Sexual reproduction in the Mediterranean solitary coral Balanophyllia europaea (Scleractinia, Dendrophylliidae)

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ABSTRACT: Balanophyllia europaea (Risso, 1826) is a common scleractinian coral living on subtidal rocky substrates in the Mediterranean Sea. The annual sexual reproductive cycle of this species is studied in specimens from an area near Leghorn (Tuscany, Italy). This study represents the first report of a reproductive cycle in a Mediterranean scleractinian coral. B. europaea is a simultaneous hermaphrodite and brooder. Polyp size at sexual maturity occurs at approximately 3 yr of age (6 to 10 mm maximum oral disc diameter). Observation of hermaphroditic polyps shows several significant differences in the distribution of spermaries and oocytes along the oral-aboral axis. Mean distance of spermaries from the oral pole is less than that of oocytes. Also, large mature spermaries tend to line up towards the mouth, while large mature oocytes tend to line up towards the polyp base. This differential gamete distribution could result in a barrier to self-fertilization, as it reduces encounters between gametes of the opposite sex produced by the same individual. Testes require 1 yr to reach maturity with a maximum spermary diameter of 500 µm. Oogenesis lasts 2 yr with a maximum oocyte diameter of 1400 µm. Fecundity varies significantly with polyp size. The rate of gonadal development increases significantly during January and February, fertilization takes place in March to June and planulation in August and September. Released larvae have a maximum diameter of 2000 µm. The annual cycle of photoperiod and water temperature appear to coincide with the reproductive cycle in B. europaea.

KEY WORDS: Coral · Balanophyllia europaea · Reproduction · Gametogenesis · Fecundity · Mediterranean

INTRODUCTION

The studies hitherto conducted on the annual cycle of sexual reproduction in scleractinian corals refer almost exclusively to tropical and subtropical species (Fadlallah 1983b, Harrison & Wallace 1990, Richmond & Hunter 1990, Richmond 1997, Lam 2000), with few data on reproduction in temperate species (Stoddart & Black 1985, Ward 1992, Beauchamp 1993, and references therein). In particular, for species living in the Mediterranean, information is available only from the 19th century, on Caryophyllia smithi, Astroïdes calycularis, Balanophyllia regia, B. pruvoti and Cladopsammia rolandi (Lacaze-Duthiers 1873, 1897), and from the last few years on B. europaea (Goffredo & Telò 1998, Goffredo et al. 2000).

Most scleractinians examined to date are hermaphroditic and reproduction is characterized by external fertilization and a 1 yr gametogenic cycle culminating in a short period during which germ cells are released (Oliver et al. 1988, Harrison & Wallace 1990, Richmond & Hunter 1990). Reproductive cycle regulation in these organisms has been associated with environmental factors (Harrison & Wallace 1990). Some studies suggest that the annual reproductive cycle is mainly regulated by seasonal variations in water temperature and photoperiod (Giese & Pearse 1974, Babcock et al. 1986,

Many authors suggest that environmental factors may influence reproduction, because they act as long- term agents exerting selective pressure on the sexuality and reproductive mode of populations (Giese & Pearse 1974, Bacci 1975, Rossi 1975, Loya 1976, Van Moorsel 1983, Szmant 1986, Tomascik & Sander 1987, Shaw 1989, Fautin 1992, Ward 1992, Fan & Dai 1995). Harrison (1985), however, suggests that sexual condition is a relatively constant feature within families, and defines Dendrophylliidae, the family to which Balanophyllia europaea belongs, as gonochoristic. According to Harrison & Wallace (1990), comparative studies on congeneric species presenting different types of sexuality are essential to an understanding of the selection of reproductive strategies.

Dendrophylliidae is a cosmopolitan family with 153 living species belonging to 21 genera (D. S. Cairns pers. comm.). A total of 94% of dendrophylliid corals studied are gonochoristic (Harrison 1985). Of 50 known species of Balanophyllia (Cairns 1977), reproductive modalities are known only for B. pruvoti at Marseilles, France (Lacaze-Duthiers 1897), B. elegans at Monterey Bay, California (Fadlallah & Pearse 1982a, Beauchamp 1993) and B. europaea at Leghorn, Italy (Goffredo & Telò 1998, Given et al. 2000). All are brooders; the first 2 species are gonochoristic and the latter is hermaphroditic.

