J. Phycol. 34, 568–577 (1998)

ENVIRONMENTAL AND ENDOGENOUS REGULATION OF CYST GERMINATION IN TWO FRESHWATER DINOFLAGELLATES[1]

Karin Rengefors[2]

Institute of Limnology, Uppsala University, Norbyvägen 20, SE-752 36 Uppsala, Sweden

and

Donald M. Anderson

Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543-1049

ABSTRACT

The role of excystment in relation to seasonal succession was investigated in two freshwater dinoflagellates, Ceratium hirundinella *(O.F. Müller) Dujardin and* Peridinium aciculiferum *(Lemmermann). Field studies and laboratory experiments were performed to determine which factors regulate the timing of cyst germination. Environmental factors (temperature, light, nutrients, and anoxia) and endogenous factors (maturation period and biological clock) were investigated. Our main results indicate that temperature and internal maturation period determine when germination can occur.* C. hirundinella *had a maturation period of 4.5 months and germinated in the laboratory and in the field at temperatures above 6° C.* P. aciculiferum *had a maturation period of 2.5 months and germinated in the laboratory and in the field at temperatures below 7° C. In addition, our results indicated that both species were regulated by a biological clock. Furthermore, anoxia prevented the germination of* C. hirundinella, *contrary to results in earlier studies. To conclude, we could explain the appearance in plankton of the two dinoflagellate species through two main factors regulating excystment, that is, temperature and maturation period.*

Key index words: anoxia; Ceratium hirundinella*; cyst; dinoflagellate; dormancy; germination;* Peridinium aciculiferum*; seasonal succession*

Regulation of phytoplankton seasonal dynamics has traditionally been explained by nutrients, grazers, and other abiotic and biotic factors acting in the pelagic zone (Reynolds 1984, Sommer et al. 1986, Blomqvist et al. 1994). By taking a closer look at the plankton community, it becomes clear that the presence of the different species in the water column is discontinuous (Boero et al. 1996). In other words, species may be present in high numbers for a short period, only to later disappear completely from the water column. This is especially true in polar, boreal, and temperate latitudes, where seasonal variations are large (e.g. Nauwerck 1963, Rahmberg 1976, Blomqvist et al. 1995). In fact, most of the phytoplankton in these lakes spend most of their life cycles resting on the lake sediments. Consequently, life cycles, especially the benthic resting stage, need to be taken into consideration to fully explain phytoplankton seasonal succession (Reynolds 1984, Hansson et al. 1994, McQuoid and Hobson 1995).

If algal life histories are important in phytoplankton succession, then the inoculum from the resting "seed bank" becomes important. In turn, the size and timing of the inoculum must be dependent on the physiological and environmental regulation of germination (Binder and Anderson 1987). Resting stage formation, on the other hand, signifies the end of the population in the pelagic zone.

Resting stages are present within all freshwater phytoplankton groups, indicating that a resting stage is a primitive evolutionary trait (Fryxell 1983). Resting propagules have been attributed a number of functions, the main one considered to be survival during unfavorable environmental conditions. Other functions include bloom initiation, dispersal, reproduction, and genetic variation (Wall 1971, Anderson and Wall 1978, Fryxell 1983, Anderson et al. 1984). Furthermore, McQuoid and Hobson (1996) suggest that resting stage formation may also be a means to avoid the death of an entire population that is about to crash because of nutrient depletion.

A group of phytoplankton that produces large and distinct resting propagules is the dinoflagellates. The ecology of dinoflagellate cysts have been studied extensively in marine environments (e.g. Anderson and Wall 1978, Dale 1983, Nehring 1996), whereas there are only a handful studies from freshwater environments (Huber and Nipkow 1922, 1923, Heaney et al. 1983, Pollingher et al. 1993, Chapman and Pfiester 1995).

Dinoflagellate cyst germination is suggested to be regulated by both internal and external factors. Cysts usually undergo dormancy, a mandatory internal maturation period (e.g. Anderson 1980) during which cysts cannot be triggered into germination. After dormancy, cysts enter a stage of quiescence during which cysts are prevented from germination by an unfavorable external environment (Pfiester and Anderson 1987). Of the environmental factors, temperature has been shown to be most important in regulating germination in a number of marine species (Huber and Nipkow 1922, Anderson 1980, Binder and Anderson 1987, Bravo and Anderson

[1] Received 18 August 1997. Accepted 25 March 1998.

[2] Author for reprint requests; e-mail Karin.Rengefors@limno.uu.se.

1994). Light, day length, and dissolved oxygen in the sediments may also affect germination (e.g. Huber and Nipkow 1923, Krupa 1981a, Anderson et al. 1987, Bravo and Anderson 1994). In addition, an endogenous annual clock has been found in marine dinoflagellates that may also control germination (Anderson and Keafer 1987).

Studies of dinoflagellates in moderately eutrophic Lake Erken, Sweden (Rengefors 1998), have shown that there is a tight coupling between the number of cysts found on surface sediments and the dynamics of vegetative cells in the pelagic zone. Germination and cyst formation events apparently set the boundaries for the seasonal appearance of the vegetative cells in the lake. Our hypothesis is that internal maturation and temperature control germination, which in turn regulates the timing of the planktonic stages of the two dominant dinoflagellate species in Lake Erken: *Ceratium hirundinella* (O.F. Müller) Dujardin during late summer and *Peridinium aciculiferum* Lemmermann during late winter under the ice cover. The phytoplankton community in Lake Erken usually is dominated by dinoflagellates in late winter, by centric diatoms in spring, by dinoflagellates and/or colonial cyanobacteria (e.g. *Gloeotrichia echinulata, Microcystis* spp.) in late summer, and by large diatoms in fall (Blomqvist et al. 1994). In the present study, we have undertaken laboratory experiments to determine the dormancy period and temperature window for germination and combined these results with the field studies presented in Rengefors (1998). Furthermore, we have tested whether cysts can germinate in the dark and studied the effect of anoxia and nutrients on *Ceratium* cyst germination.

MATERIALS AND METHODS

Study site. The field study was conducted in Lake Erken, a naturally eutrophic lake (TP ca. 25 μg $P \cdot L^{-1}$, TN ca. 700 μg $N \cdot L^{-1}$) (Pettersson 1985) located in southeastern Sweden at 59°25′ N, 18°15′ E. The lake was formed about 2000 years ago as the land rose from the Baltic Sea, resulting in lake water with high conductivity and alkalinity. Lake Erken is usually ice covered for about 18 weeks a year (January through April) and has a theoretical water residence time of 7 years. The lake area is 24 km^2, the mean depth 9 m, and the maximum depth 21 m (Håkansson 1978).

