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Differences in reproductive timing among sponges sharing habitat and thermal regime

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Abstract. The reproductive cycles of four Mediterranean demosponges (*Axinella damicornis, Corticium candelabrum, Raspaciona aculeata,* and *Chondrosia reniformis*) were investigated during 2 consecutive years. Three of the species had annual gametogenic cycles characterized by a single peak of gamete production, but members of *C. candelabrum* showed continuous oocyte production during the 2 years. The relationship between gametogenic dynamics and seawater temperature varied substantially among species, contrary to the widespread belief that gamete production is associated with seasonal water warming. The annual temperature increase (in June) concurred with oocyte production only in *C. reniformis,* although maximum temperatures were simultaneous with the production of both oocytes in *R. aculeata* and sperm in *C. reniformis*. In contrast, the annual temperature decline in October was associated with both oogenesis in *A. damicornis* and spermatogenesis in *R. aculeata*. Spermatogenesis in *A. damicornis* started after a 5-month period of low-temperature values (December–April in 2004 and November–March in 2005). Likewise, in *C. candelabrum,* spermatogenesis started after a 3-month period of low-temperature values (November–February), a period concomitant with a slow increase in oocyte production. These findings reveal that sponge species that cooccur and share similar thermal regimes may differ substantially in their timing of gamete production. If we are to predict the future effects of climate change on marine benthic communities, there is an urgent need to improve our knowledge of the species-specific relationship between timing of gametogenesis and temperature, at least for those sponges that are key species in benthic communities.

Additional key words: oogenesis, spermatogenesis, environmental factors, climate change

In nature, aquatic invertebrates are subjected to fluctuations in terms of a variety of environmental factors including light, salinity, pressure, and temperature. Temperature is probably the factor with the strongest effects on most metabolic processes (Kinne 1970). Reproduction is particularly affected by temperature (Sastry 1966; Kinne 1970; Levin & 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.

Among the lower invertebrates, sponges lack gonads and also a distinct germ cell line, with somatic cells transdifferentiating into oogonia and spermatogonia when required (e.g., Harrison & de Vos 1991). The environmental stimuli inducing such cell transformation remain poorly understood (see Fell 1974 and Simpson 1984 for reviews), although temperature is thought to be the key factor (e.g., Sarà & Vacelet 1973; Fell 1974; Simpson 1984). It has frequently been proposed that a certain temperature threshold must be attained to initiate gametogenesis in some sponges (e.g., Fell 1983; Simpson 1984), and that such temperature values must hold to ensure its completion (Kaye & Reiswig 1991). A strong correlation between seawater temperature and gametogenesis has also been reported in many studies, with increasing temperatures being suggested to induce gamete production in most cases (e.g., Hartman 1958; Storr 1964; Fell 1974, 1976; Scalera-Liaci & Sciscioli 1975; Johnson 1978; Tanaka-Ichihara & Watanabe 1990; Kaye & Reiswig 1991; Fromont 1994, 1999; Fromont & Bergquist 1994; Witte et al. 1994; Ereskovsky & Gonobobleva 2000; Mercurio et al. 2007). Unfortunately, such a clear relationship

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between temperature and gametogenesis does not always emerge, not even for temperate demosponges, which may be subjected to large annual temperature fluctuations (e.g., Riesgo et al. 2007). In habitats where water temperature varies only slightly over the year, such as at very high or very low latitudes and in the deep sea, the role of temperature in inducing reproduction may not be important (Kaye & Reiswig 1991). In these situations, the photoperiod (Elvin 1976) or peaks in vertical fluxes of particles (Witte 1996) may become more important cues. 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 & Gonobobleva 2000). In other cases, gametogenesis appeared to be unrelated to the temperature cycle, such as in *Sycon ciliatum* (Sarà & Relini Orsi 1975), *Haliclona permollis* (Elvin 1976), and two species of *Tethya* (Corriero et al. 1996).

Recent findings are leading to a more realistic view of the dynamics of sponge gametogenesis, showing 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. 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 four common Mediterranean demosponge species that share habitat in a sublittoral rocky-bottom community. By assessing among-species differences in gametogenic dynamics and exploring the relationship between the timing of 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.

Methods

Species and study sites

This study dealt with four demosponge species, representing four different taxonomic orders: *Axinella damicornis* Esper 1974 (Halichondrida), *Corticium candelabrum* Schmidt 1862 (Homosclerophorida), *Raspaciona aculeata* Johnston 1842 (Poecilosclerida), and *Chondrosia reniformis* Nardo 1847 (Chondrosida). All four sponges are fairly common, sharing habitat as members of a typical Mediterranean rocky-bottom community (e.g., Ros et al. 1985) located between Blanes (2°48.12′N, 41°40.33′E) and Tossa de Mar (2°54′55.77″N, 41°42′33.25″E), on the northeastern coast of Spain.

