

# *Gloeotrichia echinulata* blooms in an oligotrophic lake: helpful insights from eutrophic lakes

CAYELAN C. CAREY<sup>1\*†</sup>, KATHLEEN C. WEATHERS<sup>2</sup> AND KATHRYN L. COTTINGHAM<sup>1</sup>

<sup>1</sup>DEPARTMENT OF BIOLOGICAL SCIENCES, DARTMOUTH COLLEGE, HANOVER, NH 03755, USA AND <sup>2</sup>CARY INSTITUTE OF ECOSYSTEM STUDIES, MILLBROOK, NY 12545, USA

<sup>†</sup>PRESENT ADDRESS: DEPARTMENT OF ECOLOGY AND EVOLUTIONARY BIOLOGY, CORNELL UNIVERSITY, E221 CORSON HALL, ITHACA, NY 14853-2701, USA

\*CORRESPONDING AUTHOR: ccc99@cornell.edu

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*Nuisance algal blooms are widely reported from eutrophic and hypereutrophic systems, but are relatively rare in oligotrophic systems. Recently, the cyanobacterium *Gloeotrichia echinulata* has begun blooming in oligotrophic to mesotrophic lakes in the northeastern USA. We explored whether existing knowledge about *G. echinulata*, derived from eutrophic systems, would be useful in predicting the recruitment dynamics of *G. echinulata* in an oligotrophic lake in central New Hampshire, USA. Our results indicate that, as in eutrophic lakes, there was a seasonal peak in both recruitment and surface abundance, with colonies recruiting from shallow (2 m) but not deep ( $\geq 5$  m) sediments. Recruitment provided approximately 20–45% of the colonies to the 2005 *G. echinulata* surface bloom, within the range reported for eutrophic lakes. Thus, we conclude that observations of *G. echinulata* in eutrophic systems may be useful in predicting *G. echinulata* recruitment dynamics in oligotrophic lakes. Moreover, recruitment from the sediments appears to be an important mechanism that allows *G. echinulata* to bloom in low- as well as high-nutrient lakes. This benthic-pelagic coupling may, over time, result in an important transfer of nutrients in such oligotrophic lakes.*

## INTRODUCTION

Understanding the causes of cyanobacterial blooms in freshwater lakes is extremely important because of their toxicity, ability to dominate summer phytoplankton assemblages and detrimental effects on aquatic food webs (Paerl, 1988). Cyanobacterial population increases typically result from two processes: recruitment from sediment resting stages (e.g., akinetes) and division in the water column (Wetzel, 2001). Recruitment, in particular, provides an inoculum for blooms and may heavily subsidize bloom formation. Consequently, an understanding of recruitment dynamics may help to predict and manage cyanobacterial blooms (Hansson *et al.*, 1994).

One cyanobacterium that subsidizes its bloom formation with recruitment is *Gloeotrichia echinulata*, a

nitrogen-fixer that produces large ( $\sim 2$  mm) colonies (Roelofs and Oglesby, 1970; Barbiero and Welch, 1992; Karlsson-Elfgren *et al.*, 2003). *Gloeotrichia echinulata* have been well-studied in eutrophic systems (Karlsson-Elfgren *et al.*, 2003). During the late summer in a eutrophic lake, pelagic *G. echinulata* sink out of the water column and produce akinetes that overwinter on the lake sediment. Some proportion of these akinetes germinate the following spring and then subsequently recruit into the water column after a developmental period on the sediment (Pettersson *et al.*, 1993; Tymowski and Duthie, 2000; Karlsson, 2003; Karlsson-Elfgren *et al.*, 2003). *Gloeotrichia echinulata*'s surface population is often, but not always, linked to the abundance of recruiting colonies (Prescott, 1951; Roelofs and Oglesby, 1970; Pettersson *et al.*, 1993). For well-studied eutrophic

systems, *G. echinulata* recruitment has been shown to supply 3–50% of the surface population (Barbiero and Welch, 1992; Forsell and Pettersson, 1995; Karlsson-Elfgren *et al.*, 2003).

Significantly, *G. echinulata* has recently started blooming in oligotrophic to mesotrophic lakes across the northeastern USA (Fig. 1). There were confirmed blooms of *G. echinulata* in at least 27 oligotrophic lakes across Maine and New Hampshire between 2002 and 2006 (Maine Department of Environmental Protection, New Hampshire Department of Environmental Services, C.C.C., personal observation). These blooms are extremely unusual, since nutrient limitation in the water column is generally thought to prevent cyanobacterial blooms in oligotrophic systems (Reynolds, 1999; Smith and Bennett, 1999; Downing *et al.*, 2001).

The consequences of *G. echinulata* blooms for an oligotrophic lake can be considerable. First, *G. echinulata* can transport a significant amount of phosphorus (P) into the water column: up to  $3.8 \text{ mg P m}^{-2} \text{ day}^{-1}$  when recruitment is high (Istvanovics *et al.*, 1993), possibly triggering other cyanobacteria to bloom (Noges *et al.*, 2004). Second, because *G. echinulata* is thought to be inedible to most zooplankton (Roelofs and Oglesby, 1970; but see Liu *et al.*, 2006), blooms of *G. echinulata* could impact pelagic food webs fueled by edible phytoplankton. Third, *G. echinulata* produces a very low concentration of microcystin-LR (Carey *et al.*, 2007).

