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Cycle of gonadal development in *Eunicella singularis* (Cnidaria: Octocorallia): trends in sexual reproduction in gorgonians

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Abstract. *Eunicella singularis* is a gorgonian species whose members are abundant in hard-bottom sublittoral communities of the Mediterranean Sea. The reproductive biology in this species has been examined to better understand the ability in this species to recover from recent mass mortality events. *Eunicella singularis* is a stable, gonochoric, iteroparous species that reproduces annually and exhibits a seasonal pattern of gametogenesis characterized by a single annual maturation of the gametes. The sex ratio did not significantly differ from 1:1. Oogenesis lasted 13–17 months, beginning between February and June, and ending with the release of 0.7 planula larvae per polyp between late May and July of the following year. The diameter of mature oocytes ranged 450–860 μm. Spermatogenesis was much shorter than oogenesis and occurred over 5–6 months. Gonadal production of both sexes increased in spring and culminated with the spawning of male colonies in late May–June. Fertilization of oocytes and development of the planula larvae occurred within the polyps of female colonies. Planula release was observed in June and July. The patterns emerging from this and previous studies on sexual reproduction of Mediterranean gorgonians suggest that investment in gonad development appears to be related to resource availability.

Additional key words: gorgonians, sexual reproduction, sex ratio, gametogenic cycle

Gorgonians play an important ecological role in sublittoral benthic communities (Wendt et al. 1985; Mitchell et al. 1992). The white gorgonian *Eunicella singularis* Esper 1794 is one of the most abundant and widely distributed Mediterranean species (Carpine & Grasshoff 1975; Weinberg 1979a, 1980). In recent years, however, several mass mortality events and their delayed effects have strongly affected gorgonian populations in the northwestern Mediterranean (Linares et al. 2005; Coma et al. 2006). As reproductive strategy plays a crucial role in the population dynamics and biogeography of an organism (Grosberg & Levitan 1992; Giangrande et al. 1994), knowledge about reproductive processes in *E. singularis* is essential to understanding the life-history patterns of the species and a preliminary step toward understanding the ability of this species to recover from disturbances. Gametogenesis plays a major role in reproduction because it not only ensures the necessary offspring to maintain populations but also provides the genetic recombination to cope with environmental change (e.g., Coffroth & Lasker 1998a; Fautin 2002).

Several aspects of the life cycle in *E. singularis* have been examined in previous studies, including larval behavior, metamorphosis, and colony growth. Although prior studies did not monitor gonad development, several characteristics of sexual reproduction were described: (1) the species appeared to be gonochoric (Theodor 1967, although without monitoring of labeled individuals, gonochoric sexuality may be confused with other sexual patterns; see Harrison & Wallace 1990); (2) each ovary contains a single oocyte within a thin layer of tissue (Weinberg & Weinberg 1979); (3) fertilization is internal (Theodor 1967), and holoblastic cleavage occurs within the female polyps (Weinberg & Weinberg 1979); (4) pink planula larvae containing zooxanthellae are released (Theodor 1967) and exhibit photopositive behavior (Weinberg & Weinberg 1979); and (5) after settlement, planula larvae metamorphose into a complete polyp in ~4 d (Weinberg & Weinberg 1979).

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This work focused on two main objectives: (a) the examination of the gametogenic cycle in *E. singularis* and (b) the synthesis of existing data on reproduction from other gorgonian species to examine whether trends emerge from species-level studies. The pattern of investment in sexual reproduction in Mediterranean species is examined within the framework of current knowledge on the patterns of sexual reproduction of gorgonian species from other environments.

Methods

Sampling

The study population of *Eunicella singularis* is located near "Tascó Gran" (Medes Islands, Northwestern Mediterranean, 43°2′30″N, 3°13′30″E) at 4–35 m depth and extends over an area of ~90,000 m^2. Samples were collected using SCUBA from the depth range where the species exhibited its highest density (15–20 m depth, unpubl. data). The height (distance from the base to the farthest point) of all colonies was measured with a ruler to the nearest 0.5 cm. Samples of apical fragments were immediately fixed in 10% formalin in seawater. Formalin-fixed samples were later transferred to 70% alcohol. In the laboratory, polyps 3–10 cm from the branch tip were dissected under a binocular stereomicroscope, and the diameters of all gonads were measured with an eyepiece micrometer on alcohol-preserved samples. Oocytes and spermaries were spherical or ellipsoidal in shape. Therefore, their volume was estimated using the formula $V = 4/3 \times \text{pi} \times (D/2)^3$ for spherical shapes and $V = 4/3 \times \text{pi} \times (D/2) \times (d/2)^2$ for ellipsoidal shapes, where V is the volume of oocyte or spermary, D the maximum diameter, and d the minimum diameter. Gonadal volume per polyp was calculated as the sum of the volume of each oocyte or spermary present in each polyp.

