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Temporal and spatial co-occurrence in spawning and larval release of *Cliona viridis* (Porifera: Hadromerida)

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This is the first report of egg release by the oviparous excavating sponge *Cliona viridis*. Adult specimens of excavating (α) and massive (β) sponge forms for the presence of oocytes were monitored from 11 May to 12 July 2000, in a shallow-rocky coast of the north-west Mediterranean. The immediate environment around the sponge was sampled for the presence of eggs. Spawning occurred synchronously in the study area at temperatures above 19°C. Oocytes were released in clusters enclosed in adhesive maternal tissue. They contained symbiotic zooxanthellae. Free, adhesive egg-masses drifted in the water or adhered to erect algae in the vicinity of the sponge. Morula stages and larval release are described.

INTRODUCTION

Sponges of the family Clionidae (Demospongiae, Hadromerida) are widespread in the coastal waters of tropical and temperate regions (e.g. Topsent, 1900; Pang, 1973; Rützler, 1974; Rosell, 1996). They colonize and excavate the calcareous substrata of both mineral and organic origins, and their contribution to coastal bioerosion is remarkable (Neumann, 1966; Wilkinson, 1983; Rosell et al., 1999). *Cliona viridis* is one of the most common species of this family in the western Mediterranean (Rosell, 1996). The species is a simultaneous hermaphrodite, since mature oocytes and spermatic cysts have been observed at the same time in sponge adults (M.P. Piscitelli, unpublished data). Several studies have elucidated the reproductive cycle, fertilization, larval dispersal and rhagon ultrastructure (Tuzet, 1930; Rosell, 1996; Mariani et al., 2000). However, certain aspects of the life history of this oviparous species remain unknown, e.g. the periodicity of both spawning and larval release and their possible co-occurrence in time and space; the stage at which symbiotic zooxanthellae are acquired by the progeny; the role of water temperature in triggering spawning and posterior larval release; and the way the eggs are released. These issues are dealt with in the present study.

MATERIALS AND METHODS

The study was carried out at the Blanes sublittoral (north-west Mediterranean, Spain), where *Cliona viridis* massively colonizes the concrete boulders of the harbour breakwater at depths of 6–11 m.

A qualitative plankton sample was taken between 11 May and 12 July 2000 (Figure 1), to assess the presence of sponge propagules near the adults. To our knowledge (see Rosell, 1996; Mariani et al., 2000), the sampling period chosen was wide enough to embrace possible spawning and larval-release events. Three hauls were performed on each sampling day. The plankton net was towed by SCUBA divers at ~30 cm from the substratum surrounding *Cliona viridis* patches (Mariani et al., 2000). Water temperature was recorded simultaneously.

During the hauls, the water around and inside the exhalant canals of *C. viridis* individuals was also sampled by drawing it into 60-ml syringes. Six qualitative samples were collected at each sampling. Syringe contents were transported to the laboratory and examined for eggs and larvae under binocular microscopes.

Mature individuals of *C. viridis* and erect algae surrounding the sponges were collected. Six fragments of α and β sponge forms (three of each) were selected at random, removed from the substratum and transported to the laboratory in sealed bowls, as were algal samples. Sponge fragments and algal samples were examined under a binocular microscope. Images of oocytes, free eggs and larvae were recorded using both video and photographic cameras connected to microscopes, and measured.

RESULTS AND DISCUSSION

Findings of oocytes, released egg masses, and larvae as well as the time course of temperature throughout the study period are depicted in Figure 1. Temperature increased with an oscillatory pattern, and the highest values were recorded during the second week of June.

Oocytes were present in the adults until 8 June (Figure 2A). From 5 June, the presence of large (average diameter 163.7 μm ±18.8; N=10) oocytes was recorded near the sponge exhalant conducts. Oocytes were grouped in masses surrounded by adhesive maternal tissue (Figure 2B). Zooxanthellae were distinguishable inside these mature oocytes.

Free egg-masses (Figure 2C) containing variable numbers of eggs (between ~5 and ~70), with a conspicuous fertilization membrane, first appeared on 8 June and were also collected the following day. Egg masses were present in the samples collected by hauls, syringes and in those adhered to algae. Sampling was not possible from 10 to 13 June. The eggs were incubated and produced larvae in the laboratory. Each egg carried ~15 zooxanthellae.

The only free larva was obtained on 14 June by the hauling method. Its presence coincided with an increase

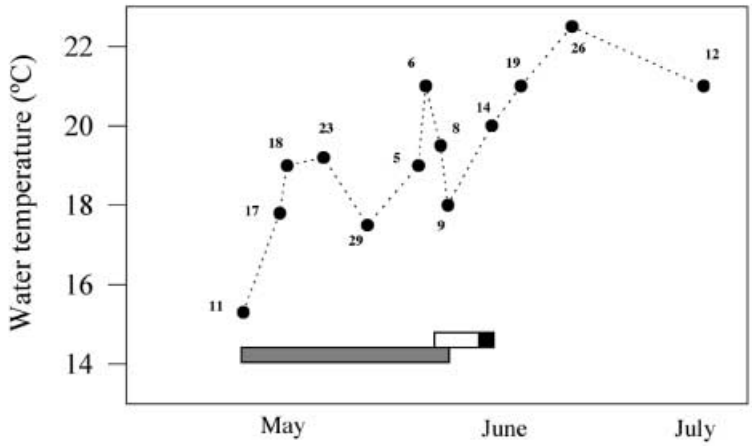

Figure 1. Temperature–time course during the study period. Sampling days are shown. The ▭, in the horizontal bar corresponds to oocytes in the sponges; □, collection of free egg masses; and ■, the free larva.

