oocytes first appeared concomitantly to the seawater temperature decline in October-November and were released in May (13ºC in 2004 and 15ºC in 2005), when the temperature rising started (Fig. 2). Although the average number of oocytes per mm^2 in the tissues increased moderately during March and April, values remained below 2 oocytes per mm^2 during the entire reproductive period (Fig. 2). Spermatogenesis concurred with late oogenesis (May or April-May) (Fig. 2). Spermatic cysts were round (Fig. 1B), measured up to 200 µm in diameter, and located homogeneously within the entire sponge tissue, though causing little disruption of the regular sponge histology (hereafter referred to as "mesohyl disruption"). During 2004, spermatogenesis initiated

immediately before the seasonal water warming, and extended only through May, when seawater temperature was 13ºC. During 2005, it also started immediately before the water warming when temperature was 13ºC again –this year in April-, and extended through May, when an increase of 2 ºC in temperature took place. During both years, 5 months of minimum temperatures (between 12-13ºC) preceded the onset of sperm production, being December to April in 2004, and November to March in 2005 (Fig. 2).

Corticium candelabrum It was a hermaphroditic viviparous sponge with oocytes and spermatic cysts (Fig. 1C-D) occurring simultaneously in the sponge tissue. During the 2 years of study about 90% of the population experienced gametogenesis, with all reproductive individuals producing both oocytes and spermatic cysts. Oocytes were oval to round, nucleolate cells (approx. 125-150 µm when mature), consistently located in the vicinity of the excurrent canals (Fig. 1C). Many nurse cells occurred around oocytes during the entire process of oogenesis. Production of oocytes extended through the entire year, but the highest production rate (approximately 7 oocytes mm^{-2} of tissue and $month^{-1}$) was recorded from October to February, that is, in the coldest months (11ºC to 13ºC) of both years (Fig. 2). Oocyte production decreased over spring months, reaching the lowest values in summer (Fig. 2), concomitantly with maximum temperatures. During 2004, oocyte production was higher than in 2005. Round to lobed spermatic cysts located close to canals in the sponge tissue, frequently around developing oocytes (Fig. 1D). In both years, cysts first appeared when seawater temperature reached its lowest values, and their production extended from February-March to June-July, before temperature reached its maximum value (Fig. 2).

Raspaciona aculeata It was a gonochoristic oviparous species (Fig. 1E-F), with a gametogenesis that extended for 5 months (from July to November) at the population level. All sampled individuals were engaged in gametogenesis, rendering a sex ratio of 1:1. Oocytes (approx. 190 µm when mature) were quite similar to those of *A. damicornis*, emitting multiple pseudopodia during early stages and becoming rounded at maturity (Fig. 1E). They were scattered throughout the mesohyl of the sponge, intermingled with choanocyte chambers. Many nurse cells occurred in the vicinity of developing oocytes. Oogenesis started in July-August, when seawater temperature reached its maximum (22-24ºC), and extended for 3 to 5 months (Fig. 2). Maximum

production rate was recorded in September 2004 and October 2005 (at 17 and18 ºC respectively), just before the abrupt seasonal seawater cooling. In both years, oocytes were released progressively during the month subsequent to the temperature dropping. Large, oval spermatic cysts (>200 µm) occurred intermingled with choanocyte chambers throughout the mesohyl of the sponge (Fig. 1F), causing appreciable tissue disruption. Cyst production started with declining temperatures, and extended for 1 or 2 months (October in 2004 and October-November in 2005) (Fig. 2).

Chondrosia reniformis This species was gonochoristic and oviparous (Fig. 1G-H). At the population level gametogenesis extended for 3 months, from June to August. About 80% of the sampled individuals produced gametes, with a female-biased 4:1 sex ratio. The oocytes were the smallest (approx. 40 µm when mature) recorded within the scope of this study. They were round to oval, nucleolate cells, connected to surrounding nurse cells by cytoplasmic bridges (Fig. 1G). Oocytes clustered in the choanosome in groups of 20-50, but never occurred in the very collagenous mesohyl areas characteristic of this sponge. Oogenesis started along with the temperature rising in June and was completed after 3 months, in late August, concurrently with the annual maximum temperature (23-24ºC) (Fig. 2).