Sample size

The minimum sample size needed to estimate gonadal volume per polyp in both male and female colonies at each time period was determined by the analysis of the standard error (SE)-sample size function according to Bross & Cowell (1987). A preliminary sampling was conducted in May 1999. An apical fragment from each of 30 male and 30 female colonies, ranging 30–40 cm in height, was randomly collected at 15–20 m depth. Samples of apical fragments were immediately fixed in 10% formalin in seawater. Formalin-fixed samples were later transferred to 70% alcohol. Sampling of a particular size class was conducted throughout the study in order to avoid the effect of size on reproduction (e.g., Coma et al. 1995a; Kapela & Lasker 1999; Beiring & Lasker 2000). Ten polyps from each apical fragment were dissected, and the diameter of all gonads measured. Variation in gonad volume per polyp among colonies was higher than among polyps within the same colony (one-way analysis of variance [ANOVA] comparing gonad volume between colonies, female colonies: $F_{29,270} = 3.14$, $p < 0.0001$, male colonies: $F_{29,270} = 35.44$, $p < 0.0001$). A plot of repeated measures of SE with increasing sample size was used to examine the degree of variation in sample estimate for the assessment of the volume of gonads per polyp in both male and female colonies (Fig. 1). A sample size of ten polyps means that all polyps were from the same colony, a sample size of 20 polyps means that samples were from two colonies (ten polyps from each), and so on up to a sample size of 150 polyps, meaning that samples were from 15 colonies. The SE as a proportion of the mean of the volume of gonads per polyp decreased with increasing sample size

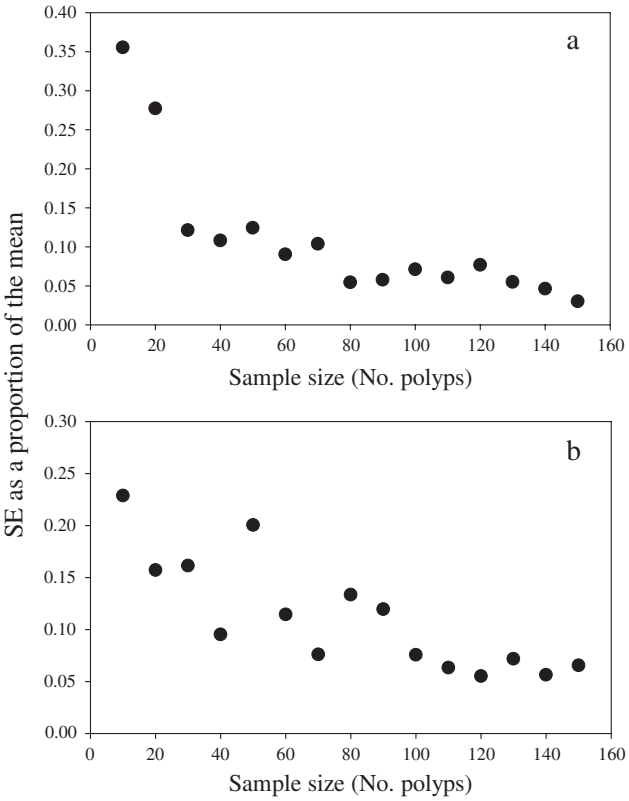


Fig. 1. Variation of the standard error (SE) as a proportion of the mean with sample size (number of polyps examined) of the volume of gonads per polyp: (a) male colonies, (b) female colonies.

(i.e., the number of polyps examined, Fig. 1). For both sexes, the curve flattens out at a sample size of ~100 examined polyps per sample where the variance becomes ~6% of the mean. On the basis of these results, we determined that examination of 100 polyps (i.e., ten polyps from ten colonies) was representative of the gonad population at each sampling period.

Reproductive cycle

To study the annual reproductive cycle, 30 colonies ranging 30–40 cm in height were tagged; from these, we monitored ten male and ten female colonies. The same ten colonies of each sex were followed over time. An apical fragment from each of these colonies was collected every 1–2 months between April 1999 and August 2000. The diameter of all gonads present on ten randomly selected polyps from an apical fragment of each colony was measured with an eyepiece micrometer under a binocular stereomicroscope. Size-frequency distributions of gonad diameter were examined using the ELEFAN program, a modification of Bhattacharya's method (Gayanilo & Pauly 1997). This method distinguishes any cohorts and produces a best-fit normal distribution for each one with a calculated mean and standard deviation (SD). Furthermore, monitoring of the labeled colonies allowed us to determine whether colonies change sex over successive breeding seasons.

