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SEXUAL REPRODUCTION IN THE MEDITERRANEAN ENDEMIC ORANGE CORAL *ASTROIDES CALYCULARIS* (SCLERACTINIA: DENDROPHYLLIIDAE)

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ABSTRACT

Astroides calycularis (Pallas, 1766) is a common endemic azooxanthellate scleractinian coral living in the southwestern Mediterranean Sea, generally in shaded habitats, below overhangs, or at cave entrances, from the surface to 50 m depth. The annual reproductive cycle of *A. calycularis* (gamete development in relation to environmental parameters, planulation timing, size at sexual maturity, fecundity, and sex ratio) was studied at Palinuro in the southern Tyrrhenian Sea (Italy) from April 2004 to September 2005. Colonies were gonochoric, were mature at 3–4 cm^2 in area, and had a sex ratio of 1:1. Polyps were sexually mature at 3–4 mm length (maximum diameter of the oral disc), and the females brooded their larvae. The maturation of spermaries took 7 mo and that of oocytes took over 12 mo. The rate of gamete development increased significantly from November to March. Fertilization occurred from April to May, with planulation in June. Mature oocytes ranged from 400 to 1590 μm and planulae size was 1850 μm (oral–aboral axis). Seasonal variation in seawater temperature and photoperiod likely play an important role in regulating reproductive events. The amount of energy devoted to male gametogenesis (quantified by gamete index) was significantly higher than female gametogenesis. In relation to other dendrophylliids, *A. calycularis* presents an intermediate reproductive strategy on the r-K continuum.

Understanding the population dynamics and dispersal in marine organisms requires knowledge of their reproductive biology (Stearns 2000), which includes sexuality (hermaphroditic or gonochoric), reproductive mode (brooding or broadcast spawning), embryonic development (coeloblastic or stereoblastic), and larval development (benthic or planktonic).

Sexual maturity, which depends on the size and age of the organism, is determined by a balance between the growth rate and mortality risk. Population growth rates are influenced by the age and size at reproduction, as well as by the sex ratio (Babcock 1991, Fujiwara and Caswell 2001). Complex interactions between intrinsic factors such as size, age, and physiological condition, as well as extrinsic factors such as density, food availability, physical disturbance, and predation regulate the timing of sexual maturity (Harvell and Grosberg 1988). Reproductive capability and mortality of scleractinian coral species generally depend on colony area (Babcock 1991, Hall and Hughes 1996).

In scleractinian corals, the most common form of sexuality is hermaphroditism, while gonochorism occurs only in 25% of the species studied (Hall and Hughes 1996, Kruzic 2008, Baird et al. 2009). In the Mediterranean Sea, sexual reproduction is described for only seven species of scleractinians. Of these, three reports are from the

19th century (Lacaze-Duthiers 1897), with the sexual reproduction of four species, *Astroides calycularis* (Pallas, 1766), *Balanophyllia europaea* (Risso, 1826), *Cladocora caespitosa* (Linnaeus, 1767), and *Leptopsammia pruvoti* (Lacaze-Duthiers, 1897), described more recently (Goffredo and Telò 1998, Goffredo et al. 2002, 2005, 2006, 2010, Kruzic 2008, Baird et al. 2009).

The cycle of gametogenesis usually culminates with a short period in which gametes are released into the environment where external fertilization occurs (Harrison and Wallace 1990, Richmond and Hunter 1990). To maximize fertilization rate and reproductive success, it is important that gamete development and release be synchronous, since the rapid dilution of gametes in the aquatic environment lowers the probability of fertile encounters (Harrison and Wallace 1990, Levitan 1996). Regulation of the reproductive cycle in corals is correlated with several environmental factors, such as seawater temperature and photoperiod (Harrison and Wallace 1990, Richmond and Hunter 1990, Soong 1991, Penland et al. 2004).

The family Dendrophylliidae is cosmopolitan and includes both solitary and colonial corals; 148 living species are described and divided into 19 genera (Cairns 1999). Seven species live in the Mediterranean Sea, and these are grouped into five genera; three of these (*Astroides, Cladopsammia,* and *Dendrophyllia*) are colonial (Minelli et al. 1995). The genus *Astroides* is made up of a single species, *A. calycularis* (Cairns 2001).

Astroides calycularis is gonochoric (male and female colonies) and brooding (planula releasing, Goffredo et al. 2010). The smaller size of peripheral polyps compared to central ones suggests that polyp budding occurs preferentially at the outskirts of the colonies, possibly increasing the competitive advantage for space utilization (Goffredo et al. 2011). Large colonies have polyps that are of a smaller size than small and medium colonies, suggesting that in larger colonies, energy is invested in increasing polyp size only up to the size at sexual maturity, rather than increasing the size of already mature polyps (Goffredo et al. 2011). *Astroides calycularis* is a Mediterranean and Ibero-Moroccan Bay endemic species and is believed to be a warm water species with narrow temperature tolerance (Zibrowius 1995, Grubelic et al. 2004, Goffredo et al. 2010). However, it has also been found outside the Strait of Gibraltar, along the Atlantic coasts of Morocco and Spain (Bianchi 2007), with some recent records in the northeastern part of the Adriatic Sea, along the coasts of Croatia (Grubelic et al. 2004, Bianchi 2007, Kruzic 2008) up to the Gulf of Venice (Casellato et al. 2007). *Astroides calycularis* is found from the surface to 50 m (Rossi 1971), but is typically found in the shallow infralittoral (0–15 m depth), on vertical walls, or inside caves (Kruzic et al. 2002). It is an azooxanthellate species (Cairns 1999, Goffredo et al. 2010), living in both light and dark, and seems to prefer elevated currents (Kruzic et al. 2002, Grubelic et al. 2004). The population density can be high, with colonies covering up to 90% of the rocky walls (S Goffredo, pers obs). Generally, the colonies have an ellipsoid shape with polyps densely crowded or separated, depending on water flow (Kruzic et al. 2002, Goffredo et al. 2010).

