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SHORT COMMUNICATION

The cyanobacterium *Gloeotrichia echinulata* stimulates the growth of other phytoplankton

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We tested the effect of the cyanobacterium *Gloeotrichia echinulata* on a diverse array of phytoplankton. We found that *Gloeotrichia* increased the growth rates of five of seven phytoplankton species up to 620% in comparison with a medium-only control after 96 h.

KEYWORDS: allelochemicals; cyanobacterial bloom; eutrophication; facilitation; stimulatory allelopathy

Biochemical interactions, either inhibitory or stimulatory, have gained attention from phycologists for their importance in structuring plankton communities (reviewed in Gross, 2003; Legrand *et al.*, 2003) by altering plankton succession, competition and bloom formation (Keating, 1977; Rengefors and Legrand, 2001). Although most research conducted on the biochemical impacts of cyanobacterial blooms indicates that they negatively affect other phytoplankton by triggering cellular paralysis or inhibiting photosynthesis, enzyme production or nucleic acid synthesis (Gross, 2003; Leflaive and Ten-Hage, 2007), a growing number of studies indicate that cyanobacteria can also stimulate the growth and division of other phytoplankton in both laboratory and field settings (Keating, 1977; Mohamed, 2002; Suikkanen *et al.*, 2005; Karjalainen *et al.*, 2007). As the incidence of cyanobacterial blooms increases worldwide due to eutrophication and climate change (Hallegraeff, 1993; Paerl and Huisman, 2008), understanding the effects of cyanobacteria on aquatic food webs is essential for predicting changes in water quality and ecosystem services.

One cyanobacterial species that may substantially affect lake ecosystems is the colonial nitrogen-fixer *Gloeotrichia echinulata* (J.E. Smith) P. Richter (Carey *et al.*, 2008). *Gloeotrichia echinulata* (hereafter, *Gloeotrichia*) is a large (1–3 mm diameter) filamentous cyanobacterium that forms surface scums in summer and produces the hepatotoxin microcystin-LR (Carey *et al.*, 2007). Although *Gloeotrichia* has historically been observed in meso-eutrophic and eutrophic lakes (Karlsson-Elfgren *et al.*, 2003), it has recently been found blooming in oligo- to mesotrophic lakes throughout the northeastern USA that have no recent (>30 years) record of previous *Gloeotrichia* blooms (Carey *et al.*, 2008; Carey *et al.*, 2009). In at least some of these low-nutrient lakes, *Gloeotrichia*

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blooms are sufficiently dense to cause lake management concerns (Carey *et al.*, 2008). Thus, understanding the factors that enable *Gloeotrichia* to dominate plankton assemblages is interesting ecologically and important for lake management in both oligotrophic and eutrophic lakes.

In this study, we conducted three laboratory experiments to examine the effect of *Gloeotrichia* on other phytoplankton taxa. A multiclonal culture of *Gloeotrichia* was obtained from akinetes isolated from Lake Erken, Sweden, in winter 2007 and spring 2008 (Karlsson, 2003). Lake Erken has experienced *Gloeotrichia* blooms for several decades, and its sediments contain up to 7800 akinetes cm^{-3} in the littoral zone (Forsell, 1998). Seven target species were studied, including cultures of the cryptophyte, *Rhodomonas lacustris* NIVA 8/82; three species of cyanobacteria: *Anabaena circinalis* NIVA-CYA 82, *Aphanizomenon* cf. *gracile* NIVA-CYA 338 and *Microcystis aeruginosa* PCC 7806; and one diatom, monospecific *Cyclotella* sp. (Kütz.) Brèb. NIVA-CYA 20, all obtained from the Norsk Institutt for Vannforskning (NIVA), Norway. In addition, one chrysophyte, *Synura petersenii* Korsh CCAP 960/3, and one dinoflagellate, *Peridinium inconspicuum* Lemmermann CCAP (Dinophyceae), were obtained from the Culture Collection of Algae and Protozoa (CCAP) in the UK. These species (hereafter denoted by their genus) have been observed to co-occur with *Gloeotrichia* in at least two separate lakes, Lake Sunapee (USA) and Lake Erken (Sweden) (Lake Erken database, unpublished data; Lake Sunapee Protective Association, unpublished data). None of these target cultures had been isolated from Lake Erken and so had not co-evolved with the Lake Erken *Gloeotrichia*.

