

Photoacclimation in the marine diatom *Skeletonema costatum*

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Abstract

Photoacclimation was examined in the marine diatom *Skeletonema costatum*, which was subjected to reciprocal shifts between irradiances of 50 (low-light) and 1,200 (high-light) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Cell chlorophyll *a* and fucoxanthin contents were higher but diadinoxanthin and diatoxanthin contents lower in cells grown at 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ than in cells shifted to 1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Cell carbon contents measured at the start of the light period were similar in both high-light and low-light treatments. However, by 6 h into the light period, the carbon contents in the high-light cells were about twofold higher than in the low-light cells. Dark respiration rates, dark Chl *a* synthesis rates, and dark cell-division rates were greater in the high-light acclimated cells than in the low-light cells. Thus, there was a greater uncoupling of carbon assimilation from cell division during the day in the high-light cells, but pigment synthesis and cell division continued in darkness. Cell-specific, light saturated photosynthesis rates, and chlorophyll *a* specific light-limited photosynthesis rates were constant during reciprocal shifts between growth irradiances of 50 and 1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Thus, differences of photosynthesis versus irradiance curves between cells acclimated to high-light versus low-light could be accounted for largely in terms of changes in cell chlorophyll *a* contents. Although the chlorophyll *a*-specific initial slope, α^{chl} , was constant, the chlorophyll *a*-specific light absorbance coefficient, a^{chl} , increased and the maximum quantum efficiency of photosynthesis (ϕ_m) declined following the shift to high light. The increase of a^{chl} was most likely due to a decreased package effect. The decline of ϕ_m was most likely due to accumulation of xanthophyll cycle pigments. Carbon-specific, light-saturated photosynthesis rates were lower in high-light than in low-light cells; this observation may indicate that control of light-saturated photosynthesis shifts from enzymes of the carbon dioxide reduction cycle (Calvin cycle) in low-light cells to the photosynthetic electron transfer chain in high-light cells.

Photoacclimation modulates photosynthesis and growth rates in all algae. This acclimation process has been studied over the past 15–20 years in the field and the laboratory, and the results have increased our understanding of the processes that regulate primary production in response to changes in irradiance (reviewed in Geider 1993). Initial laboratory studies demonstrated that reduction in the cellular contents of Chls and accessory pigments in response to an increase in irradiance is almost universal (Post et al. 1984). Later work highlighted the variations in the kinetics of photoacclimation between species (Post et al. 1984; Cullen and Lew-

is 1988) and differences in the rates of response of each molecular and physiological parameter involved in photoadaptation (Sukenic et al. 1987; LaRoche et al. 1990). More recent studies have shown that the redox state of the plastoquinone pool regulates transcription of several photosynthetic genes including those for the reaction centers of photosystems I and II and light-harvesting pigment-protein complexes (Escoubas et al., 1995; Pfannschmidt et al., 1999). The rates of photoacclimation of division rates and chl *a* per cell have been described by first order kinetics (Rivkin, 1982; Post et al., 1984). This was taken a step further by Cullen and Lewis (1988), who combined first order reactions that stimulated the rate of change of photoacclimation at a particular depth with an equation simulating turbulent mixing. They found that although the first-order kinetic model adequately described the temporal response to a shift from high to low light, the time scale of change following the transfer from low to high light was not consistent with first-order

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kinetics. A logistic model, based on unbalanced growth, was found to be more adaptable to observed differences in the rates of acclimation but it still only partially described photoacclimation.

Although experiments carried out in continuous light (LaRoche et al. 1991; Fisher et al. 1996) allow complications from physiological changes related to diel periodicity to be avoided (Chisholm et al. 1980), such experiments may not always be applicable to cells grown under a light:dark (L:D) cycle. Many species of phytoplankton actually grow more slowly under continuous light in comparison with a LD cycle (Nielsen 1992). In addition, the content of Rubisco may depend on the L:D regime under which cells are growing. For example, Hobson et al. (1985) found that Rubisco content of *T. weissflogii* was greater in cells grown on a 6:18 L:D cycle than in cells grown under continuous illumination. If results from photoacclimation experiments are to be used in developing models for interpretation of growth in nature (Cloern et al. 1995; Geider et al. 1996), then it would seem appropriate that experimental conditions simulate as closely as possible the natural environment.

The aim of this study was to examine the physiological process of photoacclimation in a marine diatom grown under a L:D cycle, after transitions from low light to high light and vice versa. Photoacclimation will be discussed not only at the cellular level but also in the context of changes in energy flux and balance (Geider et al. 1996).

Materials and methods

Cultures of the diatom *Skeletonema costatum* strain CCMP 1332 (Plymouth Culture Collection) were grown in filter-sterilized (0.2 μm -pore) seawater medium containing 500 μM nitrate, 500 μM silicate, 30 μM phosphate, and f/2 concentrations of trace elements and vitamins (Guillard and Ryther 1962) under a 12:12 L:D cycle at 15°C with constant aeration through 0.2 μm -pore filters. Light was provided by banks of cool-white fluorescent lamps (GE Lighting, F40W/33). Cells were acclimated to an irradiance of 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, as measured with a Biospherical Instruments QSL-101 quantum scalar irradiance sensor, for 7 generations prior to the experiment. Duplicate cultures were sampled at this irradiance for 5 d, after which the light was increased to 1200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Sampling continued for a further 5 d, until the cells were fully acclimated to the light shift (as indicated by the stabilization of Chl *a* cell⁻¹) and then placed back to the original light level for a further 5 d. The cultures were kept nutrient-replete by daily manual dilution with fresh medium. All variables were not measured every day.

The specific growth rate was calculated as follows:

$$\text{Specific Growth Rate} = \ln[N(T_2)/N(T_1)]/(T_2 - T_1), \quad (1)$$

where $N(T_1)$ is the cell concentration (cells ml⁻¹), at time T_1 , and $N(T_2)$ is the cell concentration at time T_2 and $T_2 - T_1$ is the time difference. Specific rates of particulate organic carbon synthesis or Chl *a* synthesis were calculated by substituting measurements of particulate organic carbon or Chl *a* for cell abundance in this equation.