Sex ratio and timing of spawning

In May 1999, eight replicate samples of thirty colonies ranging 30–40 cm in height, located along haphazardly selected 50-m-long linear transects, were examined to determine the sex ratio (240 colonies). The minimum distance between sampled colonies was 3 m. Deviation of sex ratio from parity was tested on the basis of the eight replicates using a chi-square test.

Several colonies were randomly collected from the study site in early May 1999 and 2000. Colonies were maintained in running seawater within $150 \times 70 \times 30$-cm tanks until spawning of larvae occurred. Numerous dives were conducted each year between May and July to determine whether a massive release of the sexual products occurred. Permanent plots, 40×40 cm in size, were set up within the community to determine and observe the presence of new colonies.

Results

Sex ratio

Gonads developed attached but projecting from the plane of the mesentery, and surrounded by the gastrodermis. All fertile polyps from a colony exhibited gonads from the same sex. This was the case in all examined colonies ($N = 243$) but one, which had polyps developing both sperm sacs and oocytes (hermaphroditic). Within this hermaphroditic colony, individual polyps were typically only male and only some occasional polyps developed both sperm sacs and oocytes. Although the sexes are separate, the colonies lacked any obvious secondary sexual characters and the gonads are the only guides to gender. From a total of 243 examined colonies in the 30–40-cm-height range, 96% were mature and 4% did not exhibit gonads. The recorded ratio of females to male was 1.16 (125/108), which does not significantly deviate from unity (female colonies: $\chi^2 = 2.47$, $df = 7$, $p < 0.9295$, male colonies: $\chi^2 = 3.85$, $df = 7$, $p < 0.7969$).

Annual cycle of oocyte development

Oocytes developed attached to the mesenteries by a short mesogleal stalk at the base of each polyp. Formation of new oocytes was not synchronous and took place over a 5-month period (February–June). The newly formed oocytes remained at an average diameter of 150 μm until September (Cohort 2 in Table 1). From September to February, the average diameter increased from 150 to 225 μm. Between February and May, the previous year's cohort of oocytes increased in average diameter from 225 to 600 μm. The pattern of oocyte growth is clearly shown by the size-frequency distributions of oocytes before and after spawning. The large increase in gonad volume during the February–May time period is mainly the result of the maturation of some of the oocytes from the previous year (Fig. 2). The diameter of the small oocytes changed little during this time period and was similar to that observed in August and September (~150 μm). In May, both sexes exhibited synchronization in gonad maturation, and by the end of July all oocytes >300 μm in May had disappeared. The diameters of mature oocytes exhibited a wide range of 450 and 860 μm. Therefore, the number and diameter of oocytes >300 μm in May allow an estimation of the annual production of female colonies. This allowed us to estimate the production of an average number of 0.69 ± 0.16 (mean ± SE) mature oocytes per polyp and year, which did not significantly differ between both examined years (1999 and

Table 1. *Eunicella singularis*. Variation over time of the number (N), average size (Avg), diameter in μm, and standard deviation (SD) of oocyte cohorts from ten polyps of each of ten female colonies (hundred polyps total) from a Medes Islands population between April 1999 and August 2000. Cohorts distinguished on the basis of the Bhattacharya method.

Date	Cohort 1			Cohort 2			Cohort 3		
	N	Avg	SD	N	Avg	SD	N	Avg	SD
April 1999	94	465	163	122	142	33	—	—	—
May 1999	70	528	184	82	151	44	—	—	—
May 1999	30	676	111	79	153	57	—	—	—
June 1999	18	859	318	102	141	46	—	—	—
July 1999	4	405	114	137	153	47	—	—	—
August 1999	2	340	28	182	154	50	—	—	—
September 1999	—	—	—	180	145	43	—	—	—
December 1999	—	—	—	160	176	68	—	—	—
February 2000	—	—	—	142	223	104	41	91	13
April 2000	—	—	—	98	417	154	134	140	37
May 2000	—	—	—	70	615	130	160	156	52
June 2000	—	—	—	36	829	250	195	144	48
August 2000	—	—	—	—	—	—	103	129	41

Spawning of Cohort 1 of female gonads occurred between May and July 1999; therefore em dashes from September 1999 to August 2000 indicate that Cohort 1 is no longer present because it has been released through spawning. Likewise, spawning of Cohort 2 occurred between May and July 2000. Cohort 3 was initiated in February 2000, so em dashes indicate that the cohort was not present between April 1999 and December 1999.