To better understand the recent *G. echinulata* blooms in oligotrophic lakes in the northeastern USA, we used published information about *G. echinulata* recruitment in eutrophic lakes to develop hypotheses about recruitment dynamics in low-P systems. For example, because *G. echinulata* pelagic populations exhibit a seasonal peak in eutrophic lakes (Barbiero, 1993; Istvanovics *et al.*, 1993; Forsell and Pettersson, 1995; Karlsson-Elfgren *et al.*, 2003; Karlsson-Elfgren *et al.*, 2004), we expected that there would also be a seasonal peak in *G. echinulata* surface abundance and recruitment in oligotrophic lakes. Since *G. echinulata* recruitment has been shown to depend on interacting light, temperature and nutrient conditions (Barbiero, 1993; Karlsson, 2003; Karlsson-Elfgren *et al.*, 2004), we also expected that recruitment might be temporally synchronous but spatially heterogeneous across a lake; i.e., recruitment may be triggered by a lake-wide driver, yet the magnitude of recruitment at any one site might depend on local factors such as available light or nutrients. Finally, in eutrophic lakes, *G. echinulata* recruitment primarily occurs from littoral sediments 1–2 m deep (Barbiero, 1993; Karlsson-Elfgren *et al.*, 2003). Because *G. echinulata* depends on high light levels for recruitment (Jacobsen, 1994; Karlsson-Elfgren *et al.*, 2004), we hypothesized

that recruitment from sediments at depths  $>2 \text{ m}$  is limited by light availability in eutrophic lakes with shallow Secchi depths. In contrast, we expected to see more recruitment from deep sediments in an oligotrophic lake because of the greater light penetration at depth.

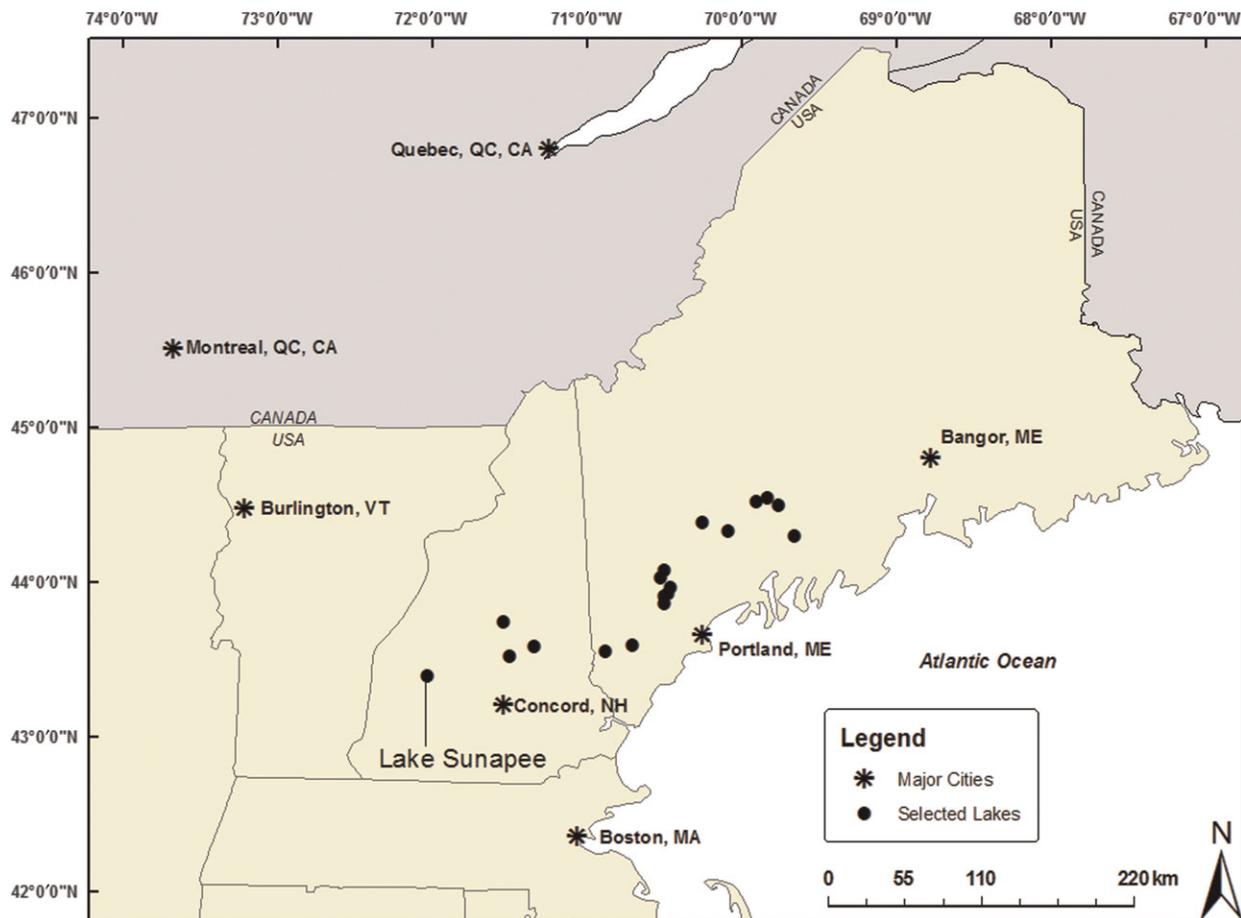
We also explored the potential influence of sediment P on *G. echinulata* germination and recruitment. Historical and paleolimnological analyses indicate that *G. echinulata* may be able to dominate lakes in transition to a more eutrophic state (Cronberg *et al.*, 1999; Bunting *et al.*, 2007), possibly because *G. echinulata* may require a threshold level of P in the sediment for recruitment (Pettersson *et al.*, 1993). Previous work suggests that P absorbed in the sediments is what fuels planktonic cell division: *G. echinulata*'s P uptake in the water column is thought to be negligible (Istvanovics *et al.*, 1993; Tymowski and Duthie, 2000). Because of the large pool of sediment P that exists even in oligotrophic lakes (Maassen *et al.*, 2005), and because of *G. echinulata*'s apparent demand for P from the sediment, we hypothesized that akinete germination and recruitment would be related to sediment P concentrations (Tymowski and Duthie, 2000).