According to Schumacher & Zibrowius (1985), Balanophyllia europaea is a solitary, non-constructional, zooxanthellate scleractinian coral that lives on rocky substrates in the Mediterranean Sea and off Spain’s Atlantic coast (Zibrowius 1980, 1983, Aleem & Aleem 1992). Owing to its symbiosis with zooxanthellae, this coral lives at 0 to 50 m depth (Zibrowius 1980), where it reaches population density peaks of about 100 individuals m⁻² (Goffredo 1999). Congeneric azooxanthellate corals have been reported at depths of up to 1100 m (Cairns 1977).

In this paper, we describe the annual cycle of sexual reproduction in Balanophyllia europaea inhabiting the eastern Ligurian Sea, near the city of Leghorn, Italy. We examine the morphological and quantitative aspects of oogenesis and spermatogenesis, the location of male and female gametogenesis in hermaphroditic polyps, the relationship between gonadal growth and relevant environmental factors, and sexual maturity and fecundity in relation to polyp size.

**MATERIALS AND METHODS**

**Sampling.** Specimens of Balanophyllia europaea were collected at Calafuria (Leghorn; 43°28.4’N, 10°20’E) during 15 sample periods from July 1997 to October 1998. The mean interval between samples was 32 d (SD = 9). Samples were collected by SCUBA divers at a depth of 6.4 to 6.8 m, where the population reaches its maximum density (Goffredo 1999). Water temperature was measured directly, and photoperiod was calculated from astronomical almanacs corresponding to the time period in which sample collection took place. Monthly samples of 20 specimens each included the full range of sizes found within the population (coral length 1 to 20 mm) (Goffredo 1999). We measured the length (L, maximum oral disc diameter), width (l, minimum oral disc diameter) and height (h, distance between the oral and aboral disc) of each collected individual and calculated body volume (V) using the formula $V = h \times \frac{L}{2} \times \left(\frac{l}{2}\right)^2$ (after Goffredo & Telò 1998).

**Histological and cytometric analysis.** Polyps were fixed in Bouin’s solution, decalcified using EDTA, dehydrated through a graded alcohol series, and embedded in paraffin; 7 to 10 µm thick transverse sections were cut in an oral/aboral serial sequence and stained with hematoxilin and eosin. We took cytometric readings of the histological samples using a LEICA Q500W for image analysis. Oocytes were not grouped into clear ovaries, thus oocytes were used as the unit of comparison with the male gonads (spermaries). Maximum and minimum diameter dimensions were measured in nucleated oocyte and spermary sections. We estimated the size of the reproductive elements by averaging the 2 diameter dimensions. Following previous methods (Beauchamp 1993, Kramarsky-Winter & Loya 1998, Kruger & Schleyer 1998, Glynn et al. 2000), spermaries were graded according to maturation stages that were distinguishable by morphological characteristics. We recorded the presence of embryos in the coelenteric cavity as well as their developmental stage according to Goffredo & Telò (1998).

**Gonadal index.** Oocytes and spermaries were ellipsoidal in shape, leading us to estimate gonadal volume using the following formula: $V = \frac{4}{3} \pi \times D/2 \times \left(\frac{D}{2}\right)^2$, where $V_o =$ oocyte or spermary volume, $D =$ maximum diameter and $d =$ minimum diameter. Total gonadal volume was calculated as the sum of spermary and oocyte volumes. A gonadal development index was
calculated as the percentage of body volume occupied by the gonads (Hall & Hughes 1996).

**Size at sexual maturity and fecundity.** In order to determine the minimum size at which specimens reached sexual maturity, we considered the length at which 50% of the specimens developed spermarys and oocytes (after Rinkevich & Loya 1979, Bianchini et al. 1998, Yoneda et al. 1998, Oh & Hartnoll 1999, Roa et al. 1999). We expressed fecundity as the number of mature oocytes per polyp per reproductive season. The formula we used to establish the number of mature oocytes in each polyp was: Fecundity = (A × B)/C, where A = length of the ‘ovary’ (based on the number of sections in which oocytes were found), B = observed frequency of mature oocytes, and C = the diameter of mature oocytes (after Kruger & Schleyer 1998).

**RESULTS**

**Sexuality and reproductive mode**

Specimens of *Balanophyllia europaea* were observed to be simultaneous hermaphrodites and brooders; no gonochoric polyp has been observed. Size at sexual maturity ranged from 6 to 10 mm in length (Fig. 1).

