

Inhibition of growth and photosynthesis of the dinoflagellate *Peridinium gatunense* by *Microcystis* sp. (cyanobacteria): A novel allelopathic mechanism

Assaf Sukenik,¹ Rachel Eshkol, Alexander Livne, and Ora Hadas

Israel Oceanographic and Limnological Research, The Yigal Allon Limnological Laboratory, P.O. Box 447, Migdal 14950 Israel

Meir Rom

Watershed Unit, Mekorot Water Company, P.O. Box 447, Migdal 14950 Israel

Dani Tchernov, Assaf Vardi, and Aaron Kaplan

Department of Plant Sciences, The Hebrew University of Jerusalem, Jerusalem 91904 Israel

Abstract

We describe a novel allelopathic interaction whereby the cyanobacterium *Microcystis* sp. inhibits photosynthesis in the freshwater dinoflagellate *Peridinium gatunense* by abolishing its internal carbonic anhydrase activity. Our analysis indicated a positive correlation between the winter presence of *Microcystis* and the timing of the annual spring bloom of *Peridinium* in Lake Kinneret (Sea of Galilee, Israel). *Microcystis* severely inhibited the growth of *Peridinium* in mixed laboratory cultures. This was attributed to the excretion of allelopathic substances rather than to successful competition for nutrients. *Microcystis*-free spent medium (MFSM) inhibited steady-state photosynthesis of *Peridinium*, as indicated by the reduced rate of oxygen evolution and by a suppressed fluorescence signal. Boosting the inorganic carbon concentration to 25 mmol L⁻¹, i.e., 10-fold higher than essential to saturate photosynthesis in the absence of MFSM, partially restored the photosynthetic capacity. MFSM-treated *Peridinium* cells accumulated a larger internal inorganic carbon pool concomitantly with reduced accumulation of photosynthetic products. The MFSM slowed the hydration rate of CO₂ (assessed from the rate of ¹⁸O exchange between ¹³C¹⁸O₂ and water), indicating that the activity of an internal carbonic anhydrase was severely depressed. Although the nature of the active component in the MFSM that inhibits CA activity has yet to be identified, the ecological consequences of its excretion to the water body are emerging as a potent allelopathic substance that may control the development of phytoplankton competitors. We propose that its allelopathic activity could control the fate and succession of the *P. gatunense* bloom in Lake Kinneret.

The phytoplankton composition of a given ecosystem is strongly affected by abiotic and biotic parameters, including physical parameters, availability, and competition for resources, selective grazing, and possibly allelopathic interactions (Reynolds 1984; Inderjit and Dakshini 1994). The latter is a mode of interspecies interaction in which chemicals produced and excreted by one organism affect the proliferation of others and thereby alter the population composition and dynamics (Keating 1977, 1978). Allelopathic interactions have been suggested for cyanobacterial and algal communities, but the exact modes of operation of many of

these chemicals are still unknown. In many cases, it is difficult to provide direct evidence for allelopathic interactions under natural conditions, because other processes hinder the ecological advantages gained through allelopathy. It is therefore essential to characterize the allelopathic interaction in a controlled system, to resolve the nature of the relevant substances and their biological target. Most of the identified allelochemicals have been characterized as algicides that directly inhibit photosynthetic electron transport in cyanobacteria and eukaryotic algae by binding to specific sites in the vicinity of photosystem II (PSII) (Keating 1999). One of the best-characterized allelochemicals is fischerellin, which has been isolated from the freshwater cyanobacterium *Fischerella muscicola* (Gross et al. 1991). As indicated by the half-times constant of fischerellin association with thylakoid membranes, it probably acts at several sites of PSII (Srivastava et al. 1998). Another allelochemical with herbicidal activity is cyanobacterin, which has been isolated from *Scytonema* (Mason et al. 1982). The activity of this compound has been shown to be associated with the oxidizing side of the quinone-B electron acceptor but not with the DCMU binding domain (Gleason et al. 1986; Gleason 1990). This was also the site for the algicidal activity found in the bloom-forming *Oscillatoria late-virens* (Bagchi et al. 1993).

¹ Corresponding author (assaf@ocean.org.il).

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Many *Microcystis* species are known to produce an array of hepatotoxic cyclic peptides, the microcystins, which inhibit protein phosphatase PP1 and PP2A (Carmichael 1994). The biological role of microcystin in the natural environment is poorly understood. Earlier reports suggested that once a population of toxic *Microcystis* forms a bloom, it could prevent the growth of other algae (Watanabe et al. 1992). Recently, Singh et al. (2001) demonstrated that microcystin caused growth inhibition of *Nostoc muscorum* and *Anabaena* BT1. This inhibition was associated with a marked reduction in both CO₂ uptake and O₂ evolution and with a severe reduction in nitrogen fixation activity. Although the environmental regulation of toxin production by *Microcystis* is currently emerging (Kaebnick and Neilan 2001), there is little information on toxin production during biotic interactions. In a recent study, Kearns and Hunter (2000) demonstrated that extracellular products of stationary phase of *Chlamydomonas reinhardtii* abolished the production and accumulation of microcystin in *Microcystis*, whereas the same exudates induced the production of anatoxin in *Anabaena flosaquae*.

