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Toxicity in *Peridinium aciculiferum*—an adaptive strategy to outcompete other winter phytoplankton?

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Abstract

Freshwater dinoflagellates may form dense blooms during winter in ice-covered lakes. Unlike their marine counterparts, freshwater dinoflagellates are rarely considered to be potential toxin producers. Here we tested whether the winter species *Peridinium aciculiferum* produces a toxin(s) and investigated the potential adaptive function of that toxin, i.e., predator defense or inhibition of competitors (allelopathy). Using traditional toxicity bioassays (*Artemia* toxicity test and hemolytic activity assay), we detected the production of a toxic substance by *P. aciculiferum* cells from both the field and from laboratory cultures. Cultures deprived of phosphorus and in stationary phase showed highest toxicity. Potential predators, such as *Daphnia galeata* (Cladocera) and *Eudiaptomus graciloides* (Copepoda), were apparently not harmed by *P. aciculiferum* toxicity. However, the naturally cooccurring competitor *Rhodomonas lacustris* (Cryptophyceae) was killed by *P. aciculiferum.* An allelopathic substance(s) caused the cells of *R. lacustris* to form blisters and subsequently lyse. We concluded that our results support the hypothesis that *P. aciculiferum* is allelopathic, but not that toxins serve as predator defense. We therefore suggest that allelopathy may be an adaptive strategy of winter dinoflagellates, which could allow them to outcompete other phytoplankton species and thereby dominate the algal biomass.

Freshwater dinoflagellates are generally considered to be nontoxic and harmless algae despite the fact that their marine counterparts may be extremely toxic and can have substantial negative effects on the systems they occur in. In freshwater phytoplankton communities, dinoflagellates are a common component and may dominate the biomass completely during late summer in temperate lakes. In addition, dinoflagellates can also form extensive blooms under ice during late winter and early spring, a phenomenon that has received little scientific attention.

The adaptive function of toxin production in phytoplankton is still debated. Possible functions may be repulsion of grazers, inhibition of cooccurring phytoplankton species (allelopathy), release of degradation products, or storage of products (Turner and Tester 1997). Regardless of which evolutionary forces have led to toxin production in marine dinoflagellates, freshwater species should be exposed to similar selective forces.

Among marine dinoflagellates, several are ichthyotoxic (Steidinger 1983; Burkholder et al. 1995), being lethal to larval, juvenile, and adult fish of several species (Turner et al. 1998). Ichthyotoxic dinoflagellates can either have a direct deleterious effect on fish or a vectorial one, i.e., intoxication through prey that have accumulated toxins through grazing, but are themselves immune to the toxin (Turner and Tester 1997). There are a few cases reporting toxic freshwater dinoflagellate blooms (Adachi 1965; Hashimoto et al. 1968; Jurgens 1953). The available evidence suggests that dinoflagellates within or related to the *Peridinium* genus are potential producers of toxins. *Peridinium polonicum* (now *Peridiniopsis polonicum*) caused fish kills in a reservoir in Japan (Adachi 1965). Another species, *Peridinium bipes*, was recently shown to have an algicidal effect on the cyanobacteria *Microcystis aeruginosa* (Wu et al. 1998). Mills et al. (1995) reported suspected fish kills due to algal toxins released by dinoflagellates in an artificially acidified lake. A potential candidate for ichthyotoxic dinoflagellates in lakes is *Peridinium aciculiferum.* This dinoflagellate is often found in large numbers in winter phytoplankton communities under ice in some Swedish lakes (Rengefors 1998). During a *P. aciculiferum* bloom, only other small dinoflagellate species cooccur at any significant biomass (Nauwerck 1963; Rengefors 1998).

It is possible that toxic blooms of dinoflagellates in freshwater occur more frequently than reported and that they affect the biota in those habitats. For example, the presence/absence of *P. aciculiferum* may be coupled to the recruitment success of vendace (*Coregonus albula*) larvae in Lake Mälaren, Sweden. Circumstantial evidence suggests

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Table 1. Sampling sites for *Peridinium aciculiferum* in two Swedish Lakes in Feb–Mar 2000; Lake Mälaren (At Ekoln and Adelsö) and Lake Erken. Lake Edasjön was sampled for copepods only.

Sampling site	Location	Date	Ice cover	*Peridinium* (cells ml^{-1})
Lake Mälaren				
Ekoln	59°45′N, 17°36′E	10 Feb	Yes	0
		24 Feb	Yes	0
		5 Mar	Yes	0
Adelsö	59°17′N, 17°31′E	11 Feb	Yes	0
		22 Feb	Yes	240
		8 Mar	Yes	0
		20 Mar	No	0
Lake Erken	59°51′N, 18°35′E	3 Mar	Yes	40,640
		7 Mar	Yes	13,480
		10 Mar	Yes	13,840
		14 Mar	Yes	16,880
Lake Edasjön	59°48′N, 17°54′E	23 Mar	Yes	