We tested our hypotheses about *G. echinulata* in an oligotrophic lake in central New Hampshire, USA, where *G. echinulata* blooms were first reported in 2003. This lake had not exhibited dense *G. echinulata* blooms during the past two decades, as indicated by long-term routine monitoring conducted by a local watershed organization [Lake Sunapee Protective Association (LSPA), unpublished data]. We investigated *G. echinulata* recruitment heterogeneity on multiple scales: temporally, during a summer season (June–September 2005), and spatially, at multiple sites at the same depth and between shallow and deep depths. In addition, we used field monitoring and a lab experiment to examine the potential influence of sediment P on *G. echinulata* germination and recruitment.

## METHOD

### Study site

We studied *G. echinulata* in Lake Sunapee, an oligotrophic system in central New Hampshire (USA) at  $43^{\circ}24'N$ ,  $72^{\circ}2'W$ . The lake has an area of  $16.55 \text{ km}^2$ , a volume of  $1.88 \times 10^8 \text{ m}^3$ , and a mean depth of 10 m and is used as a water source by some lakeside residents (LSPA, unpublished data). Most of the lake stratifies thermally from mid-June to early October at approximately 6–7 m depth, as determined by monitoring data



**Fig. 1.** Map depicting locations of selected low-nutrient, recreationally important lakes in northern New England, United States, with confirmed *G. echinulata* blooms between 2002 and 2006 (New Hampshire Department of Environmental Services; Maine Department of Environmental Protection; C.C.C., personal observation).

from deep-water sites (LSPA, unpublished data) and is covered with ice from December to mid-April. Lake Sunapee's 20-year mean summer open water total P (TP) concentration is  $0.15 \pm 0.003$  (1 SE)  $\mu\text{M}$  ( $4.8 \pm 0.01 \mu\text{g L}^{-1}$ ), mean Secchi depth is  $7.3 \pm 0.1$  (1 SE) m, and mean chlorophyll *a* concentration is  $1.7 \pm 0.01$  (1 SE)  $\mu\text{g L}^{-1}$  (LSPA, unpublished data). Significantly, there are no significant long-term (>20 year) trends in mean summer TP or chl *a* for pelagic long-term sampling locations. However, the mean summer TP concentration from Herrick Cove (see below), in which much of our sampling was conducted, has been increasing since 1986 [mean summer TP ( $\mu\text{M}$ ) =  $0.002$  (Year)  $-0.381$ ;  $r^2 = 0.30$ ,  $F_{1,19} = 16.78$ ,  $P = 0.0006$ ]. Using these long-term means and standard trophic status indices (TSI; Carlson, 1977, 1980, 1992; Carlson and Simpson, 1996; Wetzel, 2001), Lake Sunapee is oligotrophic (TSI of 27 from TP or 28 from Secchi depth) or oligomesotrophic (TSI of 37 from chl *a*) (Carlson and Simpson, 1996). There are many similar lakes in

New Hampshire (New Hampshire Department of Environmental Services Volunteer Lay Monitoring Program and University of New Hampshire Lake Monitoring Program, unpublished data).

### Spatial and temporal patterns in recruitment: field monitoring

#### Recruitment traps

We monitored *G. echinulata* recruitment in Lake Sunapee with traps designed to collect colonies as they migrated into the water column. An inverted transparent glass funnel (diameter = 8.57 cm) was attached to a 250 mL plastic collection bottle hung  $\sim 10$  cm above the sediment to trap migrating colonies. This design has been shown to be effective in preventing the lateral transport of colonies into traps (Barbiero and Welch, 1992; Hansson, 1995, 1996). On each sampling date, the recruitment trap funnel unit was stoppered underwater

and emptied into a collection bottle at the water surface. Each sample was preserved with Lugol's iodine solution in the field (Lind, 1979).

#### Lake-wide monitoring

In 2005, we monitored *G. echinulata* recruitment at eight shallow (2 m water depth) sites with organic-rich sediments around the perimeter of Lake Sunapee (Fig. 2). We selected these sites because we expected that the akinete bank would be more viable in organic-rich sediment than sandy or rocky sites (I. Karlsson-Elfgren, Uppsala, personal communication). In addition, the site locations were concentrated in the northern part of the lake because *G. echinulata* blooms were most dense in this area in 2004 (LSPA, unpublished data). Two of these sites (Herrick Cove East and Herrick Cove South, ~500 m apart; hereafter HC East and HC South) had paired recruitment traps separated by 2 m to examine spatial heterogeneity within a site (10 traps total). In addition, we placed one trap at 5 m and one at 8 m (“deep traps”) at three sites in the lake (six deep traps total). We sampled *G. echinulata* abundance in the shallow recruitment traps by snorkeling every 3–9 days from 26 June to 25 September 2005 and in the deep traps by SCUBA diving every 7 days from 8 July to 13 September 2005. We expect that this monitoring period sufficiently represented the entire bloom period of *G. echinulata* in Lake Sunapee as colonies were not observed

in the water column prior to our sampling and the bloom ended quickly in mid-September.

We also measured P at the sediment–water interface (hereafter, sediment P) adjacent to each recruitment trap approximately once a week to estimate the potential size of the P pool available to *G. echinulata* near the top layer of the lake sediment. To collect these samples, we placed a sealed 125 mL acid-washed (2 N HCl) plastic collection bottle on top of the sediment surface directly adjacent to the recruitment traps and removed its cap to fill the bottle with ~40 mL sediment and ~60 mL lakewater from directly above the sediment (Carey *et al.*, in press). The filled collection bottle was sealed underwater, brought to the surface and frozen until analysis.

Finally, we sampled *G. echinulata* surface density (colonies L<sup>-1</sup>) in the top 1 m of the lake at each shallow site using a plankton net (80 µm mesh, 30 cm diameter, 1.5 m length).