Field study: Phytoplankton sampling. Phytoplankton samples were taken weekly in the water column from February 1995 until September 1996. A 2-m-long tube sampler (2.0 L) was utilized to collect water at 2-m intervals from 0- to 14-m depths. Depth-integrated samples taken from three locations in the lake were combined, and 1 L was subsampled. Subsamples were preserved with Lugol's solution. Counts were made on each sampling occasion using an inverted microscope and a 25-mL sedimentation chamber. Temperature was measured weekly at 12-m depth in the central part of the lake while sampling phytoplankton.

Sediment cyst sampling. Sediment samples were taken monthly from September 1995 until September 1996 at a 16-m depth. Four replicates were taken at one location with a gravity core sampler. The surface sediments (0–2 cm) were preserved and stained with primuline according to the methods of Yamaguchi et al. (1995) and then counted in a Sedgewick-Rafter counting chamber in a Nikon epifluorescence microscope with a filter for blue light excitation (a 450- to 490-nm excitation filter and a 520-nm barrier filter).

Collection and storage of cysts. Cysts of *C. hirundinella* (hereafter referred to as *Ceratium*) were obtained from concentrated vegetative cells (about to encyst) and cysts in the water, collected with a 40 μm plankton net on 28 August 1995. The sample was first sieved through a 120 μm net to remove larger phyto- and zooplankton and then sieved through 20 μm to remove small particles. The cysts and cells were finally resuspended in filtered lake water that was spiked with EDTA, vitamins, and metals (ingredients and concentrations according to DM medium [Beakes et al. 1988]). The suspension was incubated in the laboratory at about 15° C at a 14:10 L:D (light:dark) cycle (approximate L:D cycle in nature during this season) to allow for encystment of remaining plankton cells. Ten days later, the cysts that were obtained were concentrated and stored in 3-mL cryovials with a hole in the lid. A 20 μm net was placed between the vial and the lid. The cryovials were subsequently submerged in lake sediment in tightly sealed plastic jars. These were stored in the dark at 4° and 10° C. Cysts and plankton cells of *P. aciculiferum* (hereafter referred to as *Peridinium*) were collected with a 20 μm net in April 1996 and were prepared as above but were incubated at 7° C at a 12:12 L:D cycle (approximate L:D cycle in nature during this season), which reflects spring conditions in the lake. *Peridinium* cysts were stored as above but only at 4° C because of a lack of sufficient material.

Laboratory studies: Maturation and storage effect. Maturation experiments were performed on *Ceratium* cysts once a month starting on 11 September 1995. On each occasion, two 96-well plates were prepared by adding 200 μL of sterile DM medium to each well. At least 30 cysts were picked out manually, using capillary tubes with drawn-out tips, from each of the cyst suspensions stored at 4° and 10° C and were placed in individual wells. The plates were then incubated at 7° C (ranging from 5° C at night to 10° C during the day) at 14:10 L:D. Plates were checked after 1, 2, 5, 10, 15, and 30 days for empty cysts. *Peridinium* cysts were treated like those of *Ceratium*, except that the experiment was not started until the cysts were 2 months old. *Peridinium* cysts were placed in a 3° C incubator at a 12:12 L:D cycle.

Temperature and light experiment. To study the effect of temperature and light on germination of mature cysts, we used a temperature gradient bar, as described in Watras et al. (1982). This device consists of a aluminum bar with a heat source connected at one end and a cooling device at the other. The bar contains holes for the insertion of large test tubes arranged in 20 rows of four between the two ends of the bar. The heater and cooler were regulated to attain a temperature gradient ranging from < 0° to 20° C. Light was supplied by two white fluorescent lamps set at a 10:14 L:D cycle, positioned under the test tubes. For a detailed description, see Blankley and Lewin (1976).

The test tubes were filled with 25 mL of sterile DM medium. For each row (representing one temperature, ±0.5° C), two test tubes were randomly picked out to be darkened by enclosing in aluminum foil. The test tubes were acclimatized during 2 days previous to starting the experiment, and the temperature was recorded for each one before cysts were added. Cysts were taken out from storage (4° C) and resuspended in DM medium. The cell concentration was adjusted to about 500 cysts·mL^{-1} through cell counts and by dilution with DM medium. Additions of 1 mL per test tube were done in random order, including 10 tubes to be used for initial values. All work was performed at night, with a red lamp as the only light source. After placing all the test tubes in the temperature gradient bar, the experiment was run for 14 days (based on results from maturation/germination studies) to allow for maximum excystment. Temperature was recorded for each test tube at the end of the experiment.

The same setup was used for *P. aciculiferum*, except that the temperature range was set between 0° and 15° C, as preliminary results indicated that this species germinates at lower temperatures. To keep the temperature low, we placed the temperature gradient bar in a 10° C walk-in incubator. The day length was set to a 12:12 L:D cycle, which reflects light conditions when cysts presumably germinate in the field. Only about 100 cysts were added to each test tube because of a lack of material. The experiment

was run for 17 days (based on results from maturation/germination studies) to allow for maximum excystment.

The difference between light and dark treatment was tested using a one-way analysis of variance (ANOVA), using SuperANOVA. The effect of temperature on germination was tested using graphical statistics where mean values of remaining cyst numbers outside two standard variations (5% confidence level) from the original mean cyst number were considered to have germinated.

Effect of anoxia. Ceratium and *Peridinium* cysts were incubated at four different degrees of oxygen concentration: fully oxygenated (normoxic), 0.5 mg·L^{-1} oxygen (low oxygen), 0.1 mg·L^{-1} oxygen (very low oxygen), and anoxic. Anoxic conditions were produced using the methods of Lutz et al. (1992) with some modifications. Sterile DM medium was prepared according to Beakes et al. (1988) and was buffered with Hepes and set to pH 6.9. Hypovials (100 mL) were filled with 30 mL of cold medium and cyst suspension (ca. 500 cysts·$vial^{-1}$) and were capped with BELLCO rubber stoppers. Vials were kept on ice to prevent initiation of germination during the preparation time. The vials were made anoxic by bubbling with argon through spinal needles inserted through the septum and into the medium. Shorter needles were also inserted into the headspace to serve as vents. After 45 min, the spinal needle was pulled up into the headspace, the vent needle was removed, and the headspace was pressurized to 1 atm. The spinal needle was then removed.