**Spermarys and oocytes**

Spermarys were observed as groups of germ cells delimited by a mesogregar envelope (Fig. 2). Diameters varied from 20 to 500 µm. We identified 5 distinct developmental stages of spermarys containing: I, spermatogonia (Fig. 2A,B); II, spermatocytes surrounded by spermatogonia (Fig. 2A,C); III, spermatocytes (Fig. 2D); IV, spermatocytes peripherally and spermatids and sperm centrally (Fig. 2E,F); V, sperm (Fig. 2G,H).

The diameter of clearly identifiable oocytes varied from 20 to 1400 µm. Oocytes were found centrally in the mesenteries, surrounded by a mesogregar layer (Fig. 3). During the early stages of oogenesis, the centrally located nucleus was surrounded by a homogeneous cytoplasm (Fig. 3A–C) while in later stages the ‘U’-shaped nucleus was in the periphery of an heterogeneous ooplasm filled with small yolk plates (Fig. 3D–G). The nucleus/oolasm size ratio decreased in later stages.

Distribution along the oral-aboral axis of the hermaphroditic polyp varied significantly between spermarys and oocytes (Fig. 4). Spermary size decreased with distance from the oral pole, while oocytes increased in size with distance from the oral pole. Even though spermary and oocyte spatial distributions overlapped, the mean distance of spermarys from the oral pole (60.92%, SE = 0.15) was less than that of oocytes (68.34%, SE = 0.29; Student’s t-test p < 0.001; Fig. 4).

**Annual sexual reproductive cycle**

Gonad size increased 5-fold in the winter months from January to February. Photoperiod increased during this time following the annual minimum (Fig. 5). In samples collected during these months, we observed 2 distinct oocyte stocks, one made of small cells (20 to 440 µm), the other of larger cells (440 to 1400 µm). We also observed an acceleration in spermatogenesis indicated by many spermarys at an intermediate stage of maturation (Fig. 6).

Fertilization occurred from March to June. Water temperature increased during this period following the annual minimum (Fig. 5). Large-sized oocytes disappeared in the samples collected during these months, while spermarys had reached full maturity; early and intermediate embryos were observed in the coelenteron (Fig. 6).

During the months immediately following fertilization (June to August), we observed a growth of small-sized oocyte stock, the recruitment of new oocytes, the beginning of spermary ripening and the incubation of embryos (Fig. 6).

Planulation took place between August and September. Water temperature began to decrease during this period following the annual peak (Fig. 5). During these months, mature embryos disappeared from the coelenteric cavity (Fig. 6).