#### Laboratory analyses

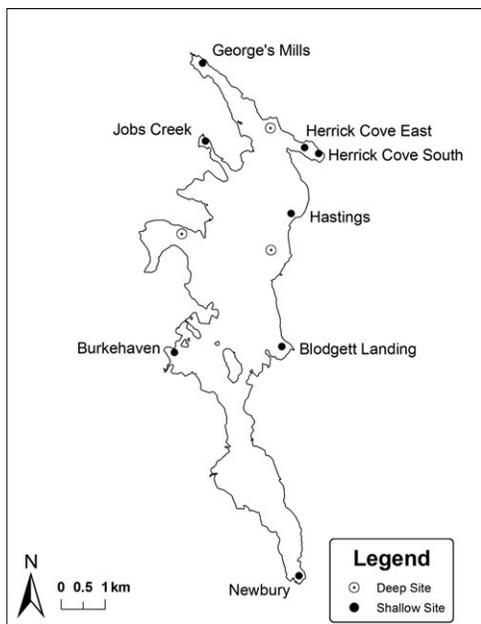
All *G. echinulata* colonies were identified and enumerated with an Olympus SZH10 dissecting microscope. We calculated *G. echinulata* recruitment rate as the number of *G. echinulata* colonies migrating into the water column between sampling dates (colonies cm<sup>-2</sup> of lake sediment day<sup>-1</sup>).

Immediately prior to analysis, sediment P samples were thawed, vigorously shaken and filtered through Whatman GF/F filters. The filtrate was digested using potassium persulfate digestion and analyzed colorimetrically using the molybdenum blue method (Method 4500-P, APHA, 1980). Variation using this analytical protocol was ± 0.16 µM.

#### Data analysis

We assessed spatial and temporal heterogeneity in recruitment using a variety of statistical analyses in SAS 9.1 (SAS Institute, Cary, NC, USA). First, we used a Welch one-way ANOVA to quantify the differences in recruitment between the 10 shallow (2 m) and six deep (5 and 8 m) sites. Because the shallow and deep traps were not sampled at the same intervals, we performed this analysis on total number of colonies collected in each trap throughout the sampling season, rather than on individual sampling dates.

To evaluate the synchrony of recruitment from shallow sites across Lake Sunapee, we calculated Spearman rank correlations of *G. echinulata* recruitment rate across all pairs of shallow traps throughout the sampling period. This follows the general approach for estimating temporal coherence among sites developed



**Fig. 2.** Lake Sunapee, New Hampshire, USA. The black dots and their corresponding names demarcate the 2 m shallow sampling locations and the white circles with enclosed dots demarcate 5 and 8 m deep sampling locations.

by Kratz *et al.* (Kratz *et al.*, 1987), but takes the extreme non-normality of our data into account.

To explore spatiotemporal variability within a particular small cove of the lake, we performed univariate repeated measures ANOVA (RM-ANOVA) in SAS PROC GLM, which allowed comparison of the two replicated shallow sites in Herrick Cove across sampling dates. Only sampling dates with non-missing data for all traps were included in this analysis. We adjusted degrees of freedom with the Greenhouse-Geisser correction because the sphericity assumption was not met (Quinn and Keough, 2002).

Finally, we looked for pairwise associations between the time series for several focal variables using Pearson product-moment correlations. We compared mean *G. echinulata* surface abundance to mean *G. echinulata* recruitment rate on the same sampling dates. We also evaluated whether the mean sediment P was associated with mean *G. echinulata* recruitment rate by comparing these variables at time lags from 0 to 30 days as the approximate time it takes for *G. echinulata* to germinate and recruit into the water column is 3–4 weeks (Tymowski and Duthie, 2000; Karlsson, 2003).

### Contribution of recruitment to the surface bloom

We estimated the contribution of recruiting *G. echinulata* colonies to the surface population maximum after Karlsson-Elfgren *et al.* (Karlsson-Elfgren *et al.*, 2003). First, we calculated the total number of *G. echinulata* colonies in the lake during peak bloom conditions from the mean surface abundance data measured at our sampling locations and the known volume of the top 1–2 m of the lake. We extrapolated our surface abundance data to the top 1 and 2 m of the lake separately because *G. echinulata* colonies were distributed primarily at the water column surface during the bloom peak and we wanted to check the sensitivity of our bloom estimates to model assumptions. Second, Karlsson-Elfgren *et al.* (Karlsson-Elfgren *et al.*, 2003) determined that one *G. echinulata* colony can remain in the water column for 14–30 days before it sinks out of the pelagic zone. Using a 14 day estimate, we calculated the mean number of colonies that could recruit out of the 0–2 m sediment stratum daily (using our lake-wide mean recruitment trap data from 2 m to calculate the number of colonies recruiting each day). The area of the lake stratum used for this estimate was conservative: from littoral surveys, we determined only 25% of the total lake 0–2 m stratum to be suitable habitat for *G. echinulata* recruitment because most of the littoral zone has at least some rocky substrate (LSPA, unpublished data).

We repeated this measurement for a 30 day pelagic residence time and used the range of the 14 and 30 day estimates to derive the approximate contribution of recruitment to the bloom maxima. Finally, this number was divided by the total number of *G. echinulata* colonies in the entire lake at the bloom peak (as calculated above) to determine the recruitment-dependency of the surface population.

