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Effects of Temperature on the Germination of Marine Phytoflagellate Cysts

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ABSTRACTS

The effect of temperature on the germination of some phytoflagellate cysts was studied in two different temperature conditions, step-gradient temperature and constant temperature. The highest number of newly germinated cell of *Scrippsiella* spp., *Alexandrium* spp., *Protoperidinium* spp., *Gyrodinium* spp., *Gymnodinium* spp., and *Diplopelta* spp. was observed after incubation 2 or 3 days when the initial temperatures were 13 and/or 19 °C, but it took at least 5 days at the initial incubation temperature of 10 °C. The germination rates of phytoflagellate cyst under optimum window should be faster than the other temperature ranges. The optimum temperature for cyst germination of *Alexandrium* spp., *Protoperidinium pellucidum* Bergh and *Protoperidinium* spp. was considered to be at 16 °C. For *Scrippsiella* spp., *Gymnodinium* spp. (small) and *Gymnodinium* spp., the optimum temperature for their cyst germination was considered to be 13 °C, whereas that for *Chattonella* spp. could be as high as 25 °C.

Key words: *Chattonella* spp., cyst germination, phytoflagellate, *Protoperidinium pellucidum*, temperature

INTRODUCTION

Many phytoflagellates produce resting cysts as part of their life histories. These resting cysts may survive several seasons before germination (Anderson *et al.*, 1983; Keafer *et al.*, 1992). Germination is considered to be the important mechanism of the outbreak of phytoflagellate red tides. One of the several roles attributed to cysts is that of inoculating vegetative cell into the water column to initiate blooms (Prakash, 1967; Imai and Itoh, 1987; Park and Hayashi, 1993). The germination of marine phytoflagellate cysts in natural environment has been regulated by several factors. The most important factor is the requirement for a period of development or maturation prior to which the cysts can not germinate (Anderson *et al.*, 1987). However, several reports suggested that water temperature was a major environmental factor regulating the germination of phytoflagellate cysts (e.g. Fukuyo *et al.*, 1982; Endo and Nagata, 1984; Park and Hayashi, 1993). Since deposited cysts were buried well below the sediment surface by bioturbation or sedimentation (Anderson *et al.*, 1982), cyst may not germinate during the optimal conditions for germination if the resuspending of that cyst did not occur. The evidences that many viable cysts remained in the sediments at times when temperatures were within the optimal ranges for

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germination have been reported by a number of authors (e.g. Heaney *et al.*, 1983; Anderson *et al*., 1983; Lewis *et al.*, 1985). Imai and Itoh (1987) suggested the turbulence due to bioturbation and/or tidal flow in the bottom layer to be the factor that enhanced the resuspension of phytoflagellate cyst. The other environmental factors which could regulate the germination of phytoflagellate cysts were considered to be light intensity, dissolved oxygen concentration, salinity level and nutrient concentrations, and a circannual internal rhythm (Anderson and Wall, 1978; Binder and Anderson, 1986; Anderson *et al*., 1987; An *et al*., 1992; Meksumpun, 1994; Montani *et al*., 1995; Perez *et al*., 1998). In contrast, some published data reported that the light had little or no effect on the germination of *Peridinium* sp. (Endo and Nagata, 1984; Sako *et al*., 1984) and *Alexandrium tamarense* (Perez *et al*., 1998) cysts. The requirement of light for the germination of cysts may thus depend on species of the phytoflagellates. In this study, it was attempted to determine the effects of temperature on the germination of marine phytoflagellate cysts from the sediment. The results might provide more information for understanding the mechanism of the out break of red tide.

MATERIALS AND METHODS

Cysts were recovered from natural sediments collected from 42 m-deep in the Harima Nada Sound and 27 m-deep in the Hiketa Bay, the Seto Inland Sea, Japan (Figure 1). The sediment was collected by a gravity core sampler. Sediment core samples were extruded on board ship and the overlying water and oxygenated surface sediment (upper 2 cm) was removed into plastic box. Samples were transported to laboratory and then stored at 10 °C in the dark for six months before used in experiments. In preparation of the cysts, ten grams of sediment from the Harima Nada Sound were suspended in 100 ml of filtered seawater. These cyst suspensions were sonicated for 1 min to separate cysts from attached substrates. The

