

Elena Litchman

Population and community responses of phytoplankton to fluctuating light

Received: 24 September 1997 / Accepted: 8 July 1998

Abstract Light is a major resource in aquatic ecosystems and has a complex pattern of spatio-temporal variability, yet the effects of dynamic light regimes on communities of phytoplankton are largely unexplored. I examined whether and how fluctuating light supply affects the structure and dynamics of phytoplankton communities. The effect of light fluctuations was tested at two average irradiances: low, $25 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ and high, $100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ in 2- and 18-species communities of freshwater phytoplankton. Species diversity, and abundances of individual species and higher taxa, depended significantly on both the absolute level and the degree of variability in light supply, while total density, total biomass, and species richness responded only to light level. In the two-species assemblage, fluctuations increased diversity at both low and high average irradiances and in the multispecies community fluctuations increased diversity at high irradiance but decreased diversity at low average irradiance. Species richness was higher under low average irradiance and was not affected by the presence or absence of fluctuations. Diatom abundance was increased by fluctuations, especially at low average irradiance, where they became the dominant group, while cyanobacteria and green algae dominated low constant light and all high light treatments. Within each taxonomic group, however, there was no uniform pattern in species responses to light fluctuations: both the magnitude and direction of response were species-specific. The temporal regime of light supply had a significant effect on the growth rates of individual species grown in

monocultures. Species responses to the regime of light supply in monocultures qualitatively agreed with their abundances in the community experiments. The results indicate that the temporal regime of light supply may influence structure of phytoplankton communities by differentially affecting growth rates and mediating species competition.

Key words Temporal heterogeneity · Light fluctuations · Phytoplankton · Community structure · Diversity

Introduction

Light is a major factor influencing phytoplankton and has a complex pattern of spatial and temporal variability. Temporal frequencies of light variation range from fast fluctuations caused by surface waves (on the order of 10 Hz) to seasonal changes of irradiance (10^{-7} Hz). The light levels experienced by phytoplankton cells can vary from the complete darkness in the aphotic zone to irradiances greater than $1500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at the surface. Phytoplankton are sensitive not only to the absolute average light level but also to the pattern of light supply, i.e., constant versus fluctuating light (e.g., Ferris and Christian 1991; Prezelin et al. 1991; Reynolds 1994). As phytoplankton experience a dynamic versus constant light supply, changes may occur in the biochemical composition (Gibson and Foy 1988; Kroon et al. 1992; Ibelings et al. 1994) and optical properties of cells (Stramski et al. 1993), and in the rates of photosynthesis (Harris and Piccinin 1977; Marra 1978; Grobbelaar et al. 1992; Kroon et al. 1992) and respiration (Beardall et al. 1994).

The majority of studies on the effects of fluctuating light supply have concentrated on the biochemical and physiological levels. However, light fluctuations may affect higher levels of biological organization, up to the population and community levels. Physiological changes due to light fluctuations potentially can be translated into changes in long-term growth rates. Several studies

E. Litchman¹
Department of Ecology, Evolution and Behavior,
University of Minnesota,
1987 Upper Buford Circle,
St. Paul, MN 55108, USA

Present address:

¹Smithsonian Environmental Research Center,
P.O. Box 28, Edgewater, MD 21037, USA
e-mail: litchman@serc.si.edu, Fax: +1-301-2617954

have demonstrated differential growth-rate responses of phytoplankton to fluctuating light (Quéguiner and Legendre 1986; Gibson and Foy 1988; Stramski et al. 1993; Ibelings et al. 1994). If these growth-rate responses are species-specific (cf. Ibelings et al. 1994), then light fluctuations are likely to affect the community level as well, i.e., cause changes in dominance patterns, species dynamics and diversity (Tilman et al. 1982).

These potential community-level effects of light fluctuations remain largely untested. Brzezinski and Nelson (1988) showed that fluctuating light (light:dark cycle) facilitated the coexistence of two species of planktonic diatoms competing for ammonium, probably by temporally separating nutrient uptake by the two species. Similarly, van Gemerden (1974) showed that two strains of sulfur bacteria were able to coexist under fluctuating light but not under constant light. The generality of these results is unknown, as well as how differences in variability of light supply would influence more complex multispecies phytoplankton communities such as those found in nature.

A question of particular interest for community structure is whether fluctuating light supply can promote coexistence and increase species diversity. The study of Brzezinski and Nelson (1988) provides an example of coexistence of two diatoms facilitated by light fluctuations at non-limiting levels. For limiting irradiances, theory predicts competitive exclusion under a constant light supply (Tilman 1982; Huisman and Weissing 1994). Fluctuating supply of a limiting resource may, however, increase the number of coexisting species as was shown theoretically by Levins (1979) and Armstrong and McGehee (1980). This was confirmed experimentally for nitrogen- and phosphorus-limited phytoplankton (Turpin and Harrison 1979; Sommer 1984, 1985; Grover 1991a). However, it is unknown whether a fluctuating light supply of either limiting or non-limiting level can promote diversity of multispecies phytoplankton communities.

The goal of this study was to investigate whether the structure and dynamics of multispecies communities are sensitive to the degree of variability in light supply. In particular, I sought to determine whether the taxonomic composition, dominance patterns, dynamics of individual species, and total biomass of the community depend on the temporal regime of light supply and whether light fluctuations can enhance diversity. These questions were explored using a laboratory multispecies assemblage of freshwater phytoplankton. In addition, because the community and species responses to environmental fluctuations may depend on species richness (Tilman 1996), I performed the same experiment with a community consisting of only two species, selected based on their high abundance in the multispecies experiment. Because community responses should depend on the differential responses of individual species, I also examined the effects of the same light regimes that were used in community experiments on growth rates of the two species in monocultures. This study thus examined

the effect of temporal variability in light supply at three levels of complexity: in monocultures, and in two- and multispecies communities.