Sample processing and analyses

For long-term monitoring of reproductive activity in the studied populations, we tagged five presumably mature (according to size) individuals of each species. These individuals were sampled monthly during 2 consecutive years (from November 2003 to November 2005) by scuba divers (8–15 m depth). A small piece of tissue ($\sim 1 \times 0.5 \times 0.5$ cm) of each sponge was collected using surgical scissors at each sampling time. In no case did tissue collection cause death. In 2005, when samples revealed that gametogenesis activity was about to peak in the populations (the month immediately before spawning in each case), we increased the number of randomly sampled individuals to 25. Sex-ratio estimates were made using both tagged and untagged individuals ($n = 30$) when possible.

Tissue samples for optical microscopy were maintained in ambient seawater for transportation to the laboratory (1–2 h), and then fixed in 4% formaldehyde in seawater for 24 h. Samples of *C. candelabrum* were desilicified with 5% hydrofluoric acid for 1.5 h; 5 h were required for desilicification of *R. aculeata,* and *A. damicornis*. Samples of *C. reniformis* were not acid-treated, because they lacked spicules. Tissues were subsequently rinsed in distilled water, dehydrated through a graded ethanol series (70%, 96%, and 100%), cleared in toluene, and embedded in paraffin before cutting them into 5-μm-thick sections using an Autocut Reichert-Jung microtome 2040 (R. Jung GmbH, Nubloch, Germany). After deparaffining with xylene, sections were stained with hematoxylin-PAS and examined through a Zeiss Axioplan II compound microscope (Carl Zeiss, Oberkochen, Germany) connected to a spot-cooled color digital camera.

To obtain oocyte number per unit tissue area and to document the occurrence of spermatic cysts, we took three pictures (final magnification of $\times 100$) of each of two non-serial sections per individual, which rendered a total surveyed area of 7 mm^2 per individual. Pictures were taken ≥ 240 μm from each other to avoid the overlapping of oocytes leading to overestimation. On the histological images, we counted the number of oocytes and, using the program ImageJ (http://rsb.info.nih.gov/ij/index.html), we measured their diameters. We also estimated the average density of oocytes (mean number per $mm^2 \pm$ SD) for each species. We recorded only the occurrence of

spermatic cysts in the tissue over the 2 years, because sperm investment cannot accurately be inferred from cyst area and cyst density. Estimates of sperm investment would require counts of spermatozoa per cyst, but they were too densely packed to separate them visually and count them.

To investigate the potential relationship between temperature and the timing of reproductive events, we measured seawater temperature (± 0.5°C) during each monthly sampling. Readings were taken by placing an underwater electronic thermometer (Suunto, Vaanta, Finland) on the rocky walls where the sponges grew, usually between 10:00 and 12:00 GMT. Monthly temperature values were plotted versus the estimated density of oocytes and the presence/absence of spermatic cysts.

We usually did not detect temperature differences between 8- and 15-m-deep sites. On the two occasions that we did (September 2004 and October 2005, <1°C difference), it was due to mixing processes subsequent to the disruption of the local summer thermocline by severe storms. Therefore, although more accurate temperature monitoring could have been conducted with more frequent sampling, we assume that our data depict the main annual pattern of temperature change and are suitable to be related to slowly changing physiological and gametogenic processes.