Prior to the experiments, *Gloeotrichia* colonies and stock cultures were grown in a modified-WC medium (MWC; Guillard and Lorenzen, 1972) for a minimum of 14 days at 20°C at 20 μmol photons m^{-2} s^{-1} with a 14:10 light:dark cycle (Rengefors and Legrand, 2007). We measured the incident light with a LI-COR (LI-250A) light meter placed adjacent to the microdishes and assume that a large proportion, if not all, of the incident light reached the phytoplankton because the microdish lids were transparent. We reproduced these temperature, light intensity and photoperiod conditions in all experiments. We harvested only one mature *Gloeotrichia* colony from each germinated akinete to maintain multiclonal *Gloeotrichia* cultures. We transferred these colonies from their germination cultures to a new MWC medium for incubation prior to an experiment once they became mature (to synchronize the age of the colonies used in the experiments). We determined colony maturity by the formation of a central core consisting of terminal heterocytes, germinated akinetes in spore sheaths and vegetative cells (Karlsson, 2003). The target species used in the experiments were obtained from MWC stock solutions in exponential growth phase.

Table I: Biovolumes (μm^3, calculated from measuring >10 different cells of each species) and concentrations of seven different autotrophic target species used in Experiment 1

Species	Cell biovolume ± 1 S.E. (μm^3)	Target concentration (cells mL^{-1})
Anabaena circinalis	22 ± 5	6818
Aphanizomenon cf. *gracile*	35 ± 7	4220
Cyclotella sp.	438 ± 105	342
Microcystis aeruginosa	13 ± 3	11 540
Peridinium inconspicuum	1161 ± 53	200
Rhodomonas lacustris	15 ± 2	10 000
Synura petersenii	185 ± 47	810

Biovolumes and standard errors were calculated according to Blomqvist and Herlitz (Blomqvist and Herlitz, 1996).

First, we tested the allelopathic effect of *Gloeotrichia* on the phytoplankton taxa listed above. To compare the responses of the seven target species, biovolume equivalents for each species were used that corresponded to 10 000 *Rhodomonas* cells mL^{-1} (Table I), calculated according to Blomqvist and Herlitz (Blomqvist and Herlitz, 1996).

We examined the effect of three treatments (live *Gloeotrichia* colonies, *Gloeotrichia* cell-free filtrate and a medium-only control) on each target species in 24-well Nunclon™ microdishes (2 mL final volume in all treatments), except for the cyanobacterial species, where only the effects of live *Gloeotrichia* colonies and a medium-only control were tested. This sterile microdish set-up has been used in several studies examining phytoplankton interactions in laboratory settings (e.g. Rengefors and Legrand, 2001; Rengefors and Legrand, 2007). The *Gloeotrichia* cell-free filtrate was collected from MWC medium incubated with *Gloeotrichia* colonies for 1 week at a density of 100 *Gloeotrichia* colonies L^{-1} (~100 mg L^{-1}), within the range of bloom densities observed in nature (Carey *et al.*, 2007), before filtration with GF/F (0.7 μm pore size) Whatman filters. We initiated the treatments immediately after the target cells were placed in the microdishes. All of the live *Gloeotrichia* addition treatments received 2 mL of fresh MWC medium and one *Gloeotrichia* colony of similar biomass (~1000 μg), except for the *Peridinium* wells, which received three colonies. The biomass of an individual *Peridinium* cell was considerably higher than the biomass of the other target species; consequently, to match biomass equivalents with the other species yet