To isolate the effect of the degree of variability of light supply from the effect of the total irradiance, I contrasted light regimes of the same total irradiance dose, with and without fluctuations. For simplicity, I used only one type of fluctuation (square wave), although in nature many types of temporal fluctuations are superimposed on each other. Underwater irradiance varies not only in intensity, but also in spectral composition, and there is experimental evidence that spectral fluctuations can be important (e.g., Anderson 1993). However, a consideration of their possible effects on phytoplankton was beyond the scope of this paper.

I used fluctuations of two periods, 1 h and 8 h, so that the time scales of experimental light variation were similar to time scales of vertical mixing in the epilimnion of a typical lake (Imberger 1985). The chosen fluctuation periods are also comparable to two important photo-physiological time scales of phytoplankton (less than 2 h, associated with electron transport and greater than 5 h, associated with pigment turnover) (Neale and Marra 1985) and thus are likely to affect physiology and possibly species dynamics. The effects of fluctuations were investigated at two different levels of irradiance, one within the range of growth-limiting irradiances, and the other closer to saturating levels, because theoretical and experimental studies suggest that the effect of light fluctuations of the same frequency might depend on the average level of irradiance (Thornley 1974; Dromgoole 1988; Stramski et al. 1993). The amplitude of fluctuations was ± 70 –80% of the average irradiance for both irradiance levels. The frequency and amplitude of experimental light fluctuations are comparable to those of fluctuations resulting from phytoplankton movements in the thermocline due to internal wave mixing (Denman and Gargett 1983).

Materials and methods

Multispecies community experiment

To investigate the effect of light fluctuations at the community level, a laboratory community of 18 species of freshwater phytoplankton from three classes (green algae, diatoms and cyanobacteria) (Table 1) was grown at six different light regimes. At each of the two average levels of irradiance, 25 and 100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, there were three treatments with the same total irradiance: constant irradiance and square-wave high-low light fluctuations with periods of either 1 h or 8 h. This design allowed separation of the effect of fluctuations per se from the effects of the total irradiance dose. There was no dark period in any of the treatments. The periods of low and high irradiance were of equal duration. At low average irradiance (25 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$), light varied between 15 and 35 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, growth-limiting and subsaturating irradiances as inferred from growth-irradiance curves of selected species (E. Litchman, unpublished work). At high average irradiance (100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$), light fluctuated between 65 and 135 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, subsaturating and saturating irradiances, respectively. Fluctuations in irradiance

Table 1. List of species used in the multispecies experiment (*ATCC* American Type Culture Collection, *LCCC* the Loras College Culture Collection, Iowa, *U of MN* University of Minnesota Plant

Biology Department, *UTEX* the Culture Collection of algae at the University of Texas, Austin, *Squaw L.*, *WI* isolated by the author)

Species	Source	Culture number	Abbreviations in figures	Initial biovolume, $10^5 \mu\text{m}^3 \text{ml}^{-1}$
Bacillariophyceae				
<i>Asterionella formosa</i> Hassal	LCCC	384	Ast	0.6 ± 0.2
<i>Cyclotella meneghiniana</i> Kutzing	LCCC	868	Cycl	4.3 ± 1
<i>Fragilaria crotonensis</i> Kitton	LCCC	369	Frag	6 ± 0.6
<i>Navicula</i> sp.	U of MN		Nav	2 ± 0.4
<i>Nitzschia</i> sp.	U of MN		Nitz	12 ± 2
Cyanophyceae				
<i>Anabaena flos-aquae</i> (Lyng.) Brebisson	ATCC	22664	Anab	6 ± 0.9
<i>Gloeocapsa</i> sp.	U of MN		Gloeo	0.4 ± 0.3
<i>Phormidium luridum</i> var. <i>olivace</i> Boresch	UTEX	426	Phorm	20 ± 3
<i>Oscillatoria tenuis</i> Ag.	UTEX	1566	Osc	2 ± 0.4
Chlorophyceae				
<i>Chlamydomonas reinhardtii</i> Dang.	UTEX	89	Chlam	2 ± 0.5
<i>Cosmarium botrytis</i> Meneghini	UTEX	301	Cos	0.02 ± 0.01
<i>Mougeotia</i> sp.	UTEX	LB 758	Moug	12 ± 2
<i>Pandorina morum</i> (Müller) Bory	UTEX	788	Pand	21 ± 3.6
<i>Pediastrum boryanum</i> (Turp.) Menegh.	Squaw L., WI		Ped	0.6 ± 0.1
<i>Scenedesmus quadricauda</i> (Turp.) Bréb.	UTEX	614	Scen	14 ± 3
<i>Selenastrum capricornutum</i> Printz	UTEX	1648	Sel	1 ± 0.2
<i>Sphaerocystis Schroeteri</i> Chodat	U of MN		Sph	30 ± 4.7
<i>Staurastrum gracile</i> Ralfs	UTEX	LB 562	Staur	8 ± 1.6

were achieved by periodically turning on and off additional light sources. Light was provided by Philips "cool white" fluorescent tubes. Irradiance was measured with a spherical quantum sensor (LI-COR 185 B).

The algae were grown in WC freshwater medium (Guillard 1975) in 1 l Erlenmeyer flasks (300 ml culture volume) at 20°C, a temperature suitable for most freshwater species (Reynolds 1987). Each treatment had three replicates. Species were inoculated to have the same order of magnitude biovolume contribution to community. However, due to cell clumping in the inocula of some species, initial biomasses of species differed more than expected (Table 1). The initial cell or filament densities, due to cell size differences, also varied significantly among species (see Results). Once per day, 90 ml of the culture was replaced by fresh sterilized medium (dilution rate of 0.3 day^{-1}). Cultures were swirled several times daily. Optical densities of cultures were measured at 750 nm with a spectrophotometer (Shimadzu UV160, 1 cm path length) to estimate light attenuation in the cultures. Phosphorus ($\text{PO}_4\text{-P}$) and nitrogen ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) concentrations were measured according to Strickland and Parsons (1972) after 2 and 3 weeks from the start to check for the nutrient-replete conditions. The experiment was run for 3 weeks; samples were taken once per week, preserved with Lugol's iodine and counted using the inverted microscope technique (Lund et al. 1958). For abundant species (with proportions >0.1 of the total cell counts) at least 400 cells or filaments were counted, and for rare species the entire counting chamber was screened. Biovolumes of each species were calculated from cell measurements of at least 20 individuals using appropriate geometric formulae (Wetzel and Likens 1991). Shannon diversity indices were determined for both cell densities and biomasses (expressed as biovolumes) (Sommer 1995).

