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Excystment patterns of the freshwater dinoflagellate *Peridinium bipes* (Dinophyceae) in Juam Reservoir, Korea

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ABSTRACT: We examined seasonal variations in vegetative populations and cyst germination of the dinoflagellate *Peridinium bipes* Stein in reservoir water samples collected from August 2003 to March 2005, a period that included a sudden bloom of this species (September 2003 to March 2004). Monthly variations in cyst abundance and germination were tracked, and the effects of environmental factors (water temperature, pH, light and nutrients) on cyst germination were measured in the laboratory under ambient field conditions. During the bloom period, the cyst abundance of *P. bipes* in sediment samples fluctuated from 4 to 427 cells g^{-1} (wet weight), and did not show season-dependent variation. During the same period, the number of vegetative cells of *P. bipes* in water samples varied from 0 to 9.79×10^2 ml^{-1}. Laboratory experiments revealed a maximum germination rate at 15.6°C, and effective germination was observed at the naturally occurring pH values of 6 to 8, but not at pH 9. Cysts obtained from samples collected at higher temperatures (over 15°C) germinated more quickly than those seeded at lower temperatures, while cysts collected in fall and early winter had a higher cumulative excystment rate than those collected in spring and summer, suggesting that cysts deposited at higher temperatures may act as a seed population for the winter blooms. These findings collectively indicate that germination of *P. bipes* was mainly affected by water temperature and light intensity, and not nutrient levels and pH, and further show that the bloom of *P. bipes* observed in Juam Reservoir was likely promoted by the presence of sufficient nutrients, relatively high excystment rates and active growth occurring under low temperature conditions.

KEY WORDS: Cyst germination · Dinoflagellates · Environment · Freshwater red-tide · *Peridinium bipes*

INTRODUCTION

Many dinoflagellates, including various species of *Peridinium*, form resting cysts during their sexual reproduction cycles (Fryxell 1983, Rengefors et al. 1998). Species of *Peridinium*, such as *P. bipes* Stein, occur in dense blooms in freshwater reservoirs and lakes throughout the world (Pollinger & Berman 1975, Park & Hayashi 1993, Wu et al. 1998, Yacobi 2003). *Peridinium* blooms, which are mainly initiated by the germination of benthic cysts (Pollinger & Berman 1975, Park & Hayashi 1993, Rengefors et al. 1998), may have adverse effects on domestic drinking water supplies, including clogging of filtration systems and/or unpleasant tastes and smells (Anderson 2000).

The encystments (cyst formation) and subsequent excystments (cyst germination or vegetative cell formation) of dinoflagellates are components of survival under adverse environmental conditions, as well as bloom initiation, species dispersal, reproduction, and preservation of genetic variation (Dale 1983, Rengefors & Anderson 1998, Kishimoto et al. 2001). An improved understanding of the factors underlying cyst germination may provide important new insights into the management of dinoflagellate outbreaks in marine and freshwater environments. Numerous studies have

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shown that dinoflagellate encystment can be triggered by various biotic and abiotic factors, including predation by zooplankton and fish (Burkholder & Glasgow 1995), changes in light, dissolved oxygen, temperature and nutrients (Chapman & Pfiester 1995, Perez et al. 1998, Rengefors & Anderson 1998, Kremp & Anderson 2000). To date, although 2 previous studies on *P. bipes* f. *oculatum* have examined the effects of environment on excystment (Park & Hayashi 1992, 1993), there is no general consensus on the process in *P. bipes*, due perhaps to contradictions arising from differences in species, localities and experimental methods.

Peridinium bipes may be divided into several intraspecific taxa, including *P. bipes* var. *excisum* Lef., *P. bipes* f. *globosum* Lef., and *P. bipes* f. *oculatum* (Elster & Ohle 1968). *P. bipes* populations that we worked with differed from previously studied *P. bipes* f. *oculatum* in that, while both have the apical pore complex specific to the *P. bipes* group (Stein 1883, Abé 1981), individuals we examined had 2 short spines on the ventral area of the antapical plates, while *P. bipes* f. *oculatum* does not. *P. bipes* cysts collected from Juam Reservoir were ovoid or spherical in shape (30 to 60 µm diameter), and bore oval or rod-shaped red spots (5 µm wide, 15 µm long). Notably, these cysts were aggregated with mucilaginous particles and debris, so that the samples required several minutes of sonication prior to separation.

Juam Reservoir in South Korea, which was constructed in 1992, is mesotrophic due to high organic loads from domestic and agricultural wastewaters. Nuisance algal blooms associated with the cyanobacterium *Microcystis aeruginosa* have occurred every year since construction (Kim 1996, Lee et al. 2005). Prior to 2003, *Peridinium bipes* did not represent a major portion of the phytoplankton community in the reservoir; however, a sudden bloom of this dinoflagellate occurred between September 2003 and March 2004. Here, we examine factors that may potentially control cyst germination and bloom initiation in Juam Reservoir. Germination patterns and dormancy period of benthic cysts were investigated *in vitro*, and the *in situ* seasonal relationships between cyst germination and bloom occurrence were clarified.