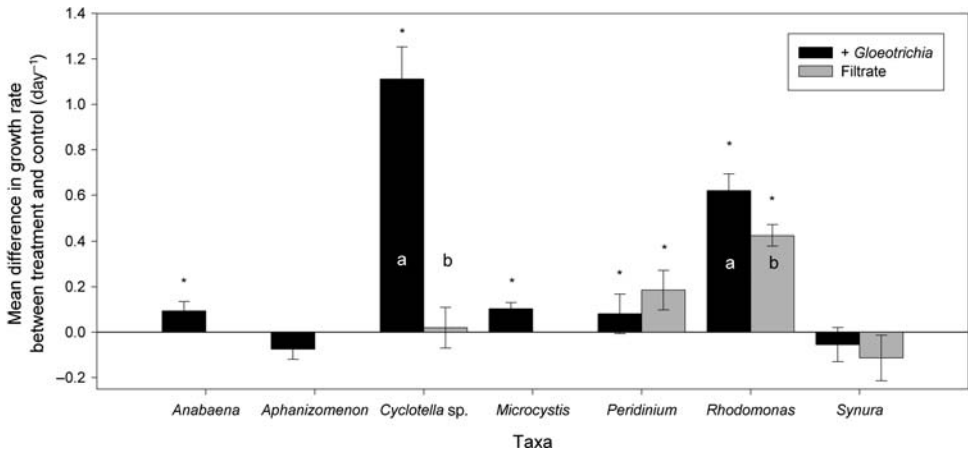

Fig. 1. The mean difference in growth rate (day^{-1}) between treatments of *Gloeotrichia echinulata* or filtrate and the medium-only control for seven phytoplankton species after 96 h ($n = 4$). Error bars represent 1 SE. Asterisks represent treatments significantly different from the control, and letters represent significant differences between the two treatments (Tukey's test).

still ensure adequate encounter rates, we increased the number of *Gloeotrichia* colonies and *Peridinium* cells per milliliter. Each treatment by species combination had four replicates, which were terminated by adding 25 μL of Lugol's solution to each well at 96 h. We counted cells directly in wells at ×40 with an inverted light microscope (Nikon Eclipse TS100).

We analyzed the differences in growth rate, *r*, among treatments over the 96-h period with a one-way ANOVA separately for each species because of the unbalanced design. We calculated the growth rate using the following equation: $r = [\ln(n_1) - \ln(n_0)]/[t_1 - t_0]$, where n is the cell density and t the time. Statistical analyses were conducted in JMP (v. 7.0, SAS Institute, 2007). Finally, we used Tukey's test ($\alpha = 0.05$) to analyze differences in target species growth rate among the treatments.

Gloeotrichia colonies significantly stimulated the growth rate of five of the seven target species relative to the medium-only control: *Anabaena*, *Cyclotella* sp., *Microcystis*, *Peridinium* and *Rhodomonas* all increased (Fig. 1, Table II). The effect of *Gloeotrichia* was considerable for some species: growth rate in the *Gloeotrichia* treatment for *Rhodomonas* and *Cyclotella* sp. was 620% and 150% greater, respectively, than in the corresponding control at 96 h. *Anabaena*, *Microcystis* and *Peridinium* each exhibited a 24–53% greater growth rate in the *Gloeotrichia* treatment than the control after 96 h. In addition, target species growth rate was substantially greater in the *Gloeotrichia* filtrate treatments for *Peridinium* and *Rhodomonas* than in their corresponding medium-only controls (100% and 420%, respectively); but not for *Cyclotella* sp. or *Synura*, the two other species that received filtrate treatments. For *Aphanizomenon* and *Synura*, there were no significant differences in the growth rate among treatments ($P > 0.09$). We were unable to detect any negative effects of *Gloeotrichia* on any of the target species, as would be expected if *Gloeotrichia* produced inhibitory compounds.

Table II: One-way ANOVA tests of treatment (Gloeotrichia echinulata live colonies, filtrate and medium-only control) of growth rate for each target taxon over the 96 h experiment

Species	d.f.	*F*-ratio	*P*-value
Anabaena circinalis	1,6	9.03	0.02
Aphanizomenon cf. *gracile*	1,6	4.00	0.09
Cyclotella sp.	2,9	95.05	<0.0001
Microcystis aeruginosa	1,6	28.45	0.002
Peridinium inconspicuum	2,9	5.15	0.03
Rhodomonas lacustris	2,9	75.92	<0.0001
Synura petersenii	2,9	1.15	0.35

Second, we tested the effect of live *Gloeotrichia* on five densities of *Rhodomonas*: 500, 1000, 10 000, 20 000 and 40 000 cells mL^{-1}. We chose to use *Rhodomonas* because of its documented sensitivity to allelochemicals (Rengefors and Legrand, 2001; Rengefors and Legrand, 2007). We established initial *Rhodomonas* densities from dilutions of the stock cultures, with standard deviations <1%. We exposed each density to three treatments (*Gloeotrichia*, *Gloeotrichia* cell-free filtrate and a medium-only control) with four replicates each in 2 mL, 24-well Nunclon microdishes. We used four microdishes for the experiment, with each microdish containing one replicate of every treatment × density combination. The live *Gloeotrichia* treatment consisted of two live non-clonal colonies of similar biomass

(~1000 μg) with 2 mL of fresh medium. The experiment ran 45 h and cells were counted as described above. We analyzed the mean difference in *Rhodomonas* growth rate among treatments with a one-way ANOVA.