**Mature oocyte size and fecundity**

Mature oocyte diameter ranged from 440 to 1400 µm (Fig. 6). Polyp fecundity varied with body size (Fig. 7).
Fig. 2. Balanophyllia europaea. Stages of spermary maturation. (A) Early stages: spermaries originate from the migration of undifferentiated germ cells (arrows) that migrate from the gastrodermis to the mesoglea. Scale bar = 20 µm. (B) Stage I: the spermary is made up of a group of spermatogonia. Note the recruitment from the gastrodermis of undifferentiated germ cells (arrow). Scale bar = 20 µm. (C) Stage II: the spermary is made up of a layer of spermatogonia peripherally and of a group of spermatocytes centrally. Note the recruitment from the gastrodermis of undifferentiated germ cells (arrow). Scale bar = 20 µm. (D) Stage III: spermaries are made up of a mass of spermatocytes. Scale bar = 40 µm. (E) Stage IV: differentiated spermatids can be observed centrally; note that a cavity has formed. Scale bar = 60 µm. (F) Stage IV: spermatocytes are visible in the peripheral portion of the spermary; spermatids may be observed centrally with their tails projecting towards the central cavity. Scale bar = 20 µm. (G) Stage V: the spermary is made up of a mass of sperm. Scale bar = 40 µm. (H) Stage V: sperm are released from the spermary and cross through the gastrodermis in order to reach the coelenteron (arrow). Scale bar = 40 µm. cc: coelenteric cavity; g: gastrodermis; m: mesoglea; o: oocyte; sdi: spermatids; sni: spermatogonia; sti: spermatocytes; szoi: sperm; I, II, III, IV, V: stage of spermary development (see ‘Results’)
Fig. 3. *Balanophyllia europaea* oogenesis. (A–B) Early stages. (A) Small oocyte located in the gastrodermis characterized by a high nucleus:cytoplasm ratio. Scale bar = 20 µm. (B) Vitellogenic oocytes. The spherical nucleus is located centrally and contains a single nucleolus. Oocytes appear to be linked by an intercellular bridge (arrow). Scale bar = 30 µm. (C–D) Intermediate stages. (C) A medium-sized vitellogenic oocyte located in the central portion of the mesentery. The cytoplasm is still quite homogeneous. Scale bar = 140 µm. (D) Differentiation of the ooplasm has begun as well as the nucleus' migration towards the peripheral portion of the cell. Scale bar = 70 µm. (E–G) Late stages. (E) The nucleus is now located in the outer portion of the oocyte and is kidney-shaped. Scale bar = 150 µm. (F) The oocyte's nucleus has begun to surround a deep invagination in the plasma membrane and in section appears 'U'-shaped. The ooplasm is full of small yolk plates. Scale bar = 60 µm. (G) Detail of the nucleus of a mature oocyte. The nucleus uniformly adheres to the invaginated plasma membrane. Location of the nucleolus coincides with the bottom portion of the invagination. Scale bar = 60 µm. cc: coelenteric cavity; g: gastrodermis; m: mesoglea; mf: mesenteric filament; o: oocyte; N: nucleus; n: nucleolus; ss: skeletal septum; yp: yolk plates; sti: spermatocytes; III: stage of spermary development (see 'Results')}
Fig. 4. *Balanophyllia europaea*. Spermary and oocyte size distribution along the oral-aboral axis of the hermaphroditic polyp. The distance from the oral pole is expressed as a percentage: 0% is the oral pole level and 100% the aboral pole level. ▲: the point at which mean spermary distance (60.92%; SE = 0.15) and mean spermary size (110.61 µm; SE = 0.52) intersect. ●: the point at which mean oocyte distance (68.34%; SE = 0.29) and mean oocyte size (241.55 µm; SE = 3.43) intersect. Note that the value ranges on the ordinate axes are different.

Fig. 5. *Balanophyllia europaea*. Variation of gonadal development, water temperature and photoperiod for the 16 mo in which samples were collected. Mean length of analyzed mature polyps: 13.93 mm (SE = 0.38; n = 59). P = planulation period; F = fertilization period.
Fig. 6. Balanophyllia europaea. Size-frequency distributions of oocyte and of the 5 spermary maturation stages in monthly samples collected off the coast of Calafuria from July 1997 to October 1998. Mean length of analyzed mature polyps: 13.93 mm (SE = 0.38; n = 59). Values reported in the graphs indicate the number of reproductive polyps/total number of oocytes or spermaries measured per monthly sample. The middle column illustrates the presence of embryos in the polyp's coelenteric cavity and their stage of development. P = planulation period; F = fertilization period.
Fig. 6 (continued)
Specimens of 8 mm in length contained a mean of 8.2 oocytes (SE = 8.0), those 13 mm in length contained a mean of 124.4 oocytes (SE = 28.5), and specimens that were 18 mm in length contained a mean 163.4 oocytes (SE = 78.7).

DISCUSSION

Polyp size at sexual maturity

In the studied sample, the size of the polyps of Balanophyllia europaea at sexual maturity was from 6 to 10 mm in length. According to Goffredo (1999), specimens of this size are approximately 3 yr old. B. elegans reaches sexual maturity at an age close to this one; according to Fadlallah (1983a) individuals reach sexual maturity at approximately 2 yr of age and at 1 to 3 yr of age according to Beauchamp (1993).

Oral-aboral distribution of gametogenic processes

The observed differential spermary and oocyte distribution could have been caused by the migrations of these reproductive elements towards the oral and aboral poles, respectively; this could occur during their maturation and would explain the overlapping of the more immature elements. The oral-aboral distribution of Balanophyllia europaea gametogenic processes is in some ways analogous to that found in other hermaphroditic polyps of freshwater hydads (Stagni 1974, Vannini 1974, Grassi et al. 1995), the octocoral Heteroxenia fuscenses (Achituv & Benayahu 1990) and the scleractinian corals Stylophora pistillata (Rinkevich & Loya 1979) and Oulastrea crispata (Lam 2000).