In the present study, we examined allelopathic interactions between *Microcystis* and the armored dinoflagellate *Peridinium gatunense*, both isolated from Lake Kinneret (Sea of Galilee), Israel. During the past 3 decades, the phytoplankton population in this lake was characterized by the spring bloom of *Peridinium* that has occurred almost every year (Berman et al. 1998). Results presented herein suggest that, in some cases where significant *Microcystis* populations were observed in the water body toward the end of the winter, the *Peridinium* bloom was either missing or delayed. Allelopathic interaction between *Microcystis* and *Peridinium* was therefore postulated and examined. A long limnological record of the phytoplankton population in Lake Kinneret (Berman and Pollinger 1974; Berman et al. 1998), as well as the availability of isolated endemic cyanobacterial species, enabled the initial phase of the study.

Materials and methods

Lake water sampling and phytoplankton counts—Sampling of water from the upper water layer was carried out weekly at a single station located in the center of Lake Kinneret. The samples were transferred to the lab for cell counting. Subsamples were fixed by acidified lugol, sedimented in aliquots of 10 or 1 ml depending on cell concentration, and counted as described by Berman and Pollinger (1974). Bio-volume was estimated according to the method of Hillebrand et al. (1999), and integrated areal biomass concentration was calculated as described by Berman and Pollinger (1974). Multiannual data (1971–1990) on the population of both *P. gatunense* and *Microcystis* sp. were divided into two series that were filtered to remove seasonal–annual effects. Bloom events of each species were recorded on the basis of the timing of their occurrence (week number during each year) and on their intensity, as was reflected by the bloom maximum (Fig. 1A). Two distinct groups were created; one group contained all the events when winter *Microcystis* presence was recorded prior to the *Peridinium*

bloom, and the second group contained events where no *Microcystis* population developed in the early winter. Variations in the timing of the peak of the bloom of *Peridinium* (number of weeks since the beginning of the year) were statistically analyzed by *t*-test and the Mann–Whitney nonparametric test (Conover 1980). In addition, the two series were cross-correlated according to Diggle (1990) to identify direct interactions between the two species.

Organisms and growth conditions—The dinoflagellate *P. gatunense*, isolated from Lake Kinneret, was maintained in laboratory cultures (Lindström 1985, 1991). The cyanobacteria *Microcystis* sp. (kll strain MG), *Microcystis* sp. (kll strain MB) were isolated from Lake Kinneret and maintained in medium BG 11 (Stanier et al. 1971).

Cells of *Peridinium* and of *Microcystis* were each grown in batch cultures in 3-liter flasks. *Peridinium* was grown in 1.5 liters of Lindström medium at 20°C. Illumination was provided by VHO fluorescence tubes at 20 μmol photon m⁻² s⁻¹, 12:12 h light:dark cycles. The cultures were neither aerated nor agitated and were maintained for up to 2 months. Maximal cell yield varied with the initial nutrient concentration. The nutrient content in 1× Lindström medium could support growth up to 2,000 *Peridinium* cell ml⁻¹, whereas 8× medium resulted in up to 25,000 cells ml⁻¹ (Lindström 1985). The cells were collected toward the end of their exponential growth by slow filtration on a 40-μm sieve, washed in a fresh medium, and their photosynthetic response analyzed as described below.

Microcystis cells were grown in batch cultures in medium BG11 at 24°C ± 1°C with continuous illumination (cool white fluorescent tubes, 50 μmol photons m⁻² s⁻¹). The cultures were mixed by aeration with air (1 L min⁻¹) and harvested after 20 days of growth (when late exponential growth phase was reached) by centrifugation (15 min at 10,000 × *g*). *Microcystis*-free spent medium (MFSM) was freeze-dried and stored at -20°C for examination of its allelopathic competence.

Photosynthetic activity—The photosynthetic activity of intact *Peridinium* cells was measured by pulse-modulated fluorometry (PAM-101; Waltz). *Peridinium* cells (~5 × 10⁴ cells ml⁻¹, corresponding to 5 μg chlorophyll *a* ml⁻¹) were resuspended in fresh Lindström medium (pH 7.8, 20 mmol L⁻¹ Hepes-NaOH buffer) in a 3-ml cuvette of the fluorometer maintained at 23°C. The light intensity of the modulated (1.6 kHz) measuring flash was 10 nmol photons m⁻² s⁻¹. Actinic white light of 40 μmol photons m⁻² s⁻¹ was used to assess steady-state fluorescence (*F*_s). The maximum fluorescence level (*F*_m) was measured with pulses of saturating white light (3000 μmol photons m⁻² s⁻¹) for 2 s.