With all growth rate data grouped together, regardless of initial *Rhodomonas* density, there were significant differences among treatments in *Rhodomonas* growth rate over the experimental period (Fig. 2, one-way ANOVA, $F_{2,57} = 6.46$, $P = 0.003$): *Rhodomonas* growth rates were significantly higher after exposure to *Gloeotrichia* filtrate than to *Gloeotrichia* colonies (Tukey's test). The effect of *Gloeotrichia* filtrate on *Rhodomonas* growth rate compared with the control growth rate was significantly greater at lower initial densities of *Rhodomonas* (one-way ANOVA, $F_{4,15} = 6.38$, $P = 0.003$).

We observed a stronger stimulatory effect of the filtrate than *Gloeotrichia* colony treatment on *Rhodomonas* (10 000 cells mL^{-1}) in the multidensity experiment and vice versa in the multispecies experiment. The filtrate treatment effect (i.e. the difference in growth rates between the filtrate and the control) was very similar between experiments: 0.41 ± 0.08 day^{-1} in the multidensity experiment and 0.42 ± 0.05 day^{-1} in the multispecies experiment. The *Gloeotrichia* treatment effect was more variable between experiments: we observed an effect of 0.12 ± 0.05 day^{-1} in the multidensity experiment and 0.62 ± 0.07 day^{-1} in the multispecies experiment. We hypothesize that the differences in the growth rate may be due to the varying physiological state of the *Gloeotrichia* in our separate experiments, which were started several days apart. It is possible that the *Gloeotrichia* colonies were providing less stimulatory effect in the multidensity experiment than in the multispecies experiment. Although differences in the growth rate between the experiments do exist, the variability within treatments for both experiments is quite low. Thus, even with the variability between experiments, the consistency of the treatment effects indicates that *Gloeotrichia*'s filtrate stimulation is valid.

Third, we tested the effect of varying biomass of live *Gloeotrichia* colonies on an intermediate density of *Rhodomonas* (10 000 cells mL^{-1}). Before the experiment began, we calculated the biomass of *Gloeotrichia* colonies in culture from measurements of colony diameter (assuming *Gloeotrichia*'s density was 1 g cm^{-3}) and grouped the colonies into six biomass classes, with each class representing one treatment. We then chose four live *Gloeotrichia* colonies from each biomass class for each treatment. The live *Gloeotrichia* colonies were added separately to 2 mL wells in 24-well Nunclon microdishes containing 10 000 *Rhodomonas* cells mL^{-1} and MWC medium and incubated for 96 h. The six *Gloeotrichia* treatments consisted of 0 μg biomass (no colony added),

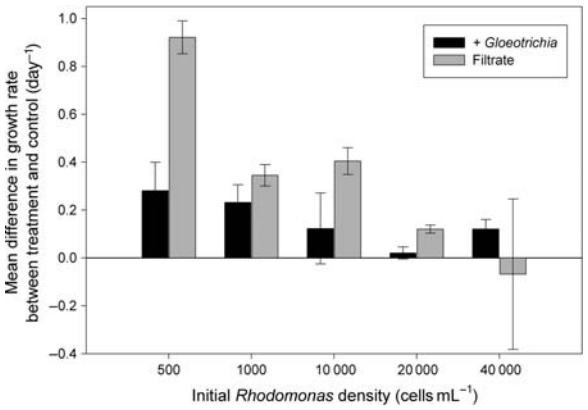

Fig. 2. The mean difference between the *Rhodomonas lacustris* growth rate in the *Gloeotrichia echinulata* or filtrate treatment and the medium-only control treatment growth rate for each of the five initial densities of *Rhodomonas* ($n = 4$). Error bars represent 1 SE.

220 ± 0, 610 ± 0, 1020 ± 0, 1890 ± 70 and 3320 ± 170 μg (1 SE).