that poor recruitment occurs where *P. aciculiferum* is present (Nyberg et al. 1998). Vendace larvae hatch at the end of April concurrent with ice-out. Yolk-sac and first-feeding larvae of various marine fish species ingest dinoflagellates and herbivorous zooplankton (Turner and Tester 1997). Consequently, if larvae emerge simultaneously or following a bloom of dinoflagellates, the phycotoxins could be potentially devastating (Hjort 1914; Lasker 1971). Planktivorous fish are likely to eat dinoflagellates, since their fatty acid content makes them high quality food (Ahlgren et al. 1992). For example, in Lake Kinneret, Israel, the fish *Tilapia galilaea* selectively feeds on *Peridinium cinctum* (Spataru 1976). Alternatively, first-feeding larvae risk vectorial ingestion of phycotoxins through intake of copepods and rotifers that may have fed on dinoflagellates. *Peridinium aciculiferum* is within the size range of a potential food source for vendace larvae or as prey for zooplankton, which then provide a food source for the fish larvae. If *P. aciculiferum* produces ichthyotoxins, this may be the cause of death for fish larvae, resulting in the poor recruitment of vendace in Lake Mälaren during the past 10 yr (Nyberg et al. 1998).

Here we present the results of a study designed to determine (1) whether the freshwater dinoflagellate *Peridinium aciculiferum* produces toxin(s) and (2) whether the adaptive function of the toxin(s) is to inhibit competitors or whether it is a defense against grazers. Our strategy was to use traditional toxicity tests to detect any toxin and then to test for acute negative effects of *P. aciculiferum* cells and cell-free filtrate on zooplankton (predators) and phytoplankton (competitors).

Methods

Sampling sites—Dinoflagellates were sampled during the winter of 2000 in two Swedish lakes, Lake Mälaren (Ekoln and Adelsö sites) and Lake Erken (Table 1). Ekoln, a eutrophic bay, has good recruitment of *Coregonus albula* (vendace), while Adelsö, located in the central less eutrophic part of the lake, does not. Lake Erken is known to have *Peridinium aciculiferum* blooms during late winter/early spring but is not populated with *Coregonus albula.*

At all sites, 10–20 liters of water were collected at 0–2 m depth below the ice. A subsample of 50 ml was taken out and preserved for phytoplankton counts with Lugol's solution. Ten liters were concentrated through a 20-μm sieve. The sieved and the unsieved fraction were filtered onto a GF/C filter in the laboratory. The filters were frozen and stored at −20°C. On one occasion (14 March 2000), cells from Lake Erken were collected, processed, and analyzed immediately.

Phytoplankton and zooplankton cultures—A strain of *Peridinium aciculiferum,* isolated from Lake Erken, was used for all the experiments. This culture has been identified as *P. aciculiferum* Lemmermann according to Popovsky and Pfiester (1990) by analyzing plate patterns through SEM (Fig. 1) in collaboration with B. Meyer (*see also* Rengefors 1998). Exponentially growing cells of *P. aciculiferum* were harvested from cultures grown at 3°C using diatom medium (DM) (Beakes et al. 1988). Cells of *P. aciculiferum* were also harvested during stationary phase in cultures grown at 2°C, in phosphate replete medium (~90 μM), and in phosphate poor medium (~2 μM). Phosphorus (P) limitation was confirmed by decreased P content and increased C : N : P ratios based on CHN and particulate phosphorus analysis respectively (Table 2). Aliquots of cultures were filtered onto GF/C filters, amounting to 1.6×10^6 cells of P-replete cells, and 0.8×10^6 P-deficient cells per filter. The filters were placed in cryovials and stored at −70°C. For long-term preservation the filters were freeze-dried for 5 h and subsequently stored at room temperature.

The other organisms used in this study are listed below. *Rhodomonas lacustris* Pascher and Ruttner isolated from Lake Erken was grown at 17°C in L16 medium (Lindström 1991). Prior to the allelopathic tests, *R. lacustris* cultures were transferred to the same conditions as *P. aciculiferum* cultures. *Daphnia galeata* Sars isolated from Lake Erken 1992 was cultured in the laboratory and fed with *Rhodo-*