Cell composition—Samples were typically collected three times a day, prior to the onset of the light period (0900), 6 h into the light period (1500) and at the end of the light period (2100). Following each light-shift, samples were collected every 3 h to closely monitor the time scale of acclimation. Cell numbers were determined using a hemocytometer. Samples for chlorophyll, particulate organic carbon and nitrogen (POC and PON, respectively), and total protein were collected by vacuum filtration onto precombusted Gelman A/E glass-fiber filters. Chlorophyll concentration was determined fluorometrically (Holm-Hansen 1978). POC and PON were determined on a Carlo Erba autoanalyzer. Total protein was measured against a bovine serum albumin standard with Folin-Ciocalteu's reagent (Sigma), in a modification of the Lowry method (Dorsey et al. 1977). All assays were performed in triplicate. Samples for pigment analysis were collected as above but only from the middle of the light period. Samples were immediately frozen on liquid nitrogen and stored at -80°C. Fucoxanthin and the photoprotective xanthophylls diadinoxanthin and diatoxanthin were determined by high-pressure liquid chromatography (Barlow et al. 1997).

Photosynthetic parameters—Photosynthesis versus irradiance (PE) curves were determined from ¹⁴C uptake by incubating 24 × 1 ml aliquots of culture enriched with 1 $\mu\text{Ci ml}^{-1}$ NaH¹⁴CO₃. The aliquots were incubated simultaneously over a gradient of irradiances in a temperature-controlled "photosynthetron" (Lewis and Smith 1983). Light was provided by two General Electric 500W quartz halogen lamps and filtered through a 2.5-cm layer of 20 g L⁻¹ CuSO₄ solution. Incubations were terminated after 30 min by adding 25 μl of glutaraldehyde, and residual inorganic carbon was driven off by shaking after acidification with 250 μl of 6 N HCl. Total dissolved CO₂ in the media was determined by alkalinity titrations. Total activity of NaH¹⁴CO₃ in the incubation was determined on 20 μl aliquots of sample taken directly into 4 ml of scintillation cocktail with 1 ml of 0.1 N NaOH. Data were fitted by least-squares nonlinear regression to the following equation (Platt et al. 1980):

$$P^{\text{Chl}} = P_s^{\text{Chl}} \left(1 - \exp\left(\frac{-\alpha^{\text{Chl}} E}{P_s^{\text{Chl}}}\right) \right) \exp\left(\frac{-\beta^{\text{Chl}} E}{P_s^{\text{Chl}}}\right) + P_o^{\text{Chl}}, \quad (2)$$

where P^{Chl} is the rate of photosynthesis, normalized to Chl *a* (g C g⁻¹ Chl h⁻¹) at irradiance E ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$); P_s^{Chl} (g C g⁻¹ Chl h⁻¹) is the maximum rate of photosynthesis in the absence of photoinhibition; α^{Chl} (g C g⁻¹ Chl h⁻¹) ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)⁻¹ is the initial slope of the P/E curve; and β^{Chl} (g C g⁻¹ Chl h⁻¹) ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)⁻¹ is a parameter describing the reduction in photosynthesis at high irradiances. P_o^{Chl} (g C g⁻¹ Chl h⁻¹) is an intercept term, subtracted from P^{Chl} so that modeled photosynthesis in the dark is always zero. The light-saturated rate of photosynthesis, P_m^{Chl} (g C g⁻¹ Chl h⁻¹), was calculated according to Platt et al. (1980):

$$P_m^{\text{Chl}} = P_s^{\text{Chl}} \left(\frac{\alpha^{\text{Chl}}}{\alpha^{\text{Chl}} + \beta^{\text{Chl}}} \right) \left(\frac{\beta^{\text{Chl}}}{\alpha^{\text{Chl}} + \beta^{\text{Chl}}} \right)^{\beta^{\text{Chl}}/\alpha^{\text{Chl}}}. \quad (3)$$

Light absorption—Optical density between 400 and 700 nm was measured by use of a Hitachi U-3000 spectrophotometer equipped with a $\varnothing 60$ integrating sphere to collect forward-scattered light. Spectra were collected using a 4-nm slit width and were not smoothed. Absorption was normalized to Chl *a* concentration to give the chlorophyll-specific absorption coefficient, a^{Chl} ($\text{m}^2 \text{g}^{-1}$ Chl *a*), after subtracting optical density at 750 nm to correct for residual scattering.

$$a^{\text{Chl}}(\lambda) = \frac{2.3[\text{OD}(\lambda) - \text{OD}(750)]}{0.01\text{Chl}} \quad (4)$$

where $a^{\text{Chl}}(\lambda)$ is the Chl *a*-specific absorption cross section ($\text{m}^2 \text{g}^{-1}$ Chl *a*) at wavelength λ (nm), $\text{OD}(\lambda)$ is optical density; 0.01 is the path length of the cuvette (m), and Chl is the Chl *a* concentration in the sample (g m^{-3}).

The maximum quantum yield of photosynthesis, ϕ_m (mol $\text{CO}_2 \text{ mol}^{-1}$ photons), was calculated from the initial slope of the *P/E* curve, α^{Chl} , and a chlorophyll-specific absorption coefficient:

$$\phi_m = 23.148 \left(\frac{\alpha^{\text{Chl}}}{a_{\text{PE}}^{\text{Chl}}} \right), \quad (5)$$

where 23.148 is a conversion factor calculated as follows: $(1 \text{ mol C}/12 \text{ g C})(\text{h}/3600 \text{ s})(10^6 \mu\text{mol photons}/\text{mol photons})$. The absorption coefficient, $a_{\text{PE}}^{\text{Chl}}$ ($\text{m}^2 \text{g}^{-1}$ Chl *a*), was weighted for the spectral output of the photosynthetron by convoluting $a^{\text{Chl}}(\lambda)$ with the spectral output of the photosynthetron in 1-nm intervals over the wavelength range 400–700 nm and integrating the product over the range.

$$a_{\text{PE}}^{\text{Chl}} = \int_{\lambda=400}^{\lambda=700} a^{\text{Chl}}(\lambda) E_{\text{PE}}(\lambda), \quad (6)$$

where E_{PE} is the spectral output, expressed in quantum equivalents and scaled to a mean value of unity between 400 and 700 nm.

Results and discussion

Cell division rate—Cell division rates were three times higher in cultures acclimated to $1,200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ than in cultures acclimated to $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Fig. 1). When cells were placed into high light, the cell-division rate increased almost instantaneously. On returning to low light, the cell-division rate decreased immediately but increased again over the next 2 d.