Figure 1. Mature oocytes and spermatic cysts of the studied demosponges. **(A)** Mature oocyte of *Axinella damicornis*, showing the nucleus (n). **(B)** Spermatic cyst of *A. damicornis*. Arrow heads mark the perimeter of the cyst. **(C)** Mature oocyte of *Corticium candelabrum* with a nucleolate (nu) nucleus (n). **(D)** Spermatic cyst of *C. candelabrum* (arrow head). **(E)** Mature oocyte of *Raspaciona aculeata*, showing the nucleolate (nu) nucleus (n). Note the dark area surrounding the nucleus, which contains multiple units of Golgi apparatus (g). **(F)** Spermatic cyst of *R. aculeata* (arrow heads). **(G)** Mature oocyte of *Chondrosia reniformis* showing the cytoplasmic bridges (cb) and the nucleus (n). **(H)** Spermatic cysts of *C. reniformis* (arrow heads). Scale bar = 100 µm.

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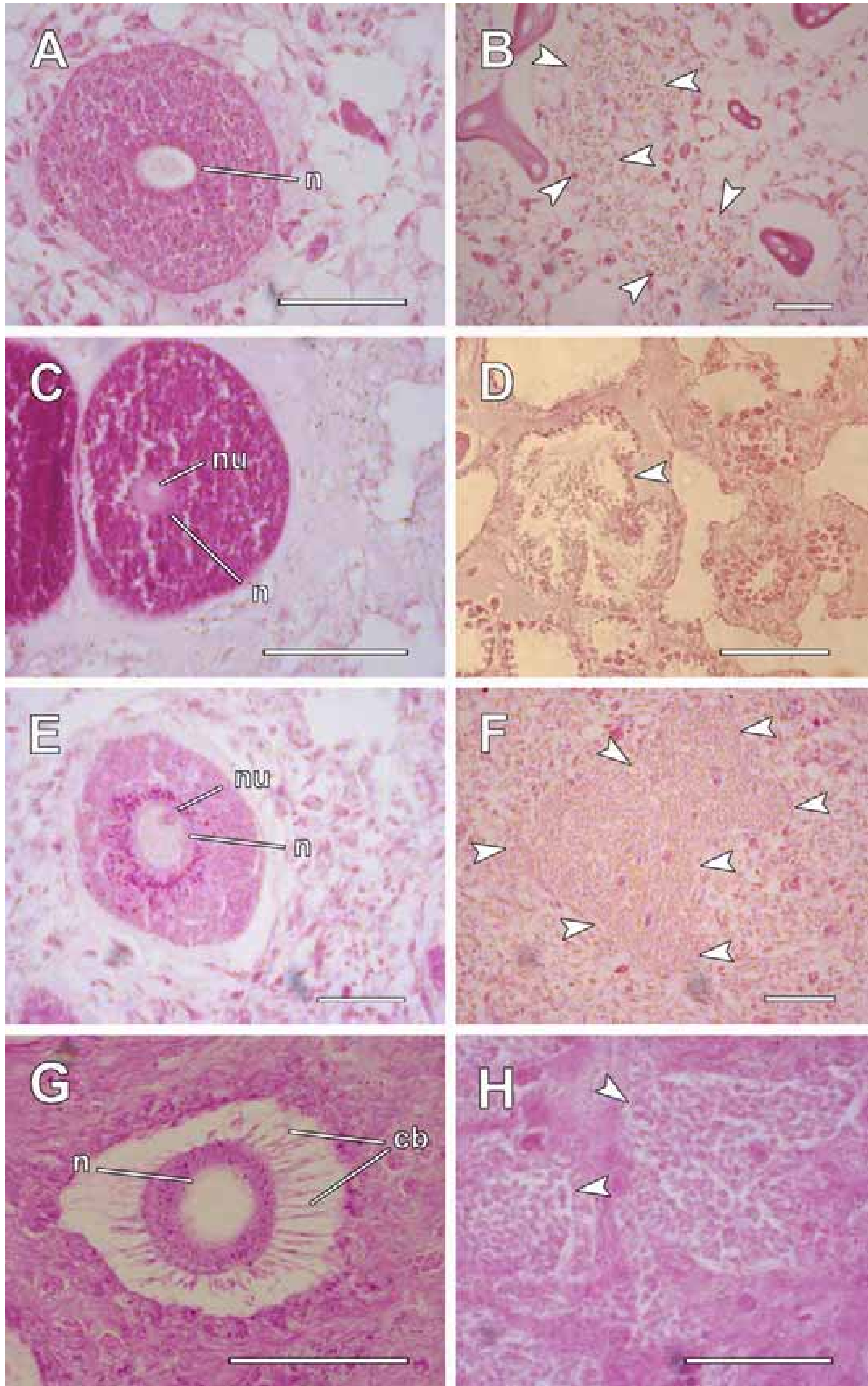
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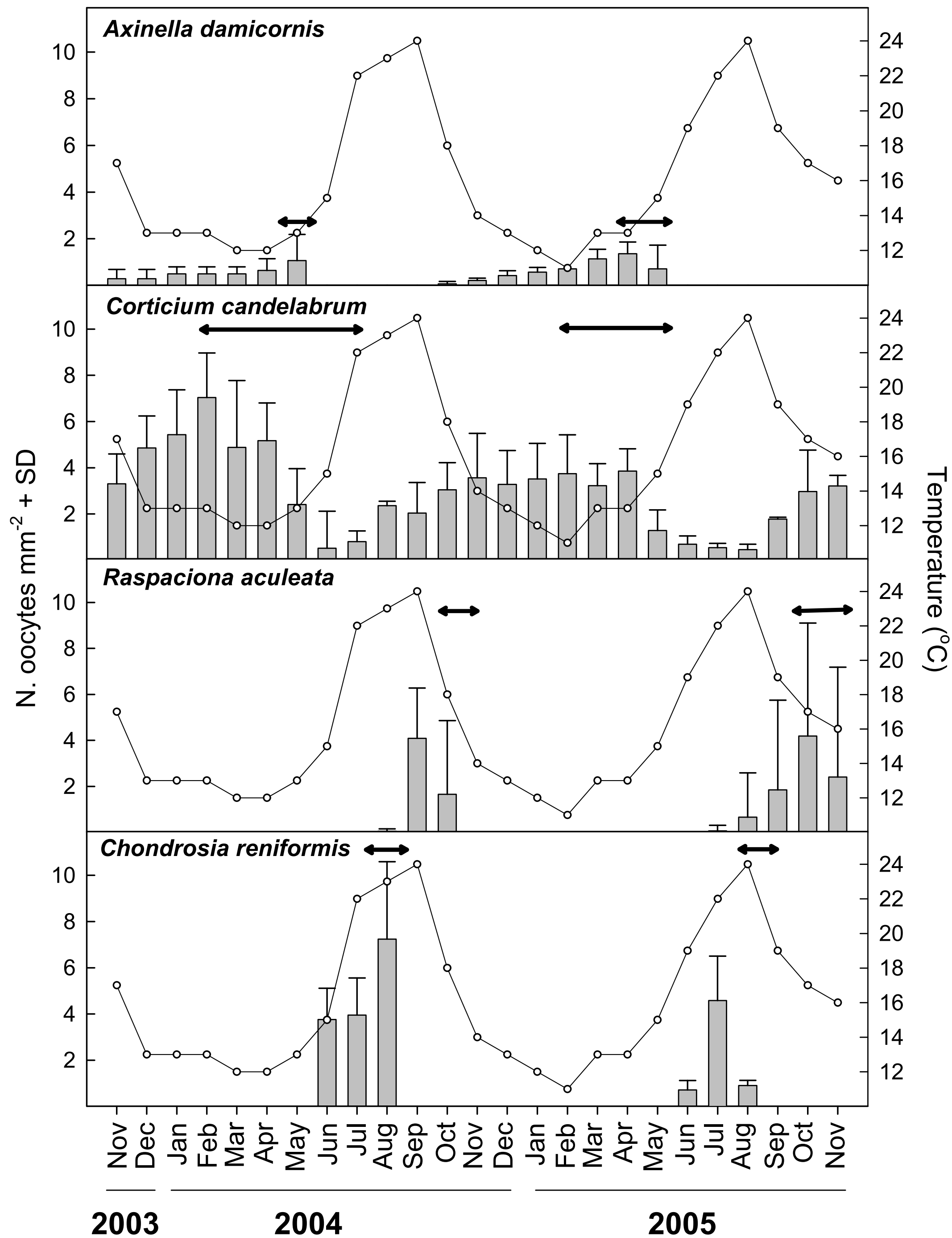