MATERIALS AND METHODS

Study area. Juam Reservoir has a surface area of 1010 km^2, and is located southwest of the Yeosu peninsula near the coast of South Korea. The reservoir has an altitude of approximately 400 m above sea level, is surrounded by mountainous valleys (Fig. 1), and is fed by 2 large tributaries of the Seomjin River, Bosung and Dongbok streams. The present field study was conducted from August 2003 to March 2005 in the vicinity of the Mundeok Bridge (35° 00′ N, 127° 11′ E), which crosses the upper part of Juam Reservoir. The sampling site was located at the point where Bosung and Dongbok streams merge; this site has an expansive watershed, hypersaturated dissolved oxygen levels, high nutrient levels and high transparency (Kim 1996, Lee et al. 2005).

Water sampling and sorting of *Peridinium bipes* cysts. Water samples were taken at the surface (~50 cm) monthly from August 2003 to March 2005 using a Van Dorn sampler (5 l, General Oceanics). Subsamples were fixed in 2% (v/v) glutaraldehyde. For enumeration of phytoplankton, fixed samples were concentrated by settling, and vegetative cells were enumerated under a differential interference microscope (Axioplan, Zeiss) using a Sedgwick-Rafter chamber. Water temperature, dissolved oxygen and pH were measured at the time of sampling using an YSI meter (6600). Ammonium, nitrate, nitrite, nitrogen and soluble reactive phosphorus were measured with an automatic analyzer (TRAACS800, Bran-Luebbe) (APHA 1992). Samples in disposable polycarbonate bottles were treated with persulfate in an autoclave at 120°C for 45 min, then total phosphorus (TP) and total nitrogen (TN) were measured.

Concurrently with each monthly water sampling, sediment samples were collected in triplicate using a hand corer. The top 2 cm of each core was transferred to a plastic vessel and stored in the dark at 5°C. Sur-

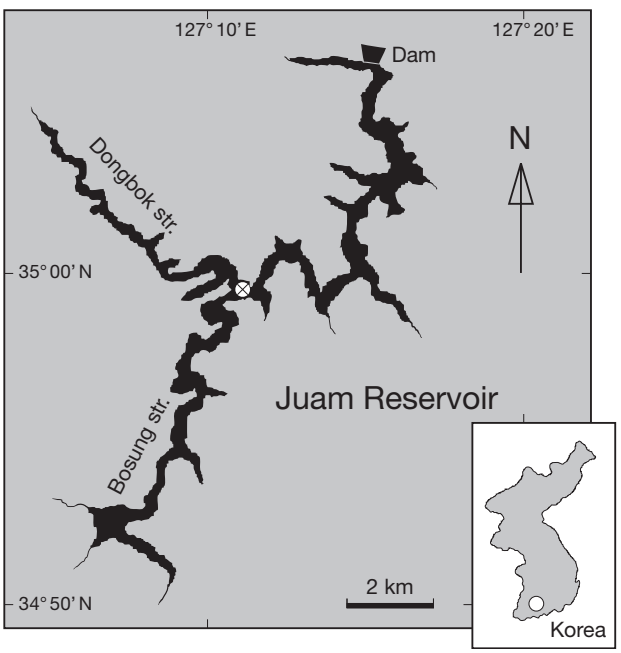

Fig. 1. Sampling site (⊗) in Juam Reservoir, South Korea, showing the Dongbok and Bosung streams

face freshwater collected was filtered (Whatman GF/C) and used during cyst isolation and culture. For cyst sorting, each sediment sample was thoroughly mixed and 1 g (wet weight) was transferred to cooled, filtered water (Matsuoka et al. 1989). This cyst suspension was sonicated for 30 s (UT 53N, Sharp) in order to separate the cysts from sediment, and each sample was sieved through 100 and 20 µm mesh filters. The 20 to 100 µm fraction that passed through the 100 µm mesh and was retained by the 20 µm mesh was transferred to filtered water and concentrated to a final sample volume of 10 ml. For simultaneous cyst sorting and counting, 1 ml of each sample was placed in a Sedgwick-Rafter chamber, and intact cysts were counted and sorted by micropipetting into a capillary pipette under an inverted microscope (Axiovert 100, Zeiss). Only intact cysts with full cytoplasm and red spots were counted. In general, the number of empty cysts varied with sampling date. Cyst germination was characterized by morphological events, i.e. bursting of the cyst, hatching, and movement of new vegetative cells with flagella. A positive germination score was given when we simultaneously observed both empty cysts and swimming cells. Cumulative excystment (%) was calculated as the ratio of cumulative germinated cysts to the number of inoculated cysts in the *in vitro* studies.