All vials were prepared in the same way. Some vials were taken out for air additions through a spinal needle that was pushed back into the medium. Normoxic conditions were produced by introducing 3 × 30 mL of air into the liquid phase and then adding an additional 30 mL to the headspace to obtain overpressure. To the low-oxygen treatments, 0.5 and 2.0 mL of air was added to each set of vials. The methods for obtaining anoxia and low oxygen concentrations were tested prior to start by gas chromatography.

The tubes were incubated in the dark for 21 days at 20° C. At the end of the incubation period, all vials were measured for oxygen content using a gas chromatograph (Chrompack Micro TCD, column: 7538 Molsieve 5A) with argon as a carrier gas. Calculations of final oxygen concentrations in the medium were made according to Lutz et al. (1992). One vial of each treatment was used for testing germination of remaining cysts. Cysts were transferred to 3-cm petri dishes containing fresh DM medium and were incubated at 20° C at 12:12 L:D period for another 3 weeks. The other hypovials were used for cyst counts and were preserved with Lugol's solution. The samples were counted in 25-mL sedimentation chambers with an inverted microscope. The experiment was analyzed using a one-way ANOVA followed by Scheffe's multiple comparison test (SuperANOVA).

Effect of nutrients. Ceratium cysts were incubated in four different media treatments: replete DM (an artificial medium), P (PO_4^{-3}) deplete medium, N (NH_4^+, NO_3^-) deplete medium, and N and P deplete medium. Cysts were taken out from storage and sieved and cleaned with sterile N and P deplete medium and then resuspended to about 500 cysts·mL^{-1}. One mL of cyst suspension was added to each of 40 large Pyrex test tubes, each containing 20 mL of medium. Four replicates were made for each treatment, and eight tubes were prepared for initial counts. The test tubes with cysts were incubated for 4 weeks, in 14:10 L:D cycle, at 10° C. The four different media were analyzed for nitrate and phosphate spectrophotometrically. At the end of the incubation period, test tubes were preserved with Lugol's solution and samples counted in 25-mL sedimentation chambers using an inverted microscope. *Peridinium* cysts were not tested because a sufficient cyst number was not available for this experiment. The experiment was analyzed using a one-way ANOVA followed by Scheffe's multiple comparison test (SuperANOVA).

RESULTS

Field Study

The field study showed that the point in time when *Ceratium* vegetative cells first appeared in the water column and cyst number decreased in the sediment corresponded to a temperature of 6°–7° C (Fig. 1a). On the other hand, *Peridinium* vegetative cells were first encountered in the water in December, when the temperature at 12 m had fallen below 4° C (Fig. 1b). During this time, cyst number in the surface sediments also dropped, indicating excystment.

Laboratory Experiments

Age and storage effect. The dormancy period of *Ceratium* cysts stored at 4° C was about 4.5 months (20 weeks). The cysts started to germinate at an age of 148 days (January 1996) after 10 days of incubation (Fig 2a). The excystment rate was 29% at this time. The excystment rate increased until a maximum of around 90% was reached at an age of 200 days in late March and April. By the end of May (age 250 days), a drop in excystment rate was noted. However, the following months the germination rate stayed high (above 85%) until the experiment was terminated in March 1997, when the cysts were 550 days old.

Ceratium cysts stored at 10° C also had a dormancy period of 4.5 months. They started germinating after 148 days, following a 10-day incubation period, with a germination rate of 42% (Fig. 2a). Maximum excystment rate (97%) was reached in late April (230 days). As of June, at an age of 300 days, the germination rate for these cysts started to drop, reaching a minimum of 10% at an age of 360 days. After this minimum, excystment increased again to 75% by March 1997, when cysts were 350 days old.

The median incubation time (time interval when most cysts germinated) could be determined as cysts were checked for germination after every 2–3 days for 30 days. *Ceratium* cysts stored at 4° C changed with time. Early in the winter, most cysts germinated after 10 or 14 days. By March most germination occurred in the 3- to 5-day interval. The shortest median incubation time was in April and May, when 50%–60% of the cysts germinated after 1–2 days incubation. From June onward, most cysts germinated after 6–10 days incubation. Ranges of median incubation time are listed in Table 1.

The median incubation time was different for *Ceratium* cysts stored at 10° C than those stored at 4° C (Table 1). In January and February, most cysts germinated during the 6- to 10-day interval. During March and April, the median incubation time decreased, and most cysts (50% and 90%, respectively) germinated after 5 days incubation. The median incubation time increased to 6–10 days during the summer and then eventually to 10–14 days during the fall and the following winter.

The maturation period of *Peridinium* was shorter than that of *Ceratium,* 2.5 months versus 4.5 months (Fig. 2b). When 3 months old, *Peridinium* cysts needed at least 14 days of incubation at 3° C before germinating. After 4 weeks of incubation, 55% of the