Cell carbon content and C:N ratios—Cell carbon content remained constant throughout the light and dark periods in cells growing at $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, indicating that cell division and net carbon assimilation proceeded in parallel. In contrast, cell carbon content increased during the light period in cells exposed to $1200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, indicating that cell division and net photosynthesis had become uncoupled under these circumstances (Fig. 2A,B). Carbon levels in these cells then declined to the same level as the previous day after the dark period (Fig. 2A,B). Reductions in cell carbon will arise from either “dilution” by cell division and/or respiration. The relative contributions of di-

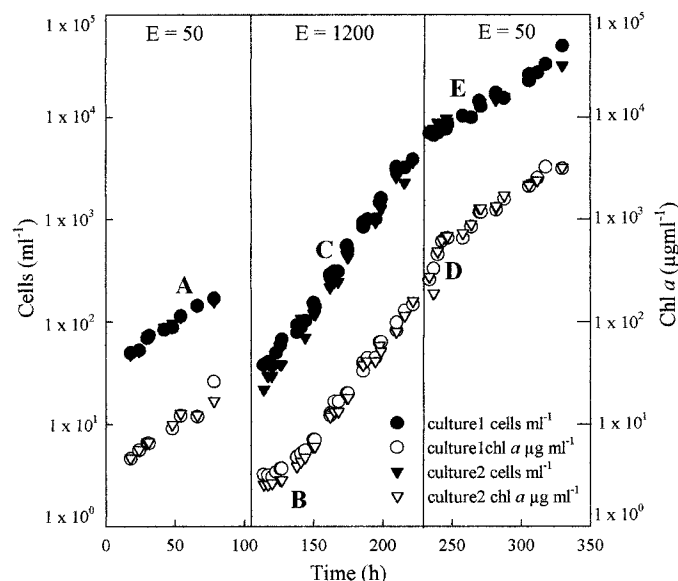


Fig. 1. Growth curves for cell abundance and chl *a* concentration in duplicate cultures constructed from observations corrected for periodic dilution. Regions A, C, and E show the exponential growth rates at the different irradiances. B and D show regions of unbalanced growth after each light shift. E = irradiance, $\mu\text{mol m}^{-2} \text{ s}^{-1}$.

lution and respiration can be evaluated by examining changes in cell abundance and POC concentrations during the dark periods. Cell abundance increased by $\sim 80\%$ during the dark period in cultures acclimated to $1200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ lower than during the light period.

The C:N ratio remained constant (4.72) in cells exposed to $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ but showed a significant increase to 5.67 (Table 1) at $1200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Thus, the uncoupling of carbon assimilation from cell division was accompanied by uncoupling of carbon assimilation from nitrogen uptake.

Cell Chl *a* content and Chl *a*:C ratios—Chl *a* cell $^{-1}$ decreased 2.5-fold ($1\text{--}0.4 \text{ pg cell}^{-1}$) when cells were put into high light and approached a new steady-state level after 5 d (Fig. 2C,D). There was a rapid increase in Chl *a* cell $^{-1}$ when cells were returned to low light, approaching the original level in 3 d. The rate of increase in Chl *a* cell $^{-1}$ during the high to low light transition mirrored the rate of decrease in growth rate. Our results (Fig. 1B,D) suggest that change of cell Chl *a* content after the shift to high light is not regulated by the rapid degradation of Chl *a* (Riper et al. 1979) but is controlled by cell division and reduction in the rate of pigment synthesis at high light, as found by other investigators (Post et al. 1984). This is supported by the observation of net Chl *a* synthesis of the high-light-acclimated cells during the dark period (Fig. 3D), with average synthesis rates of 0.03 h^{-1} . Chl *a* cell $^{-1}$ did not change because of continued cell division during the dark period (data not shown).

Changes in Chl *a*:C followed the same temporal trend as Chl *a* cell $^{-1}$ (Fig. 4A). The roles of photosynthesis and Chl *a* synthesis in determining the time course of variations in Chl *a*:C can be seen by examining the time dependencies

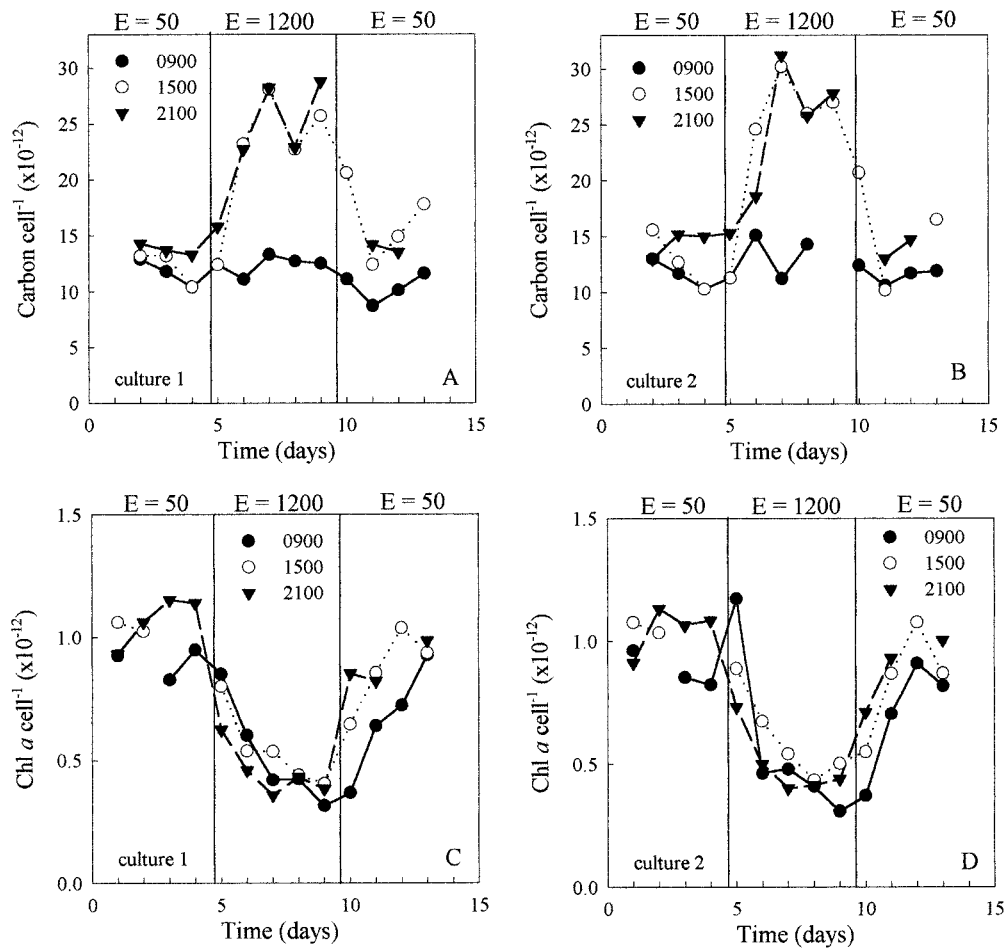


Fig. 2. Diel changes in cellular carbon and chl *a* abundance with time. E = irradiance, $\mu\text{mol m}^{-2} \text{s}^{-1}$.

of POC and Chl *a* concentrations after the shifts of irradiance. There was a significant increase in POC concentration for the 12 h following transfer of cells from 50 to 1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 3A). Particulate carbon increased at a specific rate of 0.08 h^{-1} , whereas Chl *a* levels showed very little change (Fig. 3B).