Excystment studies. Cysts were isolated as described above, washed in cool, sterile filtered water and separately inoculated into 96-well tissue culture plates (Nunc) filled with the same cool, filtered water. This preparation was completed within 1 wk of sediment sampling. The isolated cysts were incubated at the temperature of the bottom water layer at the sampling station (Fig. 2A), under 20 µmol photons m^{-2} s^{-1} cool-white illumination with a 12:12 h L:D cycle. For the first week, cyst germination was examined daily under an inverted microscope. Cysts that had not germinated within 1 wk were checked at 2 to 5 d intervals thereafter. The germination ratio was calculated as the ratio of cumulative excystment over 30 d versus the number of inoculated cysts. Each experiment was performed in duplicate with 30 to 60 cysts. We also examined germi-

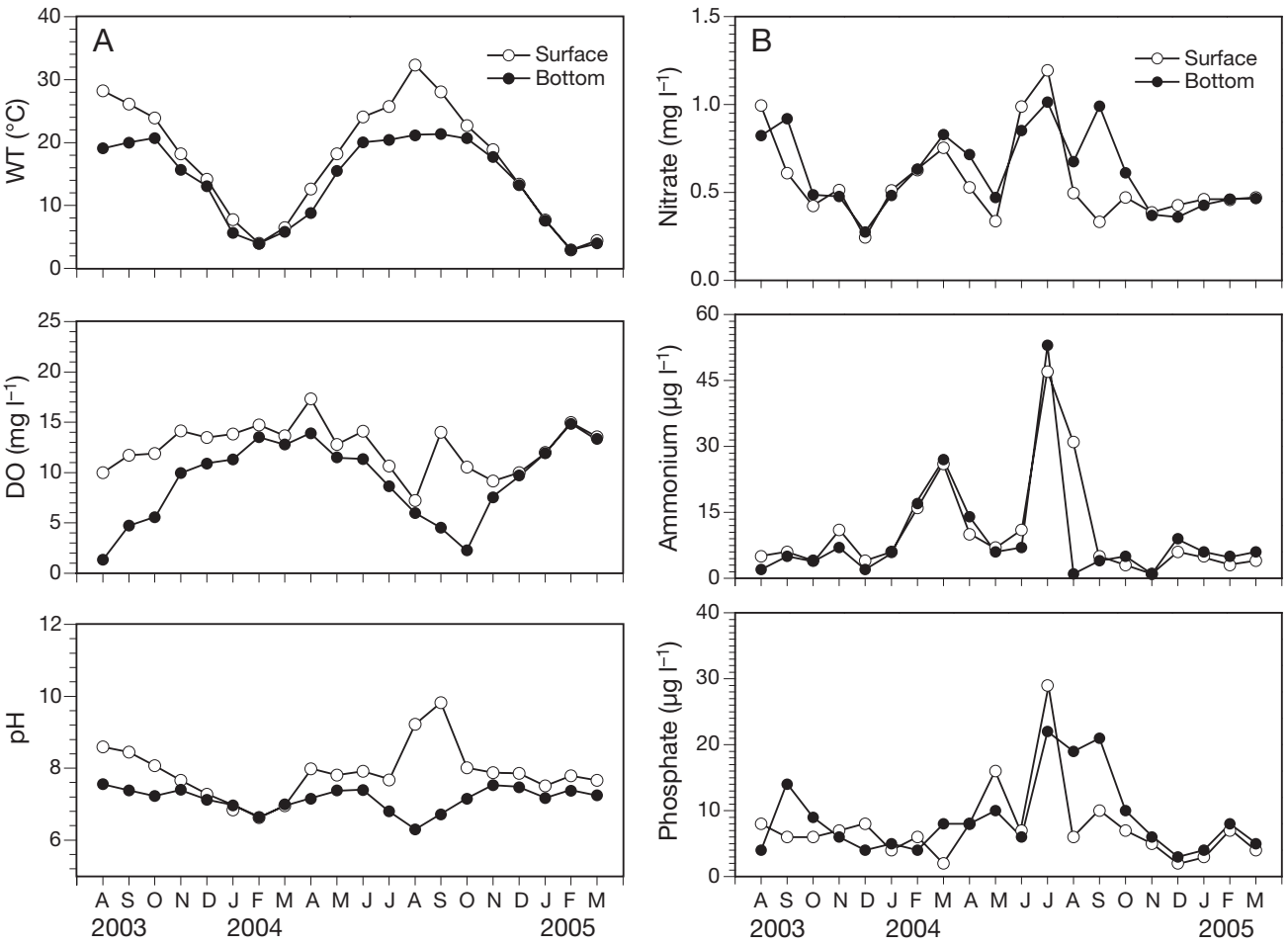

Fig. 2. Physico-chemical characteristics of Juam Reservoir over time. (A) Water temperature (WT), dissolved oxygen (DO) and pH; (B) phosphate, nitrate and ammonium concentrations

nation times (the period between onset of the environmental triggering and the first germination event) at similar temperatures across different seasons to determine whether the germination time was season- or water temperature-dependent. The isolated cyst populations were stored in 3 mL perforated cryovials in the dark at 4°C for 1 to 2 wk, and were resuspended in filtered lake water prior to use. To test the effects of light level, cyst germination was measured at 20°C under 0.2, 2, 10, 20, 50, 80 and 100 µmol photons m^{-2} s^{-1} cool-white illumination with a 12:12 h L:D cycle. Cyst germination was also measured at pH values of 6, 7, 8 and 9 at 20°C under 20 µmol photons m^{-2} s^{-1} cool-white illumination with a 12:12 h L:D cycle. To test the effects of nutrients, cyst germination was measured in natural water (NW), diatom medium (DM; Beakes et al. 1988), nitrogen-phosphorus deficient medium (DM-NP), nitrogen deficient medium (DM-N) and phosphorus deficient medium (DM-P) at 20°C under 20 µmol photons m^{-2} s^{-1} cool-white illumination with a 12:12 h L:D cycle. Germination was examined over 30 d, under an inverted microscope (Axiovert 100, Zeiss), and all experiments were performed in triplicate.

