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Instantaneous effect of dibromomethane on metamorphosis of larvae of the sea urchins *Strongylocentrotus nudus* and *Strongylocentrotus intermedius*

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Abstract

A volatile chemical, dibromomethane (DBM), produced from red coralline algae is known as a chemical inducer of larval metamorphosis of the sea urchin *Strongylocentrotus nudus*. We performed experiments exposing DBM to the larvae of *S. nudus* and *Strongylocentrotus intermedius* through a hydrophobic membrane. Metamorphic rates resulting from different diluted DBM solutions and exposure times were ascertained. The highest metamorphic rate, more than 80% in both species, was found after 1 h exposure to 1/2 diluted DBM. With this dilution, more than 80% of *S. nudus* and *S. intermedius* larvae metamorphosed 1 h after start of the experiment after only 10 and 5 min exposure, respectively, which corresponded to the low concentrations of 52–61 ppm and 34–43 ppm DBM by GCMS analysis, respectively. These findings suggest that DBM has an instantaneous effect on high success of metamorphosis of larvae of *S. nudus* and *S. intermedius*.

Keywords: Sea urchin; Metamorphosis; Dibromomethane; Larvae; *Strongylocentrotus nudus*; *Strongylocentrotus intermedius*

1. Introduction

Communities of crustose coralline red algae with no large erect macrophytes in subtidal rocky habitats are called “Barren ground” (Pearse et al., 1970), “Coralline flat” (Ayling, 1981), “Isoyake area”(Hagen, Hagen, 1983), “Deforested area” (Harrold and Pearse, 1987), “Heavy grazing bottom” (Keats et al., 1990), or “Urchin barren” (Coyer et al., 1993). In these communities, a specific benthic animal community consisting of gastropods, limpets and sea urchins as herbivores is established (e.g. Andrew and Choat, 1982).

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It is considered that the high density of sea urchins in crustose coralline communities is attributed to the high success rate of recruitment involving metamorphosis, high survival rate of juveniles and/or migration after settlement. However, juvenile migration is generally discounted as a causative factor due to their limited movement and physical barriers such as sand (Rowley, 1989; Watanabe and Harrold, 1991). Metamorphosis of echinopluteus larvae is induced by chemicals produced from crustose corallines. The larvae of *Strongylocentrotus purpuratus*, *S. franciscanus* (Cameron and Schroeter, 1980; Rowley, 1989), *Strongylocentrotus nudus* (Sano et al., 1998) and *Strongylocentrotus droebachiensis* (Balch and Scheibling, 2000) metamorphose abundantly on crustose corallines. Taniguchi et al. (1994) found that two articulated coralline algae *Serraticardia mazima* and *Calliarthron yessoense*, the crustose coralline alga *Lithophyllum yessoense* (dominant species in coralline communities), and the green alga *Ulvella lens* all induce larval metamorphosis of *S. nudus*. They ascertained that dibromomethane (DBM), a volatile chemical produced by all these algae (Itoh and Shinya, 1994; Ohshiro et al., 1999), induced 100% of larvae to metamorphose within 2 h. In that experiment, larval metamorphic rate was examined in petri dishes with seawater in which DBM was dissolved to a relatively high concentration of approximately 700 ppm. Moreover, the effect of exposure time of the larvae to DBM was not examined.

The fully developed 8-armed larvae contact the surface of the corallines just before metamorphosis (Taniguchi et al., 1994). Hence, metamorphosis appears to be induced by immediate reception of DBM, constantly released from the corallines (Itoh and Shinya, 1994). To simulate the relation between echinopluteus larvae and crustose corallines, we designed a new system that diffuses DBM through a hydrophobic membrane. In the present study, the optimum exposure time to DBM and the optimum concentration inducing larval metamorphosis were quantified.

2. Materials and methods

2.1. Metamorphosis by different DBM dilutions

The experiments on inducing larval metamorphosis of *S. nudus* and *Strongylocentrotus intermedius* were conducted at Fukushima Prefectural Fish Farming Center in October 2001 and 2002 and at Hokkaido Institute of Mariculture in September 2002, respectively. Larvae of both species were reared at a density of approximately 1 individual/ml in 0.5-m^3 rectangular tank at the flow rate of 0.8 l/min and fed *Chaetoceros gracilis* with 50,000 cells/ml/day for ca. 1 week at water temperatures of 17–18 °C. Light and dark conditions were controlled every 12 h. They grew to 8-armed competent larvae with fully developed urchin rudiments. Filtered seawater to 5 μm (250 ml) was added to a light protected 500-ml erlenmeyer flask covered with black tapes that had a glass stopper. Twenty five grams of dibromomethane (DBM, CH_2Br_2) (Wako Pure Chemical Industries Ltd.) was added and dissolved by stirring for 24 h. As DBM is likely to chemically resolve under light condition for a long time, the light protected one was used. Seawater with insoluble DBM at the bottom was considered to be a saturated solution. To establish metamorphic rate at different concentrations of DBM, the saturated solution was diluted with filtered seawater to 1/8, 1/4, 1/3 and 1/2. The larvae of *S. nudus* were examined also at 1/16 diluted solution. The experimental vessel was a filter holder (SUS316, Shibata Scientific Technology Ltd.) cut into upper and lower halves and held upright in an acrylic stand (Fig. 1). Ten millilitres of each diluted DBM solution were added to the lower vessel and sealed with a silicon rubber plug. A filter (Shibata Support screen: 47 mm) was embedded immediately on the seal and a hydrophobic PTFE membrane (Advantec polymer: 47 mm diameter, 0.2-μm pore size, Toyo Roshi Kaisha, Ltd.) was placed on it. The upper vessel was clamped onto the lower one. Filtered seawater to 5 μm (28 ml), with ca. twenty 8-armed larvae, was added to the upper vessel to initiate the experiment. Thus, DBM diffused from the lower vessel to larvae in the upper vessel through the hydrophobic membrane. After 1 h, the DBM solution in the lower vessel was discarded by removing the rubber plug.

A plastic plate (35 × 40 mm) covered with the green alga *U. lens* was added to a petri dish (5.5 cm diameter, 3.0 cm deep) containing 28 ml of filtered seawater to 5 μm and ca. 20 larvae and served as a positive control. A petri dish with only filtered seawater to 5 μm was a negative control (blank). After 1, 2, 4, 8, 16 and 24 h from the start of the experiment,