We conducted model selection in the R statistical package (R Development Core Team 2008; http://www.R-project.org) to determine the most appropriate regression model describing the relationship between *Rhodomonas* growth rate and *Gloeotrichia* biomass. We tested four possible models commonly used to describe algal dynamics, two linear and two non-linear saturating functions: mean ($y = a$), linear ($y = ax + c$) and Michaelis–Menten with an intercept term ($y = c + ax/(s + x)$) and without an intercept term ($y = ax/(s + x)$) (Briggs and Haldane, 1925) to ascertain if *Gloeotrichia* linearly or non-linearly affected *Rhodomonas* cell density. We solved for maximum likelihood estimates for each model parameter using a simulated annealing algorithm, a global parameter optimization procedure, with 10 000 iterations, using a normally distributed error term. We used the Akaike Information Criterion (AIC) to select the most parsimonious model, i.e. the best model fit for the fewest parameters (Burnham and Anderson, 2002).

Similar to the first two experiments, we observed a stimulatory effect of *Gloeotrichia* colonies on *Rhodomonas* growth rate in comparison with the control treatment; in this case, *Rhodomonas* growth rate increased as a linear function of *Gloeotrichia* biomass (Fig. 3). We chose the linear regression model (over mean and non-linear models) because it exhibited the lowest AIC value (Table III; Burnham and Anderson, 2002).

We found that *Gloeotrichia* exhibited the greatest stimulatory effect at low densities of *Rhodomonas* and at high *Gloeotrichia* biomasses. The most likely explanations for these results, similar to Rengefors and Legrand (Rengefors and Legrand, 2007), are that at a low density of a target species, more *Gloeotrichia* exudates are

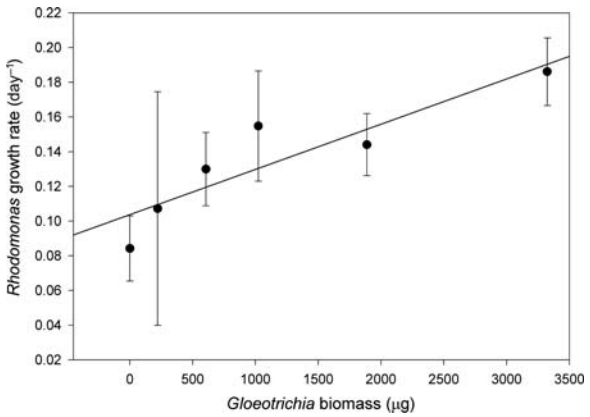

Fig. 3. Growth rate of *Rhodomonas lacustris* increased linearly with *Gloeotrichia echinulata* biomass. There was non-significant lack of fit for the linear model ($F_{4,16} = 0.61$, $P = 0.66$). Error bars represent 1 SE.

Table III: A list of the regression models tested in Experiment 3 in descending order of best fit, as determined by the lowest AIC value

Model type	Model equation	Parameters with two-unit support intervals	R^2	Corrected AIC
Linear	$ax + c$	$a = 0.10$ (0.09–0.12) $c = 0.03$ (0.01–0.06)	0.35	−72.5
Michaelis–Menten with intercept term	$c + ax/(s + x)$	$a = 0.12$ (0.08–0.15) $c = 0.08$ (0.07–0.10) $s = 0.04$ (0.03–0.06)	0.40	−72.0
Mean	a	$a = 0.14$ (0.12–0.16)	0	−64.9
Michaelis–Menten without intercept term	$ax/(s + x)$	$a = 0.18$ (0.15–0.21) $s = 0.18$ (0.04–0.43)	0	−60.4

available for each target cell. This finding suggests that *Gloeotrichia*'s stimulatory effect on phytoplankton may be greatest when *Gloeotrichia* biomass in the water column is high (during blooms). Despite that the Michaelis–Menten model's AIC value was similar to the linear model's AIC, we did not observe saturation in *Rhodomonas* growth rate across the wide range of *Gloeotrichia* biomasses tested. Only 35% of the variation in the stimulatory effect on *Rhodomonas* was explained by *Gloeotrichia* biomass, which may be explained by differential production of exudates in the non-clonal *Gloeotrichia* colonies. Differences in the allelochemical effect among clones of the same species have also been observed in dinoflagellates (Tillmann *et al.*, 2009).

