Quantification of sexual reproduction in the marine benthic hydroid *Campanularia everta*

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**Abstract** *Campanularia everta* is an epiphytic hydroid that may form dense populations on the macroalga *Halimeda tuna*. The main objects of this study were to quantify sexual reproduction and estimate sexual reproductive output of this hydroid. Sexual reproduction occurred from mid-October to mid-December 1991 along the Spanish Mediterranean coast. During this period, male and female colonies formed gonangia. Four oocytes mature in each female gonangium, and embryonic development starts probably after internal fertilization occurs. Planulae complete their development within a mucus sheath attached to the top of the gonotheca (acrocyst). Eight successive gonangia with a life-span of \( \approx 1 \text{ wk} \) each were formed over a 2 mo period. The fertile period was characterized by high initial production of gonangia followed by a progressive decline resulting from a decrease both in the number of fertile colonies and in the gonangia density of fertile colonies. Annual production was estimated at \( \approx 42,000 \text{ gonangia m}^{-2} \), representing 83,000 oocytes m\(^{-2}\). The high fertilization rates observed (77 to 100\%) yielded a minimum production of 64,000 planulae m\(^{-2}\).

Reproduction in *C. everta* is characterized by: (1) a high number of larvae produced m\(^{-2}\); (2) formation and gradual release of larvae throughout the sexual reproduction period; (3) direct formation of planulae with no intermediate medusa stage; (4) low dispersive ability of the planula. All these mechanisms are part of a reproductive strategy designed to ensure the permanence of the population in its habitat.

**Introduction**

When and how medusoid or planuloid forms are generated by hydroids have mainly been described from laboratory experiments (e.g., Berrill 1949, 1950; Yoshida 1954). Many species of Hydroidomedusae have undergone an evolutionary adaptation resulting in the progressive loss of the medusoid stage (e.g., Boero et al. 1992; Corneliūs 1992). In such species, reproduction may be asexual by stolonization or budding, or sexual by the formation of medusae. Whereas asexual reproduction among colonies seems to take place all year round and plays a major role in the permanence of the colony, sexual reproduction is, in temperate waters, typically restricted to a specific season of the year (e.g., Calder 1990; Llobet et al. 1991a) and may introduce genetic variability into the population (Hughes 1989). Early studies of Hydroidomedusae described gametogenesis and subsequent development of sexual reproductive structures (Weismann 1883; Goette 1907; Kühn 1910; Teissier 1926), but not until recently has there been great interest in the high morphological and environmental variability of their sexual reproductive patterns (Miller 1973; Campbell 1974; Fautin et al. 1989; Bouillon 1995).

The features most commonly reported in the literature regarding hydroid reproduction deal with timing and duration of sexual reproduction (e.g., Teissier 1950; Millard 1975; Corneliūs 1979, 1982; Boero and Fresi 1986; Calder 1991; Llobet et al. 1991b), but very few papers deal with the quantification of the reproductive output. To estimate reproductive output, the present study monitored such parameters as gonangial longevity, number of generations of gonangia, and number of oocytes generated, in different populations of *Campanularia everta*, a common hydroid in benthic communities in the north-west Mediterranean Sea. The study also examines the importance of sexual reproductive features to the population dynamics of this species in an attempt to quantify the role of sexual reproduction.
among marine benthic hydroids. Knowledge of the number of offspring and, especially, its relationship with the size and biomass of the progenitor colonies, is the first step to evaluating the reproductive effort and the dispersion capability of benthic species.

Materials and methods

The cycle of sexual reproduction in Campanularia everta was studied at the Medes Islands (42° N–3° 13' E; north-west Mediterranean). C. everta forms epiphytic populations on the chlorophytoan alga Halimeda tuna, whose thalli are composed of discoid articles and usually grow over rock walls at 12 to 17 m depth. Here, C. everta (described initially as Orthopyxis crenata by Cornelius 1982), forms colonies that attain life-spans of 4 to 6 wk during autumn–winter, the maximum period of growth (Llobet et al. 1991a). The population density and artihode and gonotheca of C. everta was determined from the presence of somatic and reproductive polyps in field colonies. Samples of 40 thalli of H. tuna were collected randomly by SCUBA divers during May 1990 to December 1991, and were immediately fixed in 4% formaldehyde solution in sea water.

Campanularia everta colonies were examined under a stereomicroscope, and their abundance on each Halimeda tuna thalli was recorded. We distinguished between somatic polyps (hydranths) and reproductive polyps (gonangia). Estimates of the alga surface area (cm²) were made from length measurements of the articles (cm) and the regression equation provided by Llobet et al. (1991b): surface area = 0.628 length 1.637 (r = 0.989, p < 0.001). The densities of hydranths and gonangia (number of polyps cm⁻²) were computed based on the estimates of alga surface area. Differences in densities throughout the year were tested by analysis of variance.