Results

Axinella damicornis

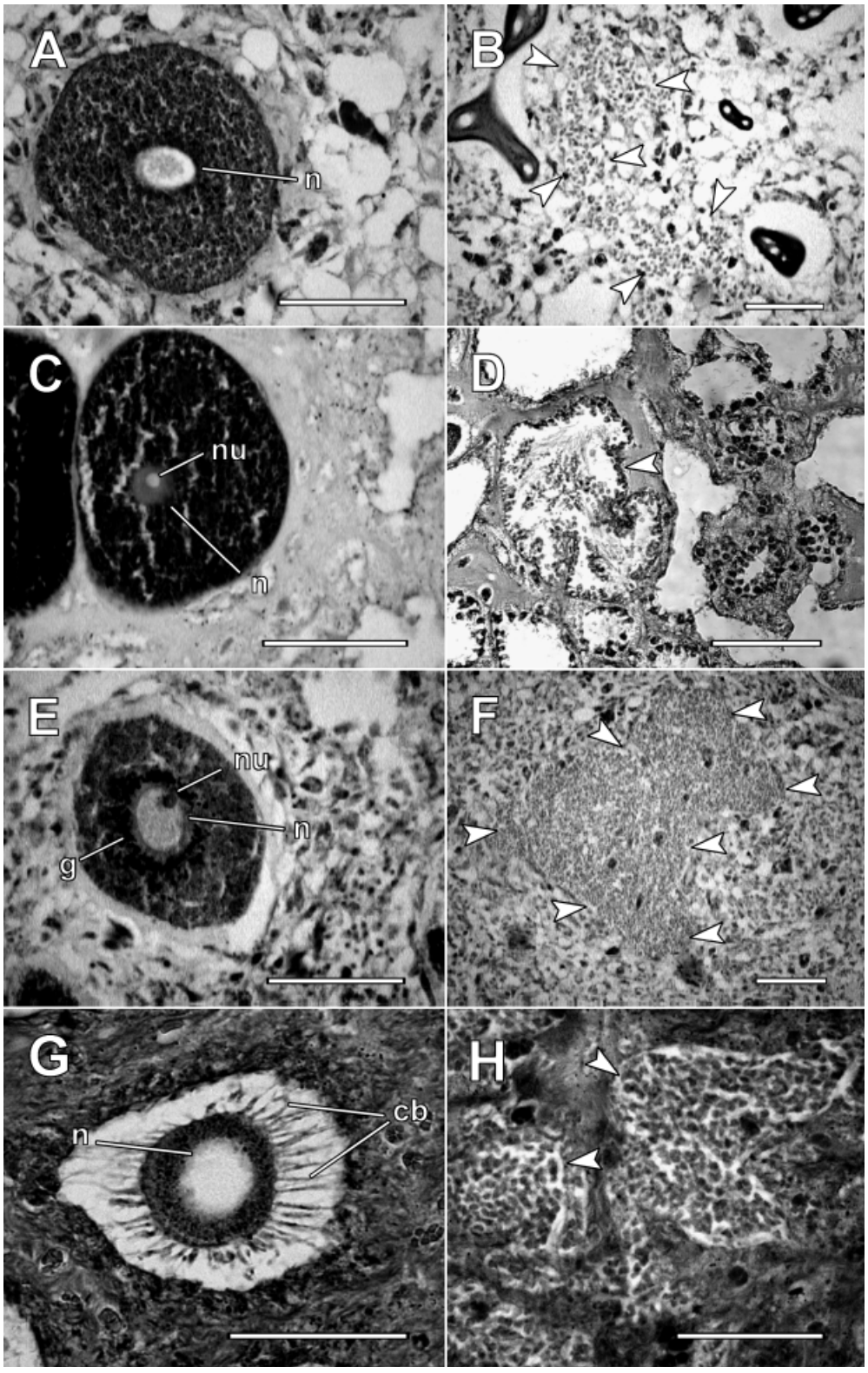
Members of *A. damicornis* were gonochoristic and oviparous (Fig. 1A,B) with a gametogenic period that extended for 7–8 months at the population level (Fig. 2). In 2005, we recorded three females, one male, and one non-reproductive individual ($n = 5$ tagged individuals), but, in 2005, two of the same five individuals were not reproductive, leaving two females and one male ($n = 5$). The sex ratio in this small sample was ~2:1. We could only use the tagged individuals to estimate the sex ratio due to a fixation problem in the samples of the untagged individuals, where males were not reliably identifiable. In females, oocytes were distributed uniformly throughout the mesohyl. Young oocytes were amoeboid, with evident pseudopodia (not shown), and measuring 23.7 ± 0.1 μm (Fig. 3). Mature oocytes, measuring ~150 μm (Fig. 3), became round, showing a nucleolate nucleus (Fig. 1A). Scarce nurse cells occurred around the oocytes during their development.

In both years of study, oogenesis was confined to the coldest period of each year. Oocytes first appeared during the seawater temperature decline in October–November, with virtually all oocytes growing at a similar speed in all the studied individuals. Oocytes were released synchronously in May (13°C in 2004 and 15°C in 2005), when the annual temperature increase started (Fig. 2). Although the average number of oocytes in the tissues remained <2 oocytes mm^{-2} over the entire reproductive period, it increased moderately in March and April, during the cool period of both years (Fig. 2).