When the cells were shifted from high to low irradiance, there was a sharp increase in the Chl *a* levels, at a specific rate of 0.077 h^{-1} (Fig. 3D). The POC levels increased throughout the day (Fig. 3C) but at only half the rate (0.04 h^{-1}), compared with that observed for the shift from low to

high light. Thus, acclimation to low irradiance involved acceleration in the rate of Chl *a* synthesis relative to that for net carbon assimilation and conversely for acclimation to high irradiance.

Light-saturated photosynthesis—Cell-specific, light-saturated photosynthesis rates (P_m^{Cell}) measured at the midpoint of the light period did not vary during the experiment (Fig. 4D). However, there was some diel variability, indicated by the significant increase of P_m^{Cell} at the midpoint of the light

Table 1. Average values for protein cell^{-1} , P_m^{protein} , and P_m^{cell} at the three different sample times at the two different irradiances. Daily variability for the above measurements was tested for the two different irradiances, and the significance is indicated by the P values in the last column. Average values for C:N at the midlight period at the two irradiances is also shown. E = irradiance, $\mu\text{mol m}^{-2} \text{s}^{-1}$.

	0900 \bar{x}		1500 \bar{x}		2100 \bar{x}		Significance levels for time of day effect $P < 0.95$	
	$E = 50$	$E = 1200$	$E = 50$	$E = 1200$	$E = 50$	$E = 1200$	$E = 50$	$E = 1200$
Protein cell^{-1}	1.31	1.55	1.64	2.06	1.57	1.84	0.06	0.055
P_m^{Cell}	2.42	2.06	3.74	3.34	3.14	2.46	<0.001	<0.001
P_m^{protein}	0.18	0.16	0.23	0.15	0.17	0.132	0.026	0.22
C:N (molecule mol^{-1})			4.72	5.67				

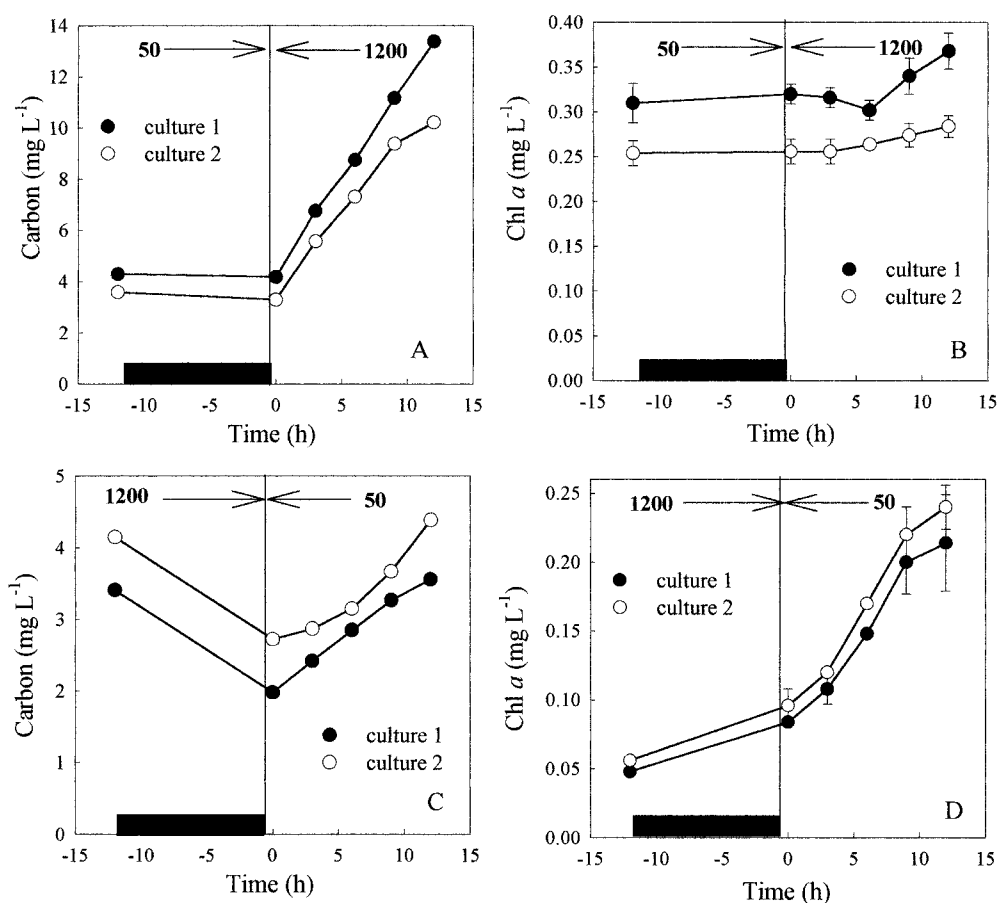


Fig. 3. Initial changes in chl *a* and carbon after a shift in irradiance from (A and B) 50 to 1,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and from (C and D) 1,200 to 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Error bars indicate ± 1 SE.

period, in comparison with that measured at the beginning and end of the day (Table 1) at both irradiances.

P_m^{Cell} represents the maximum rate of photosynthesis that can be achieved at saturating irradiance. It does not represent the actual rate of photosynthesis at the growth irradiance, which is designated P^{Cell} . This value can be obtained from the P^{Cell}/E curves shown in Fig. 5B. For cells grown at the low-growth irradiance ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$), P^{Cell} at that growth irradiance was 0.8 pg C h^{-1} (see arrow on Fig. 5B), whereas for cells grown at $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$, P^{Cell} at that irradiance was 2.8 pg C h^{-1} (see arrow on Fig. 5B). Thus, cells grown at $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ were not at saturating irradiance, whereas those at $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ were. What the similar values for P_m^{Cell} obtained at the growth irradiance of $50 \mu\text{mol}$ and at the saturating irradiance (Fig. 5B) do suggest is that low-light-acclimated cells placed into high-light-acclimated cells during a 30-min incubation (Fig. 5B), suggests that the cellular abundance of *Rubisco* remains the same at both irradiances.