Figure 2. Density of oocytes (number $mm^{-2} \pm$ SD) and presence of spermatic cysts (arrows) in the sponge tissue of 5 tagged individuals of 4 different sponge species over 2 years (November 2003 to November 2005) plotted versus seawater temperature.

During 2004, oocyte production rate increased from approximately 4 oocytes per mm^2 in June-July to 7.2 ± 3.3 oocytes per mm^2 in August, in parallel to the temperature rising, which culminated with a temperature maximum in late August, immediately prior to oocyte release. However, in 2005, oocyte production followed a different pattern. It increased from 0.7 ± 0.4 oocytes per mm^2 in June to 4.6 ± 1.9 oocytes per mm^2 in July, then, decreased progressively to 0.9 ± 0.21 oocytes per mm^2 in August. This pattern revealed an earlier and slower oocyte release in 2005 than in 2004, presumably caused by 1ºC warmer temperatures in July 2005. Lobed spermatic cysts took most part of the choanosome (Fig. 1H), causing such a mesohyl disruption that virtually no choanocyte chamber was found during spermatogenesis. Spermatogenesis started in August simultaneously with the temperature maximum (23-24ºC), and probably lasted less than a month (Fig. 2).

Discussion

All 4 species experienced gametogenic activity in the 2 years of study. *Axinella damicornis*, *Raspaciona aculeata*, and *Chondrosia reniformis* were gonochoristic and oviparous, while *Corticium candelabrum* was hermaphroditic and viviparous. As the sponge individuals were tagged during the monitoring, we confirmed that none exhibited sex reversal during the 2-year study. Some cases of sex-reversal have been reported in freshwater demosponges (van de Vyver and Willenz 1975; Gilbert and Simpson 1976).

In all the studied species most individuals (80-100%) of the studied populations contained gametes. A situation similar to that reported in the oviparous demosponge *Geodia cydonium* (Mercurio et al. 2007) and the viviparous *Mycale* sp. (Reiswig 1973), *Halisarca nahatensis* (Chen 1976), and *Latrunculia magnifica* (Ilan 1995). It is worth noting the case of one individual of *Axinella damicornis*, which was reproductively active during 2004 but not in 2005. Because the reproductive pause only affected one out of the several studied individuals, we suspect the involvement of endogenous (e.g., nutritional status, disease, etc) rather than exogenous factors - which should influence the entire population otherwise. Sex ratio was about 1:1 in *Raspaciona aculeata* and *A. damicornis*, but 4:1 in *Chondrosia reniformis,* with female overabundance. An ample diversity of sex ratios has been recorded in sponges, ranging from 1:1 to overwhelming

predominance of one sex (Scalera-Liaci and Sciscioli 1979; Fell et al. 1979; Kaye 1991; Mercurio et al. 2007).