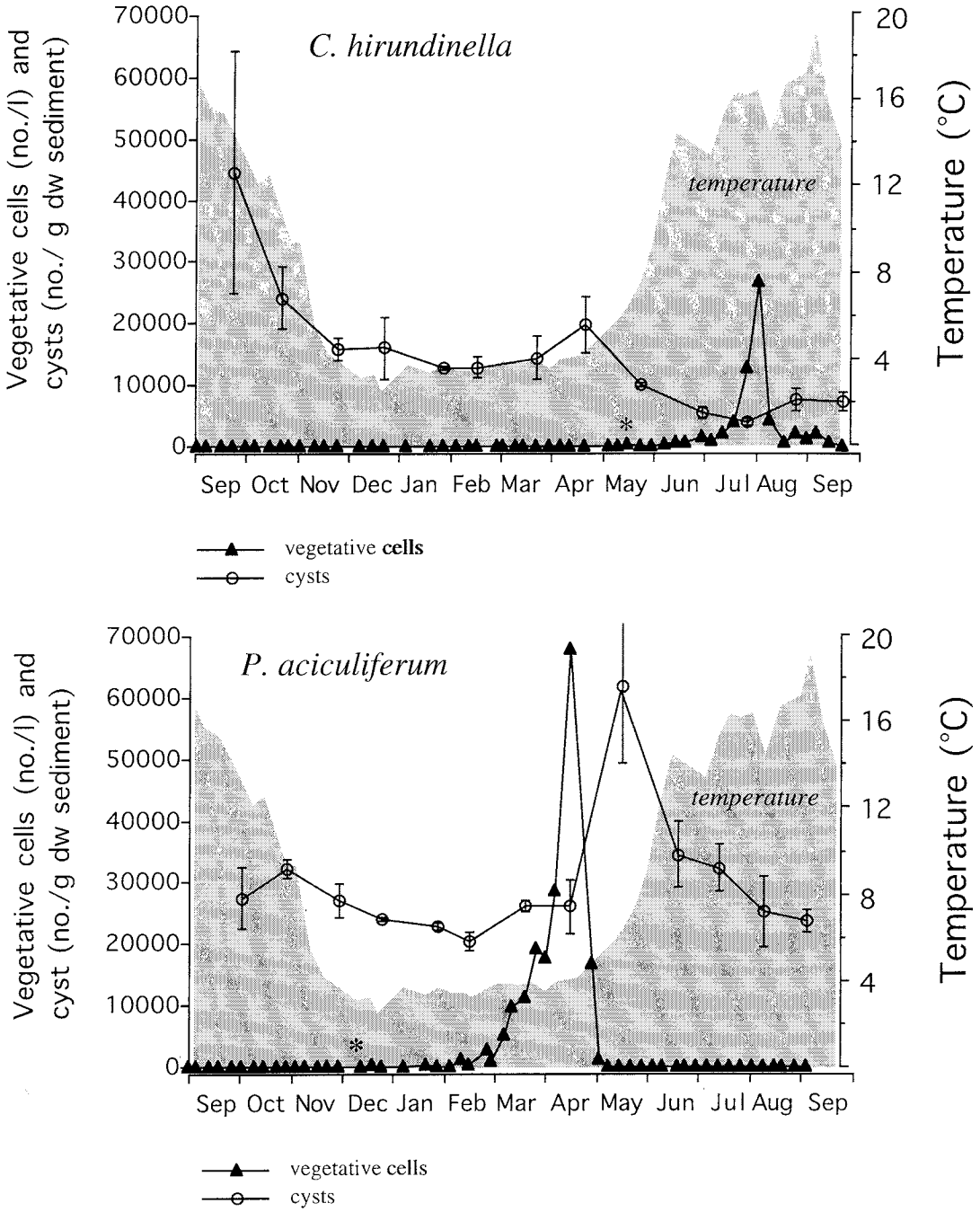

FIG. 1. Dynamics of dinoflagellates in the water column and corresponding cysts in surface sediments, coupled to temperature development at 12 m depth in Lake Erken, from September 1995 to September 1996. A) *C. hirundinella*, vegetative cells (▲) as cells·L^{-1} and cysts (○) as cysts·g^{-1} dry weight (dw) sediment. * indicates when the first vegetative cells occurred in the water. B) *P. aciculiferum*, vegetative cells (▲) as cells·L^{-1} and cysts (○) as cysts·g^{-1} dw sediment. * indicates when the first vegetative cells occurred in the water.

cysts had germinated. The percentage of cysts that germinated increased until October and November (cysts 230 days old), when 100% germinated. When cysts were 260 days old and onward, the germination rate decreased until it reached below 15% in March 1997 (age 350 days).

The median incubation time for *Peridinium* also changed with time. Initially, most cysts needed 18 days or more to germinate. By October and November, most cysts germinated after 10 days of incubation. In December, the median incubation time started increasing again, and by January 1997 most cysts needed 18–30 days to germinate. Ranges of median incubation time are listed in Table 1.

Temperature and light effects. The temperature gradient experiment showed that there was a threshold temperature above which *Ceratium* cysts germinated. No (or little) excystment occurred between 0° and

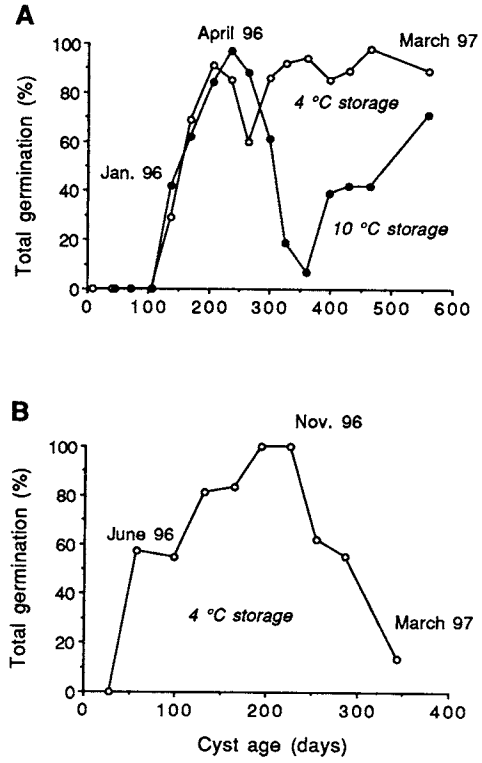

FIG. 2. The length of the maturation period (in days) and the total germination rate (in %). Cyst age describes age of cysts when placed in incubation, not when actual excystment took place. A) *C. hirundinella* cysts stored at 4° (○) and 10° C (●). Cysts started germinating in January 1996 at an age of 4.5 months, and maximum excystment occurred in April the same year. B) Cysts of *P. aciculiferum* (○), stored at 4° C, started germinating in July 1996 (start of incubation in June) at an age of 2.5 months.

6° C. At 7° C, cysts in tubes exposed to light started to germinate (significantly different from the initial value at the 5% level) (Fig. 3a). From 11° C and up to 21 °C, more than 95% of the cysts incubated in light germinated.

Light had a positive effect on the excystment of *Ceratium*. Cysts incubated in the light had a significantly (one-way ANOVA, $P = 0.01$) higher rate of germination than those incubated in darkness (between 7° and 21° C). Also, at 7° C cyst number incubated in light was significantly lower than initial cyst numbers, indicating excystment, whereas those in the dark were not. Further evidence that germination had occurred was indicated by the presence of empty cysts and vegetative cells in these samples. Cysts in the dark germinated at 8° C and above.

The temperature gradient experiment showed that mature *Peridinium* cysts germinate at low temperatures (Fig. 3b). Cysts did not germinate when test tubes were frozen (indicated by 0° C in the graph). Between 3° and 7.5° C, cysts incubated in the light germinated, and these results were significantly different from initial cyst numbers at the 5% level (Fig. 3b). Cysts incubated in the light at 9° C and above did not germinate. Cysts placed in dark test tubes did not germinate at any temperature, as indicated by final cyst numbers, which did not differ significantly from initial cyst numers.

Anoxia experiment. Oxygen concentrations measured at the end of the experiment were lower than the original calculated concentration. Hypoxic vials had 0.01 mg $O_2 \cdot L^{-1}$ instead of 0.5 mg $O_2 \cdot L^{-1}$ and < 0.001 mg $O_2 \cdot L^{-1}$ instead of 0.1 mg $O_2 \cdot L^{-1}$. In fact, oxygen concentrations in the 0.1 mg $O_2 \cdot L^{-1}$ treatment were as low as in the anoxic treatment at the end of the experiment. Normoxic vials had a concentration of 7.1 mg $O_2 \cdot L^{-1}$ instead of a calculated 8.7 mg $O_2 \cdot L^{-1}$.

