Short Contribution

Effects of Phytoplankton Abundance on Excystment of Tintinnid Ciliates from Marine Sediments

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I have examined the effects of abundance of two species of phytoplankton, Pavlova lutheri and Heterocapsa triquetra, on the excystment of tintinnids from sediments. The total abundance of tintinnids excysting from sediments over five days increased with increasing concentrations of both phytoplankton species. This tendency was particularly notable for Eutintinnus tubulosus and Helicostomella longa. Concentrations $\geq 5.4 \times 10^4$ cells ml$^{-1}$ of P. lutheri inhibited the excystment of Tintinnopsis beroidea. The optimum concentration of P. lutheri at which excystment of tintinnid species is enhanced is $\geq 1 \times 10^4$ cells ml$^{-1}$, depending on the species of tintinnids.

Keywords: Tintinnid, ciliate, excystment, phytoplankton concentration, marine sediment.

1. Introduction

Several reports have presented data on the occurrence of tintinnids during certain limited periods of the year (Krsinic, 1987; Paranjape, 1987; Graziano, 1989). This phenomenon depends on the ability of tintinnids to form cysts, allowing them to survive under unfavorable conditions (Reid and John, 1978; Paranjape, 1980). Hence, tintinnid cysts probably play an important role in the population dynamics of tintinnid assemblages in coastal waters (Kamiyama and Aizawa, 1992).

To understand the significance of the cysts on the population dynamics of tintinnids, a simple method is needed for counting tintinnid cysts in marine sediments. Kamiyama (1996) found that the most probable number (MPN) method is effective for enumerating viable tintinnid cysts in sediments. When several serial 10-fold dilutions of a sediment sample are prepared with filtered seawater in the MPN method, one examines whether the target organisms are present or absent in the dilutions incubated under standard conditions. This method is advantageous as it estimates the number of viable cysts only, without a quantitative count of total cysts.

To use this method, it is necessary to clarify the optimum environmental conditions that induce excystment of the tintinnid cysts. Temperature is an important factor for excystment of each species, and it is species-specific (Kamiyama and Aizawa, 1992). Light irradiance of $\geq 6.9 \mu$E m$^{-2}$sec$^{-1}$ is also necessary for excystment (Kamiyama et al., 1995). Further, Kamiyama (1994) reported that extracellular products from phytoplankton stimulate excystment of Eutintinnus tubulosus and Helicostomella longa, suggesting that the existence of phytoplankton is essential in causing the excystment of these tintinnid species from sediments. However, the effects of phytoplankton abundance on the excystment of tintinnids have hitherto been unclear.

In this study, I have examined the effects of abundance of two species of phytoplankton, Pavlova lutheri and Heterocapsa triquetra, on the excystment of tintinnids from sediments.

2. Materials and Methods

Two sediment samples were collected on 17 February 1989 and 21 February 1996 at a coastal station (34°16'N, 131°16'E, 10 m deep) in Hiroshima Bay using a gravity core sampler (inner diameter 7.3 cm: Yamada and Kayama, 1987). The upper 3 cm of sediment, composed almost entirely of silt (weight percentage of particles less than 63 $\mu$m: 90%) was taken off each core, and was stored during 2 days in 1989 and 5 days in 1996 under cold (5°C) and dark conditions until the subsequent experiments were performed.