There were clear between-species differences in the duration of the gametogenic cycles, despite the fact that the 4 species shared habitat and were subjected to nearly identical environmental stimuli. Short cycles of oogenesis were developed by *Raspaciona aculeata* (3-5 months) and *Chondrosia reniformis* (3 months) consistently during both years of study, although the reproductive cycle of the latter one was previously described to occur during a longer time (Scalera-Liaci et al. 1971, 1973). Such quickness in the maturation of oocytes has been described in several other demosponges, as *Aplysilla rosea* (Lévi 1956), *Erylus discophorus* (Scalera-Liaci & Sciscioli 1970), *Haliclona loosanoffi* (Fell 1976a), *Halisarca nahatensis* (Chen 1976), *Mycale contarenii* (Corriero et al. 1998), *Iophon piceus* (Ereskovsky 2000), *Cinachyra tarentina* (Lepore et al. 2000) or *Chondrilla nucula* (Usher et al. 2004). *Axinella damicornis* experienced oogenesis during 8 months, as reported for *Ircinia fasciculata*, *Ircinia variabilis*, *Pellina semitubulosa* and *Spongia officinalis* (Scalera-Liaci & Sciscioli 1975). The sexual reproductive cycle of *A. damicornis* was first examined by Siribelli (1962), finding that oogenesis extended for 5 months (from February to June), which contrasts with our results. However, it is important to remark that the monitoring in Siribelli's study started in February; hence she may have missed the first months of the oogenesis (from November to February). *Corticium candelabrum* experienced an unconventional pattern of oogenesis, with continuous production of oocytes during the entire year. Although some sponges are known to produce oocytes during most months of the year,- e.g., *Haliclona ecbasis* (Fell 1974), *Hippospongia lachne* (Storr 1964), *Halisarca dujardini* and *Mycale contarenii* (Corriero et al. 1998)-, the case of *C. candelabrum* is different because new oocytes are produced continuously during the entire year. Since oocyte growth was completed in 7-8 months, the fate of young oocytes appearing when sperm was already mature remains unclear. They may have been used for nourishing of mature oocytes, zygotes, or early embryos, as it has been postulated for other sponges (Sarà 1955; Maldonado et al. 2005).

Oocytes of all species were relatively similar in morphology, but showed a great interspecific variation in size at the end of the cycle, independently of their oviparous or viviparous nature, as described before in sponges (Fell 1974) and in many other invertebrates (Lopo 1983).

Spermatogenesis, when compared to oogenesis, was consistently a shorter process in all 4 species. Duration of spermatogenesis at the individual level did not vary much between species, being generally completed in few weeks. Duration of spermatogenesis at the population level ranged from 1 to 5 months, and depended on the reproductive mode of the sponge and the level of inter-individual asynchrony. Spermatogenesis was rapid (from 1 to 2 months) and synchronous in *Axinella damicornis*, *Raspaciona aculeata* and *Chondrosia reniformis*, as it is also the case in most oviparous species (see Reiswig 1983 and Boury-Esnault and Jamieson 1999 for reviews). Our findings regarding the duration of spermatogenesis were consistent with previous studies on the spermatogenic cycle of *A. damicornis* (Siribelli 1962) and *C. reniformis* (Scalera-Liaci et al. 1973b). In the viviparous species *C. candelabrum*, sperm production at the population level lasted for 4-5 months, resulting in an asynchronous release of sperm during, at least, 3 months. Such a spawning pattern, which is coupled to asynchronous oocyte maturation, is thought to minimize the risk of sperm loss derived from a single spawning event under unfavourable hydrodynamic conditions, therefore increasing the chances of fertilisation at the population level.

