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Role of cyst germination in the bloom initiation of *Alexandrium tamarense* (Dinophyceae) in Masan Bay, Korea

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ABSTRACT: The role of cyst germination in the bloom initiation of the toxic dinoflagellate *Alexandrium tamarense* was examined in Masan Bay, Korea. Germination success was measured by the incubation of cysts isolated monthly from natural sediments and compared with vegetative cells and environmental factors (temperature, salinity and dissolved oxygen) in the water column. Germination maxima (80 to 90%) were observed during the period of decreasing water temperature in December 1996 and November 1997, while little or no germination occurred in summer. The seasonal germination exhibited an opposing pattern with temperature and similar seasonalities with salinity and dissolved oxygen, respectively. The bimodal nature of *A. tamarense* blooms, a large bloom in spring and a much smaller bloom in fall, was observed. Excysted cells in early spring can initiate the spring bloom and then proliferate to the bloom peak in increasing temperatures. Massive germination in fall contributes directly to the small bloom in fall. A temporal discrepancy between the peak of germination success and of vegetative population was found in *A. tamarense* dynamics from Korean coastal waters.

KEY WORDS: *Alexandrium tamarense* · Cyst germination · Vegetative population

INTRODUCTION

Phytoplankton species can be present in high numbers for a short period in polar, boreal and temperate latitudes, where seasonal variations in environmental conditions are large (Blomquist et al. 1995). Dinoflagellate blooms are particularly important due to their deleterious effect on fishing industries and public health. Blooms of *Alexandrium tamarense*, a toxic dinoflagellate known to produce paralytic shellfish poison (PSP), occur regularly in many temperate coastal waters throughout the world (Therriault et. al. 1985, Méndez et al. 1996, Sekiguchi et al. 1996). Outbreaks of PSP and *A. tamarense* blooms along Masan Bay, a major portion of the southeastern coast of Korea, have been nearly annual events for many years (Han et al. 1992, 1993, Kim et al. 1996). However, the influence of environmental factors, including physical and chemical parameters, on the bloom of *A. tamarense* is not fully understood.

Life cycles, especially the resting stage, need to be taken into consideration to fully explain seasonal succession of dinoflagellate blooms. The ecology of dinoflagellate cysts, especially *Alexandrium tamarense*, has been studied extensively in marine environments. Blooms are initiated by the germination of benthic cysts during spring warming (Anderson & Wall 1978, Anderson & Morel 1979).

However, there exist different patterns of bloom initiation of dinoflagellates. A temporal discrepancy between the peak of vegetative cells and of cyst germination rates was demonstrated in *Scrippsiella trochoidea* and other dinoflagellate species in Japan (Ishikawa & Taniguchi 1996, 1997). An inverse relationship between the germination ratio and the abun-

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dance of vegetative cells was found in Korean coastal waters (Kim & Han 2000). The germination strategy of *S. trochoidea* thus showed a similar pattern in different geographical regions.

The present study examines the factors that could potentially control cyst germination and affect bloom initiation of *Alexandrium tamarense*. Germination success and patterns were investigated by culturing naturally occurring cysts. The seasonal relationships between germination and blooms of *Alexandrium tamarense* were clarified in this study.

MATERIALS AND METHODS

Study area. The sampling station was located in Masan Bay on the southeastern coast of Korea (Fig. 1) that is heavily eutrophicated because of river runoff, which includes domestic and industrial wastewaters. Masan Bay has been known to be an area that commonly has spring blooms of *Alexandrium tamarense*. Average water depth at the station is 12 m.