Low oxygen levels had a negative effect on germination of *Ceratium* cysts (ANOVA, $P = 0.0001$). On average, 96% of the *Ceratium* cysts germinated in the normoxic treatment (Fig. 4). In the low-oxygen treatment, 94% had germinated. The difference between normoxic and low-oxygen treatment was not significant (Scheffe's test, $P = 0.9970$) at the 5% level (Fig. 4). In the very low oxygen treatment and in anoxia, respectively, 86% and 76% of the cysts remained unhatched after incubation. In these treatments, the germination frequency was significantly lower (Scheffe's test, $P = 0.0001$) than the normoxic control (Fig. 4). When the remaining cyst number was compared to the original number add-

TABLE 1. *Dormancy period and median incubation time for mature* P. aciculiferum *and* C. hirundinella *cysts when incubated at 3° C with a 14:10 L:D cycle and 7° C with a 12:12 L:D cycle, respectively.*

Species	Storage temperature (° C) before incubation	Dormancy period (months)	Median incubation time: range (days)	Occurrence of minimum median incubation time (months)
C. hirundinella	4	4.5	1–14	April–May
C. hirundinella	10	4.5	5–14	March–April
P. aciculiferum	4	2.5	10–31	November

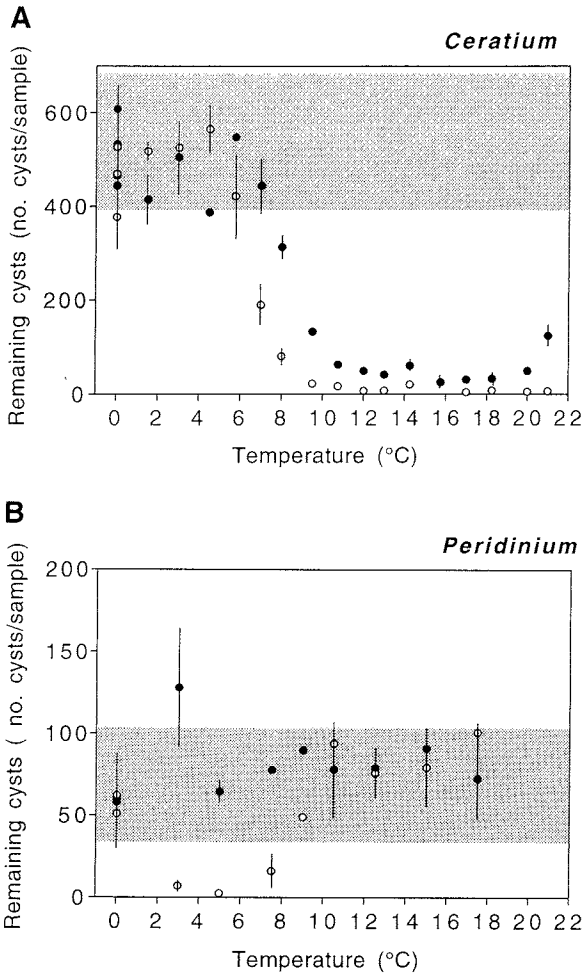

FIG. 3. Effect of temperature on the excystment of dinoflagellate cysts. The graphs show the number of remaining (ungerminated) cysts per sample versus temperature (0°–22° C), where 0° C indicates frozen samples. Cysts were incubated in the light (○) and in the dark (●). Circles designate mean and bars show range at each treatment. The grey area indicates interval of the mean of initial values (cyst number prior to incubation) plus two standard deviations (5% significance level). A) *Ceratium hirundinella*, B) *Peridinium aciculiferum*.

ed, a decrease of cyst number had occurred in all treatments.

Results from the anoxia experiment on *Peridinium* were excluded because the lack of light prevented cysts in both control and low-oxygen treatments from germinating.

Nutrients. The presence of N and P in the incubation medium appeared to have small but significant effects on germination (ANOVA, $P = 0.0277$) (Fig. 5). The germination rate of cysts incubated in replete DM medium was above 99%, in N deplete medium 96%, and in P deplete medium 95%. Cysts with no N or P added to the medium had an average germination rate of 92% (Fig. 5). The results showed a slightly lower but significant excystment frequency in medium without N or P than in the nutrient replete control (Scheffe's test, $P = 0.0289$).

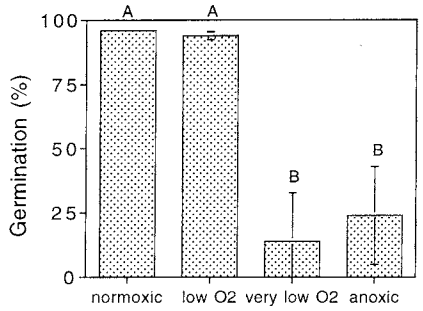

FIG. 4. Effect of anoxia on the excystment of *C. hirundinella* cysts. Total germination (in %) at four different treatments: normoxic (8.7 mg $O_2 \cdot L^{-1}$), low oxygen (0.5 mg $O_2 \cdot L^{-1}$), very low oxygen (0.1 mg $O_2 \cdot L^{-1}$), and completely anoxic (<0.001 mg $O_2 \cdot L^{-1}$). The letter A designates that treatments are not significantly different from each other at the 0.05 level; B signifies a significant difference between treatment and control.

DISCUSSION

In this study we investigated various environmental and endogenous factors that regulate the cyst germination of two common freshwater dinoflagellates, *C. hirundinella* and *P. aciculiferum*. The two main factors determining the timing of germination for the two species were internal maturation processes and water temperature, although environmental factors such as anoxia and darkness could suppress germination.

We observed that both *Ceratium* and *Peridinium* had relatively long dormancy periods (20 and 11 weeks, respectively) following cyst formation. It is unclear what determined the length of the dormancy period, although temperature has been suggested in marine studies (Anderson 1980, Endo and Nagata 1984). However, *Ceratium* did not show any difference in maturation period whether stored at 4° or 10° C. These findings are consistent with those of Binder and Anderson (1987) for *Scrippsiella trochoidea*. The results suggest that the dormancy period

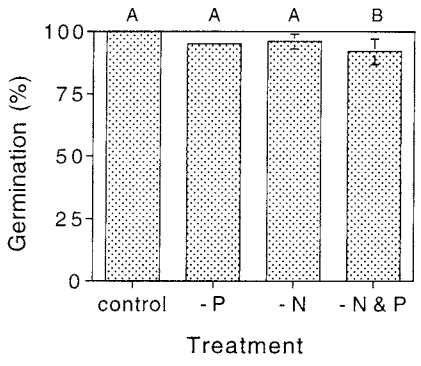

FIG. 5. Effect of nutrients on the excystment of *C. hirundinella* cysts. Total germination (in %) at four different treatments: control (replete DM medium), −P (P deplete medium), −N (N deplete medium), and −P&N (P and N deplete medium). The letter A designates that treatments are not significantly different from each other at the 5% level; B signifies a significant difference between treatment and control.

in *Ceratium* is not governed by metabolic activity or some other temperature-dependent process.