The Chl *a*-specific light-saturated rate of photosynthesis (P_m^{chl}) at midday increased almost threefold after cells were placed in high light and subsequently fell to original values after 3 d of returning to the low irradiance (Fig. 4B). The change of P_m^{chl} could be accounted for exclusively by the decline of Chl *a* cell⁻¹ at high light (Fig. 2C,D).

The carbon-specific light-saturated photosynthesis rate (P_m^{C}) at midday showed the opposite pattern, with P_m^{C} values decreasing more than twofold during the high-light period and increasing to original levels when placed back into low light (Fig. 4C). As with P^{Cell} , the carbon-specific rate of photosynthesis (P^{C}) at the growth irradiance was still greater at high light than in low light (see arrows on Fig. 5C). However, when one looks at the two P^{C}/E curves, cells at the lower irradiance have the ability to photosynthesize at a greater rate than cells at the higher irradiance. This may well be attributed to the increase in cellular carbon (i.e., an accumulation of energy reserve polymers) throughout the day at the high irradiance (because of the diel variability in photosynthesis), resulting in a dilution of photosynthetic catalysts (Fig. 2A,B). The capacity of a cell to increase its carbon content may also be related to its size. Many cells increase their volume at high irradiance (Thompson et al. 1991), and if this occurred in *Skeletonema* then it would have been necessary for the cells to increase their ability to synthesize additional ribosomes and molecules required for rapid growth.

Another factor that may determine the carbon-specific rate at high light is the regulation of photosynthesis by *Rubisco* or electron transport. It is possible that at high irradiances there may be a shift in control of light-saturated photosyn-

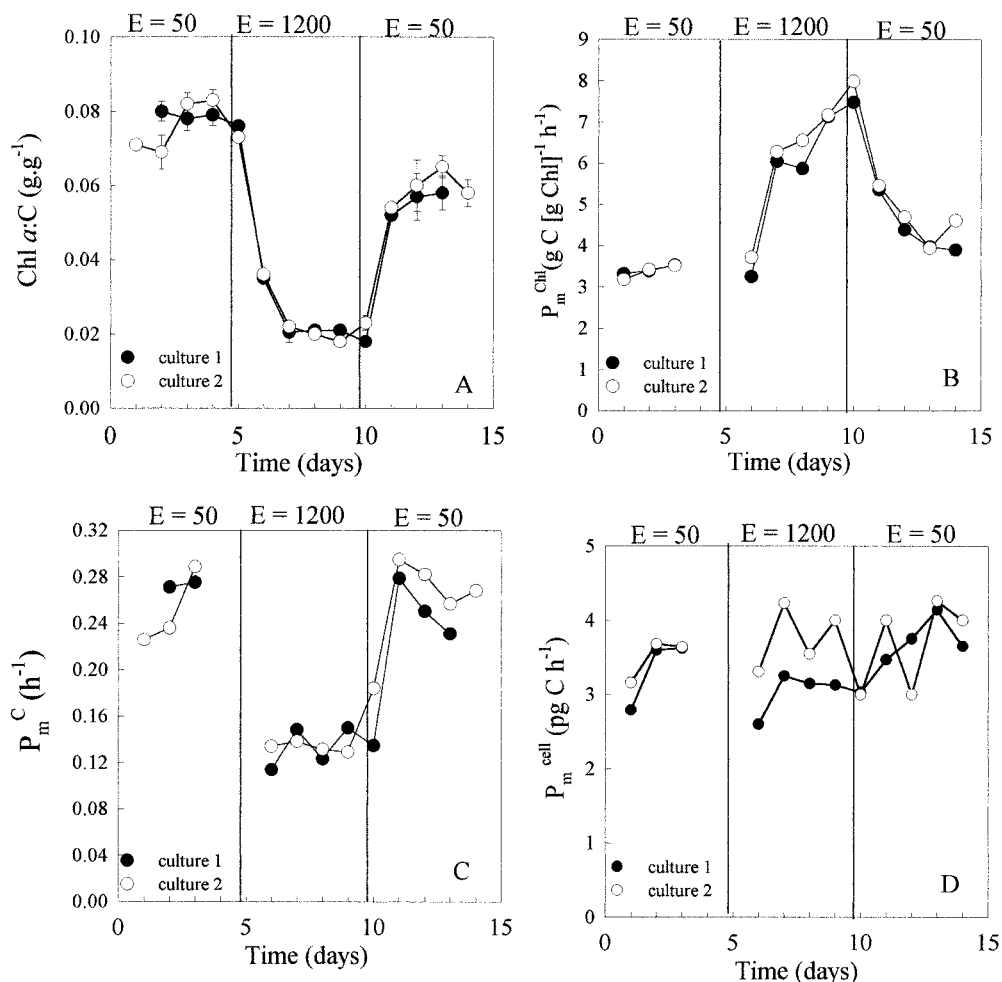


Fig. 4. Temporal changes in (A) Chl α :C, (B) P_m^{Chl} , (C) P_m^{C} , and (D) P_m^{cell} . Error bars indicate ± 1 SE. E = irradiance, $\mu\text{mol m}^{-2} \text{s}^{-1}$.

thesis from the Calvin cycle to the photosynthetic electron-transfer chain. This would require P^{C} to be proportional to the rate at which electrons are drawn from the electron transfer chain by the Calvin cycle (Geider et al. 1996) and for Rubisco abundance to remain constant (indicated by the similarity in protein cell⁻¹ and P^{Protein} , Table 1). However, more work will need to be carried out to investigate this further.

P/E curves not only provide the basis of information for primary productivity models (Fasham et al. 1990) but also reflect the underlying biophysical, biochemical, and metabolic processes that regulate photosynthesis (Falkowski and Raven 1997). However, interpretation of the curves, especially the light-saturated rate of photosynthesis, can vary depending on the variable to which photosynthesis is normalized. The most common way of expressing photosynthesis is on a per-unit chlorophyll basis. Taking the P_m^{Chl} results from this experiment, one would conclude that cells acclimated to a high irradiance will always have a greater ability to photosynthesize at saturating irradiance than cells grown at a low irradiance (Fig. 4B). In contrast, if photosynthesis is normalized on a per-cell basis, then one would conclude that low-light cells have the same ability to photosynthesize

at saturating irradiance as high-light cells, albeit with some photoinhibition. A further interpretation is made when normalizing photosynthesis to carbon, where results suggest that low-light cells outperform high-light-acclimated cells at saturating irradiance. Because of the diel variability of carbon during the high light period (Fig. 2A,B), P/E curves taken at the onset of the light period can also suggest that low-light and high-light cells have the same photosynthetic capacity (figure not shown).