Statistical analysis. Pearson product-moment correlation analyses were used to identify the environmental variable(s) likely to be associated with the population dynamics of *Peridinium bipes* cysts in the sediment and vegetative cells in the water column, as well as the variables associated with the germination of cysts separated from each sediment sample. The statistical significances of the correlation coefficients were determined by a sequential Bonferroni procedure using a table-wide significance level of $p = 0.05$ (Holm 1979). Differences in excystment between presence and absence of light, pH and nutrients were tested with a 1-way analysis of variance (ANOVA) and regarded as statistically significant at $p < 0.05$.

RESULTS

Physico-chemical variables

Between August 2003 and March 2005, the water temperature and concentration of dissolved oxygen at the surface and bottom of the study site displayed a clear seasonal cycle. Temperatures varied from 4.0 to 32.3°C in the surface layer and from 4.0 to 21.4°C in the bottom layer (Fig. 2A). During periods when the surface temperature was high, from 25.0 to 32.3°C (i.e. from August to October 2003 and from June to October 2004), the bottom temperature was variable, fluctuating between 19.0 and 20°C. During periods when the surface temperature was low, from 4.0 to 8.0°C (i.e. from January to April 2003 and from January to March 2005), the bottom temperature was very similar to that at the surface. The concentration of dissolved oxygen (DO) in the surface and bottom layers varied seasonally, in an inverse relationship with temperature (Fig. 2A). In the bottom layer, the concentration of DO was >7.0 mg l^{-1} during the colder season from November to June, but ranged between 1.3 and 5.9 mg l^{-1} during the warmer months of August and September in 2003 and 2004. The pH of the surface layer varied between 7.0 and 9.8, while that of the bottom layer varied between 6.3 and 8.9. The nitrate and phosphate levels gradually decreased from September 2003 to March 2004, while the concentrations of nitrogen species (nitrate and ammonium) found in both surface and bottom layers were higher from June to August 2004 (Fig. 2B). With increasing water temperature, ammonium levels rose from 16.0 to 47.0 µg l^{-1} between February and August 2004. The concentration of phosphorous in the surface layer varied between 2.0 and 29.0 µg l^{-1}, while that in the bottom layer varied between 3.0 and 22.0 µg l^{-1} (Fig. 2B). The reservoir was never completely ice-covered during the study period.

Seasonal distribution of *Peridinium bipes*

High densities of *Peridinium bipes* vegetative cells (>100 cells ml^{-1}) were present continually in the surface water (depth = 0 to 1 m) during a 6 mo period when the water temperature was between 18.2°C (September 2003) and 4.0°C (March 2004) (Fig. 3). The maximum density of vegetative cells (ca. 1.0×10^3 cells ml^{-1}) occurred in January 2004 (at 7.7°C). Thereafter, there was a rapid decrease in vegetative cells, beginning in April 2004 (at 12.6°C), and no vegetative cells were observed between June 2004 and March 2005, regardless of water temperature. The abundance of *P. bipes* cysts in

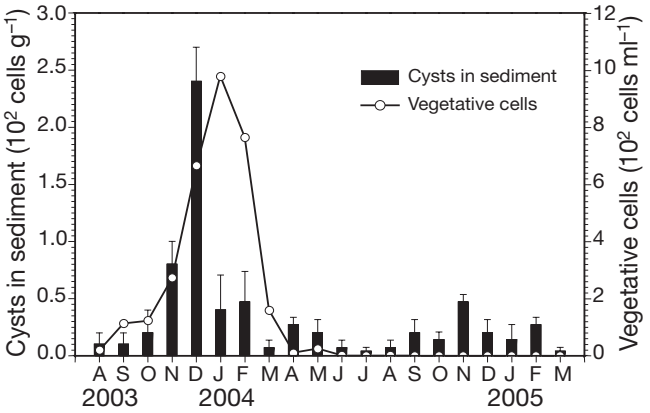

Fig. 3. *Peridinium bipes*. Seasonal changes in vegetative cell abundance at the surface (0 to 1 m depth) and cyst abundance in the sediment from August 2003 to March 2005. Values are mean + SD (n = 3)

the sediment samples ranged from 4 to 240 cysts g^{-1} and showed a seasonal pattern similar to that of the vegetative cells (Fig. 3), except that some cysts (~25 cysts g^{-1}) were found in samples taken between September 2004 and March 2005, when no vegetative cells were seen. The cyst abundances peaked at 80 and 240 cysts g^{-1} in November and December 2003; higher cyst abundances (>40 cysts g^{-1}) occurred approximately 1 to 2 mo before the peak in vegetative cell levels, and remained elevated during the period of highest vegetative cell density (November 2003 to February 2004).