The photosynthetic activity was also assessed from the rate of oxygen evolution measured by a Clark-type oxygen electrode (Hansatech). *Peridinium* cells were resuspended in 4× Lindström medium supplemented with 25 mmol L⁻¹ N-tris[hydroxymethyl] methyl-3-amino-propanesulfonic acid-NaOH (pH 8.5), to a cell density of 5 × 10⁴ cells ml⁻¹, and placed in the oxygen electrode chamber. The cell suspensions (2 ml) were shortly bubbled with a stream of N₂, to reduce the dissolved O₂ concentration. The chamber was

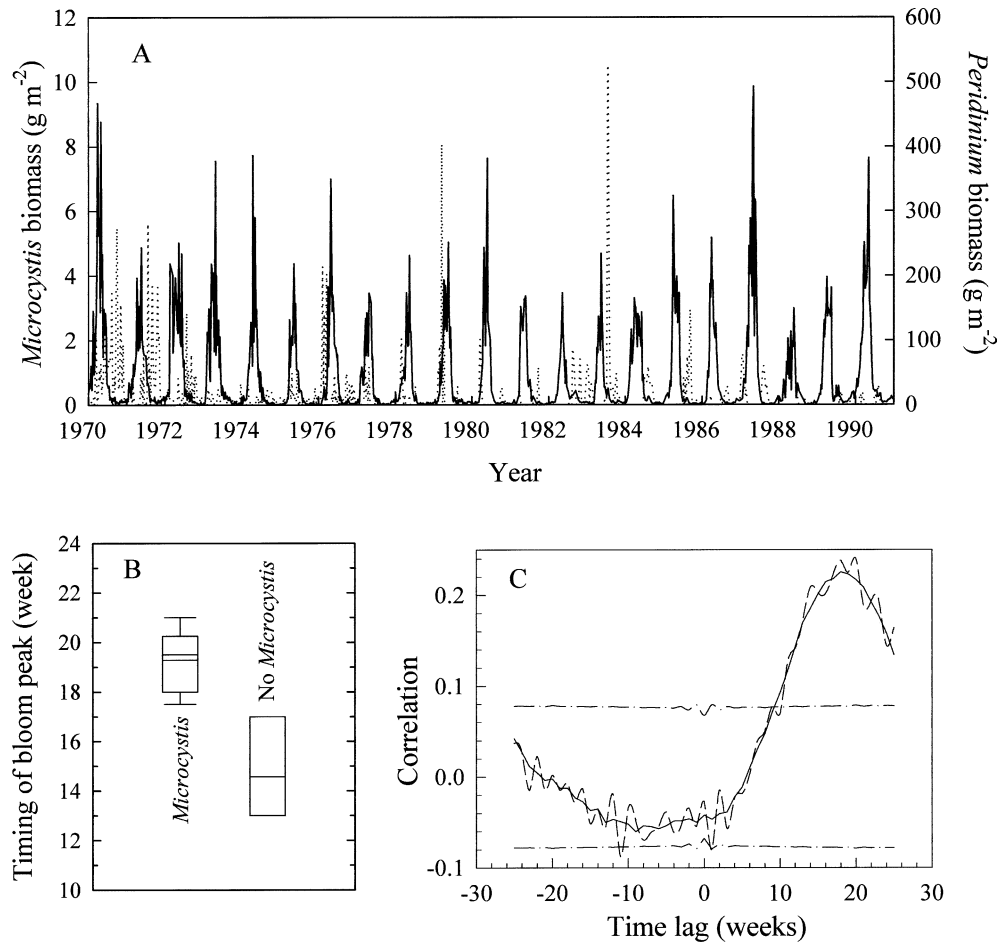


Fig. 1. (A) Multiannual variations in the abundance of *Peridinium* (solid line) and *Microcystis* (dotted line) areal biomass ($\text{g wet weight m}^{-2}$) between 1970 and 1991. (B) Box plots for the timing (week of year) of the annual *Peridinium* bloom over a period of 20 yr. Data were grouped on the basis of the presence or absence of *Microcystis* prior to the *Peridinium* bloom. The lower boundary of the box indicates the 25th percentile, a line within the boundary marks the median, and the upper boundary of the box indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. (C) Cross-correlation function (correlogram) of weekly areal biomass density for *Peridinium* vs. *Microcystis* in Lake Kinneret during the years 1970–1991. Calculated values (dashed line) and smoothed data (solid line) are presented, together with dashed horizontal lines that correspond to 99% tolerance limits, under the assumption that the underlying cross-correlation function is zero at all lags (Diggle 1990). No significant correlation was found at lag 0, whereas correlations that correspond to lags of 12–24 weeks (3–6 months) were significant.

then closed and covered with black cloth to measure dark respiration (O_2 uptake). The rate of gross photosynthesis before and after the addition of MFSM to the *Peridinium* samples was measured under a light intensity of $210 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, a constant temperature of 22°C , and saturating inorganic carbon (Ci) concentration (2.5 mmol L^{-1}).

Uptake of Ci—Accumulation of Ci and photosynthetic products were assessed by use of the filtering centrifugation technique (Kaplan et al. 1988). *Peridinium* cells were harvested as described above, and the cells were allowed to utilize the Ci in the medium within the O_2 electrode chamber until the O_2 compensation point was reached. Aliquots (0.1 ml of $8 \times 10^4 \text{ cells ml}^{-1}$) were then introduced to a centrifuge tube that contained (from bottom to top) 0.02 ml of 3.0

M NaOH and 0.07 ml mixture of 7:1 dioctyl phthalate and dibutyl phthalate (Fluka). Incubation of the cells (for a desired duration) in the presence of a known concentration of $^{14}\text{C-Ci}$, under saturating light intensity, was terminated by centrifugation. Assessment of the internal Ci concentration and photosynthetic products were performed as described elsewhere (Kaplan et al. 1988).