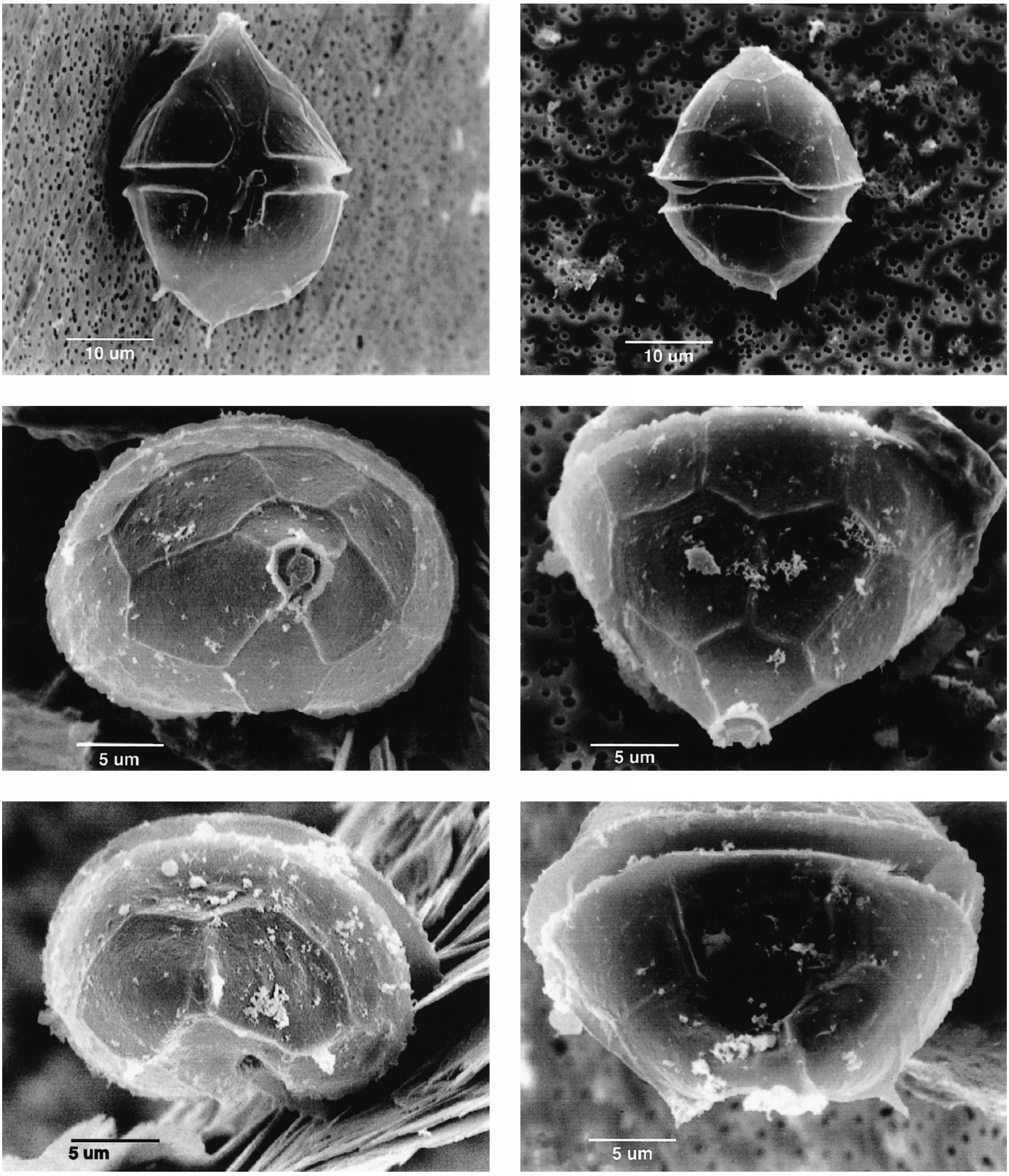

Fig. 1. SEM micrographs of *Peridinium aciculiferum* Lemmermann showing the tabulation pattern allowing for identification. The cell is shown in ventral and dorsal views, of the whole cell in the top row, the epitheca in the second row, and hypotheca in the bottom row.

Table 2. Phosphorus, nitrogen, carbon content per cell, and C : N : P (molar) ratio of P-limited and P-replete stationary phase cultures of *Peridinium aciculiferum.* Mean ± SD, $n = 5$. Note that the C : N : P ratio for the average nutrient replete algal cell is considered to be 105 : 16 : 1.

Treatment	Carbon content (ng cell^{-1})	Nitrogen content (ng cell^{-1})	Phosphorus content (pg cell^{-1})	C : N : P
P-replete	2.0 ± 0.52	0.17 ± 0.030	87 ± 14	62 : 4 : 1
P-limited	2.1 ± 0.33	0.2 ± 0.057	30 ± 6.7	176 : 15 : 1

monas lacustris. Copepods (mostly *Eudiaptomus graciloides* Liljeborg) were collected in Lake Edasjön with a 100-μm net tow under a 50-cm ice cover. Live copepods were concentrated and kept for 24 h in the dark at 2–3°C prior to toxicity tests. Eggs of *Artemia salina* were hatched according to Vanhaecke et al. (1981) using deionized water with a salinity of 5‰ and pH 8.0. The tolerance of *A. salina* to the different filtrates (lysed cells) or live cells of *P. aciculiferum* was examined on nauplii 48 h after hatching began.

Hemolytic activity—Stationary phase cells retained on filters were extracted with methanol (100%), sonicated (10 × 10 s pulses), and filtered through a 0.2-μm filter. Exponential phase cells and lake water samples were concentrated by centrifugation (3,000 g at 4°C, 15 min). After centrifugation the pellet was transferred to a 1.5 ml microfuge tube and extracted with methanol (200 μl to an original of 50 ml culture). The final extracts (filtrates) were stored at −20°C prior to analysis of hemolytic activity (HA). The HA test was performed as reported in Johansson (2000). The test was done in triplicate, and methanol was used as an optical blank. The HA of the cells was determined as saponin equivalents per cell (SnEq cell^{-1}). Hemolytic activity was also measured using fresh fish blood from rainbow trout in order to test toxicity against an organism that occurs in lakes. The same protocol was followed as for horse blood, except that fish blood was treated with heparin to prevent coagulation. The test was performed on stationary phase cultured cells only.