So is there an advantage of using one unit of biomass over another to normalize photosynthesis to? This will vary depending on whether one is interested in investigating solely the mechanisms of photoacclimation or whether the interest lies in obtaining absolute values for carbon fixation. Results from this experiment suggest that P/E curves normalized to chlorophyll are really only informative when investigating light-limited photosynthesis rates (α^{Chl}), since these are controlled in part by pigment content. The conclusion that high-light cells have a greater ability to photosynthesize at saturating irradiances may be misjudged, since the lower chlorophyll concentration at a high irradiance may overemphasize the increase in P_m^{Chl} at high light. Since light-

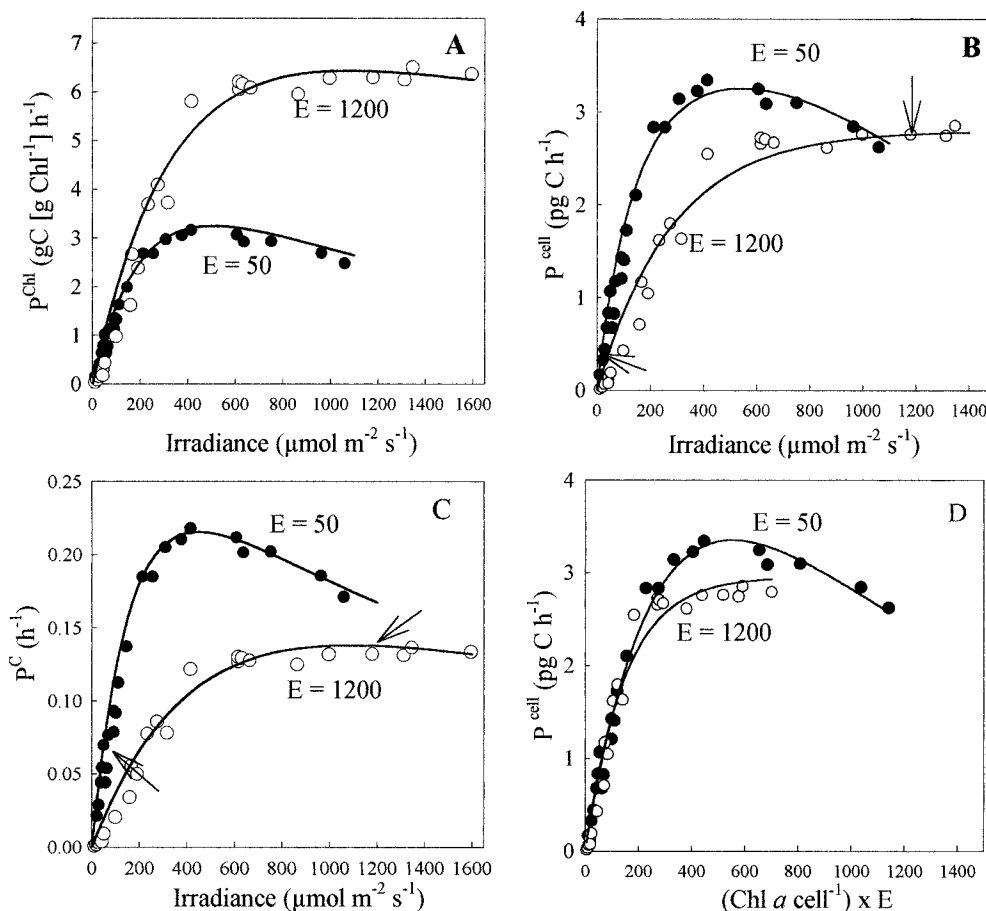


Fig. 5. Photosynthetic responses of cultures grown at two irradiances (50 and 1,200 $\mu\text{mol m}^{-2}$) normalized to (A) chl *a*, (B) cell numbers, and (C) carbon. Panel (D) shows P^{cell} plotted against chl *a* per cell multiplied by irradiance (*E*).

saturated photosynthesis is limited by something other than light absorption, information on how maximum photosynthetic rates are controlled may be better obtained by normalizing to another variable.

The advantage of expressing photosynthesis on a per-cell basis is that it gives an ecological perspective for competition and survivorship, because the smallest unit of a population is a cell. In addition, as shown in this experiment, it allows conclusions to be drawn on some mechanisms of photoacclimation, which cannot be obtained from the P_m^{chl} data. Normalizing per cell is regularly used in laboratory experiments because of the ease of measuring cell abundance. It becomes more difficult in the field if investigating the variability in carbon fixation among individual phytoplankton cells within mixed-species assemblages. In addition to this, it is also difficult to measure changes in cell size in the field, which is necessary when interpreting cell-specific photosynthetic performance.

The advantage of normalizing photosynthesis to carbon is the ability to compare the specific rate of carbon fixation at growth irradiance to rates of cell division (Geider et al. 1997). P_m^{C} has the advantage of often showing the least variability in response to changes in growth irradiance (Geider et al. 1997) and is therefore a useful parameter to normalize

to. However, this response is not universal, as demonstrated in this experiment, and changes in carbon content under different growth regimes makes it difficult to assess any physiological responses.

Although the merits and drawbacks of using different biomass units have been discussed, it is probable that chlorophyll will continue to be the preferred parameter for normalization of photosynthesis, since it can be measured by satellite and is present in all phytoplankton. Even though it may not be the best parameter to use when studying the mechanisms that control light-saturated photosynthesis, absolute values for carbon fixation at the natural growth irradiance (necessary for estimating oceanic global carbon production) can still be obtained from these P/E curves. That is, providing the spectra from the natural environment and the photosynthesis incubator are corrected.