Carbonic anhydrase activity—Carbonic anhydrase activity was assessed from the rate of hydration/dehydration of CO_2 by following the exchange of ^{18}O in $^{13}\text{C}^{18}\text{O}_2$ with ^{16}O from the surrounding water by use of a quadrupole membrane (sylastic silicone tube; Dow Corning) inlet mass spectrometer (MIMS, Balzers QMG 421), as described elsewhere (Sukenik et al. 1997; Tchernov et al. 1997). *Peridinium* cells

were placed in a temperature-controlled chamber (2.8 ml) at 22°C and illuminated with two optic fibers at the desired light intensity. CO₂ (in the form of ¹²C¹⁶O₂ or ¹³C¹⁸O₂), various inhibitors, and the concentrated MFSM were introduced to the *Peridinium* culture via a special injection port in the closed chamber. The MIMS was configured to peak switch between m/e 32 (¹⁶O₂), 44 (¹²C¹⁶O₂), 45 (¹³C¹⁶O¹⁶O), 47 (¹³C¹⁸O¹⁶O), and 49 (¹³C¹⁸O₂) in the *Peridinium* culture. Simultaneous measurements of argon and nitrogen concentrations were used to correct for variations or drifts in the system due to biological formation or consumption of O₂ and CO₂, small changes in the rate of stirring or temperature, and gas consumption by the mass spectrometer.

Results

Phytoplankton population in Lake Kinneret—Long-term records of the biomass of Lake Kinneret indicated late winter–early spring blooms of *P. gatunense* as the major constituent of the phytoplankton population (Fig. 1). These observations also suggested possible disturbance by occasional winter blooms of the cyanobacterium *Microcystis* sp. (1976, 1978, and 1979 in Fig. 1A). In 14 of 20 years shown in Fig. 1A, a winter population of *Microcystis* developed prior to the *Peridinium* spring bloom. Statistical analyses of these events (by *t*-test and the Mann–Whitney nonparametric test) indicated a significant ($P < 0.001$) delay in the peak of the *Peridinium* bloom (by ~4 weeks) relative to years when no winter population of *Microcystis* was developed (Fig. 1B). Very low correlation was found, however, between the size of the winter population of *Microcystis* and the intensity of the *Peridinium* bloom that followed or between summer *Microcystis* and the development of the preceding spring *Peridinium* population. The statistical analyses suggested that the presence of a winter *Microcystis* population could affect the timing of the development of the *Peridinium* bloom. Cross-correlation analysis of the two population series also suggested the occurrence of a *Microcystis* population in the summer, significantly ($P < 0.01$; $r^2 = 0.2$) delayed by 18 weeks behind the spring bloom of *Peridinium* (Fig. 1C).

Growth experiments revealed allelopathic inhibition of Peridinium by Microcystis—Growth of *P. gatunense* was substantially inhibited by the presence of the *Microcystis* sp. isolated from Lake Kinneret (Fig. 2A). The extent of inhibition increased with the initial number of *Microcystis* cells but was hardly affected by the initial *Peridinium* inoculum used here (200–1,500 cells ml⁻¹, not shown). Growth of *Microcystis* in the same cultures, on the other hand, was scarcely affected by the presence of *Peridinium* unless a relatively high inoculum (1,500 cells ml⁻¹) of the latter was used (Fig. 2B). Retardation of *Peridinium* growth by the presence of *Microcystis* could be due to either competition for resources, such as nutrients, or to allelopathic interaction. To examine the former possibility, *Peridinium* growth was followed in single and mixed cultures with *Microcystis*, supplemented with various nutrient levels. In single *Peridinium* cultures, raising the nutrient level up to eightfold resulted in a higher maximal growth rate (around twofold) and five times higher cell yield (Fig. 2C). By contrast, in the mixed

Peridinium–Microcystis cultures, raising the nutrient level only slightly affected *Peridinium* growth and could not alleviate the inhibition imposed by *Microcystis* (Fig. 2C). The higher nutrient level stimulated the growth of *Microcystis* (Fig. 2D), but, unlike the case of *Peridinium*, similar enhancement was observed in the single and mixed cultures. The overall biomass formed in the mixed cultures (Fig. 2C,D) was significantly smaller than that obtained when the two organisms were grown separately, largely because of the reduced *Peridinium* cell yield. This finding ruled out the possibility that retardation of *Peridinium* growth in the mixed culture was due to nutrient limitation.

The possibility that allelopathic interaction rather than competition for resources led to the inhibition of *Peridinium* growth by *Microcystis* (Fig. 2) was strongly supported by results showing that MFSM inhibited *Peridinium* growth (Fig. 3). The addition of nutrients to MFSM or to spent medium from *Peridinium–Microcystis* culture could not alleviate the growth inhibition. The growth of *Peridinium* was also inhibited when initiated in its own spent medium, but complete recovery was obtained after the addition of nutrients (Fig. 3). These data strongly supported the notion that allelopathic substance(s) excreted by *Microcystis* inhibited *Peridinium* growth.