Statistical analyses

To test the effects of average light level and degree of variability in light supply on density of each species in the community, I used two-way ANOVA on log-transformed densities with "fluctuation regime" and "average light level" as main factors. If the effect of fluctuation regime was significant, within each of the two average light levels cell densities were compared among different fluctuation

regimes separately for each species using one-way ANOVA. The Student-Neuman-Keuls test was used for multiple pairwise comparisons. A similar analysis was performed on biovolumes of major taxonomic groups and on diversity indices.

Two-species community experiment

Because the sensitivity of species in a community as well as of aggregate community characteristics to fluctuations might depend on species richness (e.g., Tilman 1996), I tested the effects of the same light regimes in a simple community consisting of two species that were dominant in the multispecies community experiment, *Phormidium luridum* and *Nitzschia* sp. These two species were grown under the same six light regimes that were used in the multispecies community experiment. The initial species densities were also similar to their initial densities in the multispecies experiment ($c. 10^3 \text{ ml}^{-1}$). Species densities and their relative abundances in each treatment were determined after 2 and 3 weeks from the start. Counts were done using a Sedgewick-Rafter chamber, and at least six random transects were counted for each sample (Wetzel and Likens 1991). The effects of average irradiance and fluctuation regime on species abundances and diversity were tested as in the multispecies community.

Growth rate experiment

The effect of light fluctuations on growth rates of the two species that were dominant in the multispecies community experiment, *Phormidium luridum* and *Nitzschia* sp., was investigated in batch culture experiments (Kilham 1978). The imposed fluctuations, medium, temperature and light sources were as in the community dynamics experiments. Monocultures of each species were grown in 1-l Erlenmeyer flasks (750 ml culture volume) with three replicates of each treatment. The initial cell densities were $c. 50 \text{ cells ml}^{-1}$. Experiments were run for 6 or 7 days, and samples were taken every day or every 2 days. Growth rates were determined as slopes of a least-squares linear regression of the ln-transformed cell concentrations versus time and compared among treatments using two-way ANOVA. To test whether the observed differences in

growth rates are associated with differences in chlorophyll concentration (Richardson et al. 1983), chlorophyll *a* concentration of monocultures and cell biovolumes were determined for each treatment in the end of experiment (Wetzel and Likens 1991). Chlorophyll *a* concentrations per cell and cell biovolumes were compared among treatments using two-way ANOVA.

Results

In all three experiments (monocultures, two-species and multispecies communities) both average light level and its temporal variability affected various population and community characteristics. These effects will be described below for each experiment type. However, because the main goal was to test the effects of temporal variability of light supply, the results will be presented to highlight those effects (e.g., separate graphs for two average light levels).

Multispecies community experiment

Taxonomic composition and total biovolume

The temporal regime of light supply had a significant effect on the taxonomic composition of the community. Biovolume contribution of green algae and diatoms, but not cyanobacteria, depended on the fluctuation regime. At low average irradiance after 2 weeks, green algae had higher absolute biovolume in constant than in fluctuating light (two-way ANOVA, $P < 0.05$). In contrast, diatoms at low average irradiance had significantly higher biovolume under fluctuating light, becoming the dominant group under those light regimes (Fig. 1). At high average irradiance, diatoms were the least abundant taxon in all three light regimes, and had their lowest abundance under constant light (Fig. 1). Green algae and cyanobacteria had similar biovolumes in the community in all three fluctuation regimes (Fig. 1). At high average irradiance there were no statistically significant differences in biovolumes among different fluctuation regimes for any of the taxonomic groups (Fig. 1). The effect of the average light level was significant ($P < 0.05$) for all taxa after both 2 and 3 weeks from the start.

The temporal regime of light supply did not have a significant effect on total biovolume. Total biovolume after 2 weeks was, however, significantly higher ($P < 0.05$) under high average irradiance than under low average irradiance: on average it increased from $1.5 \times 10^7 \mu\text{m}^3 \text{ml}^{-1}$ at the start of the experiment to $2 \times 10^7 \mu\text{m}^3 \text{ml}^{-1}$ at low average irradiance and to $4.4 \times 10^7 \mu\text{m}^3 \text{ml}^{-1}$ at high average irradiance. From 2 to 3 weeks, average total biovolume did not change significantly at low average irradiance ($P > 0.05$) and declined ($P < 0.05$) at high average irradiance to the average of $2.9 \times 10^7 \mu\text{m}^3 \text{ml}^{-1}$, so that the differences between high and low irradiance levels were no longer significant.