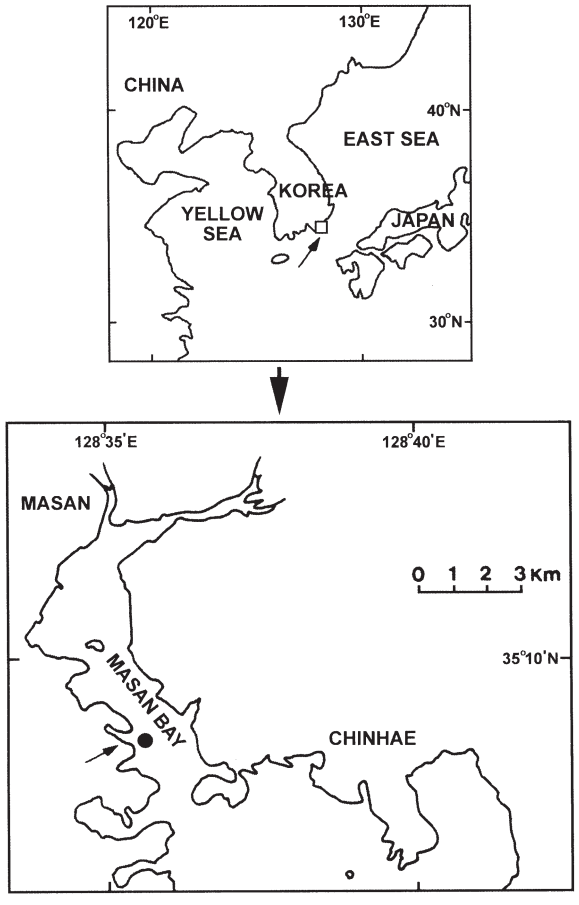

Fig. 1. Sampling site in Masan Bay on the southeastern coast of Korea

Water sampling and processing. Water samples were taken monthly from November 1996 to December 1997. A Van Dorn bottle (5 l) was utilized to collect water at 2 m intervals from 0 to 12 m depths. Subsamples were fixed in 2% (v/v) glutaraldehyde. Depending on the density of cells, 20 to 100 ml aliquot of the fixed samples were concentrated by sieving through 20 µm mesh. The vegetative cells were enumerated under a differential interference microscope (Zeiss, Axioplan) using a Sedgwick-Rafter chamber. Identification of *Alexandrium tamarense* was confirmed by dissecting thecal plates in 5% sodium hypochloride solution. Water temperature and salinity were recorded with a digital bathythermograph. Dissolved oxygen was measured by the Winkler method (Parsons et al. 1984).

Sediment sampling and processing. Sediment samples were collected monthly using a hand corer at the same time as the water samples. The top 2 cm of 3 cores were transferred to a plastic vessel and stored in the dark at 5°C until the cyst isolation. Surface seawater collected during each sampling time was filtered on Whatman GF/F filters, and used during the cyst isolation and culturing. The sediment in the vessel was mixed and 1 g wet weight was transferred into cooled, filtered seawater. This cyst suspension was sonicated for 30 s (Sharp UT 53N) to separate cysts from sediment, and sieved through 100 and then 20 µm mesh. The 20 to 100 µm fraction was transferred into filtered seawater and concentrated to a final sample volume of 10 ml. The cyst isolation and counting were done simultaneously. One ml of the fraction was placed in a Sedgwick-Rafter chamber and then intact cysts were counted and isolated by micropipetting using a capillary pipette under an inverted microscope. Only intact cysts with full cytoplasm and a red body were isolated and counted.

Cyst germination experiments. The isolated cysts were quickly washed in cooled, sterile filtered seawater and inoculated one by one into wells of tissue culture plates filled with the same filtered seawater. After which, they were placed on a coolant bag during the sorting. This preparation was completed within a week after sediment sampling. The isolated cysts were incubated at the water temperature measured at 12 m depth at the sampling station (Fig. 2A) and at 20 µmol photons m^{-2} s^{-1} cool-white illumination with a 12:12 h light:dark cycle. For 10 d, germination of cysts was confirmed daily under an inverted microscope. Cysts that had not germinated were checked at 2 to 5 d intervals. The germination success was calculated as a ratio of cumulative excystment over 30 d compared to the total number of inoculated cysts. Each experiment with 20 cysts was performed in triplicate.

To distinguish *Alexandrium tamarense* from *A. catenella* cysts based on morphological features is impossible. However, since *A. catenella* was not detected at the sampling site, it is assumed that all cysts examined can be described as *A. tamarense*.