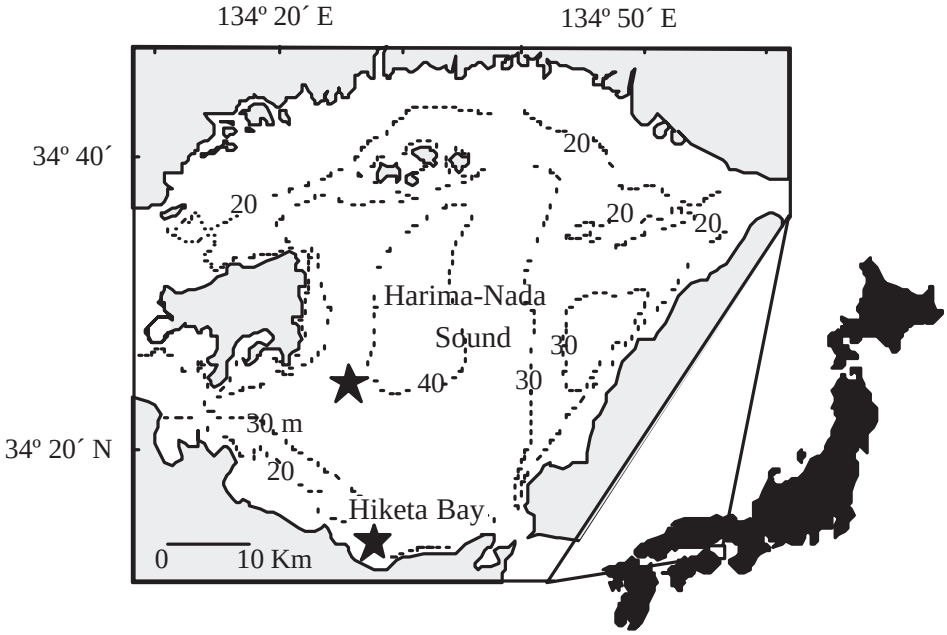

Figure 1 Map of the Harima Nada Sound and the Hiketa Bay. Locations of sediment collected stations illustrated with the stars.

samples were then sieved once through a 125 µm mesh screen and again through a 20 µm mesh screen. The 20-125 µm fractions were resuspended in 50 ml filtered seawater and transferred to 100-ml Erlenmeyer flasks. These flasks were incubated under the step-gradient temperatures. The temperature was started at 10 °C. Illumination was provided by cool-white fluorescent lamps at an irradiance level of approximately 80 µmol. $m^{-2}.s^{-1}$ on 14:10 hours LD cycle. Each treatment was done in duplicate. The subsamplings were done by removing the entire overlying water in the flasks off and then replaced with new 50 ml filtered seawater. The newly germinated cells in overlying water were observed under an inverted light microscope. The subsamplings were continued until the newly germinated cells could not be observed in the overlying seawater. The incubation under the constant different temperatures were also performed by using the sediment from the Hiketa Bay. The temperatures were controlled at 10, 13, 16, 19, 22, 25 and 28 °C. Subsampling procedure was done similarly to those of the step-gradient experiments until the newly germinated cells could not be observed in the overlying seawater.