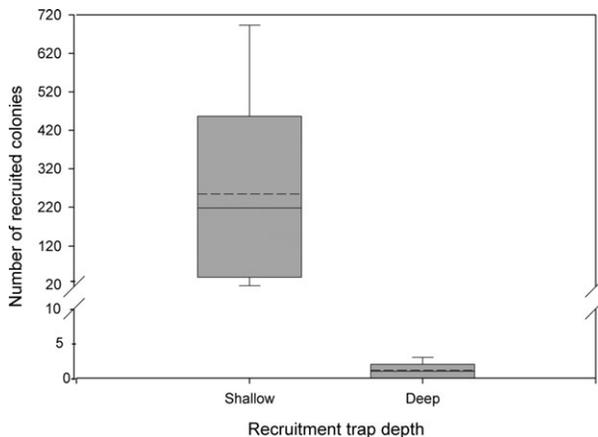
### P addition laboratory experiment

On 19 April 2005, we collected sediment samples from Lake Sunapee for an experiment to determine germination response to P addition. We collected these samples from the top 2 cm of the lake sediment in Herrick Cove (Fig. 2) with a Wildlife Supply 196T Ekman grab. The sediment was kept in the dark at 4°C to prevent germination prior to the experiment (Karlsson, 2003). Twenty-three dead colonies containing akinetes were isolated from the sediment samples and placed in individual 17 mL well plates with 1 mL of homogenized lake sediment and 10 mL of distilled water. The plates were incubated at a constant temperature of 18°C on a 12 h light/dark cycle and examined for germinating akinetes every 24 h for 26 days. We tested for differences in germination rate between distilled water controls ( $n = 10$ ) and a 3.23  $\mu\text{M}$  P (100  $\mu\text{g P L}^{-1}$ ) treatment ( $n = 13$ , prepared from a  $\text{KH}_2\text{PO}_4$  solution) with Fisher's exact test. We also compared the time to germination for the plate wells that germinated using a median test due to skew in the data for both treatments.

## RESULTS

### Spatial and temporal patterns in recruitment

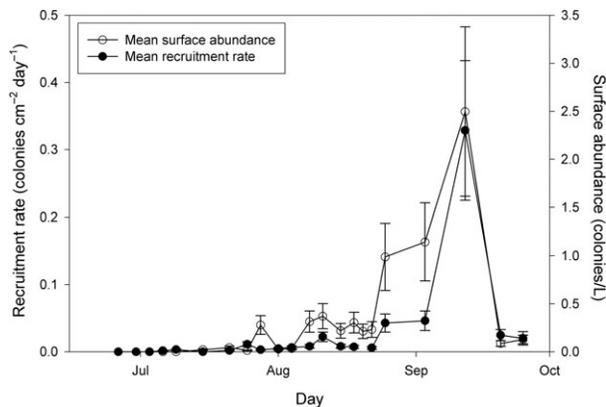
*Gloeotrichia echinulata* recruited into the water column in Lake Sunapee throughout most of our sampling period: we detected recruits from 3 July to 25 September 2005. Shallow water (2 m) recruitment traps collected a total of  $228 \pm 23$  (mean  $\pm 1$  SE) colonies, 200 $\times$  more colonies than the deep (5 or 8 m) sites (Fig. 3; Welch one-way ANOVA,  $F_{1,9} = 8.96$ ,  $P = 0.015$ ). The temporal pattern of *G. echinulata* density in the top 1 m of the lake closely matched the mean recruitment rate across sites ( $r = 0.92$ , Fig. 4). Both recruitment and surface abundance peaked on 12 September, when the mean recruitment rate among shallow sites was  $0.37 \pm 0.09$  colonies  $\text{cm}^{-2} \text{day}^{-1}$ . After this peak, the surface population decreased and *G. echinulata* was not found in the water column by the end of September.



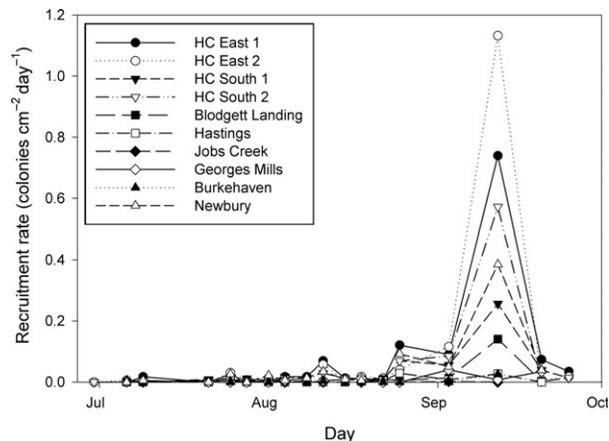
**Fig. 3.** Comparison of the total number of recruited *G. echinulata* colonies captured in the 10 shallow recruitment traps versus the six deep traps during June–September 2005 (mean, dotted line; median, solid line; whiskers, 10th and 90th percentiles), Lake Sunapee, NH, USA.

Overall, the shallow recruitment traps exhibited considerable temporal synchrony in recruitment. Seven of the 10 traps, from five of the eight unique locations (Blodgett Landing, Hastings, HC East, HC South, and Newbury), exhibited similar patterns of recruitment over time (Fig. 5, Table I; median pairwise Spearman rank correlation = 0.667, 17 out of 21 comparisons significant at  $\alpha = 0.05$ ). In contrast, the Burkehaven, Georges Mills, and Jobs Creek sites seemed to follow quite different trajectories, both from the other five sites (median, absolute value of the pairwise Spearman rank correlation = 0.140, 0 out of 18 comparisons significant at  $\alpha = 0.05$ ) and from each other (Table I).

At the two replicated sites in Herrick Cove, recruitment was temporally (RM-ANOVA, Time,  $F_{14,28} = 25.21$ ,



**Fig. 4.** Mean ( $\pm 1$  SE) *G. echinulata* surface population in Lake Sunapee, NH at the shallow sites (HC East 1, HC East 2, HC South 1, HC South 2, Blodgett Landing, Hastings, Jobs Creek, Georges Mills, Burkehaven and Newbury) and the recruitment rate at 2 m depth in June–September 2005.



**Fig. 5.** *Gloeotrichia echinulata* recruitment rate at 10 lake-wide shallow traps, Lake Sunapee, NH, from June–September 2005.