2000, repeated measures ANOVA, $F_{1,9} = 0.0194$, $p < 0.8924$). Members of *Eunicella singularis* display a multi-year cycle of oogenesis in which the oocyte development takes 13–17 months to complete. The period of oocyte development, together with the yearly formation of oocytes, produced the coexistence of two cohorts of oocytes during most of the year (Fig. 2, Table 1). However, female gonadal production occurs mainly between December and May (Fig. 2). Within this time period, the rate of gonadal production increased from $1.43 \times 10^{-4}\,mm^3$ per polyp per day (December–February) to $11.15 \times 10^{-4}\,mm^3$ per polyp per day (April–May, Fig. 2).

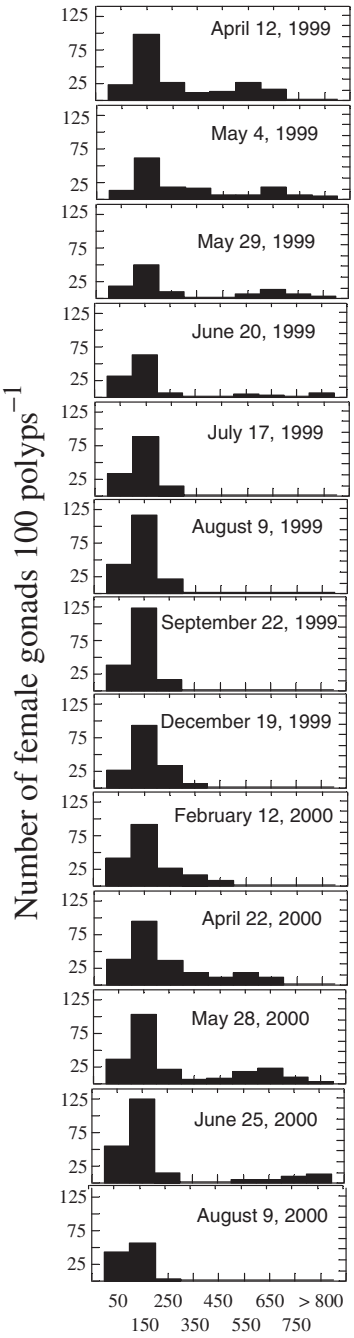


Fig. 2. Variation over time of the size-frequency distributions (diameter in μm) of the total number of female gonads from ten polyps of each of ten female colonies (100 polyps in total) between April 1999 and August 2000.

Annual cycle of spermaries development

By February, the number of new spermaries (~150 μm in diameter) per polyp increased (Fig. 3, Table 2). From February to May, spermary number and diameter increased exponentially. Ripening of spermaries was highly synchronized in May. From then on, spermary volume declined sharply, coinciding with the end of spermatogenesis and release. Thus, spermatogenesis took between 4 and 6 months to complete and was much shorter than oogenesis. However, the rate of spermary production increased from $0.45 \times 10^{-4}\,mm^3$ per polyp per day (December–February) to $42.92 \times 10^{-4}\,mm^3$ per polyp per day

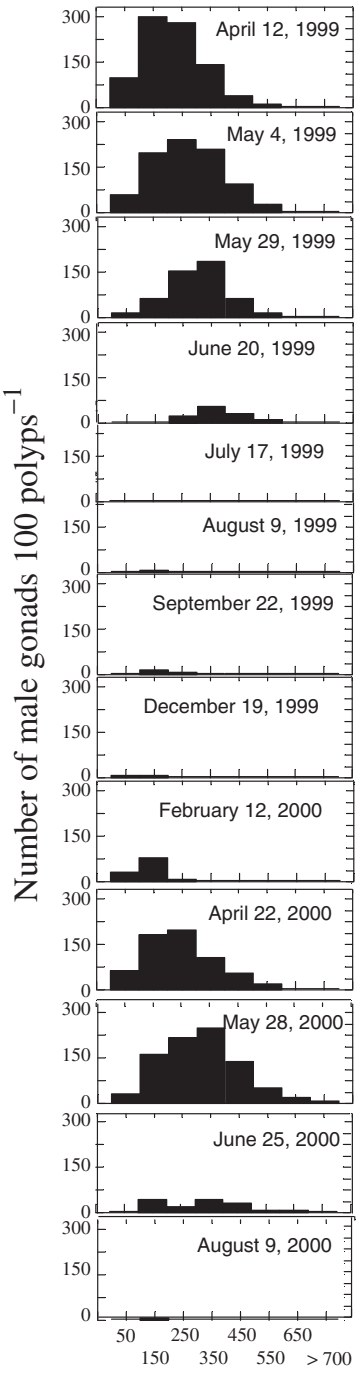


Fig. 3. Variation over time of the size-frequency distributions (diameter in μm) of the total number of male gonads from ten polyps of each of ten female colonies (100 polyps in total) between April 1999 and August 2000.