Spermatic cysts were extremely different in their size between all species, showing different tissue occupancies depending on the species. Largest cysts were found in *R. aculeata*, but *C. reniformis*' cysts occupied most of the sponge choanosome, leaving no space for choanocyte chambers. Mesohyl disruption during gametogenesis has been reported in, for instance, *Halichondria okadai* (Tanaka-Ichihara & Watanabe 1990), *Aplysina cauliformis* (Tsurumi & Reiswig 1997) and *Halisarca dujardini* (Ereskovsky 2000). However, timing of spermatogenesis in the latter two species differed greatly, so that mesohyl disruption may affect differently to the sponges. While in *H. dujardini* spermatogenesis occurred during 3 months (from January to March), in *A. cauliformis* sperm was present in the tissue only during one month (April). In *C. reniformis* virtually no chambers were present in the tissue during August. It is possible that in some oviparous sponges (such as *A. cauliformis* and *C. reniformis*) spermatogenesis had been confined to few weeks because of the necessity to recover as soon as possible the choanosome structure (choanocyte chambers) and subsequently the ability for feeding.

Within the scope of our study, between-species differences in duration of both oogenesis and spermatogenesis appeared to depend primarily on physiological

processes undergone by gametes rather than on environmental conditions. In the case of oogenesis, it appears that, when nurse cells got involved significantly in vitellogenesis, oogenic cycles shortened relative to those cases in which elaboration of yolk mostly relied on the oocyte itself, with little participation of nurse cells (Nørrevang 1968). The former situation appears to occur in both *Raspaciona aculeata* and *Chondrosia reniformis*. In contrast, an oogenesis primarily based on auto-synthesis of yolk by the oocytes appears to be the case of *Corticium candelabrum* and *Axinella damicornis.*

There were not only between-species differences in the duration of gametogenesis, but also in the relationship of gametogenic dynamics with temperature. Water warming appeared to be related to the onset of oogenesis in *Chondrosia reniformis,* and maximum temperatures were concurrent with the initiation of oogenesis in *Raspaciona aculeata.* Minimum temperatures apparently triggered the oogenesis and enhanced oocyte production in *Axinella damicornis* and *Corticium candelabrum,* respectively. All these patterns of relationship between oogenesis and temperature had been previously recorded in other demosponges, although induction of oogenesis by water warming has been more often reported (e.g., Fromont 1994, 1999; Fromont and Bergquist 1994; Witte et al. 1994; Mercurio et al. 2007) than induction by water cooling (Ereskovsky 2000).

Notable heterogeneity has also been recorded regarding the relationship between temperature and spermatogenesis onset. Maximum temperature values appeared to trigger sperm production in *Chondrosia reniformis*, while minimum values did it in *Corticium candelabrum*. The former relation has been established in many cases before (see Simpson 1984 for a review), but the latter has only been identifed in *Halichondria panicea* (Witte et al. 1994), *Halisarca dujardini, Myxilla incrustans,* and *Iophon piceus* (Ereskovsky 2000). Interestingly, all the 4 latter species are cold-water sponges. The autumn gradual decrease in seawater temperatures appeared to induce the onset of spermatogenesis in *Raspaciona aculeata*, a pattern previously recorded in *Desmacidon fruticosum* (Lévi 1956), as well as in *Tectitethya crypta* and *Verongula gigantea* (Reiswig 1973). The case of *Axinella damicornis* was fairly distinct. In the 2 studied years, spermatogenesis may have been induced not by reaching a threshold of minimum temperature but a long exposure to low temperatures, since sperm production started after a 5-month period of cold temperatures (13ºC).

That sponges sharing habitat are differently affected by a same thermal stimulus suggests a disparity in species adaptive responses, which remains little understood to date. Modifications of thermal ranges because of the climate change may result in populations initiating a physiological response at the wrong time in relation to calendar date (Lawrence and Soame 2004). This may entail variations on recruitment success and population dynamics, which can deeply alter species life-cycles and the interrelationships between relevant community members (Fromentin and Planque 1996; Saetre et al. 1999; Walther et al. 2002). Therefore, given that sponges are key components in many marine communities, we are urged to assess their phenology, with special attention to climate-driven shifts in physiological processes that are susceptible to alter recruitment and population structure.