$P = 0.04$ ), but not spatially (Site,  $F_{1,2} = 9.88$ ,  $P = 0.09$ ; Site  $\times$  Time,  $F_{14,28} = 3.85$ ,  $P = 0.19$ ) variable.

Finally, recruitment rate appeared to have a relationship with sediment P (Fig. 6). Based on our sampling, there appeared to be a lake-wide pulse in sediment P on 18 August, 25 days before the maximum *G. echinulata* recruitment rate, in 6 out of 10 sites. Statistically, there were significant ( $\alpha = 0.05$ ) correlations between mean *G. echinulata* recruitment rate and mean sediment P at lags of 7 ( $r = 0.91$ ,  $n = 5$ ), 11 ( $r = 0.89$ ,  $n = 4$ ) and 25 ( $r = 0.96$ ,  $n = 3$ ) days.

### Contribution of recruitment to the surface bloom

Recruitment contributed to the surface bloom in Lake Sunapee on 12 September 2005 (Fig. 4). Assuming that no colonies left the water column before 14 days and that recruitment from sediments  $> 2$  m depth was negligible (as suggested by our recruitment data, Fig. 3), we estimate that recruitment from the sediments was responsible for 40–45% of the pelagic *G. echinulata* population if the surface bloom resided solely in the top 1 m of the water column and, more conservatively, 21–23% if the bloom extended into the top 2 m of the water column.

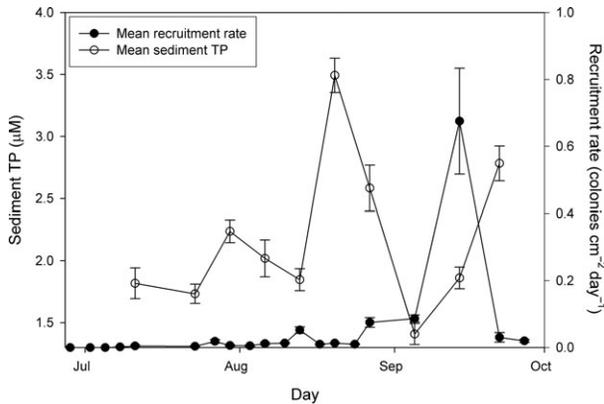
### P addition laboratory experiment

In the laboratory, P addition significantly increased the germination rate (Fisher’s exact test,  $P = 0.04$ ) and reduced the time to germination (median test,  $P = 0.04$ ) relative to the distilled water control. Akinetes within dead colonies in the +P treatment germinated at a higher frequency (76.9 versus 30%) and earlier in the

Table I: Pairwise Spearman rank correlations for *G. echinulata* colonies recruiting from 10 shallow sediment traps, Lake Sunapee, NH

	Blodgett Landing	HC East 1	HC East 2	Hastings	Jobs Creek	Georges Mills	Burkehaven	HC South 1	HC South 2
HC East 1	<b>0.664</b> , $P=0.0069$ , $n=15$								
HC East 2	<b>0.680</b> , $P=0.0075$ , $n=14$	<b>0.666</b> , $P=0.0049$ , $n=16$							
Hastings	<b>0.677</b> , $P=0.0157$ , $n=12$	0.487, $P=0.1086$ , $n=12$	<b>0.673</b> , $P=0.0232$ , $n=11$						
Jobs Creek	0.186, $P=0.5622$ , $n=12$	-0.051, $P=0.8743$ , $n=12$	0.049, $P=0.8803$ , $n=12$	0.297, $P=0.4039$ , $n=10$					
Georges Mills	0.179, $P=0.5402$ , $n=15$	0.456, $P=0.0874$ , $n=15$	0.140, $P=0.6184$ , $n=15$	-0.108, $P=0.7511$ , $n=11$	0.221, $P=0.4893$ , $n=12$				
Burkehaven	-0.065, $P=0.8333$ , $n=13$	-0.023, $P=0.9369$ , $n=14$	0.005, $P=0.9877$ , $n=13$	-0.349, $P=0.2924$ , $n=11$	-0.232, $P=0.4918$ , $n=11$	0.018, $P=0.9538$ , $n=13$			
HC South 1	0.509, $P=0.0629$ , $n=14$	<b>0.717</b> , $P=0.0018$ , $n=16$	<b>0.525</b> , $P=0.0446$ , $n=15$	0.378, $P=0.2524$ , $n=11$	0.143, $P=0.6758$ , $n=11$	0.376, $P=0.1849$ , $n=14$	0.245, $P=0.4199$ , $n=13$		
HC South 2	<b>0.667</b> , $P=0.0066$ , $n=15$	<b>0.714</b> , $P=0.0013$ , $n=17$	<b>0.745</b> , $P=0.0009$ , $n=16$	0.566, $P=0.0551$ , $n=12$	0.139, $P=0.6676$ , $n=12$	0.230, $P=0.4095$ , $n=15$	0.049, $P=0.8667$ , $n=14$	<b>0.672</b> , $P=0.0044$ , $n=16$	
Newbury	<b>0.804</b> , $P=0.0005$ , $n=14$	<b>0.920</b> , $P<0.0001$ , $n=14$	<b>0.792</b> , $P=0.0007$ , $n=14$	<b>0.647</b> , $P=0.0313$ , $n=11$	-0.061, $P=0.8577$ , $n=11$	0.316, $P=0.2717$ , $n=14$	-0.168, $P=0.5824$ , $n=13$	<b>0.639</b> , $P=0.0138$ , $n=14$	<b>0.661</b> , $P=0.0073$ , $n=15$

The first entry in each cell is the correlation coefficient; values that are significant at  $\alpha = 0.05$  are bolded. The second entry is the  $P$ -value ( $P$ ) and the third is the number of shared sampling dates ( $n$ ). The shaded cells show the pairwise correlations for sites we believe that show temporal coherence in recruitment rate.