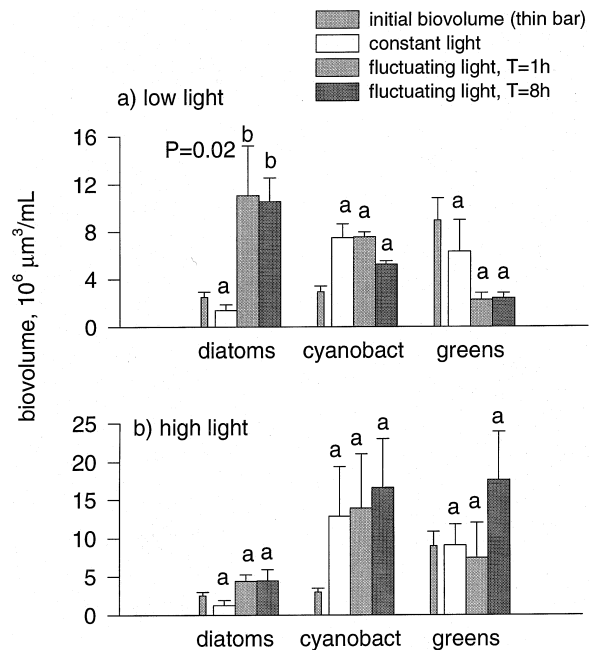


Fig. 1a,b. Biovolumes of three taxa in the multispecies experiment at the start and after 3 weeks under different light regimes: **a** low average irradiance ($25 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$); **b** high average irradiance ($100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$)

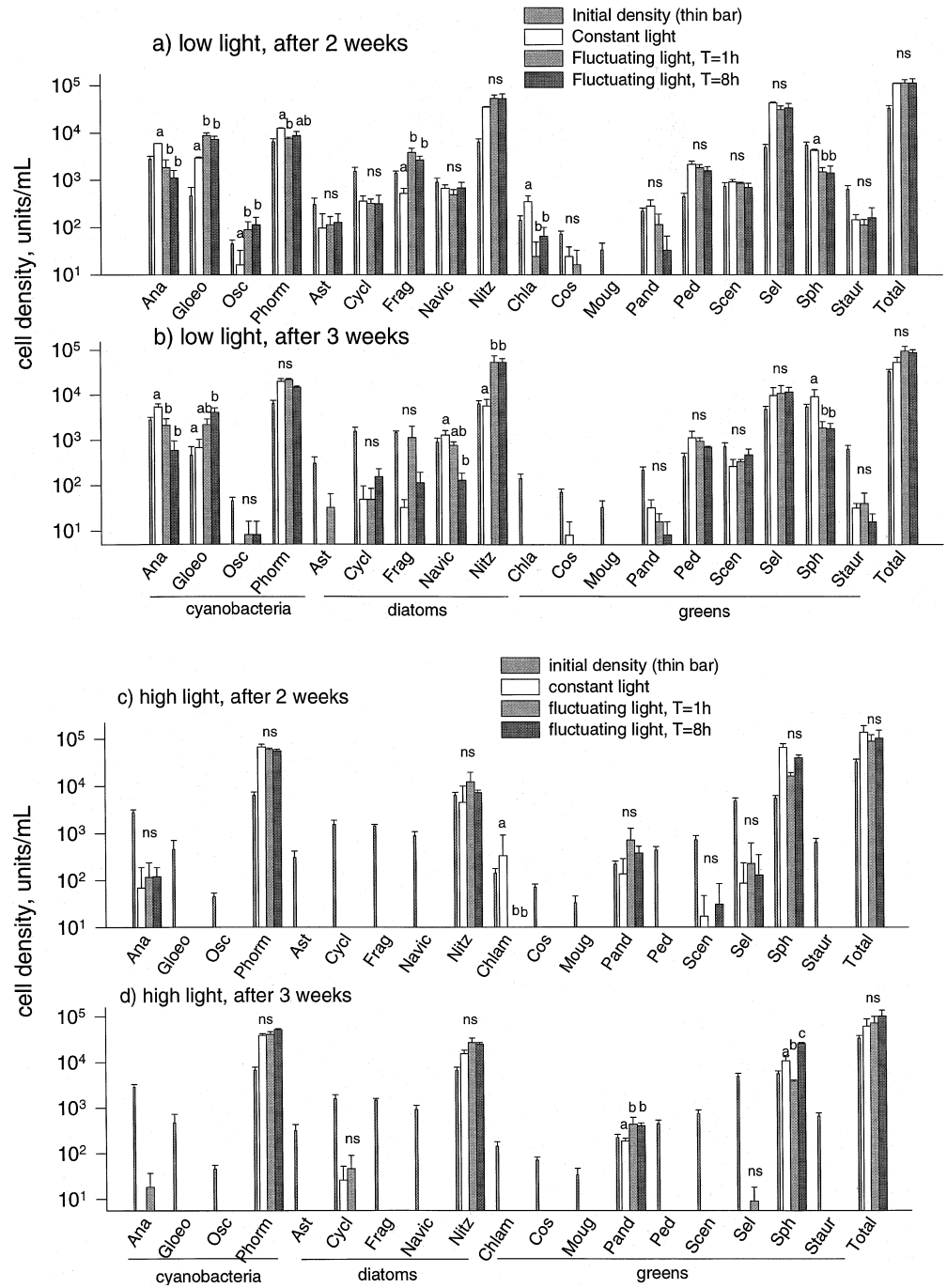
Species abundances

Densities of all species depended significantly on the average irradiance level, as determined by two-way ANOVA. Some species were also sensitive to the degree of variability in light supply and the interaction of these two factors. At low average irradiance light fluctuation regime significantly affected densities of seven species after 2 weeks and of five species after 3 weeks (Fig. 2a,b). At high average irradiance, the effect of light fluctuations on species densities was less pronounced: only one species after 2 weeks and two species after 3 weeks were sensitive to the degree of variability in light supply (Fig. 2c,d).

Species diversity and richness

Species diversity in the community depended significantly on the degree of variability in light supply. At low average irradiance, species diversity measured by Shannon index (calculated based on biomass) declined during experiment and was significantly lower under fluctuating light than under constant light (Fig. 3). At high average irradiance diversity declined even more rapidly, but, in contrast to low irradiance treatments, diversity was significantly higher under fluctuating light (Fig. 3). Shannon diversity index calculated from cell densities exhibited similar trends. The number of species present (species richness) in the samples was not significantly different (compared by one-way ANOVA) between the fluctuating and constant light treatments at low or at high average irradiance.

