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Larval release and settlement of the marine sponge *Hymeniacidon perlevis* (Porifera, Demospongiae) under controlled laboratory conditions

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ABSTRACT

The insufficient supply of wild sponge biomass, i.e., "the supply problem," critically limits the development of sponge-derived bioactive natural products and other applications. Intensive aquaculture of sponges through artificial seed rearing may provide an alternative sustainable supply of sponge biomass. To develop the technology of sponge aquaculture, protocols for artificial seed production need to be established. To understand larval release and settlement under artificial controlled environments, a model marine sponge *Hymeniacidon perlevis* was investigated under controlled laboratory conditions. The larval release of *H. perlevis* is an asynchronous event in the laboratory-controlled environment. Sponge explants attached on substrata release 5 times more larvae than unattached sponge explants. Over the course of 12 days of release, the mean release rate was 7.2 larvae g^{-1} wet sponge day^{-1} for attached sponges. Over the course of 7 days of release for unattached sponges, the mean release rate was 2.6 larvae g^{-1} wet sponge day^{-1}. Light (6000 lx) stimulated the sponges to release more larvae than did dark incubation. The highest number of sponge larvae (195.8 larvae g^{-1} wet sponge) was released at 18 °C, while only 48 and 51.7 larvae g^{-1} wet sponge were released at 14 °C and 25 °C, respectively. Larval settlement was favored in dark condition. The highest percentage of larvae settled at 22 °C, among all temperatures tested.

1. Introduction

Sponges are by far the richest sources of bioactive natural products among marine invertebrates (Duckworth and Battershill, 2003). Due to the insufficient supply of wild sponge biomass, the progress and development of sponge-derived drugs have been hindered at the preclinical phase, preventing further investigation (Pomponi, 1999). Cultivation of sponge fragments under controlled conditions could be an effective option to resolve this problem; however, mass cultivation in bioreactors has not been successful (Osinga et al., 1999; Belarbi et al., 2003; Hausmanna et al., 2006). This may be due to the use of sponge fragments that come from wild harvested sponges. As an alternative, the cultivation of eggs, reduction bodies and larvae may represent a better approach (Osinga et al., 1999). Intensive aquaculture of economical marine animals through seed (eggs or larvae) rearing, including seed production and rearing of juveniles, has achieved great success for biomass production. Extensive studies have focused on improving the production and survivorship of seeds and juveniles, in fishes (Chakraborty and Mirza, 2007), abalone (Najmudeen and Victor, 2004), sea cucumber (Asha and Muthiah, 2005) and bivalves (Southgate and Lee, 1998; Liu et al., 2002; Muthiah et al., 2002). These rearing successes indicate that artificial seed rearing of sponges provides a sustainable supply of seeding sponges for sponge aquaculture, and it can also minimize the ecological and environmental impacts of wild sponge harvest. To establish the sponge seed-rearing technology, the seed production protocols must be established based on knowledge of the sponge reproductive cycle; methods for artificially inducing larval or egg and sperm release; optimal conditions for egg hatching and larval settlement; and metamorphosis to the juvenile stage.

By far, the reproductive cycles of the following sponges are the most well understood: *Hymeniacidon perleve* (Stone, 1970), *Halisarca dujardini* (Ereskovsky and Gonobobleva, 2000), *Geodia cydonium* (Jameson 1811) (Mercurio et al., 2007), and *Corticium candelabrum* (Riesgo et al., 2007). It was reported that larval release can be triggered by adjusting the water flow, light, and temperature (Maldonado, 2006). Maternal sponges of *Haliclona tubifera* and *Halichondria magniconulosa* can release larvae following intense light irradiation after 12–20 h of incubation in the dark (Maldonado and Young, 1996). In the demosponge *H. (G.) Slgmadocia caerulea*, larvae release can be triggered by exposure to the air lasting for only a few seconds (Maldonado and Young, 1996). Carefully tearing mature *Crambe crambe* individuals can also cause larvae release (Uriz et al.,

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1998; Caralt et al., 2007). The rate of larvae release varies among different species and individual sponges. In addition, limited available data reveal that the duration of larval release is usually short in laboratory conditions. For example, for an individual sponge, the encrusting sponge *Mycale fistulifera* released larvae at a rate of 500 larvae day^{-1} for 5 days in laboratory conditions (Meroz and Ilan, 1995). *Ophlitaspongia seriata* released larvae at a rate of 4–5 larvae min^{-1} for an individual sponge (Bergquist and Sinclair, 1968). The settlement rate was faster, and the mortality was higher, when adults were incubated at lower temperature (10 and 15 °C) as compared to higher temperature (20 and 25 °C) (Maldonado and Young, 1996). Light was thought to be the cue for larval settlement. Larvae that show negative phototaxis prefer to settle onto shaded places for physical refuge (Woollacott, 1993; Maldonado and Young, 1996; Maldonado et al., 1997; Maldonado and Uriz, 1998). Many studies have reported sponge larval release and settlement, but to our knowledge, there are no other systematic quantitative studies on sponge seed production under controlled conditions.