In 1989, an experiment was designed to examine the effects of a small nano-phytoplankton Pavlova lutheri (cell dimension $4 \times 4 \mu$m) on tintinnid excystment from sediment. This alga is known to stimulate excystment of tintinnids (Kamiyama, 1994) and has been used as a suitable food for tintinnids in laboratory cultures (Kamiyama and Aizawa, 1987). The whole sediment sample, suspended in two 150–200 ml aliquots of filtered seawater (Whatman GF/C), was sonicated with an ultrasonic cleaner (Sharp, UT-51N; Output, 35W) for 10–20 s to separate the cysts from other particles.
and then fractionated using 20 and 125 μm mesh-size nets with filtered seawater (Whatman GF/C). About 1 l of sediment suspension consisting of the 20–125 μm size fraction was prepared and divided equally into fifteen 200 ml-flasks. The 15 flasks, each containing 64 ml of the sediment suspension, were grouped into four experimental and one control treatments. Cell suspensions of *P. lutheri* (7 ml) were added to the experimental flasks for each treatment (final concentration: $1.5 \times 10^2$, $9.1 \times 10^3$, $5.4 \times 10^4$ and $3.3 \times 10^5$ cells ml$^{-1}$). Filtered seawater (5 ml) without phytoplankton was added to the control flasks. Three flasks for each treatment were incubated at 20°C and under 2000 lux on a 14-h light:10-h dark cycle without shaking. Each day, almost all the supernatant seawater in the flasks was collected without agitation of the sediment and immediately fixed with glutaraldehyde at a final concentration of 0.4%. Filtered seawater (50 ml) and an adequate volume of phytoplankton culture to maintain the initial conditions were then added to each residual sediment sample, and the incubation was continued under the same conditions. This procedure was repeated for 5 days to allow the most excystment to occur (Kamiyama and Aizawa, 1990). Fixed samples were concentrated by settling to about 3 ml, and tintinnids identified and counted for each sample using an inverted microscope. Abundance of tintinnids was pooled for the 5 days for each flask, and the data represented as the occurrence of tintinnids per square centimeter of sediment.

In 1996, another experiment was designed to examine the effects of the addition of a large nano-phytoplankton, *Heterocapsa triquetra* (cell dimension $17 \times 23 \mu m$), on tintinnid excystment from sediments. *Heterocapsa triquetra* is used in the culture of large sized tintinnids (e.g. genus *Favella*) and metazoans (Stoecker et al., 1981; Stoecker and Evans, 1985; Stoecker and Sanders, 1985; Uye and Takamatsu, 1990). The experiment was conducted in the same manner as the first one, except for the following points. The volume of sediment suspension prepared and algal suspension added to each flask was 67 and 5 ml, respectively (final concentration: $1.0 \times 10^2$, $5.1 \times 10^2$, $1.7 \times 10^3$ and $8.5 \times 10^3$ cells ml$^{-1}$). Supernatant seawater taken from each flask every day was fixed with Lugol' iodine at a final concentration of 2%. The samples taken over the 5 days were pooled for each flask, and then concentrated by settling to 1 ml. Tintinnids were identified and counted for each pooled sample on a Sedgwick-Rafter chamber using a phase-contrast microscope.

### 3. Results and Discussion

Figure 1 illustrates the effects of concentrations of *Pavlova lutheri* and *Heterocapsa triquetra* on the excystment of tintinnid species from sediments. The total abundance of tintinnids over the five days ranged from 17.6 to 89.4 individuals cm$^{-2}$ for *P. lutheri* and 5.3 to 23.8 individuals cm$^{-2}$ for *H. triquetra* and generally increased with increasing concentration of the two phytoplankton species. This tendency was particularly notable for *Eutintinnus tubulosus* and *Helicostomella longa* under the addition of both algae and for *Tintinnopsis kofoidi* in the presence of *H. triquetra*. *Eutintinnus tubulosus* was predominant at the highest concentration of *P. lutheri* ($3.3 \times 10^5$ cells ml$^{-1}$) and *H. triquetra* ($8.5 \times 10^5$ cells ml$^{-1}$). *Tintinnopsis beroidea* was represented more than 50% of all the tintinnids under conditions of less than $10^4$ cells ml$^{-1}$ of *P. lutheri*, and reached a maximum at $9.1 \times 10^3$ cells ml$^{-1}$ of *P. lutheri*. However, the occurrence of this species was independent of the abundance of *H. triquetra*.