(April–May), similar to the previously described oocyte production. Between August and December, a few colonies exhibited some small spermaries that were eventually resorbed (Fig. 3). Gonads of both sexes developed annually over the study period.

Table 2. *Eunicella singularis*. Variation over time of the number (N), average size (Avg), diameter in μm, and standard deviation (SD) of spermaries from ten polyps of each of ten male colonies (hundred polyps total) from a Medes Islands population between April 1999 and August 2000.

Date	N	Avg	SD
April 1999	869	238	105
May 1999	830	288	121
May 1999	503	324	111
June 1999	129	387	106
July 1999	9	173	62
August 1999	11	138	29
September 1999	31	175	66
December 1999	18	123	49
February 2000	122	144	53
April 2000	632	264	128
May 2000	877	332	140
June 2000	157	350	157
August 2000	9	169	36

Spawning

The multi-year development of oocytes and the annual development of spermaries culminated in spawning of male colonies during May and June (Fig. 3, Table 2). However, the release of gametes was never directly observed for male colonies. Fertilization is internal, and a planula larva develops within the polyps of the female colonies. Planulae were observed within the polyps of female colonies in late May, June, and July. The preserved planula larvae observed within the female polyps were pink in color, exhibited an average length of 1156 μm (ranging 900–1640 μm), and were usually released during late May and June, although some have been observed to be released in July. A series of close-up photographs taken in the field in June 2000 allowed us to document *in situ* release of the larvae from the polyps of female colonies. Following release, the larvae do not remain on the surface of the parent colony. The expulsed larvae, which exhibit a low swimming capacity and negative buoyancy, fall down in a vertical position to the nearby substratum unless swept away by water movement. Weinberg & Weinberg (1979) estimated that most of the larvae explore the substratum within a distance ranging 2–40 m from the mother colony. Crawling of the larvae over the substratum was observed, but settlement and metamorphosis of the larvae were not monitored (see Weinberg & Weinberg 1979 for a description); however, small colonies consisting of a few polyps were observed ~4–5 months after spawning both in the laboratory tanks and *in situ* on permanent-labeled plots placed within the community.

Discussion

Gametogenic cycle

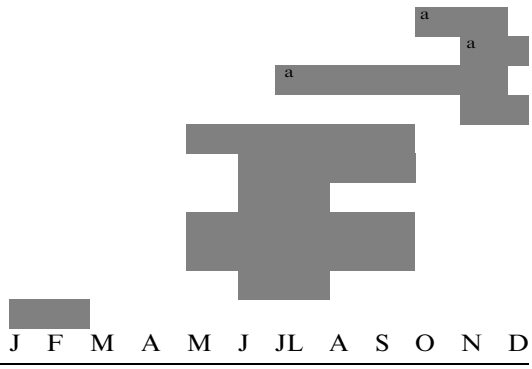
Repeated sampling of labeled colonies showed that sexual reproduction in members of *Eunicella singularis* occurred over successive annual reproductive events and that sex change did not occur either within the breeding season or between the examined breeding seasons. However, it has to be noted that less than two full seasons were examined, and further research needs to be conducted to confirm that sex change does not occur. Occasionally, a few hermaphroditic colonies were encountered, and this *E. singularis* can be considered a species that exhibits stable gonochorism (*sensu* Giese & Pearse 1974). Stable gonochorism has been observed in some cnidarian species and appears to be the result of a polyfactorial determination of sex (Fautin 1992). The observed 1:1 sex ratio is similar to that observed in most previously examined species (e.g., Vighi 1970; Grigg 1977; Martin 1982; Coma et al. 1995b; Orejas et al. 2002; Fitzsimmons-Sosa et al. 2004; Tsounis et al. 2006). Deviation from sex parity has usually been observed to be due to a higher proportion of female colonies (*Plexaura kuna,* extremely large proportion of females: Brazeau & Lasker 1989; Coffroth & Lasker 1998b; *Acabaria biserialis,* 3:4 male to female ratio: Ben-Yosef & Benayahu 1999; *Gorgonia ventalina, Muriceopsis flavida,* and *Pseudopterogorgia americana,* 1:2 male to female ratio: Fitzsimmons-Sosa et al. 2004). Higher number of male to female colonies has only been reported for *Briareum asbestinum* (2.2:1 male to female ratio: Brazeau & Lasker 1990).