**Fig. 6.** Lake-wide mean *G. echinulata* recruitment rate and mean sediment P in Lake Sunapee, NH. The error bars demarcate 1 SE.

experiment [4.5 day with an inter-quartile range (IQR) of 4 to 8.75 versus 12 days with an IQR of 11–16 days] than akinetes in the control treatment.

## DISCUSSION

Our investigations of *G. echinulata* in Lake Sunapee confirm that recruitment is an important component of the life cycle of this organism. Migration from the sediments to the water column and subsequent sinking back to the sediment may confer several advantages to recruiting *G. echinulata*. Colonies that can control their buoyancy with gas vesicles, such as *G. echinulata*, can maximize temperature, nutrient and light conditions, as well as minimize grazing (Hansson, 1996). In addition, colonies may need to recruit to divide or initiate another life cycle (Fryxell, 1983).

### Spatial and temporal patterns in recruitment

#### *Deep versus shallow sites*

Contrary to our prediction that recruitment from deep sites would contribute a greater proportion to total *G. echinulata* recruitment in oligotrophic lakes than in eutrophic lakes, recruitment occurred predominantly from shallow sites in Lake Sunapee. During our entire monitoring period, a total of just nine *G. echinulata* colonies were collected in the six 5 or 8 m traps, in comparison to the total of 2292 colonies collected in the ten 2 m traps. Thus, recruitment from deep sites did not contribute substantially to the *G. echinulata* surface population in Lake Sunapee, similar to results from other recruitment studies on both cyanobacteria and eukaryotic phytoplankton (Barbiero, 1993; Hansson,

1996; Karlsson-Elfgren *et al.*, 2003; Karlsson-Elfgren and Brunberg, 2004; Karlsson-Elfgren *et al.*, 2004; Rengefors *et al.*, 2004).

The large difference in *G. echinulata* recruitment between shallow and deep sites in Lake Sunapee may have been due to different environmental conditions between the sampled depths. Despite a mean summer Secchi depth of 7.3 m, light quantity and quality at 5 or 8 m depth in Lake Sunapee would still be considerably reduced in comparison to 2 m depth. Using Beer’s law (Wetzel, 2001), we estimate that there would be 32 or 46% more light at 2 m depth than at 5 or 8 m, respectively, assuming a 10 m Secchi depth. Thus, the absence of recruitment at deep sites may have been due to light limitation of germination or nitrogen-fixation, since *G. echinulata* is dependent on high light levels for nitrogen-fixation (Stewart *et al.*, 1967; Chang, 1979). Alternatively, the akinete banks at deep sites may be less developed than in shallow coves or akinete viability may decrease with depth (Karlsson-Elfgren *et al.*, 2004). Whichever mechanisms are acting in Lake Sunapee, *G. echinulata* recruited predominantly from shallower sites in 2005.

#### *Cove-wide and lake-wide patterns*

At both cove- and lake-wide spatial scales, *G. echinulata* recruitment appeared to be temporally synchronous across widely separated sites in the littoral zone of Lake Sunapee. Although some recruitment traps collected more colonies than others, particularly in HC East and HC South, seven of the 10 traps had very similar temporal patterns in recruitment. The timing of *G. echinulata* recruitment in Lake Sunapee seems to be largely regulated by one or more lake-wide variables, such as climate, a weather event, or a synchronous nutrient pulse. The existence of a lake-wide cue—or multiple interacting cues—is supported by the clear peak of *G. echinulata* recruitment on 12 September 2005 in 6 out of 10 shallow traps around the lake’s perimeter. Similarly, in Lake Erken, Sweden, *G. echinulata* blooms occurred synchronously during the same 2 week summer period every year from 1993 to 2002 (Lake Erken water quality database, unpublished data), indicating that *G. echinulata* might be affected by lake-wide cues in both oligotrophic and eutrophic systems.

However, the large differences in the magnitude of recruitment across sites suggest that there are biotic (such as akinete bank development and grazing risk) (Rengefors *et al.*, 1998) and/or abiotic (light, temperature, oxygen, sediment chemistry, substrate and/or nutrient availability) (Hyenstrand *et al.*, 1999; Hyenstrand *et al.*, 2001) microhabitat variables that also exert influence over recruitment. For example, the three sites that exhibited asynchronous recruitment patterns

(Burkehaven, Georges Mills and Jobs Creek) also had very low recruitment rates throughout the summer. This may have been due to environmental conditions associated with their locations on the lake (Fig. 2): all three sites are located on the western shore of the lake, with the Burkehaven site located on the leeward site of a large embayment and the Georges Mills and Jobs Creek sites at the end of long, narrow coves on the northwestern border of Lake Sunapee. The predominant winds over Lake Sunapee are from the northwest, so these three sites likely receive fewer wind-displaced *G. echinulata* colonies than the other shallow sites. With a decreased surface colony density, the *G. echinulata* akinete bank may be less developed than at other sites, resulting in less recruitment throughout the summer. However, the Hastings site also exhibited low recruitment (though in synchrony with the majority of the other shallow sites, Table I), despite its position on the eastern shore. The reduced recruitment at this site may have been due to microhabitat factors, such as a slightly sandier substrate. Thus, we propose that the size of the akinete bank at an individual site might moderate the effects of a lake-wide recruitment trigger.