Spermatogenesis occurred concurrently with late oogenesis (May or April–May) (Fig. 2). In the only male we detected in both years, all spermatic cysts were at the same stage of development during each sampling event. We anticipate synchrony at the population level as well, because eggs were also synchronously released for external fertilization. Spermatic cysts were rounded (Fig. 1B), measured ≤ 200 μm in diameter, and were distributed homogeneously within the entire sponge mesohyl, although causing little disruption of the regular sponge histology (hereafter referred to as "mesohyl disruption"). During 2004, spermatogenesis began immediately before the seasonal water warming, and extended only through May, when seawater temperature was 13°C. In 2005, it also started immediately before the water warming, when the temperature was 13°C (April) and extended through May, when the temperature was 15°C. During both years, 5 months of low temperatures (12° and 13°C) preceded the onset of sperm production (December–April in 2004, and November–March in 2005) (Fig. 2).

Corticium candelabrum

Members of *C. candelabrum* were hermaphroditic and viviparous, with oocytes and spermatic cysts (Fig. 1C,D) occurring simultaneously in the sponge tissue during part of the year. In the 2 years of study, ~90% of the population ($n = 30$) experienced gametogenesis, with all reproductive individuals producing both oocytes and spermatic cysts. Oocytes were oval to round, nucleolate cells, measuring ~125–150 μm when mature (Fig. 3). They were consistently located near the excurrent canals (Fig. 1C). Many nurse cells occurred around oocytes during the entire process of oogenesis. Oocyte production extended through the entire year, but the highest oocyte density (~7 oocytes mm^{-2} of tissue) was recorded October–February (11°–13°C) of both years (Fig. 2). Oocyte density decreased in the spring months, reaching the lowest values in summer (Fig. 2), concomitant with maximum temperatures. Round to lobed spermatic cysts were located near canals in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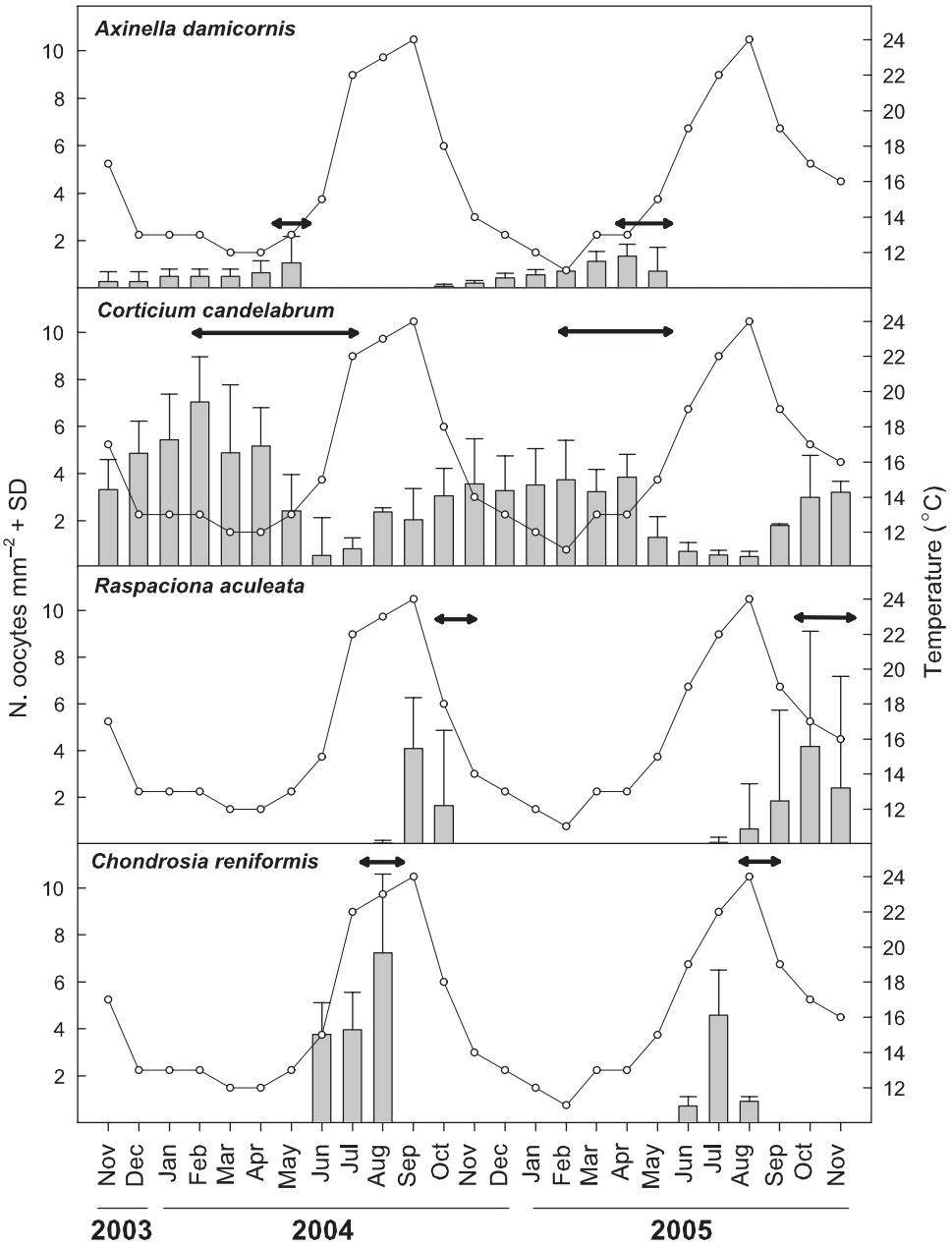