Seasonal excystment of *Peridinium bipes* under ambient temperatures

Cyst germination success varied seasonally with ambient temperature changes (Fig. 4). Between August 2003 and July 2004 (bottom temperature range 3.9 to 20.7°C), the maximum germination ratio (~81%) occurred in November 2003, when the bottom temperature was 15.6°C. Cyst germination success at intermediate temperatures (e.g. 13 to 19°C, in August, November and December 2003, and May 2004) was generally high (22.5 to 81.1%), while success rates at higher temperatures (>20°C, sampled in September and October 2003, June and July 2004) was at intermediate levels (14.0 to 33.1%), and lowest success rates (8.3 to 20.7%) occurred at lower temperatures (<10°C, sampled January to April 2004). No significant correlation was found between cyst germination rate and the water temperature of the bottom layer of the reservoir ($r = 0.31843$, $p = 0.3131$).

Our examination of germination time (the period between incubation and the first excystment event) showed that preparation time for excystment of *Peridinium bipes* was exclusively related to the sampling/culture temperature ($r = -0.9358$, $p < 0.0001$). In cysts sampled during the warm season between April and October 2004, the preparation time for excystment gradually reduced (3 d at 20.7°C) with increased exposure temperature. In contrast, during the colder season (November 2003 to March 2004), the preparation time gradually lengthened (20 d at 3.9 and 5.8°C) as the

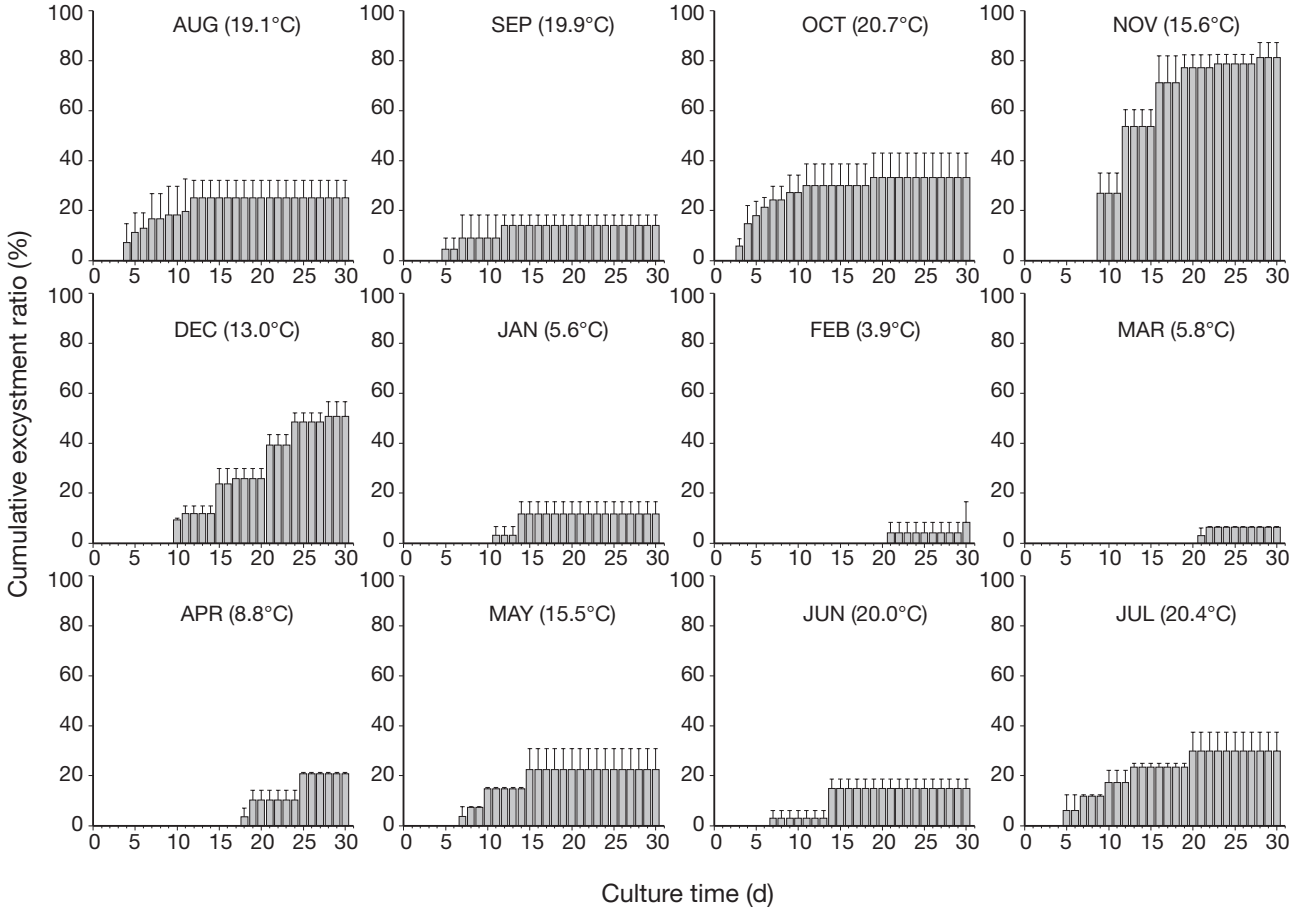

Fig. 4. *Peridinium bipes*. *In vitro* seasonal changes in excystment success (%, see 'Materials and methods' for method of calculation) during the period from August 2003 to July 2004. Samples were incubated at water temperatures matching those in the reservoir (Fig. 2A), under 20 µmol photons m^{-2} s^{-1} illumination, with a 12:12 h L:D cycle. Mean + SD, n = 3

exposure temperatures decreased. Thus, water temperature strongly affected the duration of the latent period prior to excystment, but not the success of excystment itself.