Allelopathic substances from Microcystis inhibit photosynthesis in Peridinium—We examined the effect of lyophilized MFSM on the photosynthetic performance of *Peridinium*, because alteration of the latter could have caused the growth retardation. Analysis of the fluorescence parameters, as revealed by a PAM fluorometer, showed that the MFSM treatment reduced the variable fluorescence $(F_{\max} - F_o)/F_{\max}$ in *Peridinium* (by 35%, from 0.60 to 0.39) due to a decline in F_m and a small increase in F_o (Fig. 4). These data suggested that MFSM treatment imposed slower plastoquinol oxidation in *Peridinium*, most likely because of a lower rate of photosynthetic electron transport to PSI.

Exposure of *Peridinium* cells to 2.5 mmol L⁻¹ Ci, a concentration that saturated the rate of CO₂-dependent O₂ evolution, and a saturating light intensity (250 μmol photon m⁻² s⁻¹) enabled a gross photosynthetic O₂ evolution of 2.8 μmol O₂ mg⁻¹ Chl min⁻¹ (Fig. 5). Exposure of *Peridinium* to a high (50 μg L⁻¹) concentration of microcystin-LR, the main toxin produced by this strain of *Microcystis*, scarcely affected the photosynthetic O₂ evolution (data not shown). In contrast, addition of MFSM demolished net O₂ evolution; the observed rate of O₂ uptake resembled dark respiration (Fig. 5). Raising the Ci concentration to 25 mmol L⁻¹ (i.e., 10-fold higher than that required to saturate photosynthesis in the absence of MFSM) restored O₂ evolution to a level 45% lower than in nontreated cells (Fig. 5). We could not obtain complete recovery of the photosynthetic activity by further additions of HCO₃⁻, possibly because of the harmful effect of the rising salinity. These data suggested that an allelochemical in MFSM, other than microcystin-LR, interfered with the apparent photosynthetic affinity for Ci or its acquisition in *Peridinium*.

Disturbance of the CO₂ concentrating mechanism—An increased ability of *Peridinium* to accumulate Ci within the