Fig. 2a–d Cell densities of individual species (mean \pm 1 SE) in the community in the course of experiment in low average irradiance treatments, $25 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$: **a** after 2 weeks, **b** after 3 weeks; and in high average irradiance treatments, $100 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$: **c** after 2 weeks and **d** after 3 weeks. The initial densities of each species are also shown. Species abbreviations are given in Table 1. Means with no common letters above the bars are significantly different ($P < 0.05$)



Species richness declined over the course of the experiment. At low average irradiance after 2 weeks, densities of 7 species had declined from initial densities in all three treatments (Fig. 2a), and after 3 weeks 9 species had declined or completely disappeared in one or more treatments (Fig. 2b). At high average irradiance species loss occurred faster and was more pronounced than at low average irradiance: after 2 weeks at high average irradiance, 10 species had completely disappeared from samples (Fig. 2c), and after 3 weeks, 11 species were absent from the samples and 3 more species were absent in some treatments (Fig. 2d). Species di-

versity and richness were significantly higher ($P < 0.05$) in the low light treatments than in high light treatments.

Two-species community experiment

The two-species assemblage was also sensitive to the temporal regime of light supply. After 3 weeks, the density of *Nitzschia* was significantly higher under fluctuating than in constant light at both low and high average irradiances, and its abundance was the highest under low fluctuating light (Table 2). After 2 weeks

Phormidium had significantly lower density in the 8 h fluctuating light treatment at low average irradiance; at high irradiance, however, no significant differences in filament density were found (Table 2). *Nitzschia*'s relative abundance also responded positively to light fluctuations (Table 2). At low average irradiance, after 3 weeks *Nitzschia* and *Phormidium* had approximately

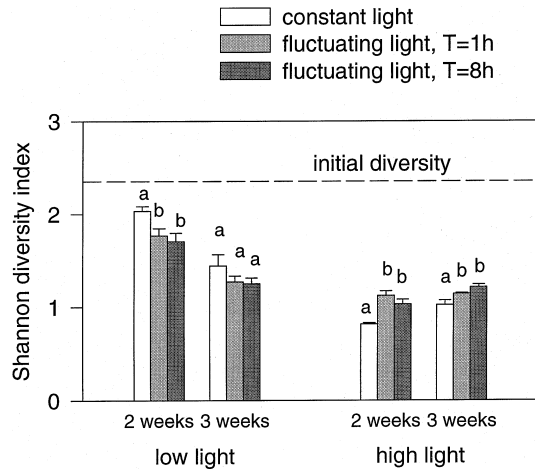


Fig. 3. Species diversity in the multispecies community experiment. Shannon diversity index (mean \pm 1 SE) was calculated based on species biovolumes. Within each light level treatments differing in fluctuation regime were compared by one-way ANOVA. Significant differences between treatments are indicated by different letters above the bars

Table 2. Results of the two-species experiment. Means of three replicates \pm SE are given (*low light* 25 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ average irradiance, *high light* 100 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ average irradiance, *CL* constant light treatment, *FL1* fluctuating light

	At the start			CL	FL1	FL8	P
<i>Nitzschia</i> density, $10^4 \text{ cells ml}^{-1}$	2.5 \pm 1	Low light	2 weeks	6.4 \pm 0.7	8.5 \pm 2	5.5 \pm 1	NS
			3 weeks	19 \pm 0.8	43 \pm 9	58 \pm 2	0.008
	High light	2 weeks	39 \pm 15	26 \pm 3	37 \pm 2	NS	
		3 weeks	7.4 \pm 2	12 \pm 0.4	15 \pm 2	0.05	
<i>Phormidium</i> density, $10^4 \text{ filaments ml}^{-1}$	2.6 \pm 1	Low light	2 weeks	9.7 \pm 1	10 \pm 1.2	4.4 \pm 0.7	0.003
			3 weeks	62 \pm 1	43 \pm 9	47 \pm 3	NS
	High light	2 weeks	51 \pm 3	47 \pm 3	50 \pm 4	NS	
		3 weeks	56 \pm 8	44 \pm 9	49 \pm 8	NS	
Proportion of <i>Nitzschia</i> (density-based)	0.49 \pm 0.01	Low light	2 weeks	0.4 \pm 0.03	0.46 \pm 0.04	0.56 \pm 0.01	0.023
			3 weeks	0.24 \pm 0.01	0.5 \pm 0.03	0.55 \pm 0.02	<0.0001
	High light	2 weeks	0.44 \pm 0.01	0.36 \pm 0.02	0.42 \pm 0.02	0.05	
		3 weeks	0.11 \pm 0.01	0.21 \pm 0.01	0.24 \pm 0.01	<0.001	
Total biovolume, $10^7 \mu\text{m}^3 \text{ ml}^{-1}$	1.3 \pm 0.1	Low light	2 weeks	4.3 \pm 0.5	4.8 \pm 0.4	2.5 \pm 0.4	0.02
			3 weeks	24 \pm 5	20 \pm 4.7	23 \pm 1.4	NS
	High light	2 weeks	23 \pm 3	22 \pm 1	26 \pm 1.5	NS	
		3 weeks	20 \pm 3	16 \pm 3	18 \pm 3	NS	
Shannon diversity index (density-based)	0.69 \pm 0.02	Low light	2 weeks	0.67 \pm 0.1	0.69 \pm 0.01	0.69 \pm 0.07	NS
			3 weeks	0.55 \pm 0.05	0.69 \pm 0.06	0.68 \pm 0.06	0.03
	High light	2 weeks	0.69 \pm 0.03	0.65 \pm 0.03	0.68 \pm 0.03	NS	
		3 weeks	0.36 \pm 0.01	0.52 \pm 0.04	0.56 \pm 0.06	0.03	
Shannon diversity index (biovolume-based)	0.74 \pm 0.03	Low light	2 weeks	0.60 \pm 0.05	0.44 \pm 0.03	0.68 \pm 0.08	0.04
			3 weeks	0.44 \pm 0.04	0.66 \pm 0.06	0.68 \pm 0.05	0.01
	High light	2 weeks	0.63 \pm 0.06	0.57 \pm 0.08	0.62 \pm 0.07	NS	
		3 weeks	0.26 \pm 0.01	0.41 \pm 0.05	0.45 \pm 0.04	0.02	