Artemia salina *bioassay*—Cells of exponential phase *P. aciculiferum* from P-deplete and -replete cultures were concentrated (10 min at 3,000 g) and then diluted to give a concentration series of P-deplete (360, 1,600 cells ml^{-1}) and P-replete (3,200 cells ml^{-1}) cultures. Stationary phase cells of *P. aciculiferum* on freeze-dried filters (GF/C) were resuspended in DM medium (at pH 10) and sonicated (10 pulse × 10 s). The liquid phase (lysed cells) was filtered through 0.2-μm filters (polyethersulfone). Filtrates obtained after the lysis and filtration of P-deplete cells corresponded to an addition of 36,000 cells ml^{-1}. Control tests were performed to rule out any effect of pH, salinity, or filters. The toxicity of a natural phytoplankton sample from Lake Erken (14 March 2000) containing *P. aciculiferum* was tested using the whole water sample (20 cells ml^{-1}) and the fraction >20 μm (1,000 *Peridinium* cells ml^{-1}).

Microwells (Falcon multiwell, 12 wells) were filled with 2 ml of *P. aciculiferum* culture, lysate with medium, or medium only. There were five replicates for each treatment. Twenty-five *Artemia* nauplii were then added to each microwell. After 24 and/or 48 h incubation at 3°C, the mortality of nauplii at each concentration was examined. Nauplii were observed under a dissecting scope and were counted as dead if no movement was observed within 10 s.

Table 3. Haemolytic activity of *Peridinium aciculiferum* cells in Lake Erken samples and cultures at different growth stages. Haemolytic activity was measured with horse and/or fish blood. Mean ± SD, $n = 3$.

Sample	Haemolytic activity (SnEq cell^{-1})	
	Horse blood	Fish blood
Lake Erken		
3 Mar 2000	2.87 ± 0.14	not measured
14 Mar 2000	not detected	not measured
Cultures		
(−P) stationary	1.61 ± 0.32	2.38 ± 0.19
(+P) stationary	1.11 ± 0.1	0.92 ± 0.11
(−P) exponential	not detected	not measured
(+P) exponential	not detected	not measured

Toxic effect on predators—Acute toxicity of P-replete and P-deplete *P. aciculiferum* cultures (400 cells ml^{-1}) and Lake Erken concentrate containing *P. aciculiferum* (14 March 2000, 1000 cells ml^{-1}) were tested on *Daphnia* cultures. Two-milliliter samples of *P. aciculiferum* were added to 2-ml wells (Falcon, multiwell, 12 wells). As controls, DM medium without cells was used. Three to four *Daphnia* were transferred to each well with five replicates for each treatment and were incubated at 3°C for 24 and/or 48 h. The control samples were incubated at 3°C and 15°C to eliminate a possible effect of temperature on *D. galeata* survival. The numbers of live and dead *Daphnia* were counted under a dissecting scope. *Daphnia* that did not move in 10 s were considered dead.

Acute toxicity of P-deplete cultures of *P. aciculiferum* was also tested on a natural assemblage of calanoid copepods

Table 4. Mortality of *Artemia salina* incubated for 24 h with live *Peridinium aciculiferum* cells or cell-free lysate, and in controls (medium only). Mortality shown as percentage, with mean ± SD. The field sample was not tested statistically, but note the difference in mortality between control and treatment. Lake Erken, 14 Mar 2000.

Peridinium treatment	Cell conc. (× 10^3 cells ml^{-1})	Mortality of *Artemia salina* (%) after 24 h exposure	
		+ *P. aciculiferum*	Control (medium)
−P, exponential, live culture	0.36	20 ± 6*	2 ± 3
−P, exponential, live culture	1.6	31 ± 10	21 ± 10
+P, exponential, live culture	3.2	25 ± 8	21 ± 10
−P, stationary, lysate	36	19 ± 8*	6 ± 6
Field sample, mixed	1	20	0

* Significantly different from the control (student *t*-test, $n = 5$, $p < 0.05$).

dominated by *Eudiaptomus graciloides.* Cells of *P. aciculiferum* (275 cells ml^{-1} final concentration) were added to each well with 1 ml of filtered lake water and 1 ml of DM medium. As controls, *Rhodomonas lacustris* cells (final concentration 100,000 cells ml^{-1}) were added in a mixture of 1 ml lake water + 1 ml DM medium. One copepod (manually picked) was added to each well with 12 replicates for each treatment. The wells were incubated in the same conditions as *A. salina* and *D. galeata.* Cultures of *P. aciculiferum* were also diluted with filtered lake water to give a dilution series of 85, 115, 170, 230, 285, and 340 cells ml^{-1} (final concentration). Six milliliters of cultures were added to 10-ml wells (Falcon multiwells, six wells). To each well, 1 ml of lake water was added along with one copepod. All treatments were incubated as in the experiments above. Wells were checked after 24 h for *E. graciloides* viability.