One of the fundamental challenges facing ecologists is to understand how natural systems will respond to environmental conditions (Harley et al. 2006). Global warming is likely to alter the phase relationship between environmental cues, such as photoperiod and temperature, that control or synchronize the reproductive cycle of many marine invertebrates, and such changes are likely to be greatest in temperate areas (Solomon et al. 2007). The potential impact of climate change on marine

invertebrate reproduction highlights the need to understand the physiological basis of reproduction in marine organisms (Lawrence and Soame 2004).

Here we report on the quantitative aspects of the annual reproductive cycle of *A. calycularis*, gamete development in relation to environmental parameters, planulation timing, colony and polyp size at sexual maturity, fecundity, and sex ratio. Morphological aspects of spermatogenesis, ovogenesis, embryogenesis, and larval development have been described elsewhere (Goffredo et al. 2010).

Materials and Methods

Sampling.—*Astroides calycularis* samples were collected at Palinuro (Italy, southern Tyrrhenian Sea; 40°01.81′N, 15°16.74′E) during 16 monthly collections from April 2004 to September 2005 at a depth of 7–10 m along a randomly placed transect line, parallel to the coast line; distance between two consecutive colonies was 2 m. The mean time interval between sampling events was 33.2 d (SE = 1.8 d). Water temperature was measured directly in the field at the depth and time of sampling using a mercury thermometer. Photoperiod data were taken from the online database Ciraci P; EuroMETEO®. Rome, Italy: Nautica Editrice Srl; 4 January, 2011, c1995–2011, 14 October, 1995. Available from: http://www.eurometeo.com.

During each sampling period, 10 colonies of *A. calycularis* were collected, fixed in saturated formalin solution (10% formaldehyde and 90% seawater; solution saturated with calcium carbonate), and transferred to the laboratories for histological analysis.

Biometric Analysis.—For each collected colony, colony length (L_C, major axis of the colony) and width (W_C, minor axis of the colony) were measured and used to compute colony area (A_C) using the formula

$$A_C = \pi \frac{L_C \cdot W_C}{4}.$$

Colony surface area was used because this is a more accurate and representative measure of colony size than colony length (Meesters et al. 2001, Nozawa et al. 2008). A biometric analysis of all of the polyps in each collected colony was performed: polyp length (L_P, major axis of the oral disc), width (W_P, minor axis of the oral disc), and height (h, oral-aboral axis) were measured and used to compute body volume (V_P), using the formula

$$V_P = \pi \frac{h \cdot L_P \cdot W_P}{4}$$ (Goffredo et al. 2002).

Histological and Cytometric Analysis.—Polyps were post-fixed in Bouin solution. After decalcification in EDTA and dehydration in a graded ethanol series from 80% to 100%, polyps were embedded in paraffin and serial transverse sections were cut at 7 μm intervals from the oral to the aboral poles. Tissues were then stained with Mayer's hematoxylin and eosin. Histological observations were made under a light microscope and cyto-histological measurements were made with a Leica Q5001 W image analyzer. To calculate the size of the oocytes in nucleated sections and of the spermaries at different stages of maturation, the largest (maximum diameter, *D*) and smallest sizes (minimum diameter, *d*) of each were measured. Gamete size was determined as the average of the two diameters. Spermaries were classified into five morphologically identified developmental stages according to Glynn et al. (2000) and Goffredo et al. (2005, 2010). Similarly, the average of the maximum and minimum diameters of the embryos was used to calculate their size (Goffredo and Telò 1998, Goffredo et al. 2005).

Gamete Index.—Oocytes and spermaries were ellipsoidal in shape, thus the volume (V_0) of oocyte or spermary was estimated using the formula

$$V_0 = \frac{4}{3}\pi\left(\frac{D}{2}\right)\left(\frac{d}{2}\right)^2$$ (Goffredo et al. 2006).

Volume of gametes was calculated as the sum of the volume of each oocyte or spermary and the gamete index was expressed as the percentage of body volume occupied by the gametes (Goffredo et al. 2006).

Size at Sexual Maturity and Fecundity.—The minimum size at sexual maturity of polyps was considered as the size at which 50% of the individuals developed either spermaries or oocytes (Oh and Hartnoll 1999, Roa et al. 1999). Fecundity was expressed both at the polyp and the colony level. At the polyp level, fecundity (F) was expressed as the number of mature oocytes produced per female polyp per reproductive season using the formula

$$F = \frac{A \cdot B}{C},$$

where A is the length of the "ovary" (based on the number of sections in which oocytes were present), B is the observed frequency of mature oocytes, and C is the size of mature oocytes (Goffredo et al. 2006). At the colony level, fecundity was calculated as the sum of the fecundity estimates for each polyp of the female colony.