RESULTS

The numbers of newly germinated phytoflagellate cell under step-gradient temperatures of 10 to 19 °C are shown in Figure 2. The highest numbers of the newly germinated cell of *Alexandrium* spp., *Protoperidinium* spp. and *Gyrodinium* spp. were observed after 9 days of the incubation when the temperature was 13 °C. For *Scrippsiella* spp., the highest number of the newly germinated cell was observed after 6 days of the incubation when the temperature was 11.6 °C. The highest number of the newly germinated cell of small *Gymnodinium* spp. (diameter; 10-15 µm) was observed after 5 days of the incubation when the temperature was 10.8 °C. *Chattonella* spp. cysts started to germinate after18 days incubation when the temperature was 16 °C. Figure 3 shows the numbers of newly germinated phytoflagellate cell under step-gradient temperatures of 13 to 22 °C. The highest number of newly germinated cell of *Alexandrium* spp. was observed after 4 days incubation when the temperature was 16 °C. The highest number of the newly germinated cell of *Scrippsiella* spp. was observed after 2 days incubation when the temperature was 13.8 °C. The number of newly germinated cell of the small *Gymnodinium* spp. markedly decreased when the initial incubation temperature was higher than 12 °C. The highest numbers of the newly germinated cell of *Diplopelta* spp., *Gyrodinium* spp. and *Protoperidinium* spp. were observed after incubation for 2 or 3 days when the temperatures ranged between 13 and 16 °C. For *Chattonella* spp., the highest number of the newly germinated cell was observed after incubation for 13 days when the temperature was 19 °C. Under step-gradient temperature ranging from 19 to 23 °C (Figure 4), the numbers of newly germinated cell of most phytoflagellate decreased remarkably compared to the numbers of newly germinated cell under the temperatures ranged between 13 to 22 °C. Cysts of *Chattonella* spp. could germinate well under this range of temperature. The highest number of the newly germinated cell of *Chattonella* spp. was observed after 6 days incubation when the temperature was 20.4 °C. Under step-gradient temperature ranging between 25 to 28 °C, the numbers of newly germinated cell of most phytoflagellate decreased markedly compared to the numbers of newly germinated cell under the temperatures of 19 to 23 °C except for *Gyrodinium* spp. The highest number of newly germinated cell of *Gyrodinium* spp. was observed after 3 days of incubation when the temperature was 25 °C. The highest number of newly germinated cell of *Chattonella* spp. was observed after incubation for 4 days when the temperature was 25 °C (Figure 5). Figure 6 shows the total numbers of the newly

germinated phytoflagellate cell of after incubation under different constant temperatures. The highest numbers of newly germinated cell of *Chattonella* spp., *Alexandrium* spp. and *Scrippsiella* spp. were obtained under the incubation at 25, 16 and 13 °C, respectively, whereas, the highest total numbers of newly germinated cell of *Gymnodinium* spp. (small), *Gymnodinium* spp., *Protoperidinium pellucidum*, *Diplopelta* spp., *Protoperidinium* spp. and *Gyrodinium* spp. were obtained under 13, 13, 16, 16-19, 16 and 19 °C, respectively.