We cannot determine the exact mechanism responsible for *Gloeotrichia*'s stimulation of other phytoplankton species in this study, but suggest three possibilities. First, *Gloeotrichia* may be releasing nutrients, such as stored phosphorus (Noges *et al.*, 2004; Carey *et al.*, 2008) or fixed nitrogen (Stewart *et al.*, 1967). We were unable to measure changes in the medium nutrient concentrations due to the low volume of medium in the microdishes (2 mL); however, due to the short-term nature of our experiments and the very high N and P concentrations in our MWC culture medium [>1 M (14 g L^{-1}) and 0.05 M (1.5 g L^{-1}), respectively], it is unlikely that the target phytoplankton were nutrient-limited. Further, as the *Gloeotrichia* were grown at an irradiance probably close to the compensation level, it is unlikely that the colonies were exuding carbohydrates because of an excess of C over N acquisition (Ana and Massimo, 2004). Second, cyanobacteria produce many bioactive secondary metabolites (Gross, 2003; Legrand *et al.*, 2003), which phytoplankton may have evolved to recognize and utilize for their own metabolism (Suikkanen *et al.*, 2004). Many phytoplankton species are capable of using dissolved organic compounds (osmotrophy; Sanders *et al.*, 1990; Tittel and Kamjunke, 2004), and thus a positive growth response to algal exudates is not unlikely. Third, *Gloeotrichia*, similar to other cyanobacteria, may produce antibacterial or antifungal compounds beneficial to other phytoplankton (Legrand *et al.*, 2003).

Our work adds to the growing literature indicating that stimulation, or facilitation, may be an important force structuring communities (Bruno *et al.*, 2003; Halpern *et al.*, 2007). Although Suikkanen (Suikkanen *et al.*, 2005) has suggested that stimulation is more likely to occur in natural communities than in laboratory experiments, our results demonstrate strong positive effects of *Gloeotrichia* on other phytoplankton in laboratory experiments. Although we do not know the evolutionary significance of *Gloeotrichia* stimulation of other phytoplankton, our findings may indicate co-evolution among phytoplankton taxa. Our results are consistent with observations from Lake Peipsi, Estonia (Noges *et al.*, 2004), where *Gloeotrichia* blooms stimulated other phytoplankton species to increase in the field. Although this stimulatory mechanism remains to be elucidated, our data suggest that *Gloeotrichia* may enhance eutrophication, particularly in oligotrophic lakes where this cyanobacterium has recently begun to bloom.

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REFERENCES

Ana, O. and Massimo, V. (2004) *Nostoc* (Cyanophyceae) goes nude: extracellular polysaccharides serve as a sink for reducing power under unbalanced C/N metabolism. *J. Phycol.*, **40**, 74–81.

Blomqvist, P. and Herlitz, E. (1996) *Methods for Quantitative Assessment of Phytoplankton in Freshwaters. Part 2. Literature and its Use for Determination of Planktic Volvocales, Tetrasporales, Chlorococcales, and Ulotrichales, and Formulas for Calculation of Biovolume of the Organisms*. Naturvårdsverkets Förlag, Stockholm.

Briggs, G. E. and Haldane, J. B. S. (1925) A note on the kinetics of enzyme action. *Biochem. J.*, **19**, 339–339.

Bruno, J. F., Stachowicz, J. J. and Bertness, M. D. (2003) Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.*, **18**, 119–125.

Burnham, K. P. and Anderson, D. R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York.

Carey, C. C., Haney, J. F. and Cottingham, K. L. (2007) First report of microcystin-LR in the cyanobacterium *Gloeotrichia echinulata*. *Environ. Toxicol.*, **22**, 337–339.

Carey, C. C., Weathers, K. C. and Cottingham, K. L. (2008) *Gloeotrichia echinulata* blooms in an oligotrophic lake: helpful insights from eutrophic lakes. *J. Plankton Res.*, **30**, 893–904.

Carey, C. C., Weathers, K. C. and Cottingham, K. L. (2009) Increases in phosphorus at the sediment-water interface may accelerate the initiation of cyanobacterial blooms in an oligotrophic lake. *Verhand. Intern. Verein. Limnol.*, **30**, 1185–1188.

Forsell, L. (1998) Migration from the littoral zone as an inoculum for phytoplankton. *Arch. Hydrobiol. Adv. Limnol.*, **51**, 21–27.