Results

Sexuality and Reproductive mode.—Histological examination of the 53 colonies revealed no signs of sexual dimorphism at either the polyp or the colony level. There were no significant differences in mean polyp and colony size between males and females (Student's t-test for L_P: t = 0.894, P = 0.373; Student's t-test for V_P: t = 0.031, P = 0.975; Student's t-test for L_C: t = 1.095, P = 0.280; Student's t-test for A_C: t = 0.486, P = 0.630; Table 1). The sex ratio of colonies was 1:1 (Chi-square test: χ^2 = 0.143, df = 1, P = 0.705, was calculated for 15 female colonies and 13 male colonies sampled in the annual period of maximum gamete expression from November to May). Fifty-nine polyps were inactive; 27 of them were from 15 female colonies (L_P = 3.66 mm, SE = 0.23; V_P = 45.40 mm^3, SE = 7.87), four were from four male colonies (L_P = 2.48 mm, SE = 0.36; V_P = 15.57 mm^3, SE = 4.90), and the remaining 28 inactive polyps (L_P = 5.13 mm, SE = 0.13; V_P = 93.17 mm^3, SE = 8.55) were from 14 indeterminate colonies collected in the summer-autumn period, from July to October. The mean size of the 27 inactive polyps from 15 female colonies was significantly smaller than the mean size of the 80 analyzed female polyps (Student's t-test for L_P: t = 7.779,

Table 1. Mean size and standard error of sexually active *Astroides calycularis* (males and females) and inactive/indeterminate polyps or colonies (L_P major axis of the oral disc of the polyp, V_P polyp volume, L_C major axis of the colony, A_C colony area, n number of polyps or colonies examined).

	Sexually active	Males	Females	Inactive polyps / indeterminate colonies
L_P (mm)	5.1 ± 0.1 (n = 123)	5.0 ± 0.1 (n = 43)	5.2 ± 0.1 (n = 80)	4.3 ± 0.2 (n = 59)
V_P (mm^3)	104.5 ± 3.9 (n = 123)	104.3 ± 6.8 (n = 43)	104.5 ± 4.8 (n = 80)	66.1 ± 6.4 (n = 59)
L_C (cm)	5.0 ± 0.3 (n = 39)	5.5 ± 0.4 (n = 13)	4.8 ± 0.4 (n = 26)	5.5 ± 0.6 (n = 14)
A_C (cm^2)	16.8 ± 1.9 (n = 39)	18.1 ± 3.0 (n = 13)	16.2 ± 2.4 (n = 26)	20.3 ± 4.2 (n = 14)

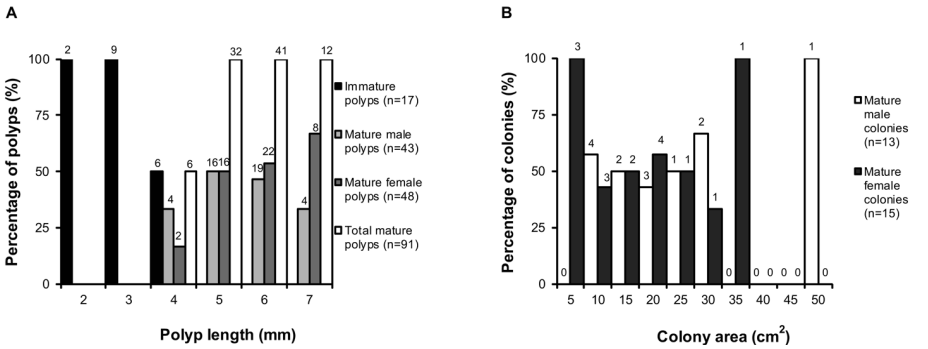


Figure 1. Percentage of sexually mature *Astroides calycularis* per size class from November to May, the period of maximum annual gamete activity. (A) Percentage of mature polyps per size class; total number of polyps measured = 108. (B) Percentage of mature colonies per size class; total number of colonies measured = 28.

P = 5.312 × 10^{-12}; Student's *t*-test for V_P: *t* = 6.305, P = 6.915 × 10^{-9}); the mean size of the four inactive polyps from four male colonies was significantly smaller than the mean size of the 43 analyzed male polyps (Student's *t*-test for L_P: *t* = 6.637, P = 3.525 × 10^{-8}; Student's *t*-test for V_P: *t* = 3.949, P = 2.731 × 10^{-4}); the mean size of the 28 inactive polyps from the 14 indeterminate colonies was not significantly different from the mean size of the 123 sexually active polyps (Student's *t*-test for L_P: *t* = 0.162, P = 0.872; Student's *t*-test for V_P: *t* = 1.241, P = 0.216). The mean size of the 14 indeterminate colonies was not significantly different from the mean size of the 39 sexually active colonies (Student's *t*-test for L_C: *t* = 0.788, P = 0.434; Student's *t*-test for A_C: *t* = 0.868, P = 0.389; Table 1). Embryos were found in the coelenteron of seven out of 10 (70%) female polyps collected in three female colonies of May 2004 and 2005. Polyps were sexually mature at 3–4 mm in length (Fig. 1A). According to biometric analyses (Goffredo et al. 2011), a polyp in this category has W_P = 3–4 mm, *h* = 3–4 mm, V_P = 17–45 mm^3. Colonies were sexually mature at 3–4 cm^2 in area (Fig. 1B). According to biometric analyses (Goffredo et al. 2011), a colony in this category has L_C = 2–3 cm, W_C = 2–3 cm.