The dry weight (DW) of hydranths and gonangia was calculated by drying four replicates of 200 hydranths and 200 gonangia of both sexes in an oven at 70°C for 24 h, and then weighing the dried material on a microbalance (precision = 0.1 µg). The organic carbon content of the hydranths and gonangia was determined in a C/N autoanalyzer after acidification of samples and was converted to mean biomass (mg dry weight and mg organic C).

The number of developmental stages and the age of the gonangia were determined in two series of colonies, hereafter named control and experimental series, surveyed in situ.

The control series consisted of eight different samples, of 35 Halimeda tuna thalli each, most of which were collected at 2 to 3 d intervals from 30 October to 21 December 1991 and immediately preserved in 4% formaldehyde solution. The thalli were examined in the laboratory following the same procedures as above, but separating male and female colonies.

The experimental series consisted of one sample of 16 thalli of Halimeda tuna bearing fertile colonies of Campanularia everta, which were removed, tagged, and attached to a PVC stake by natural fibres. The thalli were then weighted back onto the substratum using an inert mastics compound (Scotch-Calk). During surveys of the control series, the stakes and their attached thalli were also collected and transported alive to the laboratory (near the Medes Islands) in a 40-litre container with in situ sea water. Articles from these thalli-bearing fertile colonies of C. everta were also tagged in the laboratory, and the number of male and female gonangia was recorded. Subsequently, the thalli were immediately replaced in their original position in the field. The entire procedure (i.e. removal of thalli, tagging, and counting of the colonies, and replacement in the field) required < 45 min. This procedure enabled us to monitor each of the tagged thalli and its development in the field for all gonangia of 6 male and 10 female colonies over a period of 22 d. No symptoms of degeneration were observed at any time during the experiment, either in the H. tuna thalli or in the C. everta colonies.

Hydropoly density per unit Halimeda tuna surface area (Llobet et al. 1991b) was used to calculate the reproductive output of the population. Variation in individual (hydropolyps and gonangia) density was assessed for a total area of 240 cm² bi-weekly over a 1 yr sampling period. Gonangial longevity was established as described for the above experiments.

Because of the similarity of the gonothecal shape of early-stage gonangia, some were selected and crushed and their contents examined under a compound microscope to identify the gametes. In colonies with more developed gonangia, we similarly separated gonangia without embryos (male) from those with embryos (female).

Results

Reproductive biology

During its fertile period, Campanularia everta reproduces by developing gonangia consisting of polyps specialized for reproduction. The blastostyles are surrounded by a chitinous sheath (gonotheca). Colonies of this species are dioecious. At the beginning of gonangium formation, the gonotheca of C. everta has a characteristic cup shape, since the blastostyle is not yet fully developed and is small (Fig. 1a). Later the gonotheca becomes ovoid or barrel-shaped, with a narrow, raised region on the apical portion where there appears to be a small opening. At this stage the blastostyle is completely developed (ripe), taking up nearly all the available space within the gonotheca (Fig. 1a, d).

Although the male and female gonothecae are outwardly similar, male and female blastostyles develop differently.

In males, spermatogenesis takes place over the entire blastostyle, with gametes occupying almost the whole volume of the gonotheca (Fig. 1a). Later a zone devoid of gametes appears at the base of the gonangium, suggesting a polarized spermatogenesis, and resulting in the accumulation of gametes in the apical region (Fig. 1b). Spawning of spermatocytes through the apical orifice follows, leaving behind an empty, shrunken blastostyle that occupies only a tiny volume at the centre of the gonotheca (Fig. 1c). Thereafter the blastostyle degenerates and disappears, leaving only the empty gonothecae with a small orifice at its tip.

In female gonangia, oogenesis takes place at four sites in the blastostyle, the gonophores, each of which produces an oocyte (Fig. 1d). Mature oocytes remain inside the gonophores, where fertilization probably takes place. Following fertilization, the four zygotes develop and may migrate to the apical portion of the gonotheca, while at the same time a mucus envelope surrounds the zygotes (Fig. 1e). During the subsequent stage, zygotes, together with the mucus envelope (acrocyst sensu Hyman 1940) are extruded (Fig. 1f), and four planulae develop inside the acrocyst. Once they have been completely formed, the larvae are released into the water, possibly through mechanical breakage. At this point the blastostyle has degenerated, leaving the female gonothecae completely empty after the release of their larvae. The free-living planulae probably attach