The median incubation time (incubation time at which most cysts germinate) differed between *Ceratium* and *Peridinium*. *Ceratium* cysts had a median incubation time as low as 1–2 days (at 7.5° C) when the cysts were 6–7 months old. On the other hand, the incubation time of *Peridinium* was never lower than 8–10 days, which occurred when the cysts were 6–7 months old (at 3° C). The difference between the two species may be explained by differences in incubation temperature (7° vs. 3° C). Huber and Nipkow (1923) showed that the incubation time for germination of *Ceratium* cysts from Zürichsee decreased with temperature, which was also observed in this study (unpubl. observ.). The physiological explanation is that germination is preceded by a period of higher respiration, during which protein and chlorophyll *a* are synthesized (Binder and Anderson 1990), and, because these activities are temperature regulated, differences in incubation time with temperature can also be expected.

Cyst age and storage temperature had a great effect on both median incubation time and total germination (in %) of *Ceratium*. Cysts stored at both 4° and 10° C showed an increase of total germination along with a decrease in incubation period, as cysts aged from 4.5 months to 6–7 months (April and May 1996). The maximum germination rate occurred shortly before and during the time that newly emerged vegetative cells appeared in the lake. However, after this period, *Ceratium* cysts stored at 10° C showed a decrease in the total percentage of cysts that actually germinated, indicating either a reduced germination potential or that an endogenous rhythm was in action. Microscopic examination for Brownian movement suggested that these cysts were viable, although this does not rule out a lack of sufficient energy (carbohydrate) for germination. Nevertheless, germination of *Ceratium* stored in constant darkness and temperature since September 1996 increased again in March 1997, suggesting that an internal clock was operating. On the other hand, cysts stored at 4° C maintained a high germination rate throughout the year. One possibility is that the effect of continued low temperature supresses the endogenous annual rhythm.

In *Peridinium*, age and time of year also had an effect on incubation time and total germination. After reaching maturation, incubation time decreased from 18–30 days to an eventual minimum of 8–10 days when the cysts were 6–7 months old. As cysts became older, the incubation time increased again. Once again this suggests an endogenous clock, as an age of 6–7 months corresponds to the time when cysts germinate in the field. The total germination rate also changed along with incubation time. Once again, germination increased to a maximum in October and November, only to decrease again after this period, to a low in March 1997.

An endogenous clock regulating germination has not been reported previously for freshwater phytoplankton. Anderson and Keafer (1987) suggest that an annual clock regulating germination is a mechanism necessary for marine dinoflagellates that rest on sediments in deep coastal waters, where the bottom temperatures are relatively constant and photoperiodic triggering is impossible. In shallow lakes, however, temperature varies and light may reach the sediments. An endogenous clock in lakes may be a strategy that prevents cysts from germinating at the wrong time. For example, *Ceratium* cysts that have been prevented from germination by being buried too far down in the sediments may be resuspended at a later occasion. The endogenous clock prevents germination in months such as September and October, when the temperature window allows for germination but survival success is limited because of the onset of winter. However, the presence of a biological clock in *Peridinium* and *Ceratium* cannot be proven until cysts stored in the laboratory have shown to germinate at a specific time for at least 2 consecutive years.

Temperature appears to be the most important external factor regulating cyst germination. Both *Ceratium* and *Peridinium* have temperature windows within which germination is possible. *Ceratium* had a wide temperature range for excystment (from 6° to at least 21° C), whereas that of *Peridinium* was much narrower (3°–7.5° C). The temperature window for *Ceratium* agreed with the studies by Huber and Nipkow (1923). However, Huber and Nipkow (1923) also showed that cysts did germinate between 4° and 6° C but that the incubation period was 4 weeks. Because the temperature gradient experiment in this study was run for only 2 weeks (based on germination results at 7.5° C), it cannot be ruled out that germination does not occur between 4° and 6° C. However, in the field the temperature did not rise above 4° C until the end of April, and a few weeks later the temperature in the water would be well above 10° C. The fact that total germination at 7°–9° C was not as high as at 10° C or above is probably a result of slower germination at these low temperatures.

The experimental temperature window agreed well with what was seen in the field for both *Ceratium* and *Peridinium*. The opening of the germination window for *Ceratium* at 6° C corresponded to the temperature of bottom waters (12 m depth) in the spring when vegetative cells were first seen in the water samples. Also, the biggest drop in cyst number in the surface sediments occurred between April and May as the temperature rose quickly from 4° to 8° C. Likewise, cells of *Peridinium* were found in the water column when the temperature had dropped to 4° C. Cysts may have started to germinate in the beginning of November as the temperature fell below 8° C. Because of the long incubation time (8–10 days), cells were not sufficiently numerous to be

detected in the water until December. The temperature drop in the fall was followed by a decline in cyst number, which was not as clear as for *Ceratium* but was nevertheless a decline (Rengefors 1998).

Light affected the germination of the two species differently. *Ceratium* cysts incubated in the dark germinated, but to a lesser extent than those exposed to light. Presumably, darkness delayed germination but did not prevent it. This adaptation seems useful because light penetration may be limited during the spring, and, furthermore, it allows cysts from deeper parts of lakes to germinate, thus increasing the total inoculum. In contrast, *Peridinium* did need light to germinate. This result was surprising because light conditions in November, other than in the shallow areas, are poor. Despite this fact, vegetative cells were seen in the water at the time when cysts were decreasing (although weakly) in number on the sediment surface. The cysts that germinate in November are either resuspended from deeper parts of the lake prior to ice covering, are mainly germinating from shallow areas with fine sediments, or are adapted to germinate at extremely low light conditions.