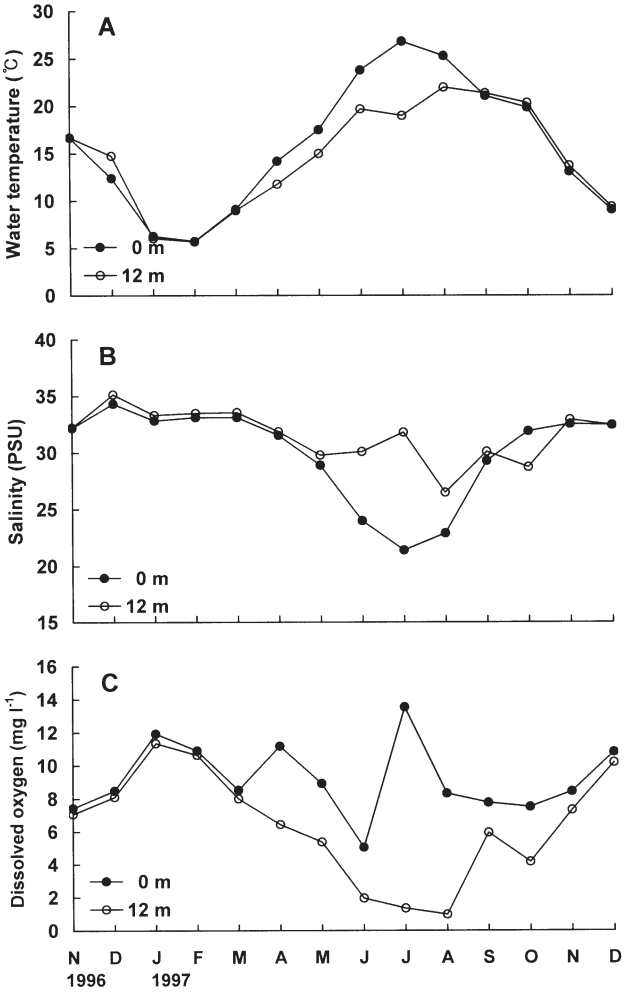

Fig. 2. Seasonal changes of (A) water temperature (°C), (B) salinity (PSU) and (C) dissolved oxygen (mg l^{-1}) at 0 (●) and 12 m (○) depth

RESULTS

Environmental conditions

Water temperature at the surface and 12 m depth displayed a clear seasonal cycle. Temperatures <10°C were recorded from January to March and >20°C from June to October (Fig. 2A). The seasonal maximum in August was 25.3°C at the surface, with a minimum of 6.0°C in January at 12 m. Moderate temperatures around 15°C were recorded in November and December in fall, and April and May in spring.

Inverse relationships were observed between salinity and temperature. Salinity varied between 22.9 PSU in August 1997 at the surface and 35.1 PSU in December 1996 at 12 m. Lower salinities at the surface from June to August were due to the summer monsoon (Fig. 2B).

Concentration of dissolved oxygen in the bottom layer (ca. 12 m) varied seasonally as an inverse of the temperature. The concentration was greater than 8 mg l^{-1} during the cold season from December to March, and as low as 1 to 2 mg l^{-1} during the warm season from June to August (Fig. 2C).

Generally, surface water conditions followed similar trends as bottom waters.

Seasonal changes in vegetative cells and cysts

The vegetative population of *Alexandrium tamarense* in the water column showed a typical spring bloom. Cells were detected from March coincident with an abrupt increase (from 5.7 to 9.1°C) of surface temperature (Figs. 2A & 3). Cell density reached a maximum (1.0×10^4 cells l^{-1} at 4 m) in May when a moderate temperature (17.5°C) was recorded. The cell density rapidly decreased from June when the water temperature was over 20°C and was followed by a summer interval with no vegetaive cells. A small population of *A. tamarense* also occurred in very low

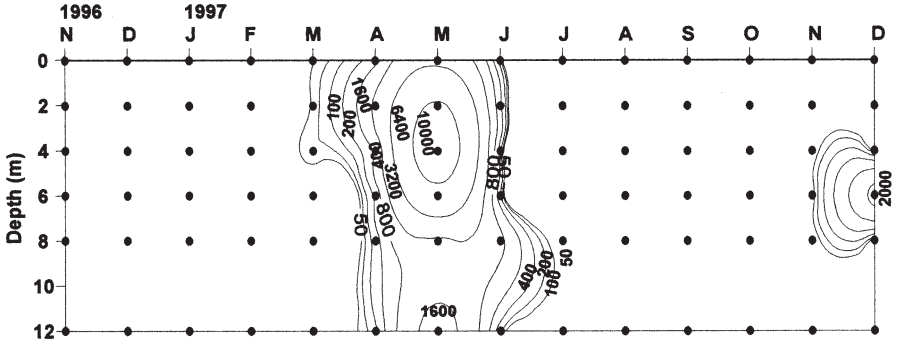

Fig. 3. *Alexandrium tamarense*. Seasonal changes in vertical distribution of vegetative cells (cell l^{-1})