Distribution of Gametogenetic Processes Along the Oral–Aboral Axis.—Gamete distribution along the polyp oral–aboral axis differed significantly between males and females (Fig. 2). While spermary size in males was not correlated with the distance from the oral pole, the size of the oocytes in females had a positive correlation. Furthermore, the mean distance of spermaries from the oral pole was significantly less than that of oocytes (Student's *t*-test: *t* = 16.737, df = 48955, P < 0.001; Mann-Whitney's U test: U = 46406652; Wilcoxon's W test: W = 1124905998, P < 0.001).

Annual Sexual Reproductive Cycle.—Gamete size increased more rapidly in males than in females from November to March, the months with shortest photoperiod and coldest water temperature (Fig. 3). In this period, females had two distinct stocks of oocytes, consisting of small (26–400 μm) or large (400–1590 μm) cells. Meanwhile, in males, there was an acceleration in spermatogenesis with a maturation from I to III/IV stage (Fig. 4, Goffredo et al. 2010). Fertilization took place from February to May, when both photoperiod and water temperature

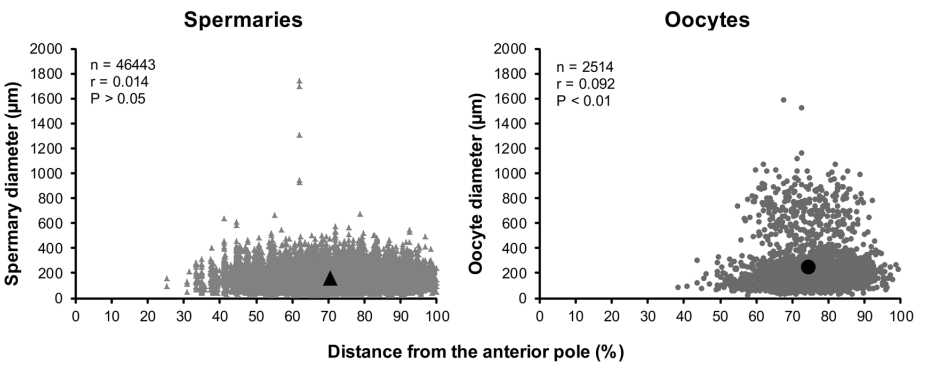


Figure 2. Distribution according to size along the oral–aboral axis of spermaries in *Astroides calycularis* male polyps, and oocytes in female polyps. The distance from the oral pole is expressed as a percentage: 0% = at oral pole level and 100% = at aboral pole level. ▲ the point at which the mean spermary distance (70.58%, SE = 0.06) and mean spermary size (160.36 µm, SE = 0.30) intersect; ● the point at which mean oocyte distance (74.64%, SE = 0.18) and mean oocytes size (242.28 µm, SE = 3.95) intersect.

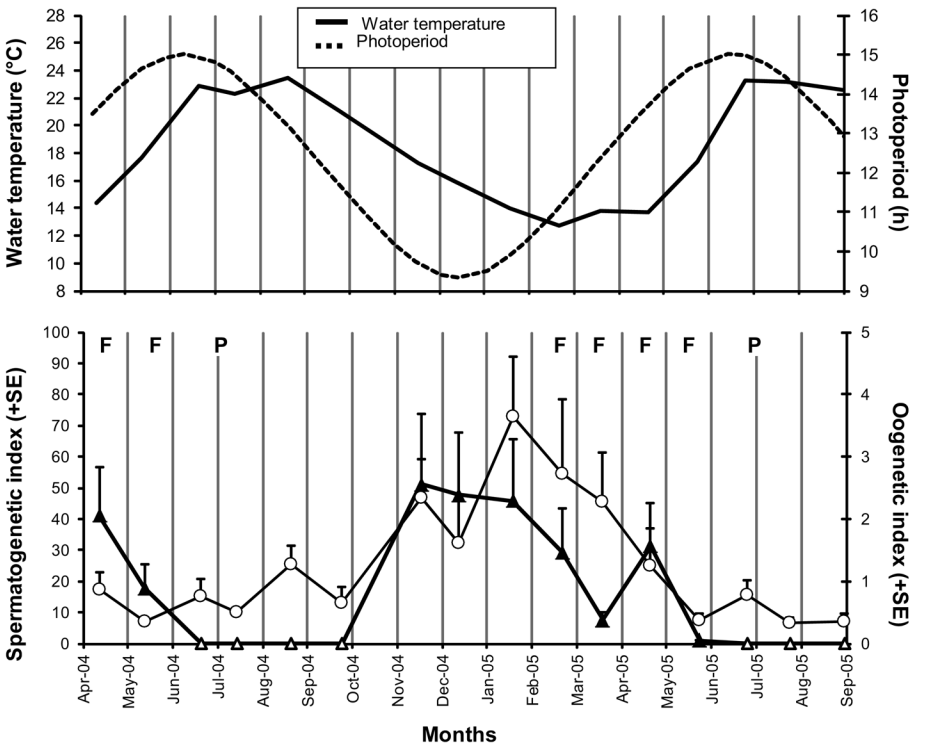


Figure 3. Variation in *Astroides calycularis* gamete development, water temperature, and photoperiod from April 2004 to September 2005 at Palinuro. Note that the value ranges on the ordinate axes are different (○ oocytes; ▲ spermaries; Δ no spermary detected; F = fertilization; P = planulation).