Light-limited photosynthesis—The cell-specific initial slope of the P/E curve was significantly lower in high-light-acclimated cells. In contrast, the Chl *a*-specific initial slope (α^{chl}) showed no significant temporal variability throughout the experiment (Fig. 6A). Thus, the differences in α^{cell} can be attributed to changes in cell Chl *a* content. The role of cell Chl *a* content in controlling the light-limited initial slope

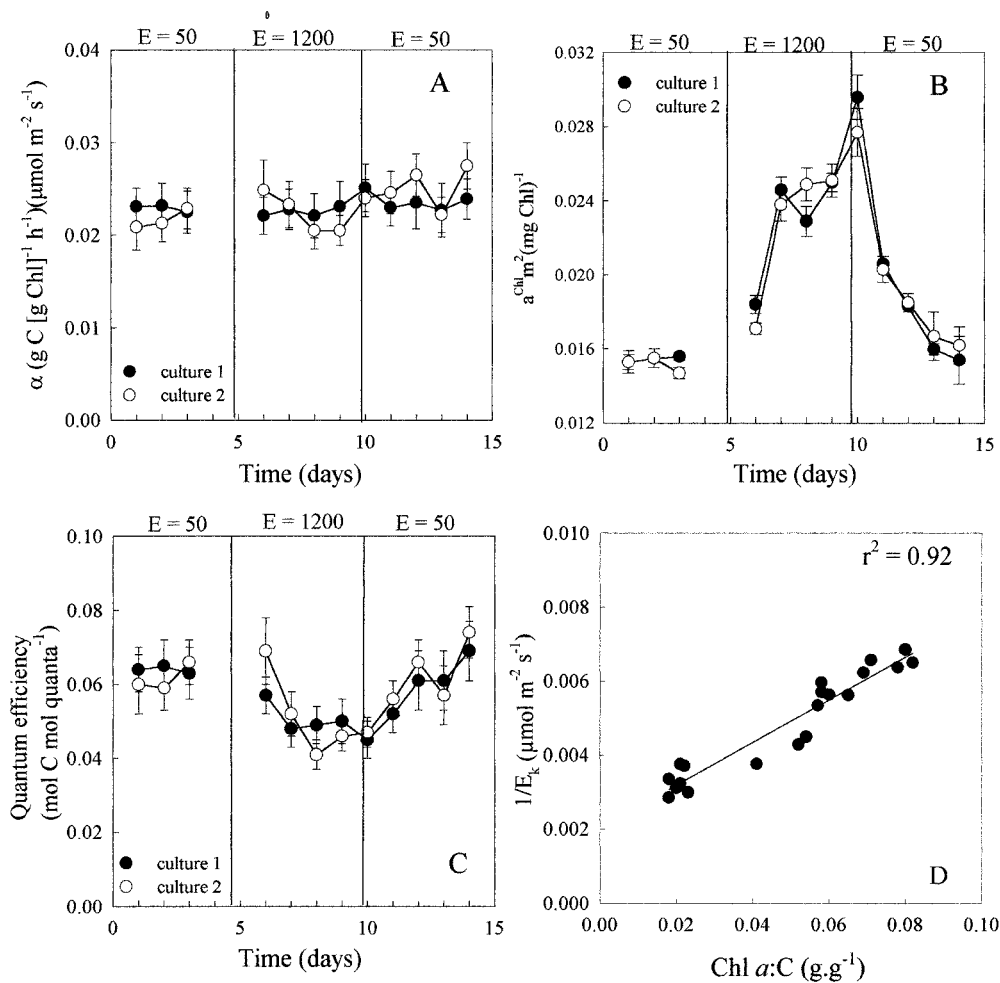


Fig. 6. Temporal changes in (A) α^{Chl} , the (B) chl *a*-specific light absorption coefficient, a^{Chl} , and (C) quantum efficiency. Error bars indicate ± 1 SE. E = irradiance, $\mu\text{mol m}^{-2} \text{s}^{-1}$. Panel (D) shows the relationship between $1/E_k$ and Chl *a*:C.

(α^{Cell}) can be seen when P^{Cell} is plotted against Chl *a* cell $^{-1}$ multiplied by irradiance (Fig. 5D). This comparison shows that once differences in Chl *a* have been accounted for, the initial rates are very similar, and the minor differences observed then relate to the maximum rates of photosynthesis.

Light absorption and pigments—The cellular abundance of the accessory pigments fucoxanthin and Chl *c* showed the same temporal changes as for Chl *a* cell $^{-1}$ (Fig. 7A,B). This is similar to the response observed in other diatoms grown at different irradiances (Goerick and Welshmeyer 1992). These pigments do not appear to be rapidly degraded during high irradiance but are diluted out subsequent to the increase in growth irradiance, as discussed for Chl *a*.

The xanthophyll cycle pigments responded rapidly to an increase in irradiance, with cell diadinoxanthin (DD) and diatoxanthin (DT) contents elevated threefold within hours of the change in the light level (Fig. 7C). This suggests de novo synthesis of the pigments and agrees with the rapid increase in both DD and DT observed in *Chaetoceros muellerii* (Olaizola and Yamamoto 1994). The increase of photoprotective xanthophylls DD and DT with an increase in

the growth irradiance may contribute to photoprotection of the reaction centers from an excess of excitation energy (Olaizola and Yamamoto 1994). After the shift to low irradiance, the DD + DT pools decreased rapidly, by 0.5 nmol cell $^{-1}$, with a consequently rapid increase in the abundance of fucoxanthin, supporting the suggestion that DD may be a precursor for fucoxanthin (Goerick and Welshmeyer 1992; Lohr and Wilhelm 1999). The accumulation of DD + DT when diatoms are exposed to high light, and rapid conversion to fucoxanthin after transfer to low light may be advantageous in phytoplankton that are subjected to large diel variations in mixed layer depth, such as those that occur during the spring bloom (Woods and Onken 1982). Cells growing in such an environment may spend several days in chronically low light below the daytime wind-mixed layer interspersed with one or more days exposed to the high light environments closer to the surface.

The cellular abundance of β -carotene was nearly constant (Fig. 7D). This accessory pigment is likely to be a precursor of DD and fucoxanthin and is also thought to play an important photoprotective role in chlorophytes (Yamamoto and Bassi 1996). However, because only DD + DT showed very

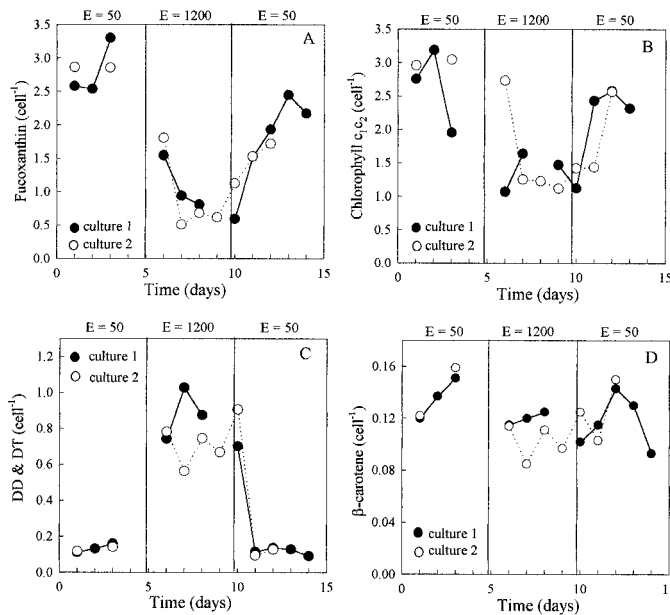


Fig. 7. Temporal changes in the cellular abundance of (A) fucocanthin, (B) chlorophyll *c*, (C) diadinoxanthin and diatoxanthin, and (D) β -carotene. E = irradiance, $\mu\text{mol m}^{-2} \text{s}^{-1}$.

large changes in abundance at high irradiance, these pigments may play a more significant photoprotective role than β -carotene.