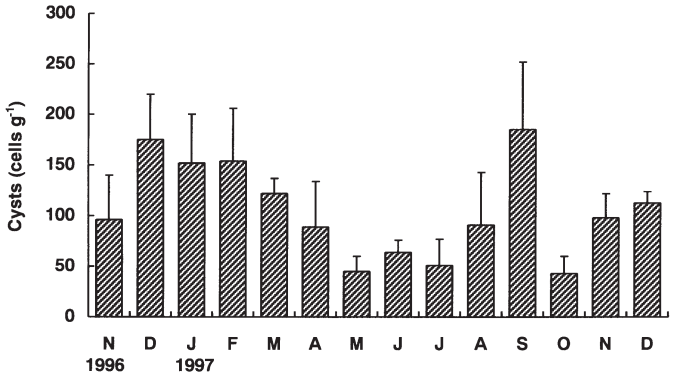

Fig. 4. *Alexandrium tamarense*. Seasonal changes in cyst abundance (cells g^{-1}) in sediment. Mean ± SD, n = 3

density (ca. 2.0×10^3 cells l^{-1}) in December 1997 at 6 m (Fig. 3). Therefore, the bimodal nature of *Alexandrium* blooms, a large bloom in spring and a much smaller bloom in fall, was observed in this study.

Mean cyst abundance varied from 43 to 185 cells g^{-1} (Fig. 4). Higher abundance above 150 cells g^{-1} was observed from December 1996 to February 1997. A decrease in cyst abundance beginning in March was found and this decreasing trend continued through the early summer until July. After July, a notable increase of cyst abundance was recorded until September.

Seasonal change in germination ratios

The monthly incubations of cysts demonstrate seasonally differing germination success. Germination abruptly increased within 1 or 2 d in November and December 1996, and reached a maximum ratio of over 70% in December 1996 (Fig. 5). From January to April, germination was delayed and gradually decreased. From May to September, germination success was significantly lower (0 to 5%). Namely, during the warmer season the cysts showed little or no germination. A slight recovery of germination occurred in October in connection with an abrupt increase in ratio over 70% in November 1997. Therefore, germination ability displayed a similar trend in the fall of 1996 and 1997.

DISCUSSION

Among the environmental factors investigated, temperature has been shown to be most important in regulating germination of *Alexandrium tamarense* (Anderson & Morel 1979, Anderson 1980). For *A. tamarense* from Cape Cod, cysts cannot germinate at cold and warm extremes. The permissive temperature window for germination ranged from 5 to 21°C (Anderson 1998). Since bottom temperatures varied from 6 to 22°C in Masan Bay, germination might be possible throughout the year except in summer when temperatures are above 21°C. Temperature effect was assessed by culturing naturally occurring cysts which resulted in higher germination at 10 and 15°C, while no germination was observed at 20 and 25°C (Park 1999). A constant germination rate of about 20% was observed throughout the year in St. Lawrence estuary, Canada, where bottom temperature was in the range of 2 to 15°C (Perez et. al. 1998). Therefore, low to intermediate temperatures (5 to 15°C) are favorable for active germination of *Alexandrium tamarense*. The germination of *A. tamarense* in Masan Bay is more successful in winter than in summer (Fig. 6). Very little germination in summer may be a result of the inhibition of germination by the high temperatures as well as the maturation time required for newly deposited cysts.

The dormancy period of *Alexandrium tamarense* cysts was 2 mo at 17°C (Turpin 1978), 1 mo at 22°C and 4 mo at 5°C (Anderson 1980). Thus, different cyst dormancy periods of the same species can be controlled by different geographic environmental conditions (Hallegraeff et al. 1998). Here, the dormancy period for Korean strains of *A. tamarense* can not be compared because of the absence of germination experiments of new cysts obtained from synchronized encystments. However, the period can be indirectly estimated by the 2 events of mass encystment in June (unpubl. data) and rapid recovery of excystment in November. The 5 mo between June and November when little or no germination has been recorded, may reflect the length of time required to achieve germination of newly formed cysts.

Oxygen is known to be another factor affecting germination by resting stages. Excystment of *Alexandrium tamarense* and other marine species was completely inhibited by anoxia (Anderson et al. 1987). Cysts cannot be germinated in anoxic sediments even when favorable temperature conditions are provided. Seasonal changes of excystment and bottom dissolved oxygen exhibited a similar curve (Fig. 7). Thus, the germination in the field might be inhibited by low dissolved oxygen as well as high temperatures that resulted in summer inactivity in Masan Bay.