It is important to note that most reports on sex ratio rely on data from a single location. In this sense, it has been observed recently that the sex ratio of a particular species may differ among locations (*Corallium rubrum,* 1:1.4 male to female: Santangelo et al. 2003; 1:1 male to female: Tsounis et al. 2006; *Paramuricea clavata,* 1:1 male to female ratio: Coma et al. 1995b; 3.3:1 male to female ratio: Cerrano et al. 2005). Differential contribution of asexual reproduction to populations between sites (Coffroth & Lasker 1998a) may contribute to the understanding of some variations in sex ratio in gorgonians. It has also been suggested that the skewing of the sex ratio toward males in *P. clavata* could be a consequence of a differential response of both sexes to perturbations such as mass mortality events (Cerrano et al. 2005). The common pattern of parity in sex ratio is in accordance with optimal resource allocation in populations with random mating (Leigh et al. 1985). Therefore, *E. singularis* is a stable gonochoric iteroparous species that reproduces annually.

Gametogenesis in *E. singularis* exhibited a seasonal pattern of development characterized by a single annual maturation of the gametes, which were released over a restricted time period (i.e., <1 month). This appears to be a common pattern in Mediterranean gorgonian and alcyonacean species examined to date (*C. rubrum*: Vighi 1970; Tsounis et al. 2006; *P. clavata:* Coma et al. 1995b; *Leptogorgia sarmentosa* and *Alcyonium acaule:* unpubl. data). The single annual maturation of the gametes observed in Mediterranean species is similar to that observed in species from other temperate environments (*Muricea californica*, *Muricea fruticosa:* Grigg 1977; *Leptogorgia virgulata:* Adams 1980; *Eunicella verrucosa:* C. Munro (pers. comm.); *Melithaea flabellifera:* Matsumoto 2004) and contrasts with the several annual maturation episodes extending over a long time period (i.e., several months) reported in some tropical gorgonian

Table 3. Spawning periods of tropical gorgonian species studied to date.

Reference	Species
Fitzsimmons-Sosa et al. (2004)	*Pseudopterogorgia americana*
Fitzsimmons-Sosa et al. (2004)	*Muriceopsis flavida*
Fitzsimmons-Sosa et al. (2004)	*Muricea atlantica*
Gutiérrez-Rodríguez & Lasker (2004)	*Pseudopterogorgia elisabethae*
Beiring & Lasker (2000)	*Plexaura flexuosa*
Kapela & Lasker (1999)	*Pseudoplexaura porosa*
Brazeau & Lasker (1990)	*Briareum asbestinum*
Brazeau & Lasker (1989), Lasker et al. (1996)	*Plexaura kuna*
Martin (1982)	*Plexaura homomalla*
Kinzie (1970)	*Pseudopterogorgia bipinnata*



[a]Presence of large oocytes within the polyps-

species. Spawning in Caribbean gorgonians exhibits a pattern of multiple bouts over consecutive days that usually repeats monthly over 2–5 months (Table 3). Three tropical species exhibit gonad maturation between monthly spawning events (*P. kuna, Plexaura flexuosa,* and *Pseudoplexaura porosa*). These three zooxanthellae-containing species exhibit the longest spawning events (over 4–5 months) in summer, and they are all broadcast spawners. These differences between temperate and tropical gorgonian species are probably related to the autotrophic contribution to the energy supply of most tropical gorgonians and to the small environmental change present in coral reef environments in contrast to the highly seasonal pattern of resource availability in temperate environments. However, it is uncertain why spawning of tropical gorgonians has been documented to occur almost over the entire year although most species spawn in the summer, and why some species exhibit a single maturation of gonads while others display several episodes of gonad maturation.

Hermaphroditism appears to be a non-important pattern of sexual reproduction in gorgonians because, although some hermaphroditic colonies have been observed in *E. singularis* and in other species (e.g., *P. clavata*), they have always represented a negligible proportion of the population (Coma et al. 1995b; this study). Hermaphroditism is present in other groups of octocorals such as Alcyonaneans (5 of 39 examined species) and Xeniids (six of 23 examined species; see Benayahu 1997 and McFadden et al. 2001 for revisions of the topic), but it is not the dominant pattern of sexual reproduction as it is in scleractinians (see Harrison & Wallace 1990; Richmond 1997 for revisions). Although there is a clear trend for octocorals to be gonochoric and for scleractinians to be hermaphroditic, studies of taxa in which both gonochoric and hermaphroditic species occur suggest that there are no genetic constraints on reproductive mode, and that hemaphroditism or gonochorism can evolve rapidly and easily in cnidarians (McFadden et al. 2001).