NS $P > 0.05$

equal densities in the fluctuating light treatments. At high average irradiance *Phormidium* was the dominant species in all treatments, but the density of *Nitzschia* was still higher under fluctuating light than under constant light (Table 2). After 3 weeks, the Shannon diversity index, which is a measure of species evenness in case of constant number of species, was significantly higher in the fluctuating light treatments at both low and high average irradiances and the period of fluctuations did not have a significant effect (Table 2). The average light level also had a significant effect on community composition: after 2 weeks, densities of both *Nitzschia* and *Phormidium*, as well as total biovolume were significantly higher under high average irradiance, as tested by two-way ANOVA.

Growth rate experiment

Growth rates of *Nitzschia* and *Phormidium* were higher at high average irradiance; they also depended on temporal variability in light supply (Fig. 4). The responses to light fluctuations were species-specific and depended on the average irradiance level (Fig. 4). In the constant light treatment, growth rate of *Phormidium* was significantly higher than the growth rate of *Nitzschia* at both low and high average irradiances ($P=0.0004$ and $P=0.02$ respectively). In the fluctuating light treatments, growth rates of the two species were not significantly different ($P > 0.05$). Interspecific similarity in

treatment of 1 h period, *FL8* fluctuating light treatment of 8 h period). Values within each light level were compared across treatments with one-way ANOVA; proportions were arcsine square-root transformed before the analysis

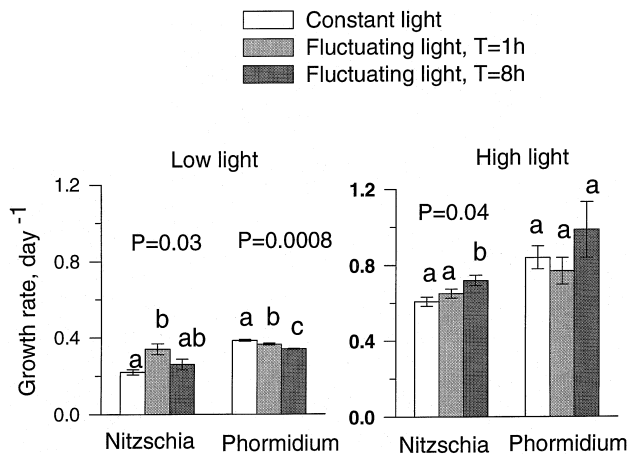


Fig. 4. Growth rates (mean \pm 1 SE) of *Nitzschia* and *Phormidium* under different light regimes. For each species growth rates within each average irradiance were compared by one-way ANOVA. Significant differences between treatments are indicated by different letters above the bars

growth rates was the greatest under low average irradiance.

For both *Nitzschia* and *Phormidium*, cell biovolumes did not differ significantly among treatments either within or between the two average irradiances. Chlorophyll *a* concentrations per cell were not significantly different among treatments within one average irradiance level. The average cellular chlorophyll *a* concentrations were, however, significantly higher under low average irradiance than under high average irradiance: $1.6 \times 10^{-3} \mu\text{g Chl } a \text{ cell}^{-1}$ versus $0.6 \times 10^{-3} \mu\text{g Chl } a / \text{cell}$ ($P=0.002$) for *Phormidium* and $1.5 \times 10^{-3} \mu\text{g Chl } a \text{ cell}^{-1}$ versus $1.1 \times 10^{-3} \mu\text{g Chl } a \text{ cell}^{-1}$ ($P=0.01$) for *Nitzschia*.

Discussion

Multispecies community experiment

Variability in light supply (constant versus fluctuating light) had a significant effect on the structure and dynamics of a laboratory multispecies community of phytoplankton. The abundance of the three taxonomic groups (diatoms, cyanobacteria, and green algae) in the community differed among treatments with the same average irradiance but different fluctuation regimes. Diatoms had higher density and biovolume under fluctuating light, reaching their maximum abundance under low fluctuating light, where they were the dominant group (Fig. 1). Diatom blooms in lakes often occur when light conditions are highly variable and average irradiances are low (Reynolds 1994), e.g., during spring and fall turnover when cells circulate through the whole water column. Better performance of diatoms (higher densities and proportions in communities) under mixing conditions is usually attributed to the positive effect of

turbulence keeping heavy-celled diatoms suspended in the water column (Reynolds 1984; Kiørboe 1993). In addition, as the experiments described above suggest, fluctuating light itself may stimulate growth of diatoms. This could be the result of an adaptation to fluctuating light associated with turbulent conditions which are favorable for diatoms (Kiørboe 1993). In enclosure experiments on artificial mixing, Reynolds et al. (1984) had found that diatoms were dominant under mixing conditions, when the light climate was highly variable, while green algae, including *Sphaerocystis*, grew better during quiescent periods. In the present study, some green algae, including *Sphaerocystis*, also had higher abundance under constant than fluctuating light of low average level.

Within each group, however, species responses to a particular light regime were not uniform: both amplitude and direction of the responses were species-specific. Among diatoms, *Nitzschia* and *Fragilaria* had higher densities under fluctuating light, while densities of *Asterionella*, *Cyclotella*, and *Navicula* either did not differ among light regimes or were lower under fluctuating light (Fig. 2b). Among cyanobacteria, *Phormidium* and *Anabaena* at low average irradiance had higher densities under constant light, but *Gloeocapsa* and *Oscillatoria* were more abundant under fluctuating light. Interestingly, *Oscillatoria* is known to respond positively to turbulence (Reynolds 1987; Klemer and Barko 1991). Moreover, Gibson (1985) found that growth rate of *Oscillatoria agardtii* was slightly inhibited by continuous versus intermittent light, which is consistent with lower abundance of *Oscillatoria* under constant light observed in this study. Among green algae, at low irradiance *Sphaerocystis* and *Pandorina* had higher densities under constant regime, while densities of *Pediastrum*, *Scenedesmus* and *Selenastrum* did not differ among constant and fluctuating light treatments. Therefore, species-specific differences should be taken into consideration when determining the effects of the light supply dynamics on species even within a taxonomic group.