Effects of environmental conditions on *in vitro* excystment of *Peridinium bipes*

Germination occurred over a wide range of light intensity from 2 to 100 µmol photons m^{-2} s^{-1} (Fig. 5A), although low light intensities (below 0.2 µmol photons m^{-2} s^{-1}) failed to induce germination. The highest observed germination ratio was >50% in samples exposed to >20 µmol photons m^{-2} s^{-1}. The germination ratios appeared saturated at approximately 50 µmol photons m^{-2} s^{-1} (maximum germination ratio = 54%), with higher intensities associated with lower germination rates. High germination success (>60%) occurred across a pH range of 6 to 8, whereas the germination ratio fell below 50% at pH 9, a pH that was seen in the water column, but not in sediment samples (Fig. 5B). The presence or absence of the tested nutrients did not significantly affect the germination of *Peridinium* cysts (df = 4, F = 4.75724, p = 0.09462 for N and P richness-DM; df = 4, F = 1.77725, p = 0.25334 for N and P deficient-DM); relatively high germination success (>60%) was observed even in cysts incubated in natural water and in nitrogen/phosphorus-deficient DM (Fig. 5C). Hence, germination rate was primarily determined by water temperature and light.

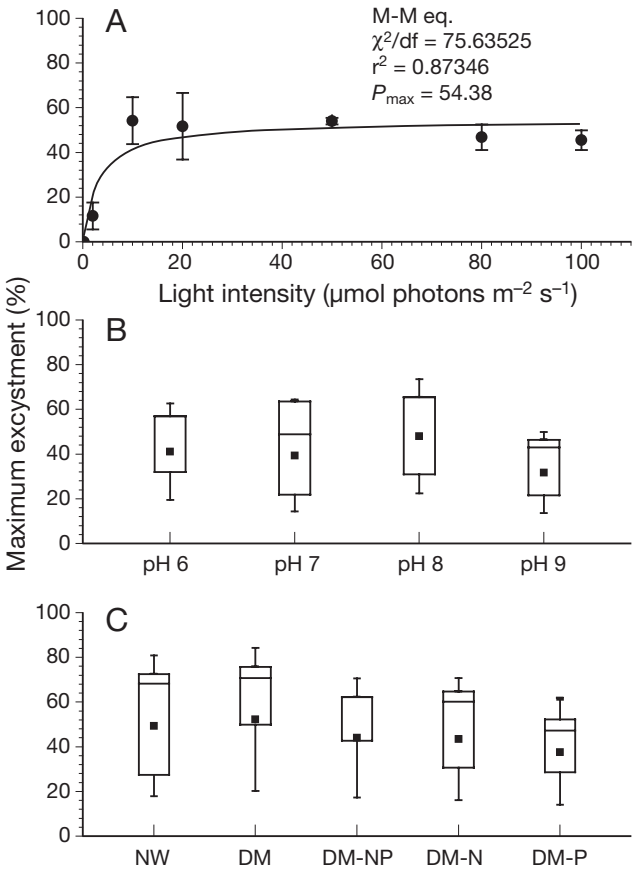

Fig. 5. *Peridinium bipes*. Effects of light intensity (A), pH (B) and nutrients (C) on *in vitro* excystment. Samples were incubated as described in Fig. 4. NW: natural water; DM: diatom medium; DM-NP: nitrogen-phosphorus deficient medium; DM-N: nitrogen deficient medium; DM-P: phosphorus deficient medium. Each value in all panels is mean (● and ■) and SD (bar). Each box in whisker plots in (B) and (C) was statistically significant at $p < 0.05$. In (A), relative excystment (P, %) of *P. bipes* on the light intensity (I) was analyzed using the Michaelis-Menten equation: $P = P_{max} \times I/(P_0 + I)$, where P_{max} is the maximum excystment in light condition and P_0 is the excystment in the dark; P_{max} = 54.37958 ± 5.5684, P_0 = 3.0663 ± 1.9438, r^2 = 0.8735, χ^2/df = 75.6353

DISCUSSION

Dense blooms of *Peridinium bipes*, including vegetative and cyst populations, occurred in Juam Reservoir over a 7 mo cold water period from September 2003 through March 2004. During this bloom, the peak of the cyst population preceded that of the vegetative population by approximately 1 mo (Fig. 3). The vegetative cell population peaked in January 2004 and rapidly decreased thereafter, and no cysts were found between June 2004 and March 2005, regardless of water temperature. Although cyst abundance was only about 20% of the peak level in January 2004, some cysts (10 to 55 g^{-1}) were detected between September 2004 and February 2005, indicating the persistence of a resting population. In general, decreased cyst concentrations in sediment are related to the development of resting cells through cyst germination (Pollinger & Berman 1975, Anderson & Wall 1978). In addition, higher excystment and encystment rates often occur in shallower regions (Park & Hayashi 1993). However, our data showed that decreases in cyst numbers in sediment did not appear to be closely related to the development of resting cells via excystment. The time profiles of the vegetative cell and cyst populations from August 2003 to June 2004 showed similar patterns, with nearly simultaneous peaks in December 2003 and January 2004 for cysts and vegetative cells, respectively. This observation seems to suggest that the accumulation of cysts in the sediment between August and December 2003 may have been induced by active settlement or encystment from vegetative cells in shallow regions.