Gross, E. M. (2003) Allelopathy of aquatic autotrophs. *Crit. Rev. Plant Sci.*, **22**, 313–339.

Guillard, R. R. L. and Lorenzen, C. J. (1972) Yellow-green algae with chlorophyllide c. *J. Phycol.*, **8**, 10–14.

Hallegraeff, G. M. (1993) A review of harmful algal blooms and their apparent global increase. *Phycologia*, **32**, 79–99.

Halpern, B. S., Silliman, B. R., Olden, J. D. *et al.* (2007) Incorporating positive interactions in aquatic restoration and conservation. *Front. Ecol. Environ.*, **5**, 153–160.

Karjalainen, M., Engstrom-Ost, J., Korpinen, S. *et al.* (2007) Ecosystem consequences of cyanobacteria in the northern Baltic Sea. *Ambio*, **36**, 195–202.

Karlsson, I. (2003) Benthic growth of *Gloeotrichia echinulata* cyanobacteria. *Hydrobiologia*, **506**, 189–193.

Karlsson-Elfgren, I., Rydin, E., Hyenstrand, P. *et al.* (2003) Recruitment and pelagic growth of *Gloeotrichia echinulata* (Cyanophyceae) in Lake Erken. *J. Phycol.*, **39**, 1050–1056.

Keating, K. I. (1977) Allelopathic influence on blue-green bloom sequence in a eutrophic lake. *Science*, **196**, 885–887.

Leflaive, J. and Ten-Hage, L. (2007) Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. *Freshwater Biol.*, **52**, 199–214.

Legrand, C., Rengefors, K., Fistarol, G. O. *et al.* (2003) Allelopathy in phytoplankton - biochemical, ecological and evolutionary aspects. *Phycologia*, **42**, 406–419.

Mohamed, Z. (2002) Allelopathic activity of *Spirogyra* sp.: stimulating bloom formation and toxin production by *Oscillatoria agardhii* in some irrigation canals, Egypt. *J. Plankton Res.*, **21**, 137–141.

Noges, T., Tonno, I., Laugaste, R. *et al.* (2004) The impact of changes in nutrient loading on cyanobacterial dominance in Lake Peipsi (Estonia/Russia). *Arch. Hydrobiol.*, **160**, 261–279.

Paerl, H. W. and Huisman, J. (2008) Blooms like it hot. *Science*, **320**, 57–58.

Rengefors, K. and Legrand, C. (2001) Toxicity in *Peridinium aciculiferum*—an adaptive strategy to outcompete other winter phytoplankton? *Limnol. Oceanogr.*, **46**, 1990–1997.

Rengefors, K. and Legrand, C. (2007) Broad allelopathic activity in *Peridinium aciculiferum*. *Eur. J. Phycol.*, **42**, 341–349.

Sanders, R. W., Porter, K. G. and Caron, D. A. (1990) Relationship between phototrophy and phagotrophy in the mixotrophic chrysophyte *Poterioochromonas malhamensis*. *Microb. Ecol.*, **19**, 97–109.

Stewart, W. D., Fitzgerald, G. P. and Burris, R. H. (1967) *In situ* studies on N2 fixation using the acetylene reduction technique. *Proc. Natl Acad. Sci. USA*, **58**, 2071–2078.

Suikannen, S., Fistarol, G. O. and Graneli, E. (2004) Allelopathic effects of the Baltic cyanobacteria *Nodularia Spumigena, Aphanizomenon flos-aquae*, and *Anabaena lemmermannii* on algal monocultures. *J. Exp. Mar. Biol. Ecol.*, **308**, 85–101.

Suikkanen, S., Fistarol, G. O. and Graneli, E. (2005) Effects of cyanobacterial allelochemicals on a natural plankton community. *Mar. Ecol.-Prog. Ser.*, **287**, 1–9.

Tillmann, U., Alpermann, T., Purificacao, R. *et al.* (2009) Intra-population clonal variability in allelochemical potency of the toxigenic dinoflagellate *Alexandrium tamarense*. *Harmful Algae*, **8**, 759–769.

Tittel, J. and Kamjunke, N. (2004) Metabolism of dissolved organic carbon by planktonic bacteria and mixotrophic algae in lake neutralization experiments. *Freshwater Biol.*, **49**, 1062–1071.