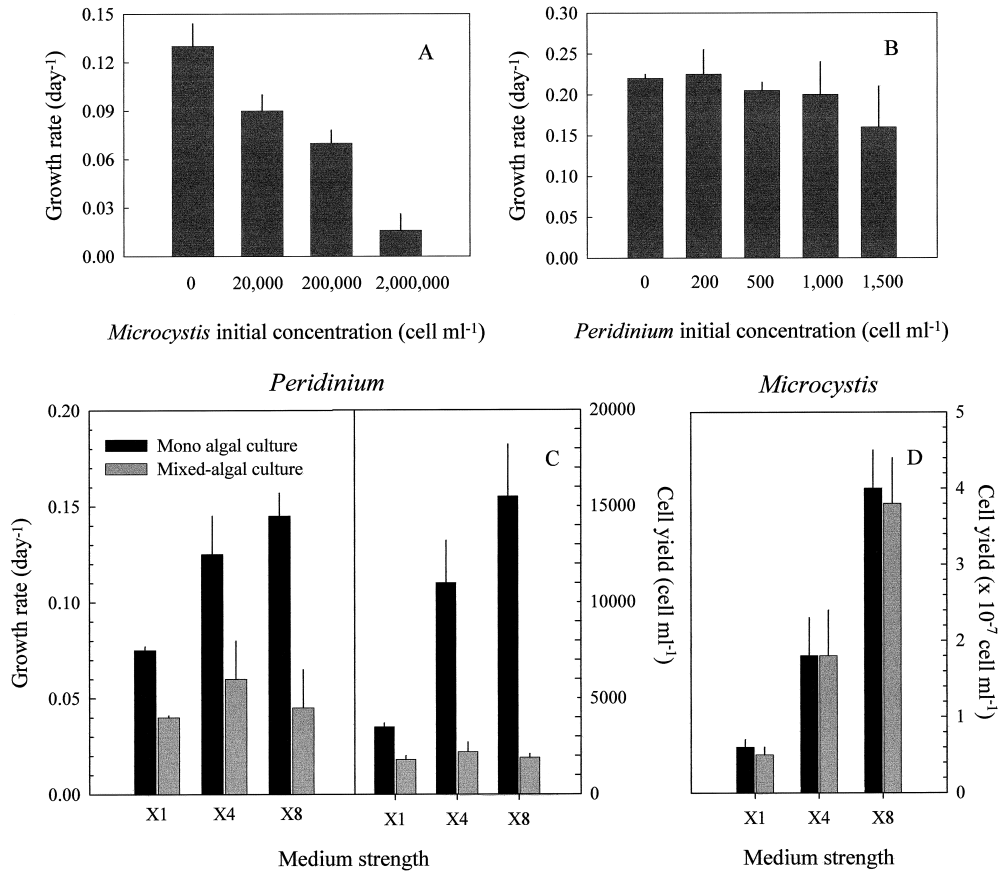


Fig. 2. Population dynamics of *Peridinium* and *Microcystis* in mixed cultures. (A) The effect of *Microcystis* inoculum size on the growth rate of *Peridinium*. The size of the *Peridinium* inoculum was 500 cells ml⁻¹. (B) The effect of *Peridinium* inoculum size on *Microcystis* growth rate. The size of the *Microcystis* inoculum was 200,000 cells ml⁻¹. (C) The response of *Peridinium* culture, growth rate, and cell yield, to the addition of nutrients. Nutrients were added 4× and 8×, relative to the base level (1×). The growth parameters of the *Peridinium* in the mixed cultures are compared with those of monoculture of *Peridinium*. The size of the *Peridinium* inoculum was 500 cells ml⁻¹ and the size of the *Microcystis* inoculum 200,000 cells ml⁻¹. (D) The response of *Microcystis* culture (cell yield) to nutrient addition (4× and 8× relative to 1×). The growth parameters of the *Microcystis* in the mixed cultures are compared with those of monoculture. The size of the *Peridinium* inoculum was 500 cells ml⁻¹ and the size of the *Microcystis* inoculum 200,000 cells ml⁻¹.

cells and a rise in CA activity were observed during its acclimation to a limiting CO₂ concentration (Berman-Frank et al. 1995). The MFSM-treated *Peridinium* cells accumulated a larger internal Ci pool (Fig. 6A) than the nontreated cells, which suggests that Ci uptake per se was not affected. Because the MFSM-treated cells exhibited a slower photosynthetic rate (Fig. 5) and accumulated far fewer photosynthetic products (Fig. 6B), the elevated internal Ci concentration could be due to reduced dissipation of the internal Ci pool via CO₂ fixation. Inhibition of an internal carbonic anhydrase (CA) could slow the dissipation of the internal Ci pool by CO₂ fixation and leak, leading to the observed rise in internal Ci pool. Similar results were obtained when the activity of the carboxysomal-located CA in *Synechococcus* PCC 7942 was reduced by either inactivation of the relevant gene or by application of specific CA inhibitors (Kaplan and Reinhold 1999).

The rate of ¹⁸O exchange between ¹³C¹⁸O₂ and water, as

measured by a membrane inlet mass spectrometer (MIMS; Tu et al. 1987), was used to compare the CA activity in control and MFSM-treated *Peridinium* cells. The cells were pretreated with a high concentration of a nonpermeant CA inhibitor, F3500, to inhibit the periplasmic-located CA (Berman-Frank et al. 1995). Rapid dissipation of mass 47 (¹³C¹⁸O¹⁶O) was observed in the control culture on illumination, concomitant with a moderate constant decrease in mass 49 (¹³C¹⁸O¹⁸O). The maximal oxygen evolution rate was reached after 1 min from light onset. During this period, mass 47 continued to decline (Fig. 7). In contrast, a short incubation of *Peridinium* with MFSM altered the hydration of CO₂ on illumination, as indicated by the initial decrease in the concentration of mass 47 followed by its slow increase (Fig. 7), whereas a constant decrease in oxygen concentration was recorded 2 min after illumination. During that period, the concentration of mass 49 gradually decreased in the MFSM treated culture in a manner similar to the control

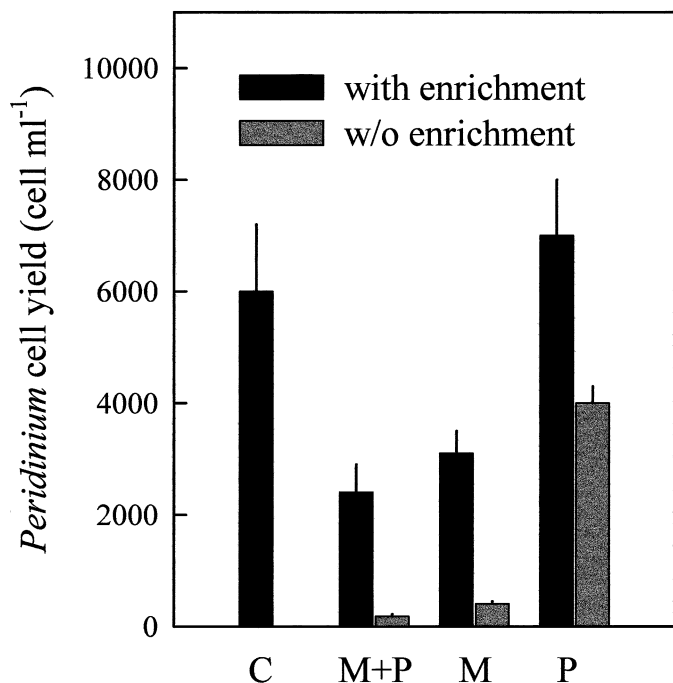


Fig. 3. Inhibition of *Peridinium* growth (expressed as cell yield) by an algal-free spent medium with and without nutrient enrichment. The examined spent media were collected from *Microcystis* culture (M), *Peridinium* culture (P), and a mixed culture of *Peridinium* and *Microcystis* (M + P). Control culture (C) was maintained in 4× Lindström medium (Lindström 1985). Additional controls were run in algal-free spent medium complemented with 4× Lindström nutrients.

(Fig. 7). These results indicated that the internal CA activity was severely depressed by the addition of the MFSM.