Fig. 2. Density of oocytes (number mm^{-2} ± SD) and the presence of spermatic cysts (arrows) in the sponge tissue of five tagged individuals of four different sponge species over 2 years (November 2003 to November 2005) plotted versus seawater temperature.

Fig. 1. Mature oocytes and spermatic cysts of the demosponges studied. **A.** Mature oocyte in *Axinella damicornis,* showing the nucleus (n). **B.** Spermatic cyst in *A. damicornis*. Arrowheads mark the perimeter of the cyst. **C.** Mature oocyte in *Corticium candelabrum* with a nucleolate (nu) nucleus (n). **D.** Spermatic cyst in *C. candelabrum* (arrow head). **E.** Mature oocyte in *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 in *R. aculeata* (arrow heads). **G.** Mature oocyte in *Chondrosia reniformis* showing the cytoplasmic bridges (cb) and the nucleus (n). **H.** Spermatic cysts of *C. reniformis* (arrow heads). Scale bar = 100 μm.

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 the temperature reached its maximum value (Fig. 2).

Raspaciona aculeata

Members of this species were gonochoristic and oviparous (Fig. 1E,F), with a gametogenic period that extended for 5 months (July–November) at the population level. All sampled individuals were engaged in gametogenesis, and the sex ratio was 1:1 ($n = 30$). Oocytes grew from ~50 μm when first detected to 160–180 μm when mature (Fig. 3). They were quite similar to those in *A. damicornis,* with multiple pseudopodia during early stages and becoming rounded at maturity (Fig. 1E). They occurred scattered throughout the mesohyl of the sponge, intermingled with choanocyte chambers. Many nurse cells aggregated in the vicinity of developing oocytes.

Oogenesis started in July–August, when seawater temperature reached its maximum (22°–24°C), and extended for 3–5 months (Fig. 2). Oocyte size increased at a similar rate in the sampled individuals. The maximum production rate was recorded in September 2004 and October 2005 (at 17°C and 18°C, respectively), just before the abrupt seasonal seawater cooling. In both years, oocytes were released progressively during the month subsequent to the temperature decline (Fig. 2).

Large, oval spermatic cysts (>200 μm) occurred, intermingled with choanocyte chambers throughout the mesohyl of the sponge (Fig. 1F), causing appreciable mesohyl disruption. Cyst production started with declining temperatures, and extended for 1 or 2 months (October in 2004 and October–November in