The light-absorption coefficients (a^{Chl}) at 440 and 676 nm increased after the increase in irradiance and decreased when the cells were placed into lower light (Fig. 6B). The increase at 676 nm from 0.012 to 0.02 $\text{m}^2 (\text{mg Chl } a)^{-1}$ can be attributed to a decline in the “package effect,” in which the efficiency of the chloroplast in absorbing light is decreased by intracellular “self-shading” because of other chloroplasts, cellular diameter, thylakoid stacking, and increases in pigmentation per cell (Berner et al. 1989).

The variation of a^{Chl} at 440 nm from 0.016 to 0.028 $\text{m}^2 (\text{mg Chl } a)^{-1}$ includes contributions from variations in the package effect and changes in the ratio of accessory pigments to Chl *a*. The 50% decrease in light absorption at 440 nm is similar to that observed for the diatom *Thalassiosira* (Geider and Osbourne 1987) and to light-absorption properties for net plankton in Delaware Bay (MacIntyre et al. 1996).

Quantum efficiency was determined by the ratio of a^{Chl} to light absorption (a^{Chl}). Because a^{Chl} was independent of irradiance but a^{Chl} increased at high light, it follows that the quantum efficiency declined in high-light-acclimated cells (Fig. 6C). This decline may be attributed to the increase in the photoprotective xanthophylls. Although these pigments absorb light, the energy is not transferred to the reaction centres and consequently acts to screen the cells from excess light. However, in doing so, the functional absorption cross-section of the associated photosystem may be reduced (Suklenik et al. 1987).

Ecological implications/applications—Although chlorophyll may not be the most accurate parameter to use as an

indicator of phytoplankton abundance (Geider et al. 1998), it is still one of the easiest parameters to measure. It is necessary to be able to convert Chl *a* concentration into phytoplankton biomass so as to accurately describe phytoplankton in carbon-budget models (Taylor et al. 1997). Consequently, knowledge of the relationship between Chl *a* and carbon under different environmental conditions is necessary. Although this relationship is easy to determine in laboratory experiments (Fig. 4A) it still remains a problem to measure photoautotrophic carbon in the natural environment. Geider et al. (1997) suggested that Chl *a*:C could be related to the light-saturation parameter E_k , a parameter that has been routinely determined in thousands of P/E measurements.

E_k describes the optimum irradiance for balance between the light and dark reactions of photosynthesis and has regularly been used as an indication of the photoacclimation state of phytoplankton (Henley 1993). Because E_k can be obtained from photosynthesis/irradiance measurements (which are widely used in the natural environment), use of this relationship may provide information on the variability of the Chl *a*:C ratio in natural populations that would otherwise be difficult to measure. E_k is determined by dividing P_m^{Chl} by α^{Chl} . If, however, one uses P_m^{C} as a description of the maximum rate of photosynthesis and the carbon-specific initial slope is described as the product of α^{Chl} and Chl *a*:C (θ) (Geider et al. 1997), then

$$E_k = \frac{P_m^{\text{C}}}{\alpha^{\text{Chl}} \times E} \cdot \theta.$$

This can be re-arranged to give

$$\theta = \frac{P_m^{\text{C}}}{\alpha^{\text{Chl}}} \cdot \frac{1}{E_k}$$

A strong relationship ($r^2 = 0.92$) between $1/E_k$ and Chl *a*:C has been demonstrated for *Thalassiosira pseudonana* (Cullen and Lewis 1988) and is consistent with the assumption that α^{Chl} and P_m^{C} remain constant (Geider et al. 1997). A good relationship ($r^2 = 0.92$) was also observed for *S. costatum* (Fig. 6D) but differs from *T. pseudonana* by failing to extrapolate through the origin. This is due to the fact that although α^{Chl} remained constant when *S. costatum* was subjected to shifts between 50 and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, P_m^{C} decreased at high light. Given the constancy of P^{Cell} and α^{Chl} in *S. costatum*, we examined the relationship between Chl *a*:Cell and $1/E_k$. The regression passed through the origin (figure not shown), although the r^2 value was only 0.72. The low r^2 value is most likely due to the relatively large errors in cell counts.

Conclusions—Our results are consistent with recent models that describe photoacclimation of cell pigment content in terms of a dynamic energy balance (Kana et al. 1997). The energy balance is determined by comparing the maximum effective rate of light absorption (calculated as $a^{\text{Chl}} \times \phi_m \times \text{Chl cell}^{-1} \times E$) with the achieved rate of photosynthesis as irradiance E (i.e., $P^{\text{Cell}}[E]$) (Kana et al. 1997). The ratio $P^{\text{Cell}}(E)/a^{\text{Chl}} \times \phi_m \times \text{Chl cell}^{-1} \times E$ is a measure of the imbalance between the rate of light absorption and the rate

at which light energy can be dissipated by photosynthesis. When this ratio equals one, light harvesting and assimilation are in balance. All of the photosynthetically available radiation is used for photosynthesis, and pigment synthesis is given high priority under these circumstances. This energy balance is achieved at irradiances that limit growth (as seen during this experiment when cells were grown at $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) where light harvesting is not constrained by the maximum assimilation rate. An increase in irradiance to a point where the rate of absorbed light is over and above the maximum assimilation rate leads to an energy imbalance and a decline in the ratio. At this point absorbed light energy must be dissipated by non-photochemical mechanisms and pigment synthesis is given a lower priority. Evidence of energy reduction was observed during this experiment when cells were grown at $1,200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. There was an increase in the abundance of photoprotective xanthophylls and a reduction in the pigment concentration by dilution through an increase in growth. This dynamic adjustment of light absorption and dissipation ensures that with time, the input of light energy balances with the demand for cellular energy.

Models are becoming increasingly complex and dynamic and require information not just on the amount of carbon fixation but on physiological changes in relation to environmental variables (Geider et al. 1997). Although laboratory experiments cannot mimic the natural environment, this study has shown that various metabolic and physiological processes that are difficult to investigate in the field can be studied and used to test model algorithms.

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