Discussion

The production of allelopathic compounds has already been observed in a wide variety of phytoplankton species (Gleason et al. 1986; Gromov et al. 1991; Gross et al. 1991; Todorova and Juttner 1996; Juttner et al. 2001). Despite their initial low abundance, organisms capable of producing allelopathic compounds may become dominant (von Elert and Juttner 1997). As an example, Keating (1978) proposed that allelopathy was essential for the ability of cyanobacteria to overcome physiological advantages of diatoms in a eutrophic lake. However, in many cases, the mode of action of allelopathic compounds and their ecological significance are poorly understood.

In the present study, we show that *Microcystis* could affect the development and intensity of the annual spring bloom of *Peridinium* in Lake Kinneret via allelopathic interactions. This was confirmed in controlled growth experiments (Figs. 2, 3) that clearly indicated that allelochemicals excreted by *Microcystis* severely inhibited the growth of *Peridinium*. It is likely that the internal CA is the primary target of the MFSM allelochemicals (Fig. 7). CO₂ limitation imposed by the inactivation of CA led to severe inhibition of the photosynthetic activity (Figs. 4, 5). The results presented herein

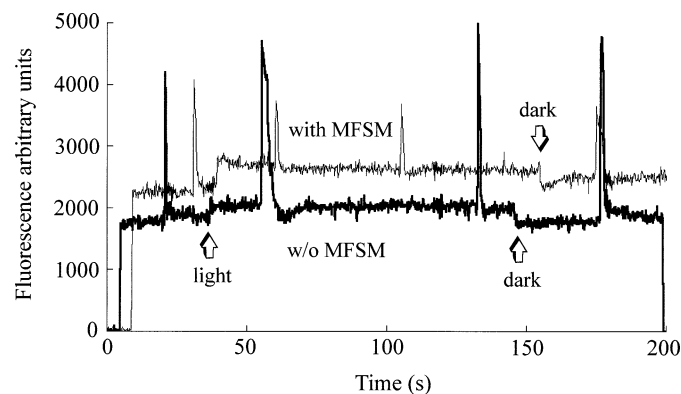


Fig. 4. PAM 101 trace of control *Peridinium* culture (thin line) and of MFSM-treated culture (thick line). *Peridinium* cells (10⁴ cells ml⁻¹, 6.5 μg Chl *a* ml⁻¹) were resuspended in 3 ml fresh medium stabilized by 20 mmol L⁻¹ Hepes buffer to pH 7.8 and incubated at 23°C in a 3-ml PAM cuvette. Reconstructed MFSM was added to the *Peridinium* suspension prior to the measurement. The actinic light intensity was 40 μmol photons m⁻² s⁻¹.

provide a novel allelopathic mechanism whereby inhibition of the photosynthetic activity is imposed by a competing species.

Members of the *Microcystis* genus are known to produce an array of hepatotoxic cyclopeptides, the microcystins (Codd 2000). Exposure of *Peridinium* to a *Microcystis* strain that does not produce microcystins resulted in growth retardation similar to that obtained with the microcystin-LR-producing strain used herein (Livne et al. unpubl. data). Furthermore, although inhibition of photosynthesis by microcystins has been reported (Abe et al. 1996; Singh et al. 2001), we found that the photosynthetic activity of *Peridinium* was only slightly affected by the presence of a high microcystin-LR concentration. These observations ruled out the possibility that retardation of photosynthesis and growth of *Peridinium* were due to the presence of microcystins. The chemical nature of the active component in the MFSM that inhibits CA activity, has yet to be resolved. Preliminary results indicated that it is thermally stable, relatively hydrophobic, and its molecular weight is <5 kD.

The ecological consequences of the allelopathic interaction described herein are emerging. The collapse of the an-

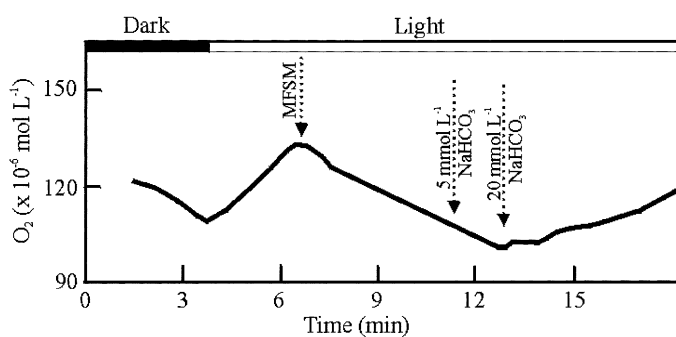


Fig. 5. The effect of MFSM on oxygen evolution by *Peridinium*. Photosynthetic oxygen evolution was promptly reduced on addition of MFSM and recovered only after the addition of 25 mmol L⁻¹ Ci.