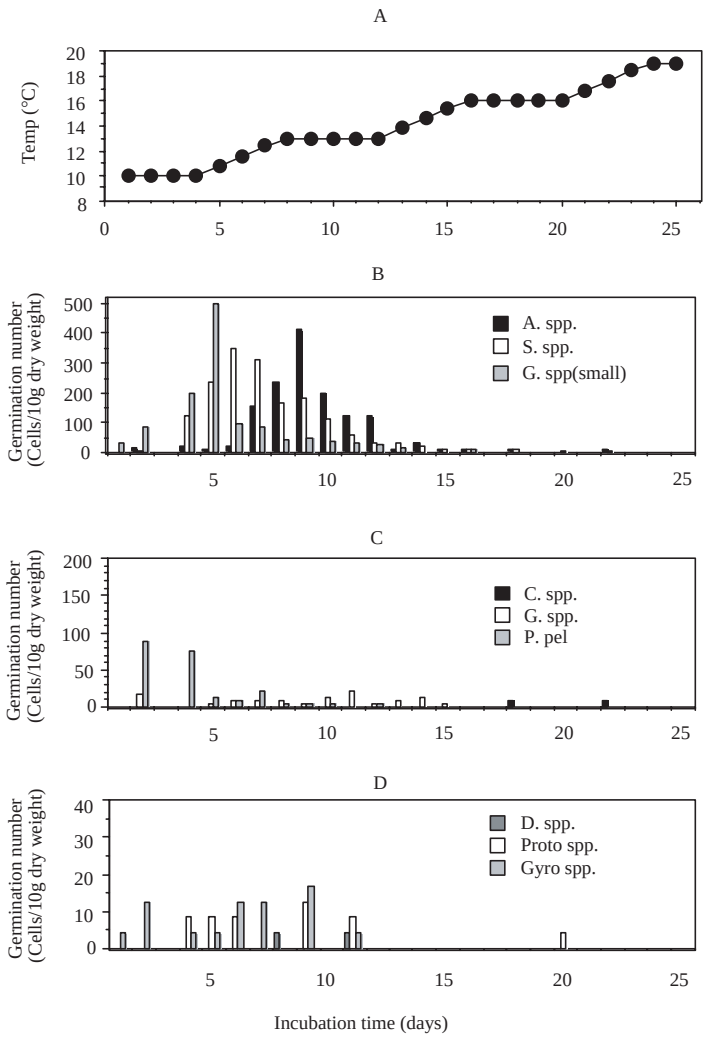

Figure 2 Numbers of newly germinated cell of phytoflagellate during incubation under step-gradient temperature ranged from 10 to 19°C; A: graph of step-gradient temperature; B: *Alexandrium* spp. (A. spp.), *Scrippsiella* spp. (S. spp.) and *Gymnodinium* spp. (small size, <20 μM) (G. spp. (small)); C: *Chattonella* spp. (C. spp.), *Gymnodinium* spp. (G. spp.) and *Protoperidinium pellucidum* (P. pel); D: *Diplopelta* spp. (D. spp.), *Protoperidinium* spp. (Proto spp.) and *Gyrodinium* spp. (Gyro spp.)

DISCUSSION

In order to explain the mechanism of the outbreak of red tide, studies on the effect of temperature on phytoflagellate cyst germination have been carried out by several workers (e.g. Park and Hayashi, 1993; Ishikawa and Taniguchi, 1996; Perez *et al.*, 1998). It has been reported that temperature had no significant effect on germination of *Alexandrium tamarense* cysts collected from St. Lawrence Estuary, Canada (Perez *et al.*, 1998). In contrast, temperature has been reported to be a major environmental factor regulating the germination of *Scrippsiella* spp.

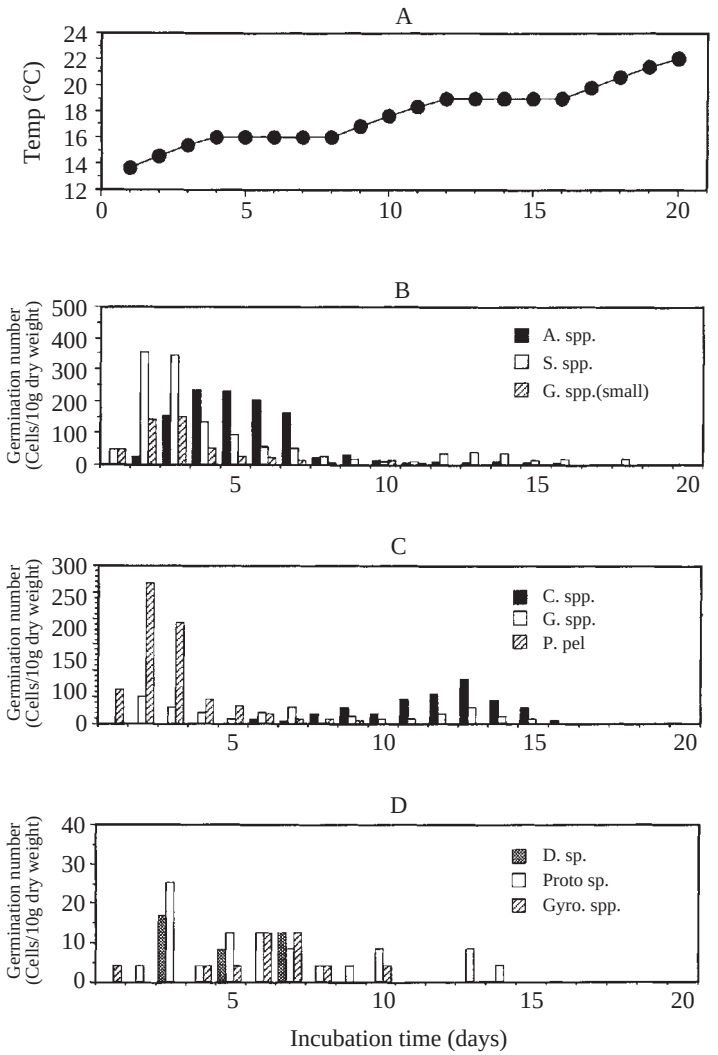

Figure 3 Numbers of newly germinated cell of phytoflagellate during incubation under step-gradient temperatures ranged from 13 to 22°C; A: graph of step-gradient temperature; B: *Alexandrium* spp. (A. spp.), *Scrippsiella* spp. (S. spp.) and *Gymnodinium* spp. (small size, <20 μM) (G. spp. (small)); C: *Chattonella* spp. (C. spp.), *Gymnodinium* spp. (G. spp.) and *Protoperidinium pellucidum* (P. pel); D: *Diplopelta* spp. (D. spp.), *Protoperidinium* spp. (Proto spp.) and *Gyrodinium* spp. (Gyro spp.)