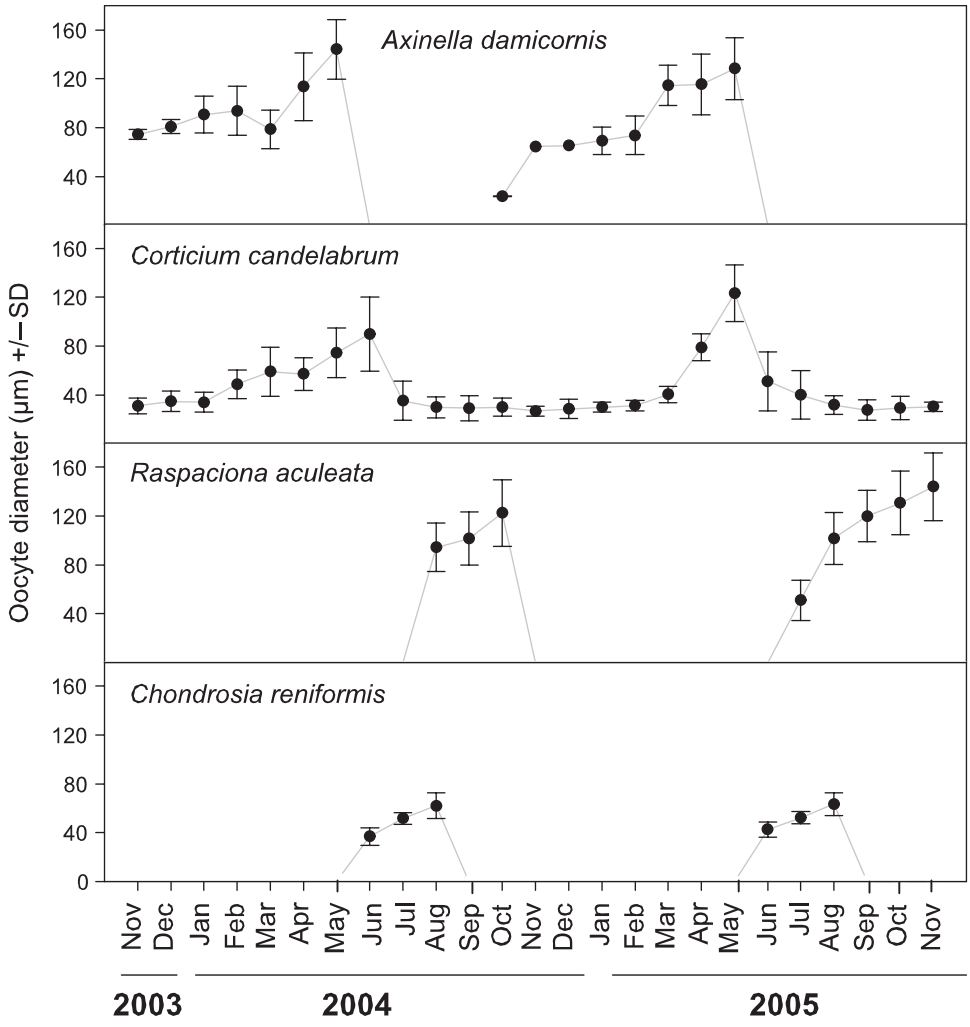

Fig. 3. Average diameter of oocytes ± SD of the four different studied species over the two sampling years (2003–2005).

2005) (Fig. 2), undergoing synchronous development within and between the studied individuals.

Chondrosia reniformis

Members of this species were gonochoristic and oviparous (Fig. 1G,H). At the population level, gametogenesis extended for 3 months, June–August. About 80% of the sampled individuals produced gametes, with a female-biased 4:1 sex ratio ($n = 30$). The oocytes were the smallest recorded in this study. When first detected, they were 35 μm in diameter, eventually growing to ~65 μm (Fig. 3). They were round to oval, nucleolate cells, connected to surrounding nurse cells by cytoplasmic bridges (Fig. 1G). All oocytes developed simultaneously in each individual and in the tagged population. 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 increase in June and was completed after 3 months, in late August, concurrently with the annual maximum temperature (23°–24°C) (Fig. 2). In 2004, oocyte density increased from ~ 4 oocytes mm^{-2} in June–July to 7.2 ± 3.3 oocytes mm^{-2} in August, in parallel to the temperature increasing, which culminated with a temperature maximum in late August, immediately before oocyte release. However, in 2005, oocyte density followed a different pattern. It increased from 0.7 ± 0.4 oocytes mm^{-2} in June to 4.6 ± 1.9 oocytes mm^{-2} in July, and then decreased progressively to 0.9 ± 0.21 oocytes mm^{-2} in August. This was an earlier and slower oocyte release in 2005 than in 2004.

Lobed spermatic cysts filled most of the choanosome (Fig. 1H), causing such a mesohyl disruption that virtually no choanocyte chamber was found during spermatogenesis. Spermatogenesis started in August during the temperature maximum (23°–24°C), and lasted < 1 month (Fig. 2), with all the spermatic cysts developing synchronously.

Discussion

All four species experienced gametogenic activity in the 2 years of study. Members of *Axinella damicornis, Raspaciona aculeata,* and *Chondrosia reniformis* were gonochoristic and oviparous, while those of *Corticium candelabrum* were hermaphroditic and viviparous. None of the tagged sponges exhibited sex reversal during the 2-year study. Sex reversal has been reported in some freshwater demosponges (Van de Vyver & Willenz 1975; Gilbert & Simpson 1976) and the marine demosponge *Spongia officinalis* (Baldacconi et al. 2007).