Germination of *Alexandrium tamarense* cysts from natural sediments in Masan Bay was also examined under several salinity conditions, namely 25 to 34 PSU, which is within the range recorded from bottom water (Park 1999). There was no significant difference of germination success under different salinities. In the case of *Alexandrium minutum* in south Australia, germination success was highest between 14 to 26‰ (Cannon 1993). It was suggested that low salinity due to freshwater inputs increased germination of the cysts. How-

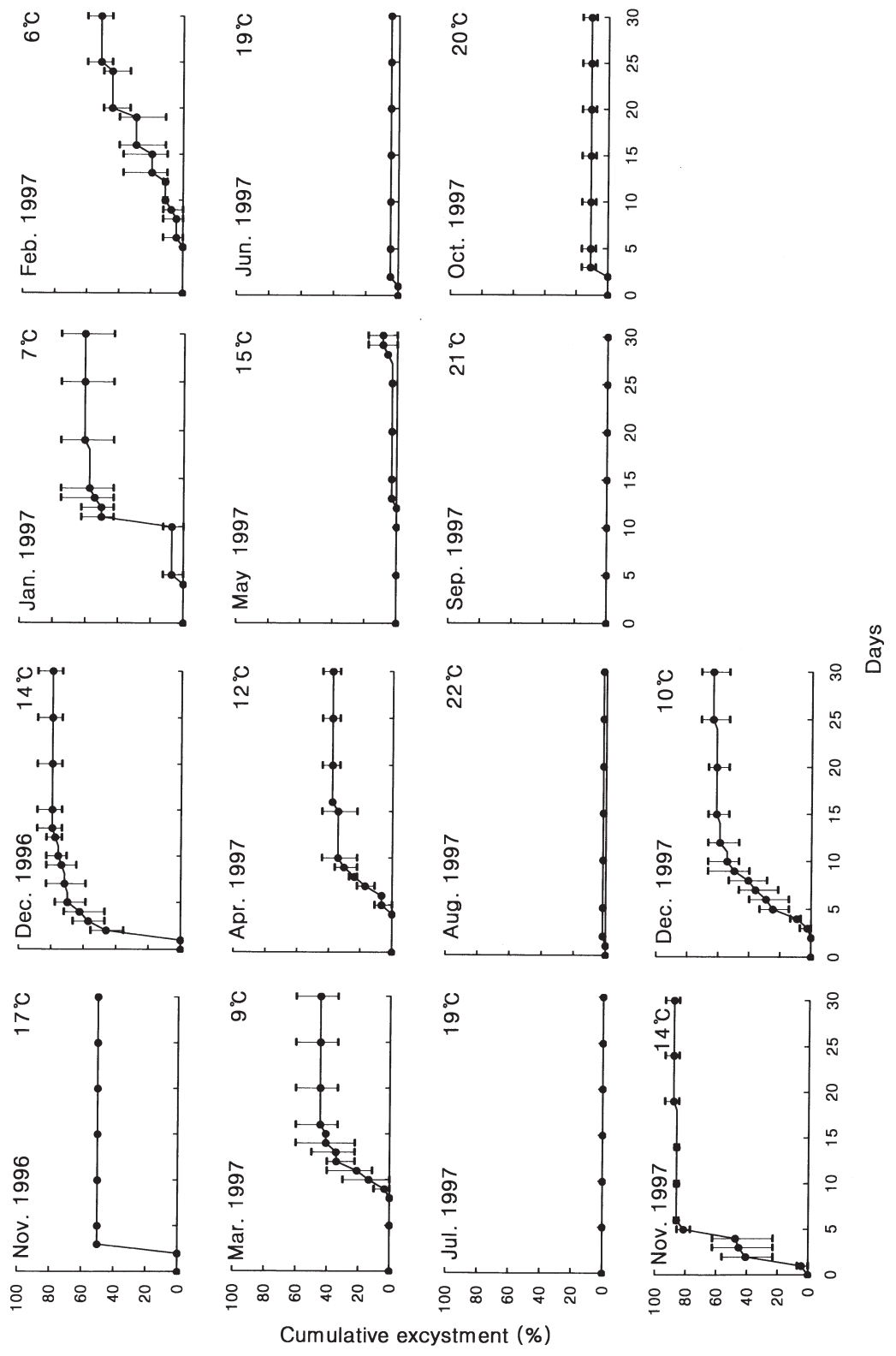

Fig. 5. *Alexandrium tamarense*. Seasonal changes in excystment of cysts collected monthly from Masan Bay and incubated at ambient (12 m) water temperatures (Fig. 2A) and 20 µmol photons m^{-2} s^{-1} with a 12:12 h light:dark cycle. Mean ± SD, n = 3