In contrast to species abundances, total biomass measured as biovolume depended only on the average irradiance, and not on the dynamics of light supply. The lower sensitivity of total biomass to environmental variation, compared to individual species sensitivity, seems to be due to differential responses of species compensating each other and thus reducing the net effect on community biomass. This may be a general property of communities (May 1974; Tilman 1996). Abundances of some species were still changing between 2 and 3 weeks (Fig. 2), so that at the end of experiment community composition possibly was not at equilibrium, at least in the low-light treatments. The goal of this study, however, was not to determine the steady-state community composition, but rather to examine whether and how a multispecies community would respond to differences in light regime over a relatively short time. As these experiments demonstrate, community composition is sensitive to the degree of temporal variability in light

supply: differences were detected as soon as 2 weeks from the start.

Species richness and diversity declined during the experiment in both high and low light treatments. Species that declined or disappeared quickly from all treatments (e.g., *Cosmarium*, *Mougeotia*, and *Staurastrum*) had low initial densities and possibly low growth rates (large-celled species tend to have lower growth rates; Reynolds 1984) and could have been washed out. However, species loss was more pronounced in the high average irradiance treatments, where the growth rates of species were higher. This suggests that some species might have been competitively excluded and that competitive exclusion occurred faster under high average irradiance. Some theoretical models also suggest that the rates of competitive exclusion may be greater when growth rates of competing species are high (e.g., Huston 1994).

At low average irradiance, light was limiting, as indicated by higher total biomass under high average irradiance than at low average irradiance. Contrary to theoretical considerations (e.g., Levins 1979) and experiments with nitrogen- or phosphorus-limited phytoplankton (Turpin and Harrison 1979; Sommer 1984), fluctuations at this limiting irradiance did not increase the number of coexisting species, and species diversity was significantly lower under fluctuating low irradiance (Fig. 3). At high average irradiance, however, diversity was higher under fluctuating light (Fig. 3).

Several explanations for lack of a positive effect of fluctuations on diversity and species richness at low irradiance are possible. A positive effect of a fluctuating light supply on diversity might have been noticeable in these experiments after a longer time, as the community approached equilibrium species composition. In the high average light treatments, where growth rates and consequently, community changes were faster than at low irradiance, fluctuations increased diversity, which agrees with this hypothesis.

An alternative explanation is that only a limited range of frequencies, amplitudes and average irradiances of light fluctuations can promote coexistence (E. Litchman and C. Klausmeier, unpublished work), and that the chosen fluctuations were not in this range. In a theoretical analysis, Grover (1991b) showed that under nutrient competition the range of nutrient fluctuations leading to coexistence may also be very narrow. In his experiments, Sommer (1984, 1995) found a maximum species diversity when phosphorus pulses or dilution disturbances had a 1-day period, comparable to the generation times of phytoplankton. Similarly, Kemp and Mitsch (1979) showed in numerical simulations that species coexistence occurred when the frequency of turbulence had approximately a 1-day period. It is possible that fluctuations in irradiance with a 1-day period (e.g., the daily light:dark cycle) could promote coexistence. Brzezinski and Nelson (1988) found that such a light:dark cycle of non-limiting irradiance level facilitated coexistence of species under nitrogen limitation. It re-

mains to be explored whether 1-day or longer periods of fluctuation in irradiance can promote coexistence under light-limited conditions.

A third explanation is that positive effects of fluctuations on diversity are greater in a system with strong competition. It is possible that in the low-light cultures, despite light limitation, competition for light was not severe, i.e., mutual shading was not high enough for species to compete strongly for light. The optical densities of the low-light cultures measured at 750 nm were in the range of 0.08–0.12. Usually higher optical densities (>0.3–0.4) are required for light competition by mutual shading (i.e., resource consumption) to be significant (J. Huisman, personal communication). Nitrogen and phosphorus concentrations were well above limiting levels, *c.* 25 $\mu\text{mol} \cdot \text{l}^{-1}$ of $\text{PO}_4\text{-P}$ and 70 $\mu\text{mol} \cdot \text{l}^{-1}$ of dissolved inorganic N (DIN; $\text{NO}_3^- \text{-N} + \text{NH}_4^+ \text{-N}$) after 3 weeks, so that competition for these nutrients was also unlikely.

In contrast to low light treatments, at high average irradiance diversity was significantly higher under fluctuating than under constant light (Fig. 3). Nitrogen and phosphorus concentrations measured after 2 and 3 weeks from the start of the experiment were above the limiting levels (*c.* 20 $\mu\text{mol} \cdot \text{l}^{-1}$ of $\text{PO}_4\text{-P}$ and 60 $\mu\text{mol} \cdot \text{l}^{-1}$ of DIN after 3 weeks), so that as in low-light treatments, limitation by these nutrients was unlikely. However, light attenuation by biomass was greater than in low-light cultures (optical densities were 0.2–0.25 after 2 weeks), so that mutual shading was greater and species might have competed for light. It is also possible that the system was carbon-limited: the average pH in cultures was around 9.5 (due to photosynthesis by a dense community in a weakly buffered medium), so that inorganic carbon was predominantly in the form of carbonate and bicarbonate ions (Stumm and Morgan 1981), which are less readily taken up by phytoplankton than is carbon dioxide (Maberly and Spence 1983). Such conditions of considerable light attenuation by biomass and low availability of inorganic carbon can be common in lakes during bloom events. Cyanobacteria were the most abundant group in all high-light treatments in contrast to low-light treatments, where average pH was below 9. These algae are known to be superior competitors for inorganic carbon at high pH (Shapiro 1973; Talling 1976). Diatoms, which generally are poor competitors for inorganic carbon (Talling 1976), had low abundance under high average irradiance. It is, therefore, possible that light fluctuations increased diversity in a system limited by another resource, by affecting competition for that resource. The importance of light fluctuations in systems limited by CO_2 has been previously discussed by Gallegos et al. (1980). Under carbon limitation, the positive effect of light fluctuations on diversity is similar to the results of Brzezinski and Nelson (1988) for a simple two-species diatom community where light fluctuations enabled coexistence of both species competing for ammonium, while constant light led to competitive exclusion. Further experiments are

needed to determine the exact mechanism of the diversity enhancement by light fluctuations.