In all four studied species, most sampled individuals contained gametes. This situation is 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 *A. damicornis,* which was reproductively active in 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. The sex ratio was about 1:1 in *R. aculeata,* but 2:1 in *A. damicornis* and 4:1 in *C. reniformis,* with female overabundance. A wide range of sex ratios has been recorded in sponges, ranging from 1:1 to an overwhelming predominance of one sex (Fell et al. 1979; Scalera-Liaci & Sciscioli 1979; Kaye 1991; Mercurio et al. 2007).

There were clear differences among the four species in the duration of the gametogenic cycles, despite the fact that they shared a habitat and were subjected to similar environmental stimuli. Individuals of *Chondrosia reniformis* had a short oogenic period (3 months). This is slightly longer than that reported in the only other well-studied chondrosid, *Chondrilla australiensis* (Usher et al. 2004). Longer oogenic periods were found in *R. aculeata* (3–5 months), *A. damicornis* (7–8 months), and *C. candelabrum* (7–8 months). In *Corticium candelabrum,* there was an unconventional pattern of oogenesis, with continuous production of oocytes during the entire year, corroborating the pattern also reported in a previous study of this species (Riesgo et al. 2007). Some other 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). However, the case of *C. candelabrum* is different because in this species new oocytes are produced continuously during the entire year despite the fact that larvae are released only during a small period of time. Only the Red Sea sponge *Siphonochalina siphonella* is known to show a similar pattern of oogenesis (Ilan et al. 2004). In *C. candelabrum,* because oocyte growth was completed in 7–8 months, the fate of the young oocytes that appeared when sperm was already mature remains unclear. They may have been used for nourishing mature oocytes, zygotes, or early embryos, as suggested for other sponges (Sarà 1955; Maldonado et al. 2005). In addition, the production

of oocytes in *C. candelabrum* was lower during the second sampling year, perhaps because of the high sampling effort (i.e., repeated tissue removal) carried out in the tagged individuals.

Although oocytes of all four species were relatively similar in morphology during the entire process of oogenesis, mature oocytes showed a considerable among-species variation in size, independent of the oviparous or the viviparous nature of the selected species, as described before in sponges (Fell 1974) and in many other invertebrates (Lopo 1983).

Spermatogenesis was a shorter process than oogenesis in all four species. The duration of spermatogenesis at the individual level did not vary much between species, being generally completed in a few weeks. The duration of spermatogenesis at the population level ranged 1–5 months, and depended on the reproductive mode of the sponge (viviparous vs. oviparous) and the level of inter-individual asynchrony. At the population level, spermatogenesis was rapid (1–2 months) and relatively synchronous in *A. damicornis, R. aculeata,* and *C. reniformis,* as is also the case in most oviparous species (see Reiswig 1983; Boury-Esnault & 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. 1973). 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, may minimize the risk of sperm loss derived from a single spawning event under unfavorable hydrodynamic conditions, therefore increasing the chances of fertilization at the population level.

Among-species differences in the duration of both oogenesis and spermatogenesis appeared to depend primarily on physiological processes undergone by gametes and histological transformations of the sponge tissue during reproduction rather than on environmental conditions. In the case of oogenesis, in species where nurse cells were significantly involved in vitellogenesis, the oogenic cycle was shorter relative to those cases in which vitellogenesis mostly relied on the oocyte itself, with little participation of nurse cells, as occurs in most animals (Nørrevang 1968). The former situation appears to occur in both *R. aculeata* and *C. reniformis*. In contrast, oogenesis based primarily on auto-synthesis of yolk by the oocytes appears to occur in *C. candelabrum* and *A. damicornis*. Regarding spermatogenesis, the quantity, size, and permanence of spermatic cysts in the tissue differed among species. In *C. candelabrum* and *C. reniformis,* cysts were smaller than in *A. damicornis* and *R. aculeata*. However, although the spermatic cysts in *C. reniformis* were small, they occupied most of the sponge mesohyl, in contrast to that observed in the rest of the species. Such massive cyst occupation (mesohyl disruption) implied that there was little space for choanocyte chambers, presumably decreasing the ability to feed. Mesohyl disruption during gametogenesis has been reported in, for instance, *Halichondria okadai* (Tanaka-Ichihara & Watanabe 1990), *Aplysina cauliformis* (Tsurumi & Reiswig 1997), and *H. dujardini* (Ereskovsky & Gonobobleva 2000). However, the duration of spermatogenesis in the latter two species differed considerably, so that mesohyl disruption may affect different sponges differently. While in *H. dujardini* spermatogenesis occurred during 3 months (January–March), in *A. cauliformis,* sperm was present in the tissue only for 1 month (April). In *C. reniformis,* virtually no chambers were present in the tissue only in August. It is possible that spermatogenesis in some oviparous sponges (such as *A. cauliformis* and *C. reniformis*) had been confined to a few weeks because of the necessity to recover as soon as possible the choanosome structure (choanocyte chambers) and subsequently the ability to feed.