The germination experiment with *Ceratium* cysts incubated at different oxygen concentrations showed that germination was prevented at 0.5% saturation and in completely anoxic conditions. Although cyst numbers in these treatments were lower than the initial values, indicating that some germination may have taken place, no empty cysts or motile cells were encountered in these samples. This leads us to conclude that no germination took place. Nevertheless, germination could occur at conditions of low dissolved oxygen concentrations (from an original 0.5 mg $O_2 \cdot L^{-1}$, with a final of 0.01 mg $O_2 \cdot L^{-1}$ = 0.1% saturation at 20° C). These results agree with those for marine dinoflagellate cysts (Anderson et al. 1987) but not with previous studies on *Ceratium* cysts (Huber and Nipkow 1923, Krupa 1981b). We believe that these earlier studies did not achieve low enough concentrations of oxygen to prevent germination. Appropriate precautions and equipment were not used, and, furthermore, it was not shown that anoxia prevailed at the end of the incubation period. Our results showed that the anoxic treatment was indeed anoxic at the end of the experiment and that oxygen had been consumed down to below detection in the very low oxygen treatment.

Nutrients may have a small positive effect on the germination of *Ceratium* cysts because there was a significant difference between the nutrient replete medium (control) and the N and P deplete medium. However, these results must be considered with caution. Although the results were statistically significant, the ecological difference between the control and the N and P deplete medium was minor. Furthermore, we noted that some samples contained several cysts that looked newly formed. These cysts stained darkly with iodine, and the stained area was spread throughout the cyst, in contrast to "old" cysts, which were lighter stained and had a concentrated stained cytoplasm. Possibly, newly germinated cells have divided and then formed new cysts because of nutrient starvation. These "new" cysts were found in all but the control samples. Even if the data are corrected for presence of "new" cysts by removing them from the total number of cysts counted, a significant difference remains. If nutrients have an effect on germination success, cysts that are preparing to germinate must be able to utilize nutrients in their environment. These data are supported by the study of Rengefors et al. (1996), which suggested that dormant cysts of the marine dinoflagellate *Scrippsiella trochoidea* are able to take up phosphorus.

Conclusions. The dormancy period, along with water temperature, determines when cysts can germinate. Together, these two factors can explain the seasonal appearance of *C. hirundinella* and *P. aciculiferum* in Lake Erken. For example, *Ceratium* germinates during April and May in the lake. Because cyst formation occurred in September (Rengefors 1998), cysts could not be induced to germinate during the fall because of the 4.5-month-long dormancy period despite temperatures allowing for germination (=6° C). The cysts were mature in January and February but remained quiescent because of the low water temperatures (2°–3° C). Once the temperature rose to 6°–7° C, both temperature and maturation allowed for germination, and this in fact occurred in April and May, when the first vegetative cells were spotted in the water samples and as cyst number was decreasing from the sediments (Rengefors 1998).

The germination of *Peridinium* during November and December can also be explained by maturation period and temperature. Cysts of *Peridinium* produced in April are mature by July. However, the cysts remain quiescent because of high water temperature. Our experiments show that *Peridinium* may germinate at 7.5° C, which is in November. This agrees with the appearance of *Peridinium* in the water column in early December and furthermore explains the decline in *Peridinium* cysts in the sediments.

However, the seasonal pattern of dinoflagellate life histories can be altered by anoxia and poor light conditions. For example, cysts buried deep in the sediments that tend to be anoxic are prevented from germination. In lakes with long periods of anoxic surface sediments, the seasonal succession of cyst-forming dinoflagellates may be altered. Likewise, in lakes with serious anoxia due to eutrophication, dinoflagellates may be lacking because of suppressed cyst germination. Moreover, species (e.g. *P. aciculiferum*) that cannot germinate without light will not germinate from lake bottoms that are not exposed to light. Because of the impediment that anoxia and light may pose on the germination of cysts, deep regions of lakes are probably not impor-

tant seed banks and inocula for future populations. Instead, the littoral zone and other shallow regions, with fine sediments, probably provide the major site of inocula. Similarly, shallow areas will also be important as seed banks for lakes that suffer from anoxia in deeper regions. Because of conditions of anoxia and/or lack of light, processes such as resuspension will be important to some species and in deep lakes.

Despite the dominance of *P. aciculiferum* (winter) and *C. hirundinella* (summer) in the phytoplankton community of Lake Erken, both species spend the major part of the year resting as cyst in the benthos. We have found that water temperature, cyst dormancy period, and perhaps an endogenous clock regulate when the cysts germinate and vegetative cells appear in plankton. These three factors together prevent bloom initiation at the wrong time of the year. Cyst formation needs to be studied to complete the picture of seasonal succession now that the timing of germination can explain when species appear in plankton. The next step will be to go beyond triggering cues and investigate the evolutionary factors behind the seasonality of different dinoflagellates.

We thank the staff at the Lake Erken Laboratory for assistance with sampling and Dr. K. Petterson for providing physical lake data. We would also like to thank Drs. P. Blomqvist, A. Brunberg, and K. Petterson and for comments on the manuscript.

Anderson, D. M. 1980. Effects of temperature conditioning on development and germination of *Gonyaulax tamarensis* (dinophyceae) hypnozygotes. *J. Phycol.* 16:166–72.

Anderson, D. M. & Keafer, B. A. 1987. An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis*. *Nature* 325:616–7.

Anderson, D. M., Kulis, D. M. & Binder, B. J. 1984. Sexuality and cyst formation in the dinoflagellate *Gonyaulax tamarensis*: cyst yield in batch cultures. *J. Phycol.* 20:418–25.

Anderson, D. M., Taylor, C. D. & Armbrust, E. V. 1987. The effects of darkness and anaerobiosis on dinoflagellate cyst germination. *Limnol. Oceanogr.* 32:340–51.

Anderson, D. M. & Wall, D. 1978. Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in initiating toxic dinoflagellate blooms. *J. Phycol.* 14:224–34.

Beakes, G., Canter, H. M. & Jaworski, G. H. M. 1988. Zoospore ultrastructure of *Zygorhizidium affluens* Canter and *Z. planktonicum* Canter, two chytrids parasitizing the diatom *Asterionella formosa* Hassall. *Can. J. Bot.* 66:1054–67.

Binder, B. J. & Anderson, D. M. 1987. Physiological and environmental control of germination in *Scrippsiella trochoidea* (dinophyceae) resting cysts. *J. Phycol.* 23:99–107.

Binder, B. J. & Anderson, D. M. 1990. Biochemical composition and metabolic activity of *Scripsiella trochoidea* (Dinophyceae) resting cysts. *J. Phycol.* 26:289–98.

Blankley, F. W. & Lewin, R. A. 1976. Temperature responses of a coccolithophorid, *Cricosphaera carterae*, measured in a simple and inexpensive thermal-gradient device. *Limnol. Oceanogr.* 21:457–61.