Allelopathy experiment—Two milliliters of P-limited cultures (1,000 cells ml^{-1}) or cell-free filtrates of *P. aciculiferum* were transferred into 5-ml microwells. Aliquots (100 μl) of *Rhodomonas lacustris* cultures were added to each well (five replicates) at two final cell densities (209 ± 5 and 1704 ± 327 cells ml^{-1}), i.e., *Peridinium*:*Rhodomonas* ratios of 1:0.2 and 1:2 respectively. As a control, *R. lacustris* cells (at the two cell densities above) were diluted with 2 ml of DM medium. All wells were incubated under the same conditions as the experiments listed above. Samples were taken after 0, 12, 24, and 48 h, and were preserved with Lugol's solution for enumeration of *R. lacustris* cells. Samples (50–300 μl) were settled and counted in an inverted microscope at 400× magnification. *Rhodomonas lacustris* cells were also scored for blistering, which is indicative of membrane disruption.

Results

Field sampling—The phytoplankton counts showed that there were no *Peridinium aciculiferum* cells at the Ekoln site in Lake Mälaren and at most 240 cells L^{-1} on one occasion (22 February 2000) at the Adelsö site (Table 1). Ice conditions at Adelsö were poor, and after 10 March 2000 this site had open water. Lake Erken, on the contrary, had a maximum of 40,640 cells L^{-1} on 3 March 2000. The >20-μm fraction in Lake Erken also contained several diatom species. As the winter of 2000 was unusually mild, ice conditions were variable and thin. The ice did not form until January on Lake Erken, but was 40 cm thick by mid-March.

Hemolytic activity—Hemolytic activity (HA) was detected both in concentrates of field samples (Lake Erken) containing *P. aciculiferum* and in laboratory cultures (Table 3). In Lake Erken samples, HA was higher on 14 March 2000 (2.9 SnEq $cell^{-1}$) than on 3 March 2000 (below detection), corresponding to a *P. aciculiferum* cell abundance of 40,640 and 16,880 cells L^{-1}, respectively. Hemolytic activity was not measured in the other field samples (e.g., Lake Mälaren), since *P. aciculiferum* concentrations were lower than 17,000 cells L^{-1}.

In *P. aciculiferum* cultures, HA was demonstrated both with horse and fish blood, with values falling within the same range. Hemolytic activity of *P. aciculiferum* was 1.1–1.6 SnEq $cell^{-1}$ (horse blood) in cultures collected during stationary phase, while HA of exponentially growing cells was below detection (Table 3). Highest HA was measured in cells grown under P-limiting conditions, amounting to 1.61 ± 0.32 SnEq $cell^{-1}$ for horse blood and 2.38 ± 0.19 SnEq $cell^{-1}$ for fish blood (Table 3).

Toxicity of P. aciculiferum *toward zooplankton*—Cell lysate of *P. aciculiferum* from cultures and field samples had a lethal effect on *Artemia salina.* Phosphorus-limited exponential phase cultures (360 cells ml^{-1}) killed 20% of the *A. salina,* which was significantly higher (Student *t*-test, $p < 0.05$) than in the control after 24 h (Table 4). On the other hand, at the higher concentration (1,600 cells ml^{-1}), mortality did not differ significantly from the control ($p > 0.05$). However, the low power of the latter test suggests that negative effects should be interpreted cautiously. Phosphorus replete cultures (3,200 cells ml^{-1}) were not toxic to *A. salina* ($p > 0.05$). In contrast, stationary phase P-limited *P. aciculiferum* lysate (36,000 cells ml^{-1}) had a significant negative effect (Student *t*-test, $p < 0.05$) on *A. salina* survival (Table 4). In addition, cells lysate from Lake Erken samples appeared to have a toxic effect as they caused 20% mortality of *A. salina,* while no mortality was observed in controls (Table 4). Repeated ANOVA of two treatments (P-deplete, 1,600 and 36,000 cells ml^{-1}) showed that time did not have a significant effect on survival of *A. salina,* which is why the 48 h data are not shown in Table 4.

Daphnia galeata and *E. graciloides* were not adversely affected by live *P. aciculiferum* cells. The mortality of *D. galeata* over 24 and 48 h did not differ between the treatments with *P. aciculiferum* and the controls. No copepods died in any of the treatments; instead they seemed to feed on the dinoflagellate cells, which was confirmed by cell findings in fecal pellets. Observation of the fecal pellets by microscopy revealed that most of the *P. aciculiferum* cells were intact (light microscopy) and bright red (epifluorescence, blue light).

Allelopathic effect—The results show that cells and cell-free filtrates of *P. aciculiferum* have an allelopathic effect on *Rhodomonas lacustris.* At a high ratio (1:0.2) of *Peridinium*:*Rhodomonas, R. lacustris* cells grew significantly poorer in *P. aciculiferum* treatments (live cells and filtrate) than in controls (Repeated ANOVA, Scheffe's Test, $p < 0.0001$, both treatments) (Fig. 2a). In the *P. aciculiferum* treatments, the number of *R. lacustris* decreased sharply after 24 h. After 48 h, the populations of *R. lacustris* cells exposed to *P. aciculiferum* were below the initial cell concentration (<209 cells ml^{-1}), while they reached 600 cells ml^{-1} in the controls.