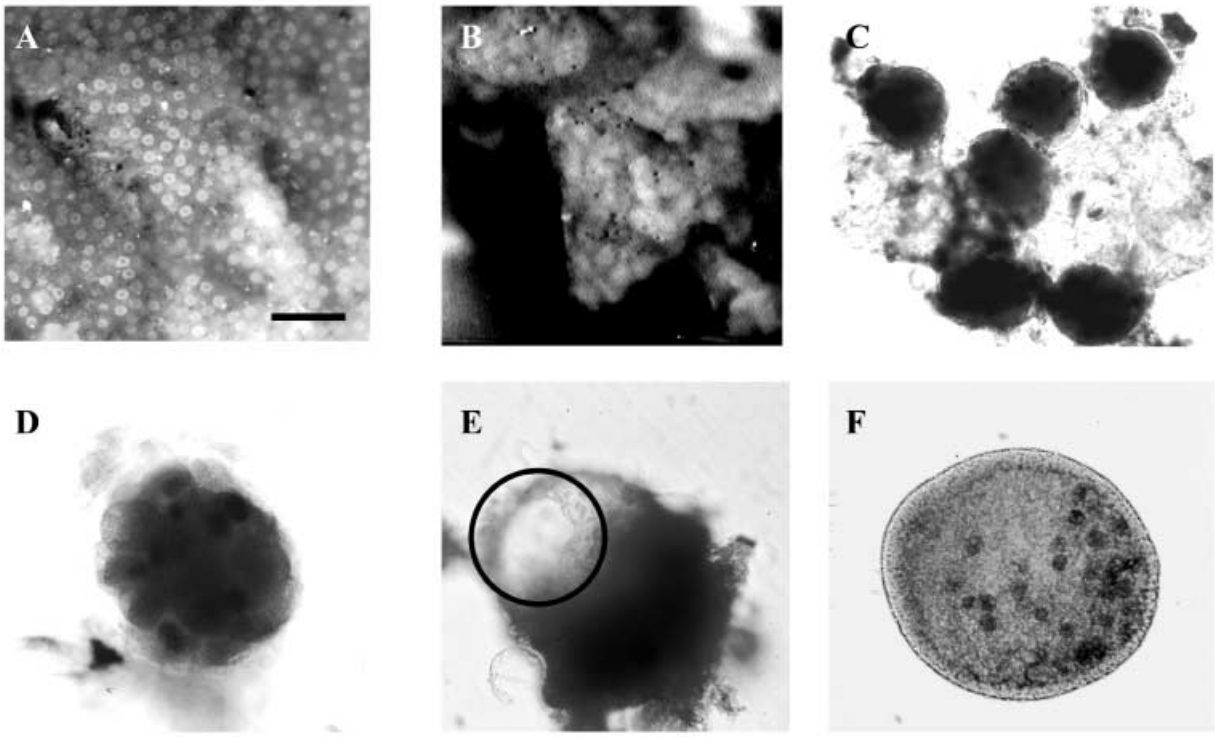

Figure 2. (A) Video image of oocytes homogeneously distributed within a mature sponge; (B) video image of mature oocytes surrounded by maternal tissue near an exhalant conduct; (C) light microscope image of a free egg mass; (D) light microscope image of a morula; (E) light microscope image of the egg capsula; encircled is the orifice through which the larva releases; (F) light microscope image of a larva. Zooxanthellae are visible in the middle-back portion of the larval body. Larval shape is distorted by the weight of the cover slip. Scale bars: A, 600 μm; B, 400 μm; C, 300 μm; D, 100 μm; E, 150 μm; and F, 120 μm.

in water temperature of ~2°C (Figure 1). No reproductive elements were found in the water after 14 June (Figure 1).

In the laboratory, most released eggs were clustered together by a transparent, sticky matrix, presumably of maternal origin, which included organic material and inorganic detritus. Egg masses were placed in plastic Petri dishes and kept at 21°C. Morulae (Figure 2D) developed after ~48 h and a variable number of zooxanthellae were distributed among the blastomers. Larvae were recognizable within the capsulae 48 h later. Larvae in the same egg-mass hatched fairly synchronously. They released the egg capsule through a narrow (~30 μm) orifice located at their anterior pole. As the orifice diameter was notably smaller than the larva diameter, the larval body constricted whilst the larva traversed the orifice. Larvae started to swim immediately after release. The empty capsules remained adhered to the sticking matrix (Figure 2E).

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Larvae were brownish-yellow, ~250 μm long, and they alternated weak swimming with crawling behaviour on the dish bottom without responding to light. Zooxanthellae were mainly grouped in the larval posterior zone (Figure 2F), where maternal cells have been described (Ereskowsky & Gonobobleva, 2000). Settlement in the Petri dishes took place 24 h after larval release.

These results confirm that both egg and larval release are synchronous annual events in *Cliona viridis* (Mariani et al., 2000). Eggs are released at temperatures above 19°C and larvae can be found in the water column at temperatures above 20°C, as reported by Mariani et al. (2000). Zooxanthellae are transferred to the larva from the maternal tissue since they are already present inside the oocytes before spawning. Eggs detach from the maternal tissue in clusters surrounded by adhesive material. Free egg-masses adhere to various materials, such as erect algae surrounding mother sponges. Similar patterns of spawning have been described for other oviparous species (Lévi & Lévi, 1976; Reiswig, 1976; Hoppe, 1988) such as *Cliona celata* (Warburton, 1958). Egg release is a synchronous event in the population studied. Temperature strongly determines egg and larval release in this species.

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