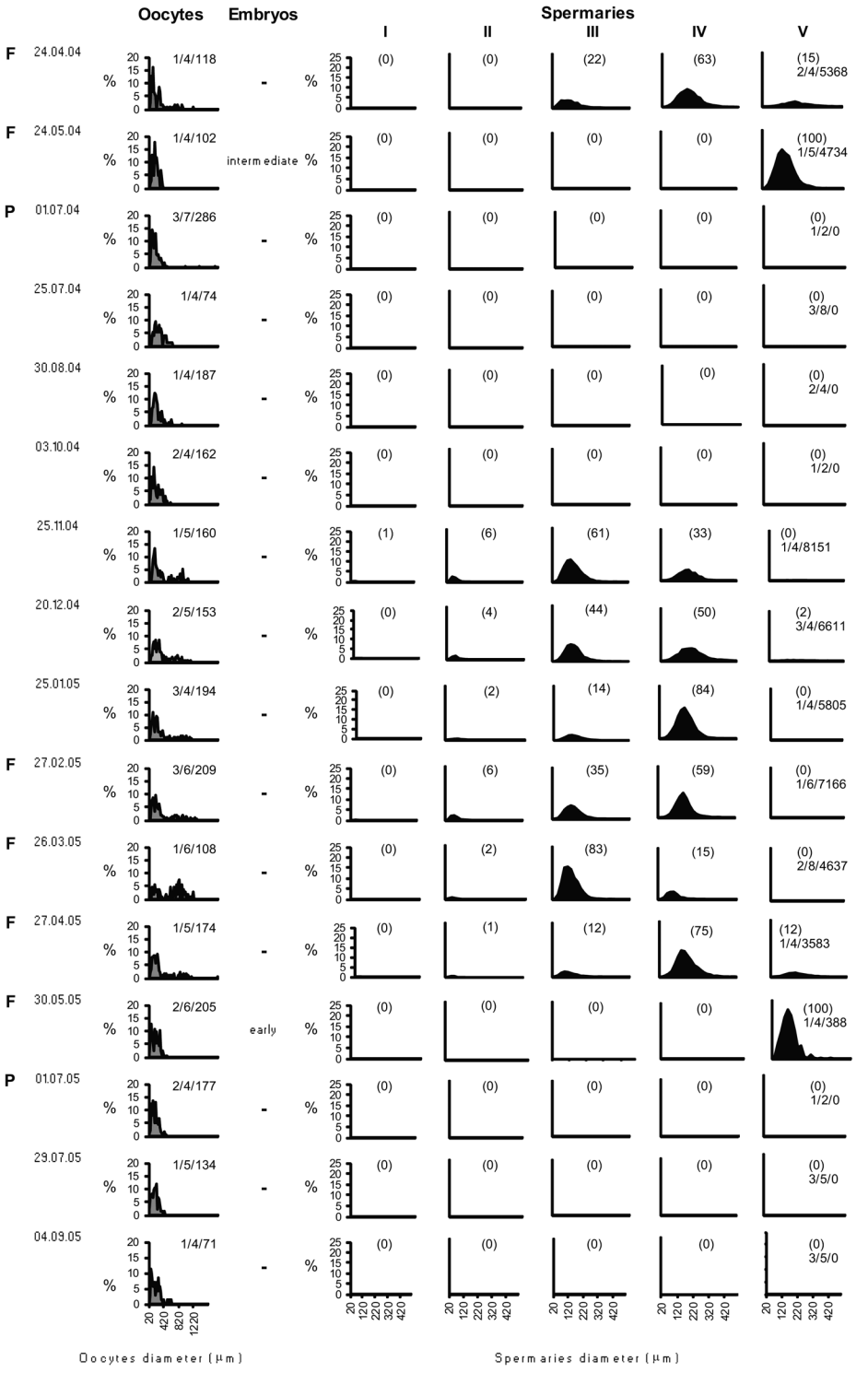
were increasing (Fig. 3). In the samples collected during these months, large-sized oocytes disappeared, while spermaries reached maturity, and early and intermediate embryos were observed in the coelenteric cavity (Figs. 3, 4; Goffredo et al. 2010). During the months immediately following the period of fertilization (June and July), we observed growth of the oocyte stock that remained after the reproductive event and the recruitment of new oocytes in female polyps. Between June and October, the spermaries in male polyps disappeared (Figs. 3, 4). Planulation took place between June and July 2004 and 2005, when photoperiod and water temperatures were at the annual maximum (Fig. 3), and was recognized when mature embryos disappeared from the coelenteric cavity (Fig. 4).

Size of Mature Oocytes and Fecundity.—Mature oocytes ranged from 400 to 1590 μm (Fig. 4). At the polyp level, a mean of 10.1 mature oocytes (SE = 1.9) were found in mean-sized female polyps of V_P = 95.1 mm^3, SE = 7.6 (L_P = 4.7 mm, SE = 0.2; W_P = 4.4 mm, SE = 0.2; h = 5.0 mm, SE = 0.2; n = 58 polyps collected during the period of maximum annual gamete development, from November to May). Polyp fecundity varied with size (Fig. 5A). Specimens of 2–3 mm length contained 0–1 oocytes (n = 13), those of 4–5 mm length contained 10–15 oocytes (n = 36), and those of 6–7 mm length contained 9–23 oocytes (n = 9). At colony level, a mean of 487 mature oocytes (SE = 66) was found in mean-sized female colonies of A_C = 13.5 cm^2, SE = 2.2 (L_C = 4.4 cm, SE = 0.5; W_C = 3.6 cm, SE = 0.3; n = 15 colonies collected during the period of maximum annual gamete development, November–May). Colony fecundity varied with colony area (Fig. 5B). Colonies up to 10 cm^2 contained a mean of 419 oocytes (SE = 101, n = 3), those of 10–20 cm^2 contained a mean of 671 oocytes (SE = 98, n = 6), and those of 20–30 cm^2 contained a mean of 710 oocytes (SE = 54, n = 2).