Environmental factors such as water temperature, light intensity and dissolved oxygen, as well as endogenous factors such as dormancy periods and annual

rhythms have been suggested as possible factors affecting excystment in dinoflagellate species (Anderson 1980, Anderson et al. 1987, Park & Hayashi 1992, 1993, Rengefors & Anderson 1998, Kishimoto et al. 2001). Of these, temperature has been identified as the most important factor regulating germination of dinoflagellate species (Park & Hayashi 1993, Chapman & Pfiester 1995, Kishimoto et al. 2001). Here, we show that *Peridinium bipes* cysts sampled from Juam Reservoir had a wide temperature range for excystment (3.9 to 20.4°C). The maximum germination ratio (~81%) occurred in cysts collected in November 2003, when the bottom temperature was 15.6°C. Notably, however, the germination ratio was far lower (<20%) in cysts collected in May 2004, when the water temperature was 15.5°C. Although we do not yet fully understand why excystment of *P. bipes* was highest during the late autumn, and showed season-specific differences at similar temperatures, cysts deposited at warmer temperatures clearly germinated more quickly than those deposited in cold water. It is also noteworthy that cyst abundance in the sediment was higher in November 2004 (at 15.6°C) than in May 2004 (at 15.5°C), November 2004 (at 17.7°C) or December 2004 (13.2°C). Thus, the large sedimentary cyst population appears to have favored excystment in the benthic environment. Size of the cyst population was closely related to high encystment rates in vegetative blooms, deposition temperature and time. Interestingly, our study revealed an excystment rate of ~10% in 3 samples taken at the lowest temperature (~3.9°C), which is below the lowest germination temperature (4 to 5°C) (Park & Hayashi 1993, Kishimoto et al. 2001). Together, these results indicate that deposition temperature and time can play an important role in determining or enhancing the excystment of *P. bipes*, and further show that cysts of this species in Juam Reservoir may germinate across an unusually wide temperature range.

Another major factor affecting germination of *Peridinium bipes* cysts is light intensity (Park & Hayashi 1993, Kishimoto et al. 2001). Previous studies have shown that marine species such as *Scrippsiella trochoidea* (Binder & Anderson 1986, Anderson et al. 1987) and freshwater species such as the cyanobacterium *Anabaena flos-aquae* (Kim et al. 2005), failed to germinate in the dark. In the present work, we found that *P. bipes* cysts could not germinate under <0.2 µmol photons m^{-2} s^{-1}, even at the optimal temperature of 15.6°C. A relatively high germination ratio (>50%) was associated with light intensities ranging from 10 to 50 µmol photons m^{-2} s^{-1}. The saturating photon irradiance was approximately 50 µmol photons m^{-2} s^{-1} (maximum germination ratio was 54.4%), with higher intensities (e.g. 80 to 100 µmol photons m^{-2} s^{-1}) yielding lower germination rates. During 2003 and 2004, the study site was shallow (~4 m depth), had a transparency (Secchi depth) of 1.0 to 1.5 m, and was generally exposed to a light intensity of 0.2 to 25 µmol photons m^{-2} s^{-1} on the bottom (Lee et al. 2005), indicating that illumination at the study site typically favored *P. bipes* cyst germination.

In addition to temperature and light, we also examined the effect of pH values ranging from 6 to 9 on germination of *Peridinium bipes* cysts. pH had little to no effect on germination across a range from pH 6 to 8, whereas exposure of cysts to media at a pH of 9 significantly decreased germination. Although higher pH values were recorded elsewhere in the water column, no pH values outside the acceptable range (6.3 to 8.9) were measured in water near the cyst-containing sediment layer, indicating that pH had little effect on *P. bipes* germination in Juam Reservoir during the study period.

N- and/or P-free media effectively induced germination of cysts, with no significant differences observed between germination rates of cysts in nutrient-deficient and nutrient-sufficient media (Fig. 5C). Numerous studies on various *Peridinium* species have shown that N-free medium promotes encystment (Sako et al. 1987, Park & Hayashi 1993, Chapman & Pfiester 1995). Although fewer studies have examined the effect of nutrient limitation on excystment or life cycles of dinoflagellates (Steidinger & Haddad 1981), Rengefors & Anderson (1998) showed that N- and P-free medium had little effect on the germination of *Ceratium hirundinella*. This is consistent with our previous finding (Kim et al. 2002) that nutrient depletion may affect encystment but not excystment in *Alexandrium tamaraense*. Collectively, these findings indicate that water temperature and light intensity were significant factors mediating cyst germination of *P. bipes* in Juam Reservoir during the study period, but that pH and nutrients were not important.