Blomqvist, P., Bell, R. T., Olofsson, H., Stensdotter, U. & Vrede, K. 1995. Plankton and water chemistry in Lake Njupfatet before and after liming. *Can. J. Fish. Aquat. Sci.* 52:551–65.

Blomqvist, P., Pettersson, A. & Hyenstrand, P. 1994. Ammonium-nitrogen: a key regulatory factor causing dominance of non-nitrogen fixing cyanobacteria in aquatic systems. *Arch. Hydrobiol.* 132:141–64.

Boero, F., Belmonte, G., Fanelli, G., Piraino, S. & Rubino, F. 1996. The continuity of living matter and the discontinuities of its constituents: do plankton and benthos really exist? *TREE* 11:177–9.

Bravo, I. & Anderson, D. M. 1994. The effects of temperature, growth medium and darkness on excystment and growth of the toxic dinoflagellate *Gymnodinium catenatum* from northwest Spain. *J. Plankton Res.* 16:513–25.

Chapman, A. D. & Pfiester, L. A. 1995. The effects of temperature, irradiance, and nitrogen on the encystment and growth of the freshwater dinoflagellates *Peridinium cinctum* and *P. willei* in culture (Dinophyceae). *J. Phycol.* 31:355–9.

Dale, B. 1983. Dinoflagellate resting cysts: "benthic plankton." *In* Fryxell, G. A. [Ed.] *Survival Strategies of the Algae.* Cambridge University Press, Cambridge, pp. 69–137.

Endo, T. & Nagata, H. 1984. Resting and germination of cysts of Peridinium sp. (Dinophyceae). *Bull. Plankton Soc. Jpn.* 31:23–33.

Fryxell, G. A., Ed. 1983. *Survival Strategies of the Algae.* Cambridge University Press, New York, pp. 1–22.

Hansson, L.-A., Rudstam, L. G., Johnson, T. B., Soranno, P. & Allen, Y. 1994. Patterns in algal recruitment from sediment to water in a dimictic, eutrophic lake. *Can. J. Fish. Aquat. Sci.* 51:2825–33.

Heaney, S. I., Chapman, D. V. & Morison, H. R. 1983. The role of the cyst stage in the seasonal growth of the dinoflagellate *Ceratium hirundinella* within a small productive lake. *Br. Phycol. J.* 18:47–59.

Huber, G. & Nipkow, F. 1922. Experimentelle Untersuchungen über die Entwicklung von Ceratium hirundinella O.F.M. *Zeitschrift für Botanik* 14:337–71.

——— 1923. Experimentelle Untersuchungen über Entwicklund und Formbildung von Ceratium hirundinella O.F. Müller. *Flora (Jena)* 116:114–215.

Håkansson, L. 1978. Erken—morfometri. *Scripta Limnologica Upsaliensia* 468. Institute of Limnology, Uppsala University, Uppsala, Sweden.

Krupa, D. 1981a. *Ceratium hirundinella* (O.F. Müller) Bergh in two trophically different lakes I. Population dynamics (with the cysts taken into account). *Ekol. Pol.* 29:545–70.

——— 1981b. *Ceratium hirundinella* (O.F. Müller) Bergh in two trophically different lakes II. Development and morphological variation of active forms and cysts. *Ekol. Pol.* 29:571–83.

Lutz, R. V., Marcus, N. H. & Chanton, J. P. 1992. Effects of low oxygen concentrations on the hatching and viability of eggs of marine calanoid copepods. *Mar. Biol.* 114:241–7.

McQuoid, M. R. & Hobson, L. A. 1995. Importance of resting stages in diatom seasonal succession. *J. Phycol.* 31:44–50.

——— 1996. Diatom resting stages. *J. Phycol.* 32:889–902.

Nauwerck, A. 1963. Die beziehungen zwischen Zooplankton und Phytoplankton im See Erken. *Symbolae Botanicae Upsalienses* 17:1–163.

Nehring, S. 1996. Recruitment of planktonic dinoflagellates: importance of benthic resting stages and resuspension events. *Int. Revue Ges. Hydrobiol.* 81:513–27.

Pettersson, K. 1985. The availability of phosphorus and the species composition of the spring phytoplankton in Lake Erken. *Int. Revue Ges. Hydrobiol.* 70:527–46.

Pfiester, L. A. & Anderson, D. M. 1987. Dinoflagellate reproduction. *In* Taylor, F. J. R. [Ed.] *The Biology of Dinoflagellates. Bot. Monogr.* 21:611–48.

Pollingher, U., Bürgi, H. R. & Ambühl, H. 1993. The cysts of *Ceratium hirundinella*: their dynamics and role within a eutrophic (Lake Sempach, Switzerland). *Aquat. Sci.* 1:10–18.

Rahmberg, L. 1976. Relations between phytoplankton and environment in two Swedish forest lakes. Ph.D. thesis, Scripta Limnologica Upsaliensia, Uppsala University, Uppsala, Sweden, 97 pp.

Rengefors, K. 1998. Seasonal succession of dinoflagellates coupled to the benthic cyst dynamics in Lake Erken, Sweden. *Archiv für Hydrobiologie Spec. Issues Advanc. Limnol.* 51:123–41.

Rengefors, K., Anderson, D. M. & Pettersson, K. 1996. Phospho-

rus uptake by resting cysts of the marine dinoflagellate *Scrippsiella trochoidea*. *J. Plankton Res.* 18:1753–65.
Reynolds, C. S. 1984. *The Ecology of Freshwater Phytoplankton.* Cambridge University Press, London, 384 pp.
Sommer, U., Gliwicz, M., Lampert, W. & Duncan, A. 1986. The PEG*-model of seasonal succession of planktonic events in fresh waters. *Arch. Hydrobiol.* 106:433–71.
von Stosch, H. A. 1973. Observations on vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* Schiller and *Woloszynskia apiculata* sp. nov. *Br. Phycol. J.* 8:105–34.
Wall, D. 1971. Biological problems concerning fossilizable dinoflagellates. *Geoscience and Man* 3:1–15.
Watras, C. J., Chisholm, S. W. & Anderson, D. M. 1982. Regulation of growth in an estuarine clone of *Gonyaulax tamarensis* Lebour: salinity-dependent temperature responses. *J. Exp. Mar. Biol. Ecol.* 62:25–37.
Yamaguchi, M., Itakura, S., Imai, I. & Ishida, Y. 1995. A rapid and precise technique for enumeration of resting cysts of *Alexandrium* spp. (Dinophyceae) in natural sediments. *Phycologia* 34:207–14.