Discussion

Sexuality and Reproductive Mode.—The sexuality found in *A. calycularis* is typical of Dendrophylliidae, in which gonochorism and brooding are the prevalent reproductive characteristics (Fadlallah 1983, Goffredo et al. 2000, 2005). This systematic pattern in dendrophylliid reproduction has been verified by recent phylogenetic and molecular analyses of the evolution on coral reproductive biology (Baird et al. 2009). Kerr et al. (2010) claim that the organism's reproductive mode (brooding vs spawning) is correlated with the evolution of its sexual system (gonochorism vs hermaphrodism). Harrison (1985) suggests that sexuality is a relatively constant feature within families of scleractinian corals, and defines Dendrophylliidae as a gonochoric family.

Szmant (1986) expected that success in fertilization of a gonochoric brooding species would depend on the population density and its sex ratio. To increase the brooding space in Caribbean coral species, incubation of embryos should yield a sex ratio that favors females. We did not observe this deviation in *A. calycularis*. The size of *A. calycularis* polyps at sexual maturity, compared with that of other solitary dendrophylliids whose reproduction is known, indicates that reproductive activity begins at an intermediate polyp size relative to the range for this family (Table 2). Colony size at sexual maturity observed in *A. calycularis* was higher than in *Tubastraea coccinea* (Lesson, 1829) (Table 2).


Oocytes
Embryos
Spermaries
I
II
III
IV
V
F 24.04.04 1/4/118 - (0) (0) (22) (63) (15) 2/4/5368
F 24.05.04 1/4/102 intermediate (0) (0) (0) (0) (100) 1/5/4734
P 01.07.04 3/7/286 - (0) (0) (0) (0) (0) 1/2/0
25.07.04 1/4/74 - (0) (0) (0) (0) (0) 3/8/0
30.08.04 1/4/187 - (0) (0) (0) (0) (0) 2/4/0
03.10.04 2/4/162 - (0) (0) (0) (0) (0) 1/2/0
25.11.04 1/5/160 - (1) (6) (61) (33) (0) 1/4/8151
20.12.04 2/5/153 - (0) (4) (44) (50) (2) 3/4/6611
25.01.05 3/4/194 - (0) (2) (14) (84) (0) 1/4/5805
F 27.02.05 3/6/209 - (0) (6) (35) (59) (0) 1/6/7166
F 26.03.05 1/6/108 - (0) (2) (83) (15) (0) 2/8/4637
F 27.04.05 1/5/174 - (0) (1) (12) (75) (12) 1/4/3583
F 30.05.05 2/6/205 early (0) (0) (0) (0) (100) 1/4/388
P 01.07.05 2/4/177 - (0) (0) (0) (0) (0) 1/2/0
29.07.05 1/5/134 - (0) (0) (0) (0) (0) 3/5/0
04.09.05 1/4/71 - (0) (0) (0) (0) (0) 3/5/0
%
20 420 820 1220
20 120 220 320 420
Oocytes diameter (µm)
Spermaries diameter (µm)

Oral–Aboral Distribution of Gametogenic Processes.—The observed distribution of reproductive elements along the oral–aboral axis in gonochoric polyps of *A. calycularis* was very similar to that observed in gonochoric polyps of *L. pruvoti* (Goffredo et al. 2006). In these two gonochoric species, the absence of a differential spermary distribution along the oral–aboral axis in males could be related to gonochorism, which ensures the physical separation of male and female gametogenetic processes in separate individuals, and in turn assures cross-fertilization. In contrast, in the simultaneous hermaphroditic polyps of the dendrophylliid *B. europaea,* mature spermaries tend to be distributed toward the oral pole, while mature oocytes are distributed toward the aboral pole (Goffredo et al. 2002). This type of arrangement may reduce the number of encounters between the gametes of the opposite sex in the same individual polyp, producing a "statistical barrier" to self-fertilization (Goffredo et al. 2005).

Annual Reproductive Cycle.—The size frequency distribution of spermaries observed in the different months suggests that spermatogenesis in *A. calycularis* follows an annual cycle, and that male germ cells take 6–7 mo to mature. In the case of females, two oocyte stocks were present, indicating that female germ cells may take longer than 12 mo to mature. Similar gametogenic cycles have been documented for the three other species belonging to the family Dendrophylliidae: the solitary corals *B. europaea* in the Mediterranean Sea (Goffredo and Telò 1998, Goffredo et al. 2002), *Balanophyllia elegans* (Verrill, 1864) along the western coast of North America (Fadlallah and Pearse 1982, Beauchamp 1993), and *L. pruvoti* in the Mediterranean Sea (Goffredo et al. 2005, 2006). Among other azooxanthellate colonial corals, the presence of two oocyte stocks has been observed in *Madrepora oculata* (Linnaeus, 1758) of the family Oculinidae (Waller and Tyler 2005). A longer maturation period for female germ cells compared to male germ cells is typical of gametogenesis in anthozoans (Acosta and Zea 1997, Goffredo et al. 2002, Schleyer et al. 2004, Guest et al. 2005, van Woesik et al. 2006, Ribes and Atkinson 2007, Hellstrom et al. 2010, van Woesik 2010).