In temperate waters, seasonal changes in temperature and dissolved oxygen (DO) typically show an inverse relationship, i.e. high temperatures and low DO coincide in the warm season, while low temperatures and high DO occur during the colder months. Numerous studies have shown that germination of dinoflagellate cysts is inhibited at lower concentrations of DO and under anoxic conditions (Anderson et al. 1987, Kida et al. 1989, Rengefors & Anderson 1998). In particular, Anderson et al. (1987) speculated that anoxia did not simply retard excystment, but instead completely inhibited the process. Consistent with this, we observed that dense blooms of vegetative cells (excystment) were closely associated with increased DO levels, although the germination ratios failed to show significant correlation with changes in DO levels (Fig. 2A). We did not specifically test the effects of

anoxia on germination, but our observation that dissolved oxygen is necessary for germination of freshwater dinoflagellates such as *Peridinium bipes* suggests that excystment should show a positive relationship with DO levels. At the study site, which is oxygen-saturated (>80%) throughout the year (Lee et al. 2005), anoxia does not occur. However, the deeper regions near the the reservoir dam site (>40 m deep) may experience more severe anoxic conditions. The bloom in Juam Reservoir bloom began at our study site, and winds and currents gradually transported cells to the center of the water body and close to the dam. Should the level of ambient dissolved oxygen in Juam Reservoir fall toward zero during the rainy season, little (or no) germination would occur.

Anderson & Keafer (1987) observed an annual germination rhythm in synchrony with the motile dinoflagellate cell blooms in the deep coastal waters of the Gulf of Maine (USA), where bottom temperatures are relatively constant at 4 to 6°C. We previously identified an annual clock in the germination rhythms of *Alexandrium tamarense* cysts in Masan Bay, Korea (Kim et al. 2002), and hypothesized that a similar timing mechanism was likely to exist for *Peridinium bipes* in Juam Reservoir. Our present results revealed that active excystment occurred prior to the blooming of vegetative populations from November 2003 to February 2004 when the surface water temperatures decreased rapidly from 15.6 to 3.9°C. *P. bipes* excysted prior to winter, and cells were then available to bloom as the water temperatures dropped below 10°C. During this time, the excystment rate was very low, but vegetative cell growth was promoted by suitable surface water temperature (>10°C, cf. Lee et al. 2005). Hence, active pre-winter excystment forms an effective seed population for the winter bloom.

A dormancy period is necessary for successful germination of cysts in numerous dinoflagellates, including *Peridinium*. Theoretically, ambient temperature is a major factor controlling the duration of this period (Anderson 1980, Perez et al. 1998). However, in the dinoflagellates *Ceratium hirundinella* and *Scrippsiella trochoidea* there are no differences in maturation period following storage at different temperatures (Binder & Anderson 1987, Rengefors & Anderson 1998), and cysts of the same species may have different dormancy periods, depending on geographic and environmental conditions (Hallegraeff et al. 1998). For example, *Peridinium* cysts have been shown to have different dormancy periods under a variety of conditions, including a 16 wk dormancy period when germinated in the light at 5 to 25°C (*P. bipes* f. *oculatum*; Park & Hayashi 1993), a 1 to 6 mo dormancy period when germinated at temperatures of 15°C or higher (*Peridinium perardii*; Sako et al. 1987), and an 11 wk dormancy period at 4°C when germinated below 7°C (*P. aciculiferum* from Lake Erken; Rengefors & Anderson 1998). Based on our observations of *P. bipes* in Juam Reservoir, we hypothesize that the dormancy period of this population is approximately 6 to 8 mo, lasting from March to October, when the bottom temperatures range from 4 to 21°C (Fig. 4). Furthermore, we showed that the exposure temperature and duration of the dormancy period affected the germination time prior to excystment, as supported by a strong negative correlation between the germination time and water temperature.

A repetitive blooming of freshwater phytoplankton, such as *Microcystis* and *Peridinium*, is not uncommon. Although *Microcystis* cyst formation is not fully understood, *Peridinium* cysts are known to form easily from the vegetative cells both during the bloom and under extreme environmental conditions (light and temperature etc.). In general, the abundance of vegetative cells is believed to be a crucial trigger for cyst formation (encystment) during the bloom period. Secondary triggers of encystment include environmental changes and allelopathic interactions with competitors. In contrast to repetitive blooms, transient blooms of *Peridinium* have been reported in waterways that are typically dominated by species of *Microcystis* (Wu et al. 1998, Sukenik et al. 2002, Vardi et al. 2002), and there is a debate as to whether the 2 organisms have a reciprocal allelopathic relationship (Sukenik et al. 2002, Vardi et al. 2002). Replacement of one of these species by the other is thought to be induced by changes in nutrient concentrations (Wu et al. 1998) and/or accumulation of algal metabolites, such as allelochemicals (Keating 1977, Vardi et al. 2002). In addition, it is generally accepted that *Peridinium* and soluble extracts containing *Peridinium*-derived fatty acids or photooxidized chlorophyll *c* are algicidal against *Microcystis* species (Uchida et al. 1988). As the conditions that lead to a transient bloom, as opposed to regular and repetitive blooms are not clear, further information is needed to identify the triggers for blooms and to develop general models of bloom relationships.

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