The reproductive cycle

The size-frequency distribution of the spermaryes observed in our monthly samples of Balanophyllia europaea suggests that spermatogenesis follows an annual cycle, and that male germ cells take 12 mo to mature. In contrast, oocyte distribution, which clearly shows the presence of 2 stocks, indicates that female germ cells take approximately 24 mo to mature. The same timing for gamete development was estimated in the only other congeneric species whose reproductive cycle has been studied in detail: B. elegans living in the temperate waters off the coast of California (Fadlallah & Pearse 1982a, Beauchamp 1993). The longer time required for female germ cells to mature is typical of gametogenesis in anthozoans (Benayahu et al. 1990, Harrison & Wallace 1990, Richmond & Hunter 1990, Coma et al. 1995, Fan & Dai 1995, 1998, Acosta & Zee 1997, Kramarsky-Winter & Loya 1998).

Seasonal variation in photoperiod and water temperature appears to play an important role in regulating the annual reproductive cycle in Balanophyllia europaea. Indeed, a significant increase in gonadal development occurs toward January-February, when the photoperiod becomes longer after its annual minimum; fertilization takes place in March to June, the period in which water temperature increases after its annual
minimum; and the planulae are released towards August-September, when water temperature starts to decrease after its annual peak. The photoperiod and/or water temperature may affect the reproductive cycle in many other anthozoans living in both temperate and tropical waters (Richmond & Hunter 1990, Soong 1991, Clayton & Collins 1992, Fadlallah et al. 1992, Beauchamp 1993, Babcock et al. 1994, Coma et al. 1995, Steiner 1995, Fadlallah 1996, Tanner 1996, Acosta & Zea 1997, Glynn et al. 2000). In *B. europaea*, an increasing photoperiod during January-February could start gonadal development by increasing photosynthetic rate in symbiotic zooxanthellae, thus giving the corals the energy needed to trigger gametogenesis (Rinkevich 1989). Furthermore, the increase and decrease of water temperature in March to June and in August-September, respectively, may serve to trigger fertilization in the former case and planulae release in the latter, so that subsequent dispersion, settlement, and metamorphosis of the larvae as well as the growth of young polyps take place in optimal environmental conditions (Giese & Pearse 1974, Fadlallah & Pearse 1982a,b, Beauchamp 1993, Holts & Beauchamp 1993). Reproduction prior to the onset of autumn and winter may avoid high mortality of young individuals due to adverse conditions, thus allowing greater reproductive success (Fan & Dai 1995).

Data on annual reproductive cycle were collected for 1.3 yr, and thus possible inter-annual variation was not revealed. However, similar stages of the reproductive cycle were observed in the period July to October, which was analyzed both in 1997 and in 1998.

**Reproductive strategies**

The diameter of mature oocytes in *Balanophyllia europaea* was 440 to 1400 μm, in contrast to 600 to 920 μm in *B. elegans* (Fadlallah & Pearse 1982a, Beauchamp 1993). In the latter species, small (100 mm³) and large (700 mm³) sexually mature individuals produced a mean of 2 and 40 oocytes, respectively (Fadlallah & Pearse 1982a, Fadlallah 1983a). Fecundity in *B. europaea* was about 2.5 to 3.0x greater (6 mature oocytes per 100 mm³ polyp and 99 mature oocytes per 700 mm³ polyp). Notable differences between the 2 species in embryonic incubation period (4 to 5 mo in *B. europaea* vs 14 to 15 mo in *B. elegans*: Fadlallah & Pearse 1982a, Beauchamp 1993), maximum planula size (2000 μm diameter in *B. europaea*, Goffredo & Telò 1998, vs 3700 μm diameter in *B. elegans*; Gerrodette 1981, Fadlallah & Pearse 1982a, Beauchamp 1993), may be associated with the above differences in fecundity values. Higher egg production has been directly associated with less parental care of embryos and the production of poorly specialized larvae (Pianka 1970, Loya 1976, Fadlallah & Pearse 1982b). Higher fecundity, shorter embryonic incubation, and the smaller size of larvae in *B. europaea* compared to *B. elegans*, in addition to hermaphroditism versus gonochorism, would seem to place the reproductive strategies of the former species towards the *r* endpoint and the latter’s towards the *K* endpoint on the *r-K* continuum (Pianka 1970, Kramarsky-Winter & Loya 1998). Future studies on population dynamics in *B. europaea* could be compared to those done on *B. elegans* (Gerrodette 1979, Fadlallah 1983a) and could help to further define the life history strategies of these congeneric species.

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