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RESEARCH ARTICLE

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Larval metamorphosis of the sea urchins, *Pseudocentrotus depressus* and *Anthocidaris crassispina* in response to microbial films

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Abstract In Japan, mass-production of sea urchin juveniles involves the culture of periphytic diatom films on plastic plates in 5- to 15-tonne tanks for the induction of larval metamorphosis. This study focused on the larval response of sea urchins, *Pseudocentrotus depressus* and *Anthocidaris crassispina*, to natural microbial films in the sea and diatom-based films formed in the tanks. The effect of diatoms and bacteria on larval metamorphosis was also examined using laboratory-cultured diatom-based films in the presence of germanium dioxide and antibiotics during film culture. Furthermore, the nature of the cue of the cultured diatom-based film was also investigated. Results showed that *P. depressus* and *A. crassispina* metamorphosed both on natural microbial films and diatom-based films in a tank. In the sea, the metamorphosis (%) of *P. depressus* increased gradually in accordance with the immersion period of film formed on glass slides, whereas the larval metamorphosis of *A. crassispina* had a bell-shaped response curve. In the tank, although the diatom-based films showed a low inducing activity for larval metamorphosis of *A. crassispina*, the metamorphosis of *P. depressus* larvae increased linearly in accordance with the diatom density. These results suggest that diatom-based films could promote the larval metamorphosis of *P. depressus*, but are less important in *A. crassispina*. In a simultaneous larval assay (May), *P. depressus* showed a higher percentage of metamorphosis than *A. crassispina*. We concluded that the former is more sensitive to diatom-based film than the latter and that this is due to differences in their natural habitats. For laboratory-cultured diatom-based film, both species of sea urchins showed a similar response, in which reduction in diatom and bacteria density resulted in a decrease in the original inducing activity. There seems to be a synergistic effect between diatom and bacteria in inducing larval metamorphosis. Films subjected to treatment with 0.1 N HCl were no longer inductive for either sea urchin, while those films treated with 40°C heat or EtOH (5% and 10% EtOH) showed a significant reduction in the inducing activity. Thus the surface-associated cues may be highly susceptible to the above treatments.

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Introduction

Most marine invertebrates have a planktonic larval stage preceding a benthic adult phase. The selection of a suitable habitat by the settling larvae often determines the long-term survival of juveniles and adults. Consequently, the larvae of a wide range of marine invertebrates do not settle and metamorphose unless they encounter specific conditions that are likely to ensure their growth and survival (Crisp 1974; Cameron and Schroeter 1980; Crisp et al. 1985; Snelgrove et al. 1999). Typically, settling larvae exhibit a specific searching behavior upon contact with a suitable surface such as microbial film, macroalgae and conspecifics. They react to the physical, chemical and biological properties of the substrate (Pawlik 1992). Many invertebrate larvae also exhibit the ability to delay metamorphosis until a suitable substrate is available (Barker 1977; Pechenik et al. 1993; Mercier et al. 2000).

The typical microbial-film formation is described as a succession of changes, beginning with the formation of a layer of organic molecules film consisting of amino acids, glycoproteins and humic materials, and advancing to the development of a complex of microbial film community (Loeb and Niehof 1975; Mitchell and