cysts collected from the Onagawa Bay, Japan (Ishikawa and Taniguchi, 1996). In this study, the results clearly showed that temperature played an important role on the germination of phytoflagellate cysts. Although almost of phytoflagellate cysts could germinate under temperature ranged from 10 to 28 °C, the results indicated that some species of phytoflagellates could germinate well under the wide ranges of temperature, but not for some species. For example, *Gyrodinium* spp. could germinate well under the temperature ranges of 10-28 °C. This result was similar to the previous report that cysts of *Peridinium bipes* could germinate higher than 50% of total cysts under temperature ranged from 5 to 20 °C (Park and Hayashi, 1993). Additionally, *Scrippsiella* spp.

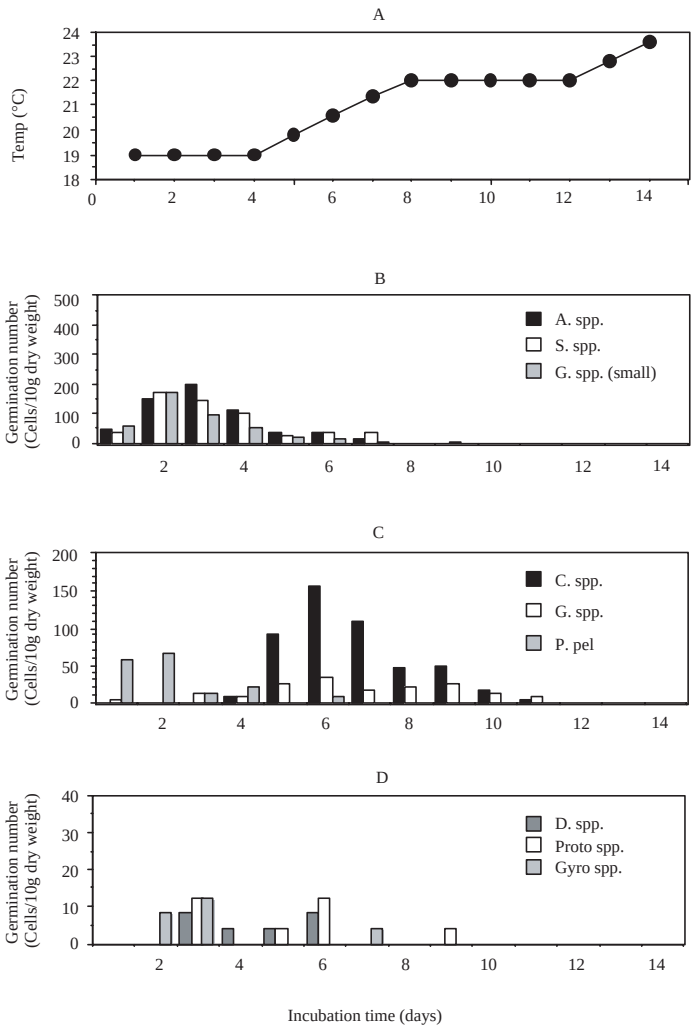

Figure 4 Numbers of newly germinated cell of phytoflagellate during incubation under step-gradient temperatures ranged from 19 to 23°C; A: graph of step-gradient temperature; B: *Alexandrium* spp. (A. spp.), *Scrippsiella* spp. (S. spp.) and *Gymnodinium* spp. (small size, <20 µM) (G. spp. (small)); C: *Chattonella* spp. (C. spp.), *Gymnodinium* spp. (G. spp.) and *Protoperidinium pellucidum* (P. pel); D: *Diplopelta* spp. (D. spp.), *Protoperidinium* spp. (Proto spp.) and *Gyrodinium* spp. (Gyro spp.)