The two-species community experiment

The responses of species in the two-species community were qualitatively similar to their responses in the multispecies community, e.g., higher density and relative abundance of *Nitzschia* in the fluctuating light treatments (Fig. 2, Table 2). Such similarity in the effects of fluctuations on species in communities with different numbers of species indicates robustness of the observed patterns. Species densities and especially relative abundances were more sensitive than total biomass to light fluctuations. Diversity (species evenness) was higher under fluctuating light, at both high and low average irradiances, because *Nitzschia* was more abundant under fluctuating light. Low light treatments in this experiment had greater light attenuation and possibly stronger competition than the corresponding treatments in the multispecies experiment, which could have led to higher diversity.

Growth rate responses to fluctuating light

The growth rates of *Nitzschia* and *Phormidium* in monocultures were sensitive to the regime of light supply. Growth-rate responses to light fluctuations were species-specific and depended on the average irradiance level and the frequency of fluctuations. Because growth rate of phytoplankton directly depends on photosynthesis, respiration and pigment turnover (Cullen 1990), fluctuations affecting each of these processes may affect growth. In the future, it would be of interest to clarify further the link between physiology and population dynamics by identifying the physiological processes that led to the observed differences in growth rates.

The lack of significant differences in cellular chlorophyll *a* concentration among different fluctuation regimes indicates that processes other than chlorophyll *a* turnover were responsible for differences in growth rates. Both species showed a decrease in cellular chlorophyll *a* concentration with increasing average irradiance, which is in agreement with the general trend observed for many microalgae (Richardson et al. 1983). This suggests that these species might acclimate to average irradiance level rather than to the maximum irradiance they experience. Similarly, Ibelings et al. (1994) found that the cyanobacterium *Microcystis* sp. responded to the total daily irradiance dose and not to the maximum irradiance. The generality of such physiological strategy across different taxonomic groups needs further investigation.

Differential responses of the growth rates of individual species to light fluctuations are likely to be the major cause for observed differences at the community level (cf. Tilman et al. 1982), assuming that loss rates were

similar for all species. There was good agreement between the response pattern of species in monoculture and their abundance in both two- and multispecies community: under low average irradiance, *Phormidium*'s growth was inhibited by fluctuations (Fig. 4) and its abundance in both communities was lower under fluctuating light (Fig. 2, Table 2). When two species are compared, there is also a good agreement between their growth rates and relative dominance in communities: at low average irradiance, under constant light the growth rate of *Nitzschia* in monoculture was significantly lower than the growth rate of *Phormidium*, while under fluctuating light, growth rates of the two species were not significantly different (Fig. 4). This agrees well with species densities in the two species experiment: *Nitzschia* had lower than *Phormidium* density under low constant light, and densities similar to the *Phormidium*'s under low fluctuating light of both periods (Table 2). In the multispecies community, this correspondence might not hold because of the influence of other species (e.g., competitive interactions). *Nitzschia* had higher growth rate in monoculture under high average irradiance but its abundance in the multispecies experiment was higher under low than under high average irradiance. A direct comparison of the growth rate experiments and the community dynamics experiments might also be complicated by the fact that the growth rate experiments were run as batch cultures, and the community experiments were run as semicontinuous cultures; also the duration of the experiments was different. It is possible that a long time-scale (>1 week) photoacclimation to a particular light regime (Geider et al. 1996) led to changes in growth rates which were not detected in the 1-week growth-rate experiments, but could have been important in the community dynamics experiments. These factors may be responsible, for example, for the discrepancy between relatively low growth rate of *Nitzschia* in batch monocultures (*c.* 0.3 day⁻¹) (Fig. 4) and its high density in the community experiments with a dilution rate of 0.3 day⁻¹ (Fig. 2, Table 2).

In summary, the results of the community and growth rate experiments demonstrate that light fluctuations may affect multiple levels of phytoplankton organization, including the population and community levels. Light fluctuations may change species abundances and dominance patterns in both simple and multispecies communities (e.g., diatom versus cyanobacterial dominance). Community changes may be caused by differential effects of fluctuations on growth rates of species and on their competitive interactions. Light fluctuations may also increase species diversity. Thus, variability in light may offer one more explanation for the "paradox of the plankton" (Hutchinson 1961), the coexistence of many species of phytoplankton in a seemingly homogeneous environment. The sensitivity of phytoplankton communities to temporal regime of light supply suggests that different regimes of vertical mixing (e.g., seasonal patterns) may cause shifts in phytoplankton communities, by altering not only the average

irradiance but light variability as well. Thus, better understanding of changes in phytoplankton communities may require an explicit consideration of temporal heterogeneity in light supply.

Acknowledgements This research was in part supported by the NSF Doctoral Dissertation Improvement Grant (DEB 94-23531), the Sigma Xi Society and Dayton-Wilkie Fund of the Bell Museum of Natural History awards. Comments by J. Huisman, C. Klausmeier, A. Klemer, H. MacIntyre, K. Schulz, J. Shapiro, R. Sterner, D. Tilman and two anonymous reviewers are greatly appreciated.

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