The life cycle of Marine sponge *H. perleve* in Langstone Harbour, Hampshire has previously been studied (Stone, 1970). *H. perleve* can produce larvae; the small red-amber granule-like embryos of *H. perlevis* develop in sponge tissues and are visible to the naked eye prior to release (Stone, 1970). *H. perlevis* is a demosponge exhibiting broad distribution throughout the coastal waters of the China Yellow Sea around Dalian City and displays an encrusting shape (Zhang et al., 2003). The growth cycle of *H. perlevis* in Dalian is similar to that observed for *H. perlevis* in Hampshire (data not published). In this paper, *H. perlevis* was selected as a model species to investigate the dynamics of quantitative larval release and settlement under laboratory-controlled conditions, in order to determine the feasibility of developing methods for artificial seed production (larval and juvenile sponges).

2. Materials and methods

2.1. Sponge collection, preparation and larval collection

Ripe *H. perlevis* were collected from the Lingshui Bay, Dalian, China Yellow Sea during September and October 2006 and 2007, with average temperature and salinity of around 22 °C and 32‰, respectively. Sponge specimens were collected from rocky substrates and immediately placed into plastic buckets filled with natural seawater. After transportation to the laboratory, sponge specimens with similar color, thickness and distribution of orange granules (as visible in sponge tissue) were chosen for the experiments. Each sponge specimen was attached onto a 4×4 cm^2 glass slide with sewing threads after the wet weight was measured using an electronic balance (BS210S, Sartorius). Three sponge specimens of 2–3 g wet weight each were placed into a 1.2 l glass tank filled with 1 l natural seawater at a temperature of 22 ± 0.5 °C and salinity of approximately 32‰ (Fig. 1). An aquarium heater (H708) and seawater cooler (HXLS 1000I) were used to control the temperature of the water bath in all experiments. In each tank, an air pump was used to supply air through an air stone at a rate of 1.0 vvm (air volume per water volume per minute) and to generate water flow with air bubbles. These tanks of sponges were used in the release experiments. The seawater was exchanged at a rate of 100% daily. Natural seawater used in these experiments was sand-filtered after being collected at a depth of 15 m from the China Yellow Sea, near Dalian City. Algae *Isochrysis galbana* was fed to maternal sponges at a concentration of 2×10^5 cells ml^{-1} once daily.

2.2. Larval release under attached and unattached conditions

To investigate the effect of sponge attachment on larval release, two tanks of attached sponges (on glass slides) and two tanks of unattached sponges were used. Tanks were placed into thermostatic water bath at 22 ± 0.5 °C and in dark conditions (light intensity of 0–30 lx). Larvae were collected using a plastic pipette from each tank and checked under light inverted microscopy (COIC IBE). When larvae were observed, photos were taken by digital camera (Olympus 3020). The number of larvae released in each tank was counted daily. The total number of larvae released by sponges in each tank during the whole experiment was calculated by summing the daily counts. The rate of larval release was calculated as the number of larvae released per gram wet sponge per day by dividing the number of larvae collected each day by the total wet weight of three sponge specimens in each tank. The mean number of larvae released per gram wet sponge during the whole experiment was calculated by dividing the total number of larvae collected from each tank throughout the entire experiment by the total wet weight of three sponge specimens. Larval release was followed until no larvae were released in the water for three consecutive days.

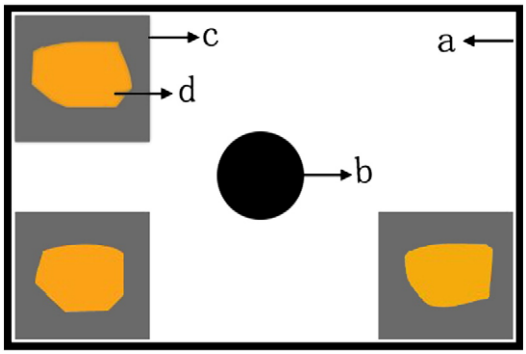

Fig. 1. Schematic drawing of the location of adult sponges in the culture tank. a: Culture tank; b: air stone; c: glass substrate; d: adult sponge.

2.3. Larval release under continuous illumination and dark incubation

To study the effect of light on larval release, three levels of light conditions were investigated: continuous dark incubation (D); 6000 lx artificial illumination at 12 h light:12 h darkness (L_{12}); and continuous 6000 lx artificial illumination (L_{24}). Three tanks of attached sponges were incubated at each light level and 22 ± 0.5 °C. Counts of released larvae in each tank were performed at 8:00am and 20:00pm each day. Larval production was calculated as described in Section 2.2. The light intensity was controlled by a set of fluorescent lamps (30 W each).

2.4. Larval release under different temperatures

To investigate the effect of temperature on larval release, attached sponges were incubated at four different temperatures (14 ± 0.5, 18 ± 0.5, 22 ± 0.5 and 25 ± 0.5 °C) in the dark (0–30 lx); two tanks of sponge specimens were used at each temperature level. Counts of larvae released in each tank were performed daily.

2.5. Larval settlement under different light intensities

Larval settlement was investigated under four different light intensities (0–30, 800–1100, 1800–2000, and 4000–5000 lx). We used 500 ml flasks containing 400 ml of seawater. This set of experiments was performed twice. In the first experiment, 40 larvae (20 larvae swimming in water and 20 larvae swimming or crawling on the bottom of the beaker) were placed in one flask at each light intensity; the experiment lasted for 68 h. In the second experiment, 20 larvae (swimming in water) were placed into one flask at each light intensity; the experiment lasted for 64 h. All larvae used in each experiment were collected from the same batch under darkness at 22 °C. A 50% water exchange was conducted every 12 h. The