Gorgonians exhibit similar proportions of broadcast spawners, brooders, and surface brooders (7, 8, and 5 species, respectively, Table 4), which contrast with broadcast spawning being the dominant mode of development in scleractinians (Harrison & Wallace 1990; Richmond 1997). The duration of oogenesis (13–17 months) and that of spermatogenesis (4–6 months) is similar to that documented previously on other Mediterranean species (*C. rubrum*, oogenesis 18–24 months, spermatogenesis 4–6 months: Vighi 1970; Tsounis et al. 2006; *P. clavata,* oogenesis 13–18 months, spermatogenesis 6–7 months: Coma et al. 1995b). Maximum oocyte size (860 μm) was similar to that observed previously in *C. rubrum* (Vighi 1970; Tsounis et al. 2006) and within the largest reported values (only oocytes from *B. asbestinum* and *Ainigmaptilon antarticum* exhibited higher oocytes, 900 μm: Brazeau & Lasker 1990; Coma et al. 1995a; Orejas et al. 2002). In contrast, the number of offspring per year was low (0.7 oocytes per polyp per year). The strategy of developing a small number of large oocytes is similar to that of *C. rubrum* (Vighi 1970; Tsounis et al. 2006) but contrasts with that of *P. clavata* (Coma et al. 1995b). Within the overall frame of gorgonian species studied so far, fecundity (i.e., the number of eggs produced per polyp) in *E. singularis* is among the lowest (Coma et al. 1995b; Kapela & Lasker 1999; Beiring & Lasker 2000; Gutiérrez-Rodríguez & Lasker 2004; Tsounis et al. 2006). It should be noted that fecundity may be affected by many factors, including reproductive season, size, depth, and polyp position within the colony (e.g., Rinkevich & Loya 1987; Coma et al. 1995a; Beiring & Lasker 2000); reported data suggest that brooding species tend to produce fewer eggs that broadcast spawners as in scleractinan corals (Harrison & Wallace 1990).

Fertilization in *E. singularis* is internal and the planula (average length = 1156 μm) develops within the polyp of the female colony until it is released into the water column between late May and July, at our study site. The behavior and metamorphosis of the larvae have been carefully examined in previous studies (Theodor 1967; Weinberg 1979b; Weinberg & Weinberg 1979).

Timing of investment in gonadal development

Investment in gonadal development occurred during the first half of the year (January–May) and exhibited a pattern of exponential increase over this time period. This timing of investment in gonadal development has also been observed in other Mediterranean octocoral species examined to date (*P. clavata:* Coma et al. 1995b; *C. rubrum:* Vighi 1970; Tsounis et al. 2006; *A. acaule:* unpubl. data). Several environmental factors including temperature, lunar periodicity, insolation, tidal surge, salinity, presence of toxic elements, and food availability have been documented to mediate and/or affect gametogenesis in marine invertebrates (e.g., Giese & Pearse 1974). However, two types of environmental factors are distinguished (Baker 1938; Clark 1979): (a) those that allow gamete development (the necessary environmental conditions) and (b) those that allow forecasting the occurrence of the necessary environmental conditions (the specific environmental

Table 4. Pattern of reproduction of gorgonian species studied to date.

	Environment			
Reproductive pattern	tropical	temperate	cold	References
Gonochoric broadcast spawners		*Leptogorgia virgulata*		Adams (1980)
	Plexaura homomalla			Martin (1982)
	Plexaura kuna			Brazeau & Lasker (1989)
	Pseudoplexaura porosa			Kapela & Lasker (1999)
	Plexaura flexuosa			Beiring & Lasker (2000)
	Pseudopterogorgia americana			Gutiérrez-Rodríguez & Lasker (2004)
		Eunicella verrucosa		C. Munro (pers. comm.)
Gonochoric brooders	*Acabaria biserialis*			Ben-Yosef & Benayahu (1999)
		Corallium rubrum		Vighi (1970)
		Eunicella singularis		Theodor (1967)
		Muricea californica		Grigg (1977)
		Muricea fructicosa		Grigg (1977)
		Melithaea flabellifera		Matsumoto (2004)
			Thouarella variabilis	Brito et al. (1997)
			Fannyella rossii	Orejas et al. (2002)
Gonochoric surface brooders	*Briareum asbestinum*			Brazeau & Lasker (1990)
	Briareum stechei			Alino & Coll (1989)
	Pseudopterogorgia elisabethae			Gutiérrez-Rodríguez & Lasker (2004)
		Paramuricea clavata		Coma et al. (1995b)
	Pseudopterogorgia bipinnata			Kinzie (1970)

signals). In cnidarians, the control of gametogenesis has been studied in Hydrozoans, and temperature and food availability were demonstrated to be the main factors conducive to gametogenesis, apart from colony age and density (see Gili & Hughes 1995 for a review). However, similar manipulative experimental work has not been performed for many cnidarian groups.