At low ratios (1:2) of *Peridinium*:*Rhodomonas,* the growth of *R. lacustris* was only slightly affected by *P. aciculiferum* cells or cell-free filtrates compared to the control. However, this difference was not significant (Repeated ANOVA, Scheffe's Test, $p = 0.06$ and $p = 0.09$), indicating a lack of allelopathic effect of *P. aciculiferum* under these conditions (Fig. 2b).

Microscopic counts revealed that the allelopathic substances produced by *P. aciculiferum* caused the formation of blisters or bubbles on the cells of *R. lacustris.* Eventually these appeared to burst, resulting in cell death. In the 1:0.2

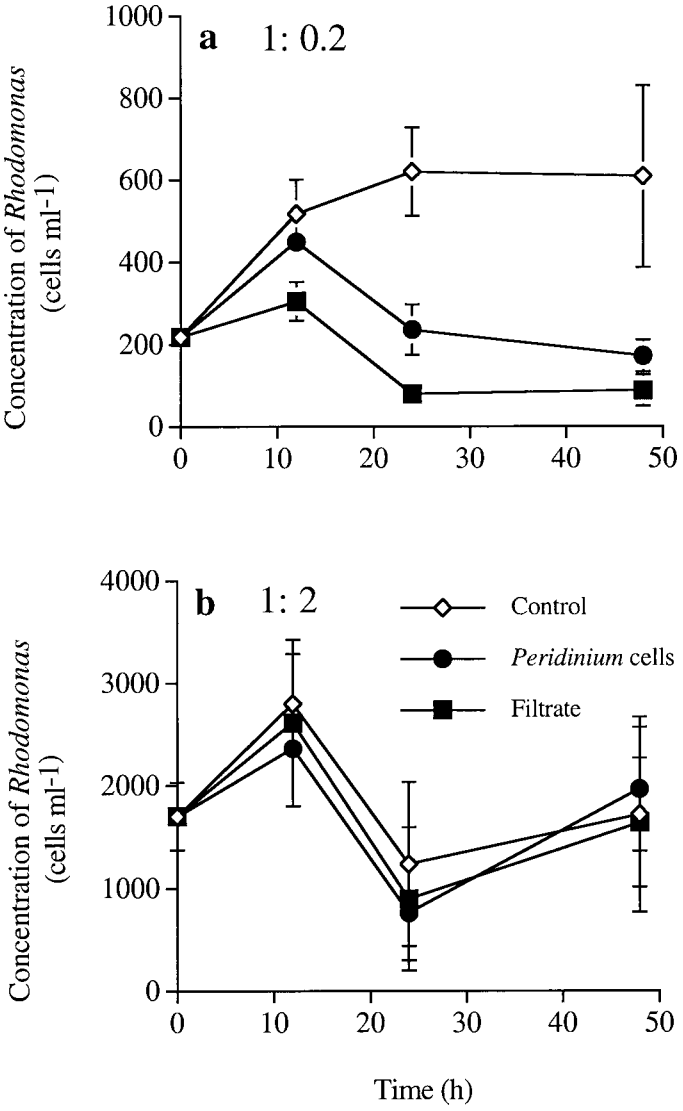

Fig. 2. Allelopathic effect of *Peridinium aciculiferum* toward *Rhodomonas lacustris* at two different treatment ratios: 1 : 0.2 and 1 : 2 of *Peridinium* : *Rhodomonas.* Vertical error bars show standard deviation ($n = 4$). (a) 1 : 0.2 *Peridinium* : *Rhodomonas.* Growth response of *R. lacustris* (209 ± 5 cells ml^{-1}) exposed to cultures and cell-free filtrates of *P. aciculiferum* (1,000 cells ml^{-1}) grown under low phosphorus concentrations. As controls, *R. lacustris* cultures were diluted with sterile filtered medium.(b) 1 : 2 *Peridinium* : *Rhodomonas.* Growth response of *R. lacustris* (1,704 ± 327 cells ml^{-1}) exposed to cultures and cell-free filtrates of *P. aciculiferum* (1,000 cells ml^{-1}) grown under low phosphorus concentrations.