The reproductive phase (gamete development, fertilization, planulation) in the annual cycle of *A. calycularis* takes place from October/November to June/July. As the period from June to October was of reproductive quiescence, these summer–autumn months are likely a trophic phase, during which polyps invest in somatic growth.

Reproductive events in this species may occur in relation to seasonal variations in water temperature and photoperiod, which could be the major factor controlling the reproductive activities of corals, as has been suggested for other anthozoans (Glynn et al. 2000, Penland et al. 2004). In winter, photoperiod and water temperature reach their annual minimum and this may act as a signal that could be correlated to gamete development. The subsequent increase in photoperiod and water temperature, during winter and spring, coincides with sperm release and egg fertilization. Recently, blue-light-sensing photoreceptors (cryptochromes) have been detected in the

Figure 4. *(Opposite page)* Size–frequency distribution of oocytes and of the five stages of spermary maturation in monthly samples of *Astroides calycularis* collected at Palinuro from April 2004 to September 2005. Values reported indicate the total number of colonies/total number of polyps/total number of oocytes or spermaries measured per monthly sample. In brackets is the percentage of the stages of spermary maturation. The middle column illustrates the presence and stage of development of embryos found in the coelenteric cavity of female polyps (F = fertilization; P = planulation).

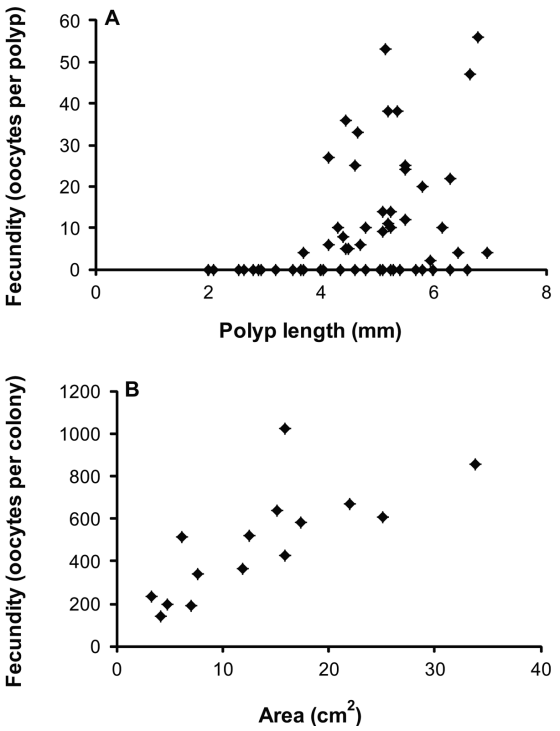


Figure 5. (A) Relationship between fecundity (mature oocytes per polyp) and polyp size (y = 4.447x – 10.952, n = 58, r = 0.369, P < 0.01) in *Astroides calycularis*. (B) Relationship between fecundity (mature oocytes per colony) and colony size (y = 22.341x + 185.360, n = 15, r = 0.759, P < 0.01).

reef building coral *Acropora millepora* (Ehrenberg, 1834) along the Great Barrier Reef. These proteins are ancestral members of the protein family potentially responsible for light perception in animals. In corals, expression patterns of genes coding for photoreceptor proteins vary in response to circadian rhythms, suggesting that mass spawning could be regulated also via photosensitive cryptochromes (Levy et al. 2007).

The period during which germ cells are released varies geographically (Harrison and Wallace 1990, Richmond and Hunter 1990). Comparison of the timing of gamete release within species among localities may reveal population responses to different environmental conditions (Babcock et al. 1994). These environmental factors could also influence reproduction by acting as long-term agents exerting selective pressure on the sexuality of populations (Acosta and Zea 1997).

In May, embryos were found inside the coelentric cavity of female polyps. Planulation occurs during the summer at maximal photoperiod and temperature. Released larvae were observed in the field on the benthos, crawling around the parental polyp, similar to the larvae of *T. coccinea*, another azooxanthellate dendrophylliid coral in the Gulf of California (Paz-García et al. 2007, Goffredo et al. 2010).

There are different interpretations of the role of photoperiod and water temperature in the regulation of the annual reproductive cycle in other dendrophylliids. For example, both of these factors are thought to play a role in regulating major reproductive events in *L. pruvoti* and *B. europaea* (Goffredo et al. 2002, 2005), while

Table 2. Characteristics of the reproductive biology of the species of dendrophylliid corals whose annual reproductive cycle is known.