can germinate at temperatures between 5 and 25 °C (Ishikawa and Taniguchi, 1996). However, these results showed that it had an existence of an optimum temperature window for cyst germination, with little or no germination below the lower and above the upper limits. Furthermore, the results indicated that the optimum temperature for cyst germination was species independented. For example, *Chattonella* spp. could not germinate under the temperature below 13 °C (Figure 6). *Chattonella* spp. could germinate well under the high temperature of 25 °C, whereas *Scrippsiella* spp. could germinate well under the low temperature of 13 °C. From the results of constant

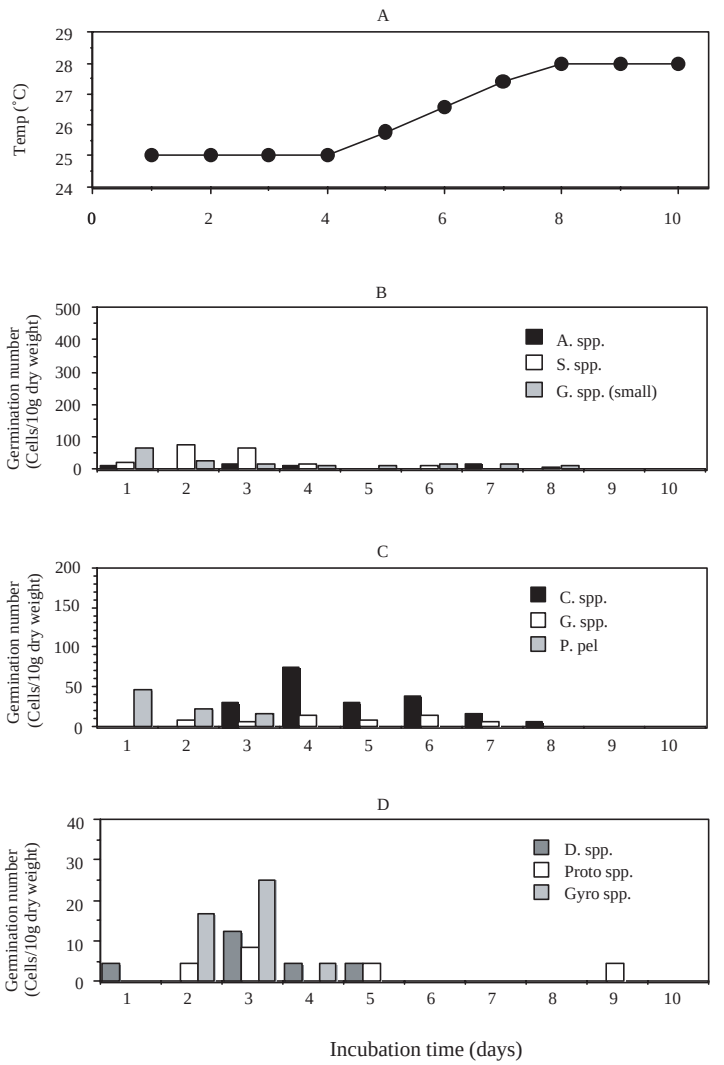

Figure 5 Numbers of newly germinated cell of phytoflagellate during incubation under step-gradient temperatures ranged from 25 to 28 °C; A: graph of step-gradient temperature; B: *Alexandrium* spp. (A. spp.), *Scrippsiella* spp. (S. spp.) and *Gymnodinium* spp. (small size, <20 ?M) (G. spp. (small)); C: *Chattonella* spp. (C. spp.), *Gymnodinium* spp. (G. spp.) and *Protoperidinium pellucidum* (P. pel); D: *Diplopelta* spp. (D. spp.), *Protoperidinium* spp. (Proto spp.) and *Gyrodinium* spp. (Gyro spp.)