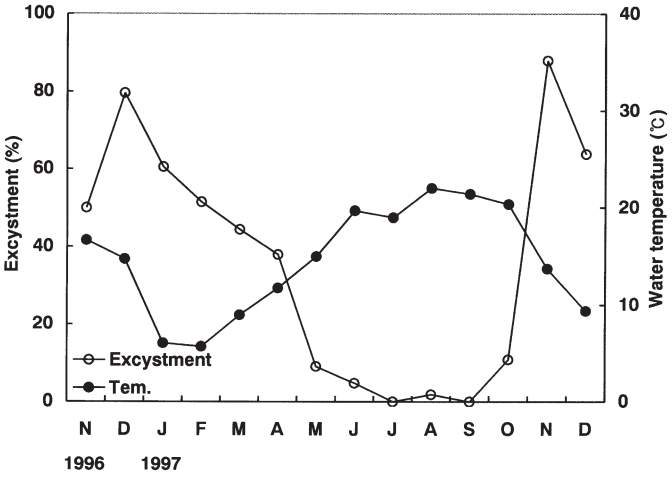

Fig. 6. *Alexandrium tamarense*. Seasonal changes in cumulative excystment (%) and bottom (12 m) temperature (°C)

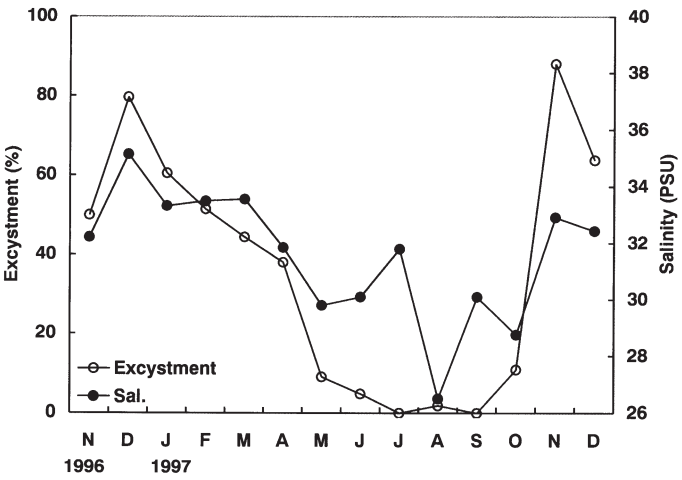

Fig. 8. *Alexandrium tamarense*. Seasonal changes in cumulative excystment (%) and bottom (12 m) salinity (PSU)

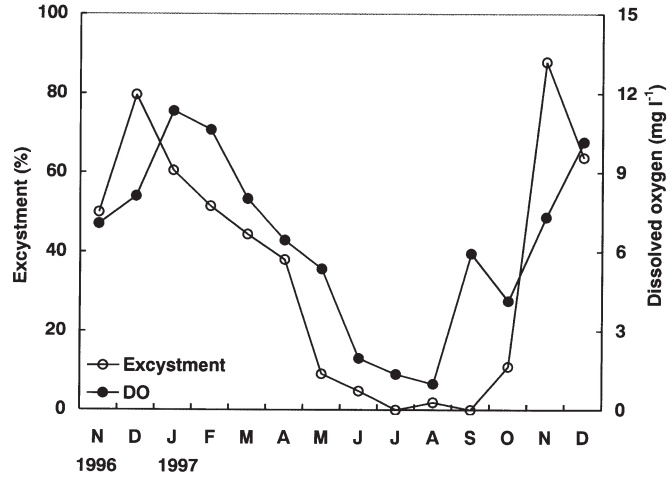

Fig. 7. *Alexandrium tamarense*. Seasonal changes in cumulative excystment (%) and bottom (12 m) dissolved oxygen (mg l^{-1})

