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Selection and viability after ingestion of vegetative cells, resting spores and resting cells of the marine diatom, *Chaetoceros pseudocurvisetus*, by two copepods

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Abstract

Feeding response of two copepods *Neocalanus flemingeri* and *Calanus sinicus* on cultures of three life-forms; vegetative cells, resting spores and resting cells, of *Chaetoceros pseudocurvisetus* was investigated. *N. flemingeri* fed heavily on the vegetative cells but scarcely responded to feed on the resting spores. *C. sinicus* showed significantly higher filtering rate on the vegetative cells and resting cells than on the resting spores. Survival of the three life-forms of *C. pseudocurvisetus* after gut passage of the copepods was also studied. The resting spores could germinate from fecal pellets of both *N. flemingeri* and *C. sinicus*; however, both the vegetative cells and the resting cells could not survive ingestion by the copepods. These results suggest that resting spore forming diatoms, such as *C. pseudocurvisetus* form spores which have a low nutritional value and during gut passage are largely indigestible due to the heavily silicified frustules and thus minimize the effects of grazing by copepods.

Keywords: Copepods; Diatoms; Grazing selectivity; Resting cells; Resting spores; Survival

1. Introduction

Marine planktonic diatoms have been shown to bloom intensively and are recognized to strongly influence the ocean carbon cycle (Smetacek, 1999). Many blooming diatom species form resting spores and/or resting cells under nutrient depletion at the termination stages of blooms (McQuoid and Hobson, 1996). Resting spores have heavily silicified frustules with distinct morphological structures from vegetative cells while resting cells have a similar appearance to vegetative cells. Both life-forms represent adaptive dormant stages characterized by depressed metabolic

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activity and storage of lipids and/or carbohydrates (Kuwata et al., 1993).

These resting stages have been shown to serve as seed banks for subsequent diatom blooms surviving while suspended within the water column under nutrient depletion (Kuwata and Takahashi, 1999) and/or settled in sediments (Itakura et al., 1997; McQuoid, 2002). For the maintenance of populations in natural waters, they also have been suggested to be able to resist attack of organisms in different trophic levels; such as grazing of zooplankton (Hargraves and French, 1983) or infection of bacteria (Kuwata and Takahashi, 1990; Oku and Kamatani, 1997). Only few studies, however, have been done to examine this important role.

To survive the effects of grazing by zooplankton, the following two strategies could be effective; avoidance of grazing of zooplankton and survival after gut passage. Zooplankton have been observed to show selective feeding behavior depending on the food quality of phytoplankton (Cowles et al., 1988; Butler et al., 1989). Resting spores and resting cells have a lower nitrogen content than vegetative cells (Kuwata et al., 1993) and the food quality of resting spores and resting cells for grazing zooplankton may be lower than that of vegetative cells. Selective feeding of zooplankton among the three life-forms can be expected to optimize their feeding efficiency. Hargraves and French (1983) observed the recovery of two diatoms, *Chaetoceros socialis* and *Detonula confervacea* from fecal pellets produced by zooplankton only if they fed on cultures that included the resting spores and suggested that the resting spores had the capability to survive gut passage of grazers. To clarify this suggestion, more detailed studies are necessary. Furthermore, no study has been conducted on survival of grazer ingestion of resting cells of diatoms.

Chaetoceros species are known to bloom (Guillard and Kilham, 1977) and almost all blooming *Chaetoceros* species form resting spores (McQuoid and Hobson, 1996). Distribution of *Chaetoceros pseudocurvisetus* is mainly restricted to warm water regions (Hasle and Syvertsen, 1997). This species has recently been frequently observed in warm waters in Japanese coastal regions with blooms occurring from autumn to spring (Takano, 1990). It has also been found in locally upwelling waters around the Izu Islands, Japan in summer (Kuwata and Takahashi, 1990). *Neocalanus flemingeri* is one of dominant large copepods in the subarctic Pacific and its marginal seas (Mackas and Tsuda, 1999) and during bloom periods has been shown to graze heavily on diatoms (Kobari et al., 2003). *Calanus sinicus* is a phytoplankton grazer and dominant copepod species in the shelf waters of the Northwest Pacific Ocean (Uye, 2000).

In this study, we examined experimentally: (1) the feeding response of copepods, *N. flemingeri* and *C. sinicus* among the three life-forms, vegetative cells, resting spores and resting cells of a coastal centric diatom, *C. pseudocurvisetus* and (2) the survival after gut passage of each life-form ingested by the copepods.

2. Materials and methods

2.1. Preparation of diatom cells

Isolation of the unialgal strain of *C. pseudocurvisetus* and maintenance conditions of the culture have been described previously (Kuwata and Takahashi, 1990). Each of the three life-forms, vegetative cells, resting cells and resting spores of this alga was prepared separately as described in Kuwata et al. (1993). Vegetative cells were cultured in f/2 medium (Guillard and Ryther, 1962) and harvested during the logarithmic growth phase. Resting spores and resting cells of this diatom were induced under nitrate depletion. Resting spores and resting cells were obtained separately by culturing in modified f/2 media with 10 μM nitrate and 150 μM silicate, and with 10 μM nitrate and 20 μM silicate, respectively. Algal samples were prepared in batch culture using 8 l polycarbonate bottles at room temperature under sufficient light for the experiment of *N. flemingeri* on board ship and 24 ± 1.5 °C under a 14 h light (*ca.* 600 μmol m^{-2} s^{-1} provided by cool-white fluorescent tubes): 10 h dark cycle for that of *C. sinicus* in the laboratory.

2.2. Collection of copepods

N. flemingeri (copepodite V stage) were collected by vertical hauls from 200 m to the surface at 0.7 m