The timing of investment in gonadal development in gorgonian species from cold temperate areas occurs in late spring and summer, which are also the periods of higher resource availability (*M. fruticosa, M. californica:* Grigg 1977; *Alcyonium digitatum:* Hartnoll 1975). In tropical areas, Dahan & Benayahu (1997) suggested that the two annual blooms of phytoplankton at Eilat supply the metabolic demand for gametogenesis of *Dendronephthya hemprichi,* and Ben-Yosef & Benayahu (1999) noted that planulation of *A. biserialis* occurred following the major seasonal bloom of its main food source. In the Mediterranean, the fact that seasonal patterns of activity and secondary production of benthic suspension feeders in general, and gorgonians in particular, are characterized by aestivation (Coma et al. 2000) and that an energy shortage related to low food availability is the basis of this phenomenon (Coma & Ribes 2003), suggests that seasonal reproduction is restricted to times of energy surplus over that required to sustain metabolism. This is consistent with studies of the trophic ecology of Mediterranean gorgonians, showing an energy surplus for investment in secondary production during winter and spring (Coma et al. 1998; Coma & Ribes 2003; Rossi et al. 2004). Therefore, although there is a lack of manipulative experiments, we suggest that investment in reproduction is related to food availability for gamete production. Long-lived species with slow oocyte development, such as octocorals, in environments with marked but predictable variations in food availability usually have gametogenic cycles that

track these variations (Clarke 1979; Giese & Kanatani 1987; Eckelbarger 1994).

Timing of spawning

It is extremely difficult to monitor male spawning *in situ,* and we did not observe it during our study, although during the numerous dives between May and July we did observe the release of planula from female polyps on a few occasions. A massive release of planula larvae was never observed in the field. The timing of spawning between late May and July is similar to that reported previously in *E. singularis* (Theodor 1967; Weinberg & Weinberg 1979). The exact date of spawning has been difficult to predict in most species of octocorals (e.g., Lasker et al. 1996) and scleractinians (e.g., Wallace 1999). Temperature and the lunar cycle could be the main external stimuli regulating spawning synchrony among colonies (Grigg 1977; Brazeau & Lasker 1989, 1990; Coma et al. 1995b); nonetheless, synchronicity in brooders may not be as crucial (Gutiérrez-Rodríguez & Lasker 2004; Lasker 2006) as in broadcast spawners (Lasker et al. 1996) to ensure fertilization.

Spawning in *E. singularis* occurred just after gametogenesis was completed, which is similar to that observed in other Mediterranean species examined to date (spawning occurring during June and July, *C. rubrum:* Vighi 1970; Santangelo et al. 2003; *P. clavata:* Coma et al. 1995b; *A. acaule:* unpubl. data) and other octocorals (Benayahu 1997). Spawning just after gametogenesis contrasts to the delay in spawning of *A. digitatum* in the cold temperate North Atlantic (gametogenesis is completed in September but spawning occurs in December–January, Hartnoll 1975). The timing of spawning is considered to be an adaptive function, as natural selection should favor those individuals releasing offspring during the season that maximizes productivity and/or minimizes mortality (Hughes & Cancino 1985). The timing of spawning of Mediterranean species maximizes the survival of offspring due to (1) decreased predation associated with summer dormancy in benthic suspension feeders and (2) the increased availability of substratum for settlement resulting from diminished growth of algae and benthic suspension feeders during the summer period (Coma et al. 2000). The delayed timing of spawning in *A. digitatum* is also a mechanism to minimize the mortality of offspring by avoiding predation (Hartnoll 1975) and by increasing substratum availability due to diminished activity during the winter period (Hughes 1989).

This work has shown that the gametogenic cycle of the abundant and widely distributed Mediterranean species *E. singularis* is characterized by: (1) seasonality with a single annual maturation of the gametes, a pattern similar to other Mediterranean and temperate species but in contrast to some tropical gorgonian species that demonstrate multiple maturational cycles; (2) a large oocyte size; and (3) oogenesis (13–17 months) longer than spermatogenesis (4–6 months), conforming to the general pattern observed in gorgonians and octocorals. These results and those of previous studies on sexual reproduction of gorgonians suggest that investment in gonad development appears to be related to resource availability.

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