Peridinium : *Rhodomonas* treatment, blistered cells of *R. lacustris* were first recorded after 24 h. Up to 50% of the cells were blistered in the filtrate treatment (Fig. 3). No blistered cells were observed in the control and were thus not reported in the figure. The percentage of blistered cells had decreased after 48 h in both the cultures and filtrate treatments, reflecting the death of blistered cells, which increases the proportional importance of healthy cells. In the 1 : 2 *Peridinium* : *Rhodomonas* treatment, blistered cells were also observed, even though no allelopathic effect was detected in the total

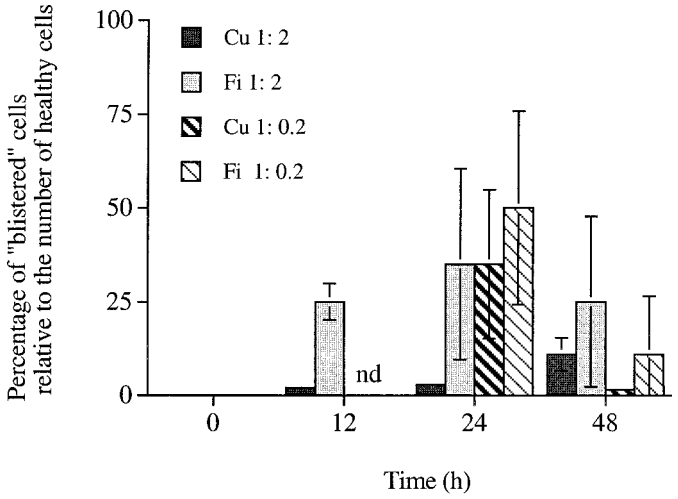

Fig. 3. Percentage of blistered *Rhodomonas lacustris* cells exposed to cultures (Cu) and cell-free filtrates (Fi) of *Peridinium aciculiferum* grown under low phosphorus concentration. Treatments with ratios of 1 : 0.2 and 1 : 2 between *Peridinium* : *Rhodomonas* are shown. Vertical error bars show standard deviation ($n = 4$). Control values are not shown on the graph since no blistered cells were observed. Note that low percentages in Cu 1 : 0.2 are due to the fact that most cells have already lysed and died.

cell counts. Blistered cells were observed only after 12 h of exposure to *P. aciculiferum* cells and cell-free filtrates, reaching 25% in the treatment with the *P. aciculiferum* cell-free filtrates (Fig. 3). In the filtrate treatment, the number of blistered *R. lacustris* cells remained at about 25% of the total number of cells throughout the 48 h of the experimental run. Remains of lysed cells were present both in the culture and filtrate treatments at both *Peridinium* : *Rhodomonas* ratios.

Discussion

In this set of experiments, we show that the freshwater species *Peridinium aciculiferum* produces a toxin or an array of toxins lethal to other planktonic organisms, particularly other phytoplankton (allelopathy). To our knowledge, this is the first winter species to be reported as allelopathic and only the second species of freshwater dinoflagellates identified as a toxin producer.

Through the use of a traditional toxicity test (hemolytic activity) we could establish that a toxin(s) is produced by *P. aciculiferum.* Phosphorus (P) limited cells harvested during stationary phase had a higher hemolytic activity than cells grown in P-replete medium, indicating that the toxin production in *P. aciculiferum* may be influenced by the nutrient status of the medium in which they are growing and/or the cellular P quotas. Nutrient limitation is known to enhance toxin content/production in marine dinoflagellates (Anderson et al. 1990), diatoms (Bates et al. 1991), and haptophytes (Johansson and Granéli 1999). Whether P limitation (low internal P quota) or growth limitation per se influences the toxicity of *P. aciculiferum* is not known. The crucial question then becomes whether *P. aciculiferum* is likely to experience P limitation in the field. Although nutrient levels

are generally high under ice, especially at the beginning of winter, *P. aciculiferum* can outstrip the pelagic zone of orthophosphate before ice-out (Pettersson 1985). Consequently, it is possible that a situation of nutrient limitation occurs when the cell density of *P. aciculiferum* becomes high. Regardless, field samples dominated by *P. aciculiferum* showed hemolytic activity, thereby supporting the assumption that toxin production occurs in the field.

Through our experiments we attempted to gain insight into the potential adaptive function of the dinoflagellate toxin. Toxin production as a predator defense mechanism was tested by exposing zooplankton to live cells and cell-free filtrates of *P. aciculiferum.* A moderate toxic effect of *P. aciculiferum* on *Artemia salina* (standard bioassay used for toxicity tests in marine organisms) provided further support for toxin production. This result, however, cannot be interpreted as the toxin being directed toward potential predators. This is because *A. salina* is a brackish species that cannot naturally cooccur with *P. aciculiferum.* The other toxicity tests showed that *P. aciculiferum* did not have an acute toxic effect on the naturally cooccurring predatory species *Daphnia galeata* (Cladocera) and *Eudiaptomus graciloides* (Copepoda). The winter period in lakes is usually a time of remarkably low densities of dinoflagellate predators, and it appears that toxicity in *P. aciculiferum* did not evolve as a defense against predators. However, it should be noted that intact cells of *P. aciculiferum* were observed in the fecal pellets of *E. graciloides,* suggesting that most of the ingested cells were not digested. This observation leads us to speculate on the potential sublethal toxic effect of *P. aciculiferum* toward grazers. If the dinoflagellate cells are not digested, are they not digested because they are toxic, and can this lead to starvation in copepods? Another issue is that since the zooplankton used in this experiment cooccur with *P. aciculiferum,* it is possible that these strains have coevolved with the dinoflagellate and are immune to its toxins. Similar tests with zooplankton from lakes without *P. aciculiferum* may render different results. These and other issues must be addressed in further experiments.