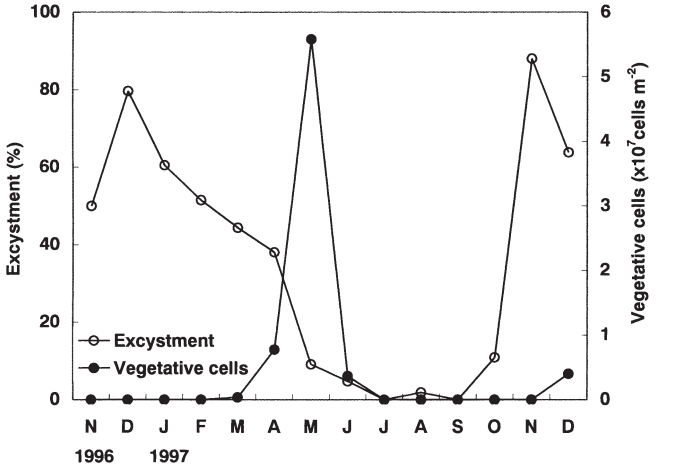

Fig. 9. *Alexandrium tamarense*. Seasonal changes in cumulative excystment (%) and vegetative cell numbers (cells m^{-2})

ever, in the present study with *A. tamarense*, germination success was very low during the summer period with lower salinity due to the summer monsoon (Fig. 8).

The scale of encystment and excystment largely affects cyst abundance in sediments. In general, it is recognized that sediment cyst concentrations of *Alexandrium tamarense* increase during or after blooms of vegetative cells in the water column and decrease prior to the bloom (Perez et. al. 1998). Similarly, there were significant decreases in cyst concentrations beginning in March, just at the time *A. tamarense* bloom begins. Mass encystment was observed in June when the spring bloom started to decline (unpubl. data). Newly formed cysts are expected to deposit on the sediments and lead to an increase of cyst abundance. Taking into consideration the sinking speed of *A. tamarense* cysts (8.6 m d^{-1}; Anderson et al. 1985), new cysts in the water column settle rapidly on the sediments as detected in the increase of cyst abundance from July. Furthermore, cyst germination in the sediments was on a minor scale from July to September in summer. The increase of cyst abundance from July to September may reflect the eventual dominance of cyst deposition over cyst germination. Therefore, the number of cysts in the sediments seems to correlate reasonably well with bloom dynamics, both during initiation and decline.

The bimodal nature of *Alexandrium* blooms in this study is similar to the results from a Cape Cod salt

pond (Anderson & Morel 1979), i.e. a large bloom in the spring followed by a summer interval with no vegetative cells and a much smaller bloom in the fall. The dynamics of the bloom populations are the same in these 2 very distant regions. In the salt pond, the higher germination success recorded in spring and fall coincided with the vegetative population blooms. Lower germination success was observed during high temperature seasons in both regions. The spring bloom of *Alexandrium tamarense* in the salt pond was triggered by germination of the overwintering hypnozygotes concomitant with a gradual increase in temperature. Also in this study, the early spring excystment in March and April, which takes place at moderate water temperatures of 9 to 15°C, might contribute to seeding the bloom. Although the germination success in early spring was not high, the excysted cells could proliferate in increasing temperatures.

A 5 mo interval between the germination and the bloom peak (Fig. 9) was in a lower temperature period (<10°C). As the optimal temperature for growth of vegetative cells in north temperate waters is ca. 15°C (Ogata et al. 1987, Anderson & Keafer 1987, Perez et. al. 1998), it is difficult to sustain the winter populations originated by massive fall germination. Predators of mesozooplankton also threaten population sustainability. Therefore, pre-winter germination is not effective as a seed for the spring bloom of *Alexandrium tamarense.* Similar germination results of *A. tamarense* were observed in Hiroshima Bay (Itakura & Yamaguchi 2001). Interestingly, the temporal discrepancy between germination and blooming was also observed in another dinoflagellate, *Scrippsiella trochoidea*, from Yongil Bay on the southeastern coast of Korea (Kim & Han 2000).

The vegetative population developed from the excysted cells in the fall was very difficult to detect in a countable biomass. We monitored the population dynamics of *Alexandrium tamarense* for 10 yr in the same study area. The occurrence of vegetative population in the fall was seldom detected in the common sampling method using the Van Dorn sampler due to much smaller cell density in the water column. Further investigation should try to find an ecological meaning of the massive fall germination and the small fall population in regards to the survival advantages of *A. tamarense*.

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