#### *Sediment phosphorus as a potential driver of recruitment*

Our laboratory as well as field results indicate that sediment P may play a role in stimulating *G. echinulata* recruitment in Lake Sunapee. Lake-wide monitoring data indicated that, on average, a pulse of sediment P occurred 25 days before the increase in *G. echinulata* recruitment rate (Fig. 6). This time lag corresponds to the amount of time *G. echinulata* needs to germinate (1–7 days) and absorb P from the sediment (2–3 weeks) before recruiting into the water column (Tymowski and Duthie, 2000; Karlsson, 2003). Consistent with our field data, additions of 3.23  $\mu\text{M}$  (100  $\mu\text{g P L}^{-1}$ ) stimulated *G. echinulata* germination in the laboratory; this treatment was slightly smaller than the mean sediment P concentration on 25 August (Fig. 6). These results, while not conclusive, are nonetheless intriguing. We suggest that more attention be paid to the role of sediment P in influencing *G. echinulata* recruitment and bloom dynamics in combination with other possible recruitment drivers such as light, temperature and sediment biogeochemistry. In addition, more research is needed to determine the causes of fluctuations in sediment P availability in the shallow sediments of Lake Sunapee as there were no storm events immediately preceding peak P concentrations and the shallow depth of the sampling sites makes anoxic P release unlikely.

#### **Contribution of recruitment to the surface bloom**

Recruitment contributed  $\sim 21$ –45% to Lake Sunapee's *G. echinulata* bloom on 12 September 2005. The synchronous patterns of surface density and recruitment demonstrate that inputs from recruitment may be an important factor in determining the abundance of the surface population. The tight coupling of recruitment and surface density patterns reflects the high buoyancy of *G. echinulata* colonies (Roelofs and Oglesby, 1970): recruitment from 2 m deep sediment to the water surface can happen in  $< 10$  min (C.C.C., personal observation).

We expect that our overall recruitment dependency calculation range may be conservative because we assumed that *G. echinulata* recruitment could only occur from non-rocky, organic-rich substrate. In addition, we extrapolated our surface density measurements to the top 1 or 2 m of the lake while calculating the number of colonies in the *G. echinulata* bloom peak. Though *G. echinulata* colonies were found throughout the water column in 2005, *G. echinulata* density was usually highest at the lake surface (C.C.C., personal observation). Thus, we most likely over-estimated the total number of colonies in the lake at peak bloom conditions and consequently under-estimated bloom recruitment dependency. We chose to use the 0–2 m stratum for estimating lake-wide recruitment rates because Forsell (Forsell, 1998) found that the highest *G. echinulata* recruitment occurred from 0 to 2 m in Lake Erken, Sweden, and that recruitment rates quickly decreased at depths  $> 2$  m. Similarly, while the majority of our shallow sites were in sheltered coves, Newbury is an open, wind-exposed site that exhibited similar recruitment dynamics as most of the more protected sites. Thus, we expected that the mean recruitment rates measured for our shallow sites to be representative of lake-wide conditions.

Interestingly, *G. echinulata*'s recruitment contribution in both oligotrophic and eutrophic lakes is higher than that reported for nearly all other migrating phytoplankton taxa ( $< 1\%$  to 17%: Reynolds *et al.*, 1981; Trimbee and Harris, 1984; Barbiero and Kann, 1994; Hansson *et al.*, 1994; Stahl-Delbanco *et al.*, 2003; Karlsson-Elfgren *et al.*, 2004; but see Hansson, 1996). Why are *G. echinulata* blooms more dependent on recruitment subsidies than other phytoplankton blooms? We cannot say definitively, but we hypothesize that it is, in part, due to *G. echinulata*'s ability to access sediment P for recruitment (Pettersson *et al.*, 1993). Past research has suggested that *G. echinulata* depends on absorbing P on the lake sediment to meet its nutritional requirements (Istvanovics *et al.*, 1993; Tymowski and Duthie, 2000). Based on P reserves and assuming no additional

P uptake, a recruiting *G. echinulata* colony can divide only  $\sim 2\text{--}4\times$  in the water column (Istvanovics *et al.*, 1993, Tymowski and Duthie, 2000), generally much lower than other phytoplankton taxa. Thus, it seems logical that recruitment is necessary for blooms to occur, but that colony division in the water column may increase substantially bloom extent.

Our finding of a moderate recruitment contribution to the 2005 bloom in Lake Sunapee suggests a previously undescribed pathway for benthic–pelagic coupling in low-nutrient systems that have several important implications. First, the migration of millions of large ( $\sim 2\text{ mm}$ ) cyanobacterial colonies from the sediments into the water column can substantially affect the cycling of organic matter and nutrients, as well as subsequent production and grazing processes (Marcus and Boero, 1998). For example, recruiting *G. echinulata* in Lake Erken (Istvanovics *et al.*, 1993) and Green Lake (Barbiero and Welch, 1992) contributed as much as 66% to those lakes' total yearly internal P load. Thus, *G. echinulata* recruitment in an oligotrophic lake such as Lake Sunapee could have considerable impacts on P translocation from the sediment to the water column. Second, our results also suggest that it is important to take into account the ecological drivers of such blooms when considering lake management strategies, especially in low-nutrient lakes. For example, because *G. echinulata* may have limited water column P uptake (Istvanovics *et al.*, 1993; Tymowski and Duthie, 2000), attempts to limit blooms by reducing in-lake nutrient concentrations (cf. Cooke *et al.*, 2005) could have little effect. Instead, efforts to prevent or reduce recruitment may be necessary for decreasing *G. echinulata* blooms.

## CONCLUSIONS

*Gloeotrichia echinulata* in low-nutrient Lake Sunapee exhibited several characteristics similar to *G. echinulata* in eutrophic lakes, which may be useful in understanding and managing the recent *G. echinulata* blooms in oligotrophic lakes in the northeastern USA. Moreover, *G. echinulata* blooms in Lake Sunapee may be supported in part by increased sediment P concentrations. As a littoral phytoplankton species, *G. echinulata* may be more sensitive to changes in nearshore conditions and therefore show an earlier response to nutrient enrichment than a pelagic species. In addition, the benthic–pelagic link provided by this species could accelerate the eutrophication process by moving nutrients from the sediments to the water column. While we currently do not have enough data to test this hypothesis in Lake Sunapee, it remains an intriguing question for future

studies as the distribution of *G. echinulata* expands across the northeastern USA.

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