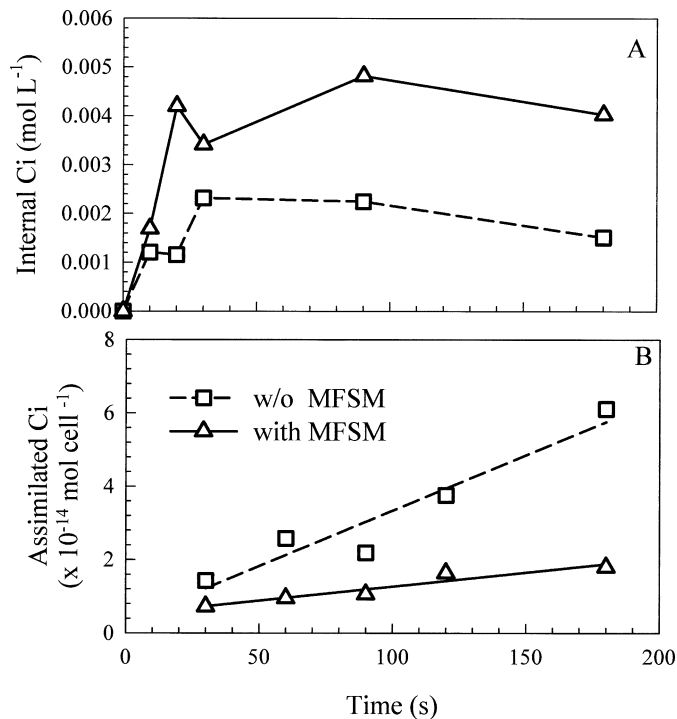


Fig. 6. The effect of MFMS on the internal pool of inorganic carbon and on photosynthetic carbon assimilation in *Peridinium*. ¹⁴C bicarbonate was introduced to MFMS-treated and -untreated cultures, and internal pools were estimated by using the filtering centrifugation technique. The experiments were initiated by adding 1.8 mmol L⁻¹ HCO₃⁻ to a suspension of 5 × 10⁴ cells ml⁻¹ and terminated after various incubation periods. Saturating light at 300 μmol quanta m⁻² s⁻¹ was provided.

nual *Peridinium* bloom in Lake Kinneret has been attributed to a process of program cell death that was induced by the development of oxidative stress toward the peak of the bloom (Butow et al. 1997; Vardi et al. 1999). The oxidative stress was imposed by CO₂ limitation because of the rising pH in the lake and, particularly in the patches of *Peridinium* (Vardi et al. 1999). Reduced availability of CO₂ within the cells, as a result of the inhibition of internal CA by *Microcystis*, could also divert photosynthetic electrons from CO₂ fixation to O₂, leading to the formation of reactive oxygen species (ROS). Indeed, the exposure of *Peridinium* to *Microcystis* resulted in fast accumulation of ROS-positive cells (Vardi et al. unpubl. data). Thus, the presence of *Microcystis*, which could induce CO₂ limitation at the beginning of the growth period, when ample ambient CO₂ is still available, mimics the limiting CO₂ conditions preceding and leading to the collapse of the *Peridinium* bloom. We propose that this is the mechanism whereby the presence of *Microcystis* inhibits growth. Indeed, substantial and fast accumulation of ROS in *Peridinium* was observed after the addition of MFMS (Vardi et al. unpubl. data), leading to programmed cell death, as described elsewhere (Vardi et al. 1999).

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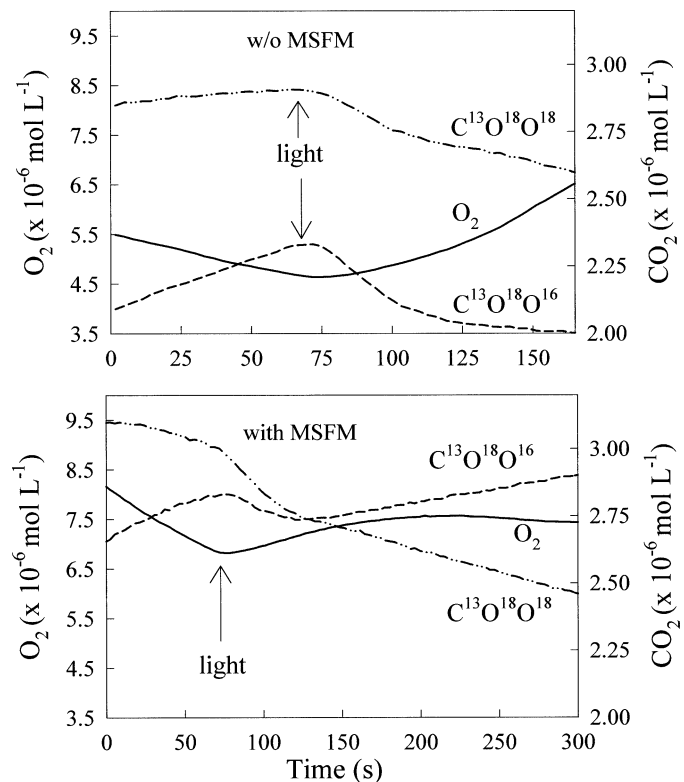


Fig. 7. Assessment of CA activity in *Peridinium* by following the replacement of ¹⁸O in ¹³C¹⁸O₂ with ¹⁶O from the surrounding medium with the aid of a quadrupole MIMS. (A) Control culture and (B) MFMS poisoned culture. Changes in mass 49 (¹³C¹⁸O¹⁸O), mass 47 (¹³C¹⁸O¹⁶O), and mass 32 (O₂) were plotted. Cultures were treated with a high concentration of F3500, a nonpermeate CA inhibitor, prior to measurement.

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