Although predator defense does not appear to be an adaptive function of the *P. aciculiferum* toxin, our results support the hypothesis that *P. aciculiferum* produces toxins to outcompete other phytoplankton. *Peridinium aciculiferum* had an algicidal effect on its competitor *Rhodomonas lacustris.* This effect is referred to as allelopathy, defined as ''the direct effect of competitors through the release of chemicals that inhibit other species'' (Lampert and Sommer 1997). In aquatic systems, freshwater cyanobacteria are known to produce allelopathic substances that can inhibit or kill other algae (Gross et al. 1991; Bagchi and Marwah 1994; Von Elert and Jüttner 1996), as are marine diatoms (Sharp et al. 1979), and haptophytes (Johansson 2000). Among dinoflagellates, both marine (Arzul et al. 1999; Uchida et al. 1999) and freshwater (e.g., *Peridinium bipes* in Wu et al. 1998) species have shown a strong allelopathic effect on cooccurring phytoplankton species.

Peridinium aciculiferum grown under P-limited conditions produces allelopathic substances. These substances are synthesized inside the cells and released in the surrounding medium either as a secondary metabolite or specifically when the *P. aciculiferum* cells encounter *R. lacustris.* At high ratios of *Peridinium*:*Rhodomonas* (1:0.2) the *R. lacustris* population crashed, while at low ratios (1:2) the dinoflagellate did not significantly affect the growth of *R. lacustris.* These results are in agreement with Johansson (2000), who showed that the haptophyte *Prymnesium parvum* had an inhibitory effect on *Rhodomonas baltica* growth only at high ratios of *Prymnesium : Rhodomonas* (10:1). The explanation for the observed effect may be that at higher densities of *R. lacustris* the amount of *Peridinium* toxin per *Rhodomonas* cell is smaller than at low densities, given that the *P. aciculiferum* density is kept the same. Consequently, higher *R. lacustris* biomass could dilute the toxicity.

The allelopathic compounds produced by *P. aciculiferum* appear to damage the cell membrane in *R. lacustris* as indicated by the presence of blistered and lysed *R. lacustris* cells in *Peridinium* culture and filtrate treatments. These results are in accordance with the cell-membrane disrupting action of the water-soluble algicidal extract of *P. bipes* (Wu et al. 1998). It should also be noted that the lysing of *R. lacustris* is homologous to the lysing of blood cells, as quantified in the hemolytic activity tests. Nevertheless, we cannot be sure that the toxin causing *Artemia* mortality or hemolytic activity is the same compound causing the allelopathic response in *R. lacustris.* The filtrate apparently caused more blistering than the intact cells, possibly due to the release of more allelopathic substances during the filtration process. Although no allelopathic effect was detected at the low ratio (1:2) of *Peridinium*:*Rhodomonas,* blistered cells were observed in these samples as well, indicating a sublethal effect of the toxin. The apparent low proportion of blistered cells after 48 h in all treatments is explained by the fact that at this time point, most blistered cells had lysed and disappeared, leaving behind only the healthy cells. As a result, the proportion of healthy cells increased, although the cell number decreased. Since the same effect was seen in both cultures and filtrate treatments, the lack of increase in blistered cells cannot be attributed to attenuation of toxicity due to degradation of the allelopathic compound.

The production of allelopathic substances by *P. aciculiferum* may be an adaptive strategy to outcompete other phytoplankton, allowing it to dominate the under-ice phytoplankton community. *Peridinium aciculiferum* cooccurs under ice along with other flagellates such as *Rhodomonas* and *Cryptomonas* spp. (Cryptophyceae). These species are typically found in low numbers throughout the winter season, making up at most 1/10 of the dinoflagellate biomass at the peak of the *P. aciculiferum* bloom in April in Lake Erken (Nauwerck 1963). As a large dinoflagellate, *P. aciculiferum* (35–40 μm) is at a disadvantage when competing with other small algae for nutrients, especially other flagellated ones (10 μm or less), which can migrate vertically just like the dinoflagellates. However, by excreting an algicidal compound, *P. aciculiferum* could prevent other phytoplankton from growing to high biomasses, thereby reducing competition for nutrients. In fact, not only is it possible to reduce the nutrient competition through allelopathy, but by causing other phytoplankton to burst and release organically bound nutrients, *P. aciculiferum* may be able to use these organic nutrients as well.

To conclude, our results show that the large winter dinoflagellate *P. aciculiferum* can produce a toxic substance(s), and that this substance could allow *P. aciculiferum* to outcompete small flagellated phytoplankton that are better at nutrient uptake and have lower nutrient requirements. Thus, the common assumption that the winter confers a period of high nutrient levels, low competition, and low predation may not be an accurate depiction of the under-ice environment. In fact, several species are able to tolerate low temperature and light levels, and nutrients may be outstripped toward the end of the ice-covered period prior to spring turnover, when nutrients are redistributed in the water. Thus, the ability to excrete algicidal substances may provide dinoflagellates with sufficient nutrients to grow, and thereby dominate the phytoplankton community.

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