There were not only differences among species in the duration of gametogenesis but also in the relationship of gametogenic dynamics with temperature. We measured only one temperature value per sampling day, and we make the assumption that the presumably slight daily temperature variations were not decisive in controlling reproductive activity. Previous monitoring of daily changes in seawater temperature at the local sublittoral water column (5–37 m depth) using a SeaBird 19Plus CTD (SeaBird Electronics, Bellevue, WA) revealed virtually no fluctuation over a 12-h cycle (8:00 to 20:00 GMT), unless heavy summer rains took place or severe storms disrupted the summer thermocline usually located 15–20 m in September–October (M. Maldonado, unpubl. data). Because of the slow change of seawater temperature values, our measurements are unlikely to be substantially different from monthly averages, as confirmed by multiple temperature readings in some months in which we dove more than once a week for different purposes.

Water warming appeared to be related to the onset of oogenesis in *C. reniformis,* and the maximum temperatures were coincidental with the initiation of oogenesis in *R. aculeata*. Minimum temperatures were apparently related to the beginning of oogenesis in *A. damicornis* and may have enhanced oocyte

production in *C. candelabrum*. These patterns of relationship between oogenesis and cold temperature had been recorded previously in other demosponges, although induction of oogenesis by water warming has been reported more often (e.g., Fromont 1994, 1999; Fromont & Bergquist 1994; Witte et al. 1994; Mercurio et al. 2007) than induction by water cooling (Ereskovsky & Gonobobleva 2000). Gamete development apparently induced by minimum water temperatures has also been reported in the Mediterranean coral *Leptosammia pruvoti* (Goffredo et al. 2006).

Notable variation has also been recorded regarding the relationship between temperature and the onset of spermatogenesis. Maximum temperature values appeared to correlate with sperm production in *C. reniformis,* while minimum temperatures were correlated with sperm production in *C. candelabrum*. The former relation has been established in many cases before (see Simpson 1984 for a review), but the latter has only been identified in *Halichondria panicea* (Witte et al. 1994), *H. dujardini, Myxilla incrustans,* and *Iophon piceus* (Ereskovsky & Gonobobleva 2000). Interestingly, all four latter species are cold-water sponges. The autumn gradual decrease in seawater temperatures coincided with the onset of spermatogenesis in *R. aculeata,* a pattern recorded previously in *Desmacidon fruticosum* (Lévi 1956), as well as in *Tectitethya crypta* and *Verongula gigantea* (Reiswig 1973). The case of *A. damicornis* was fairly distinct. In both years, spermatogenesis may have started not after reaching a threshold of minimum temperature but after a long exposure to low temperatures, because sperm production started after a 5-month period of cold temperatures (13°C).

In addition to variation in the timing of gametogenesis onset, the four studied species also varied in the timing of gamete/larval release, so that there was no among-species overlap in either gamete spawning or larval release. A similar reproductive decoupling has been observed for 13 species of Red Sea corals (Shlesinger & Loya 1985). Avoidance of overlapping in reproductive output in corals was proposed to be a mechanism to relax space competition (Shlesinger & Loya 1985). In addition, as sponges, like many other animals producing lecithotrophic embryos, have higher energetic requirements during reproduction (Bell 1980; Clutton-Brock 1984), non-overlapping reproductive periods could also result in the avoidance of peaks of intense competition for food during gamete development.

The fact that sponges sharing habitat and thermal regime differed in their reproductive timing suggests a disparity in adaptive responses, which remains little understood to date. Modifications of thermal regimes because of climate change may result in populations initiating a physiological response at the wrong time in relation to calendar date (Lawrence & Soame 2004). This may affect recruitment success and population dynamics, which can deeply alter the interrelationships between relevant community members (Fromentin & Planque 1996; Saetre et al. 1999; Walther et al. 2002). Therefore, given that sponges are key components in many marine communities, it remains important to assess their phenology, with special attention to climate-driven shifts in physiological processes that may alter recruitment and population structure.

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