Total occurrence of tintinnids under the addition of *P. lutheri* was generally higher than that under the addition of *H. triquetra*. Since the same sediment sample was not used for the two experiments, a detailed comparison of the results of these two experiments is not applicable. Occurrence of tintinnids in the control in the first experiment, however, was four-fold higher than that in the second experiment, implying that the difference of total occurrence of tintinnids was caused by the abundance of viable tintinnid cysts in the sediments collected at both time points.

The tintinnid assemblages counted in these experiments during 5 days are considered to include individuals which not only just emerged from cysts but also those which had been reproduced after excystment, because *Pavlova lutheri* and *Heterocapsa triquetra* are suitable foods for tintinnids. Hence, the number of tintinnids counted in these experiments may be larger than the real incidence of excystment under high concentrations of the algae. However, the increases of occurrence of tintinnids with increasing algae are probably caused by excystment of these tintinnids from sediments rather than their growth during the sampling intervals, for the following reasons. Growth rates of tintinnids generally saturate or decrease when food concentration increases over ca. 100 μgC l$^{-1}$ (Heinbokel, 1978; Verity, 1985). Since $9.1 \times 10^3$ cells ml$^{-1}$ of *P. lutheri* in this study converts to a carbon concentration of 150 μgC l$^{-1}$ according to Verity et al. (1992), the growth rates of tintinnids probably did not increase under food concentrations over this level. Further, although *Heterocapsa triquetra* is too large to be captured by *Eutintinnus tubulosus* and *Helicostomella longa*, the abundance of these tintinnids increased with increasing concentrations of this alga. These results confirm that extracellular products from phytoplankton induce excystment of *Eutintinnus tubulosus* and *Helicostomella longa* (Kamiyama, 1994), and suggest that excystment of the other species is also influenced by the presence of phytoplankton.

The occurrence of *Tintinnopsis beroidea* decreased in the presence of ≥$5.4 \times 10^4$ cells ml$^{-1}$ of *P. lutheri*, implying that concentrations of *P. lutheri* higher than $5.4 \times 10^4$ cells ml$^{-1}$ inhibited the excystment and/or survival of this species during the sampling intervals. An adverse influence of high
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Food concentrations on the growth of tintinnids has been observed in laboratory cultures (Gold, 1971; Verity, 1985). Further, Kamiyama and Aizawa (1992) reported that the number of this species in the field decreased in early summer, when phytoplankton are abundant, although temperature in situ was suitable for excystment of this species.

If excystment of tintinnid species can be induced under favorable food conditions, it is advantageous for rapid increases of the population size. The present experiments were an attempt to examine whether the occurrence of various species of tintinnids in sediments is influenced by the existence of the two algae. In the first experiment, the occurrence of the small tintinnids Eutintinnus tubulosus and Helicostomella longa was obviously promoted by the addition of Pavlova lutheri. This characteristic of the occurrence may explain why these species can dominate in the tintinnid community in Hiroshima Bay (Kamiyama and Tsujino, 1996). However, large tintinnid species which are able to feed on Heterocapsa triquetra were very few in number under all concentrations of this alga, probably due to the low abundance of cysts of these species in the sediment of the sampling station in Hiroshima Bay.

For the MPN method to enumerate tintinnid cysts, it is essential to set suitable conditions to assure excystment and survival and/or growth of tintinnids. The results of this study indicate that excystment of tintinnids from sediments depends on the concentration of phytoplankton. Hence, selection of phytoplankton species and adjustment of its concentration are important considerations for the MPN method. Pavlova lutheri, which can be ingested by small tintinnids, is a suitable species for use in the MPN method. Although a high abundance of this alga may be favorable in enumerating cysts of Eutintinnus tubulosus and Helicostomella longa, a concentration of $\geq 5.4 \times 10^4$ cells ml$^{-1}$ inhibited the occurrence of Tintinnopsis beroidea. Accordingly, the concentration that allows the excystment of many tintinnid species is found to be $\geq 1 \times 10^4$ cells ml$^{-1}$ of P. lutheri, depending on the species of tintinnids.

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References