temperature experiment (Figure 6), the optimum temperature for cyst germination of *Alexandrium* spp., *Protoperidinium pellucidum* and *Protoperidinium* spp. was considered to be at 16 °C, whereas these for *Scrippsiella* spp., *Gymnodinium* spp. (small) and G*ymnodinium* spp. were at 13 °C. The results showed that the highest number of newly germinated cell of *Scrippsiella* spp. could be observed after the incubation for 2 days when the initial incubation temperature was above 13 °C, but it took more than 5 days when the initial incubation temperature was 10 °C. For *Alexandrium* spp., *Protoperidinium* spp., *Gyrodinium* spp., *Gymnodinium* spp. (small), and *Diplopelta* spp., the highest numbers of newly germinated cells could be observed after the incubation for 2 or 3 days when the initial temperatures were 13 and/or 19 °C, but it took at least 5 days when the initial incubation temperature was 10 °C. This evidence was also observed in the incubation of phytoflagellate cysts which did not require light for germination. The germination rates under light condition was slower than that under dark condition (Binder and Anderson, 1986; Anderson *et al.*, 1987). The results should conclude that germination rates of phytoflagellate cysts under optimum window were faster than the other temperature ranges. Since the sediments using for step-gradient temperature experiment were in the same lot, the differences of total germinated cell of some phytoflagellate such as *Alexandrium* spp., *Chattonella* spp. and *Scrippsiella* spp. in each temperature dependent test should confirm that temperature could act as regulating factor for germination of these phytoflagellates. Overall views of this study provided more detail explaining the effect of temperature on the germination of some phytoflagellate cysts. These information may be helpful for understanding the mechanism of the outbreak of phytoflagellate red tide. Many scientists believe that germination of bottom cyst can act as potential seed population for the initiation of bloom (Dale, 1983; Park and Hayashi, 1993). However, this hypothesis seems not to be responsible for the blooms of *Alexandrium tamarense* in St. Lawrence Estuary, Canada (Perez *et al*., 1998) and for *Scrippsiella* spp. bloom in Onagawa Bay, Japan (Ishikawa and Taniguchi,

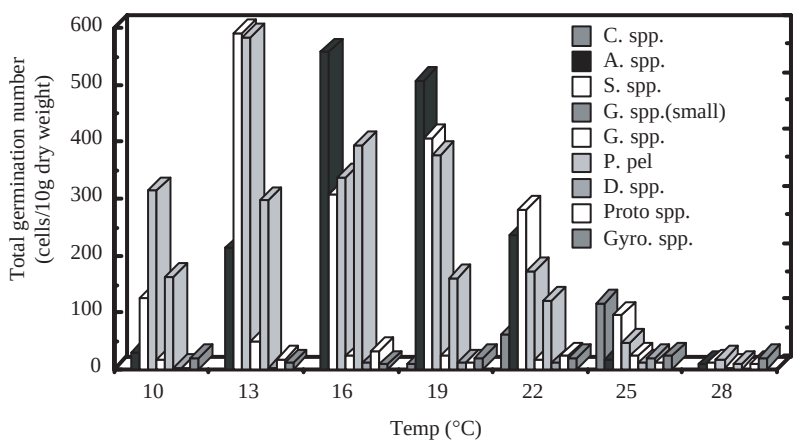

Figure 6 Total number of newly germinated cells of phytoflagellates after incubation under different constant temperature: C: *Chattonella* spp. (C. spp.), *Alexandrium* spp. (A. spp.), *Scrippsiella* spp. (S. spp.), *Gymnodinium* spp. (small size, <20 μM) (G. spp. (small)), *Gymnodinium* spp. (G. spp.), *Protoperidinium pellucidum* (P. pel), *Diplopelta* spp. (D. spp.), *Protoperidinium* spp. (Proto spp.), *Gyrodinium* spp. (Gyro spp.)

1996). In this study, the results clearly showed that some species could germinate rapidly when they suddenly exposed to the optimum temperature. Thus, in natural water when cysts are resuspended by physical factors or somewhat and exposed to optimum temperature, they can germinate rapidly to provide seed population or multiply the bloom. In the Seto Inland Sea, water temperature at bottom layer was higher than 25 °C during summer (Imai and Itoh, 1987). The results from this study showed that *Chattonella* spp. could germinate well under high water temperature (25 °C). The results could also explain why *Chattonella* red tides were capable to occur frequently during summer.

CONCLUSION

Water temperature could act as regulating factor for germination of marine phytoflagellate cysts. Different organisms were stimulated cyst germination by different water temperatures. For example, the optimum temperature for cyst germination of *Chattonella* spp. and *Gymnodinium* spp. were 25 and 13 °C, respectively. The resting cyst could serve as initiate bloom. Thus, this evidence might confirm why phytoflagellate red tides were capable to occur in different periods.

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