	Leptopsammia pruvoti	*Tubastraea coccinea*	*Astroides calycularis*	*Balanophyllia europaea*	*Balanophyllia elegans*
Sexuality	Gonochoric	Hermaphroditic	Gonochoric	Hermaphroditic	Gonochoric
Sex ratio	1:1	–	1:1	–	1:1
Polyp size at sexual maturity [fraction of maximum size (observed size, mm)]	32% (3 mm)	– (9 mm)	38% (3 mm)	38% (8 mm)	56% (6 mm)
Maximal polyp size (oral disc maximum diameter, mm)	8	–	8	21	10
Colony size at sexual maturity [fraction of maximum size (observed size, cm^2)]	–	– (1 cm^2)	6% (4 cm^2)	–	–
Maximal colony size (maximum area, cm^2)	–	–	63	–	–
Fecundity (mature oocytes 100 mm^{-3} polyp)	38–114	–	8–13	8–14	2–6
Fecundity (mature oocytes 100 cm^{-2} colony)	–	43,418–68,526	3,222–5,040	–	–
Oocyte volume output (mm^3 mature oocytes 100 cm^{-2} colony)	–	3,420–5,720	299–468	–	–
Embryonic incubation period (mo)	1–4	1–2	1	4–5	14–15
Planulae size (oral–aboral axis, µm)	1,100	1,000	1,850	2,150	4,000
Sources	Goffredo et al. (2005, 2006)	Glynn et al. (2008)	Present study, Goffredo et al. (2010, 2011)	Goffredo et al. (2002, 2004), Goffredo and Zaccanti (2004)	Fadlallah and Pearse (1982), Beauchamp (1993)

the reproductive cycle of *B. elegans* may be regulated by water temperature alone (Fadlallah and Pearse 1982, Beauchamp 1993). Additional studies are necessary to distinguish the role of different environmental factors in regulating reproductive events in these scleractinians.

In our study, information on the annual reproductive cycle was collected over an 18-mo period and therefore inter-annual variation was not examined. However, we observed similar stages in the reproductive cycle during April–September in both years (2004 and 2005). During this period, a significant number of inactive polyps and indeterminate colonies were found (the 32.4% of polyps and 26.4% colonies). The size of the inactive polyps in the indeterminate colonies was not significantly different from those of the active polyps in active colonies. Therefore, it is possible that these elements were in a state of quiescence. In particular, the 14 inactive colonies detected from July to October, when no male colonies were detected, may have been quiescent males after the period of spring fertilization.

The mean annual fecundity of *A. calycularis* (41.3 oocytes cm^{-2} of colony ± 4.6 SE) is lower than in the colonial azooxanthellate brooder dendrophylliid *T. coccinea* in the eastern Pacific (from 227.1 oocytes cm^{-2} of colony ± 1.3 SE in Costa Rica to 897.4 oocytes cm^{-2} of colony ± 0.1 SE in Panama; Glynn et al. 2008). The volume of mature oocytes produced per area unit indicates a lower fecundity for *A. calycularis* with respect to *T. coccinea* (*A. calycularis*: 3.8 mm^3 of oocytes cm^{-2} of colony ± 0.4 SE; *T. coccinea*: from 16.2 mm^3 of oocytes cm^{-2} of colony ± 0.1 SE in Costa Rica to 82.8 mm^3 of oocytes cm^{-2} of colony ± 0.1 SE in Panama; Glynn et al. 2008). *Tubastraea coccinea* has, along with *Porites panamensis* (Verrill, 1866) (Glynn et al. 1994) and *Stylophora pistillata* (Esper, 1797) (Loya 1976, Hall and Hughes 1996), a much higher annual fecundity than other colonial zooxanthellate brooder species (Harrison and Wallace 1990, Glynn et al. 2008). The colonial azooxanthellate but broadcasting species *Lophelia pertusa* (Linnaeus, 1758) has a much higher fecundity than *T. coccinea* (> 3000 oocytes cm^{-2}; Waller and Tyler 2005).

In both the gonochoric *A. calycularis* (the present study) and *L. pruvoti* (Goffredo et al. 2006), the body volume occupied by male gametes was, respectively, 17.7 and 2.6 times greater than that occupied by female gametes. In the simultaneous hermaphroditic *B. europaea,* the body volume used by male gametes is the same as that of female gametes (Goffredo et al. 2000, 2002). Thus, the proportion of energy devoted to male gametogenesis is significantly higher in the gonochoric species than in the hermaphroditic one. This difference could be related to the contrasting sexuality or fertilization biology of these three species. Cross-fertilization likely takes place in the gonochoric *A. calycularis* and *L. pruvoti*, while in the hermaphroditic *B. europaea,* fertilization could be autogamous (Goffredo et al. 2004). To assure successful mating encounters in gonochoric organisms, male sex allocation is greater than in hermaphrodites. Greater male sexual allocation in dioecism or cross-fertilization when compared to hermaphroditism or self-fertilization is common in plants (Charnov 1982, Mione and Anderson 1992, Jurgens et al. 2002).

Reproductive Strategies.—Reproductive strategies of dendrophylliids, in which the reproductive cycle has been described, seem to cover the entire range of the r–K life history strategy continuum (Pianka 1970, Stearns 2000). The gonochoric *L. pruvoti*, having higher levels of fecundity, shorter periods of embryo incubation, and smaller planula size, presents a quantitative strategy (r-reproductive strategy).

In contrast, the gonochoric *B. elegans*, having a longer delay in reaching sexual maturity, lower fecundity, longer embryonic incubation period, and larger planula size, presents a qualitative strategy (K-reproductive strategy). The reproductive strategy of the gonochoric *A. calycularis*, whose reproductive characteristics lie somewhere between the above-mentioned characteristics, is placed intermediate along the r–K continuum.

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