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Physiological responses of a marine planktonic diatom to transitions in growth irradiance

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ABSTRACT: The time course of adaptation to changes in growth irradiance was studied in the marine diatom *Thalassiosira weisflogii*. Irradiance changes were from 72 to 593 μE m^{-2} s^{-1} (L → H) and from 593 to 72 μE m^{-2} s^{-1} (H → L). In steady state, high-light (HL) grown cells were characterized by low pigment contents and a low *in vivo* absorbance. Light saturated photosynthesis, expressed on a per cell basis, was higher for low-light (LL) cells, but on a Chl *a* basis photosynthesis of HL cells was higher. PSU_{O_2} sizes were not significantly different for HL and LL cells. In both transient states the carbohydrate pool was most dynamic and responded fastest to a change in irradiance. It was concluded that the carbohydrate pool serves as an energy and carbon reservoir buffering the cells from changes in irradiance and allowing other cellular properties to adjust to the new situation. The timescales of changes in Chl *a* $cell^{-1}$ and photosynthetic activities differed considerably: by a factor of 3 in the L → H transition and by a factor of 30 in the H → L transition. Irradiance transitions did not lead to marked changes in protein $cell^{-1}$.

INTRODUCTION

The adaptation to growth irradiance in unicellular algae encompasses changes in many physiological functions (for reviews see Falkowski 1980, Richardson et al. 1983). During the adaptation process (i.e. in the transition from one steady-state growth rate to another brought about solely by changes in irradiance level), some of the general cellular processes or properties which change markedly include division rate, photosynthetic capacity, cellular pigmentation and respiration rate (Marra 1980, Prezelin & Matlick 1980, Falkowski 1984a, Post et al. 1984). In the marine diatom *Thalassiosira weisflogii* (Grunow) Fryxell Hasle, the rate of adaptation to a new irradiance level depends upon whether the change is from a higher to lower irradiance or *vice versa*. Furthermore, the rates of change for different physiological responses (e.g. chlorophyll content, growth rate) do not necessarily parallel each other (Post et al. 1984).

We undertook to study the physiological responses of growth-irradiance adaptation by following the time course of changes in light-harvesting and photosynthetic properties as well as changes in pigment, carbohydrate and protein pools in *Thalassiosira weisflogii*. We reasoned that, as a first approximation, those processes or properties which changed first in response to changes in irradiance levels are physiologically linked to processes or properties which change later and so on. By following the kinetics of these processes or properties we could establish a 'cascade' of events which could lead to a clearer understanding of the molecular mechanisms involved in adaptation to growth irradiance.

Growth-irradiance adaptation may affect light-utilization efficiency (α) as well as the maximal rate of photosynthesis. During a transition from one growth irradiance level to another there may be a mismatch between growth rate and carbon fixation. During a low- to high-light transition, for example, a cell may fix 'excess' carbon (i.e. carbon over and above that needed to maintain its former steady-state growth rate). This carbon might be directed towards carbohydrate, lipid or protein pools. Therefore, it seems likely that during photoadaptation cellular pool sizes of (for example) carbohydrates and proteins respond to a change in light irradiance. The concept of 'overflow metabolism' (e.g. Myers 1980) like photorespiration (Tolbert 1974)

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and enhanced dark respiration (Falkowski et al. 1985) has evolved from short-term transient studies at high light. During adaptation to conditions of low light, division can proceed at the expense of reserve polymers (Loogman 1982), thereby buffering the cell from the effects of relatively fast changes in irradiance levels.

In this paper we examine how light-harvesting efficiency, photosynthetic activity and cellular pool sizes of carbohydrates and proteins respond to changes in growth irradiance. Changes in cellular pigment levels and division rates during light-shade adaptation in marine phytoplankters have been shown to obey first order kinetics (Falkowski 1980, 1984a, Rivkin et al. 1982a, Post et al. 1984). We investigated (1) whether changes in other cellular properties and processes followed similar kinetics, and (2) the sequence of response of the aforementioned variables to changes in growth irradiance.

MATERIALS AND METHODS

Thalassiosira weisflogii, clone T-VIC, was grown in a 3.2 l continuous culture vessel in natural seawater, enriched with F/2 nutrients (Guillard & Ryther 1962) at 18 °C. The culture was operated in a turbidostat mode as described by Post et al. (1984). Continuous illumination was provided by 2 banks of three 32 W, cool-white HO fluorescent tubes (Sylvania F24 T12). Growth irradiance was adjusted by switching on various combinations of tubes and changing the distance between the light banks and the turbidostat. Vessel irradiance was measured as scalar PAR with a 4 π quantum sensor (Biospherical Instruments QSL 100) in the center of the water filled turbidostat. Irradiances used were 72 μE $m^{-2} s^{-1}$ (LL) and 593 μE $m^{-2} s^{-1}$ (HL). Changes in irradiance were made in a 1-step operation, either low to high (LL → HL) or high to low (HL → LL).

Cell numbers were estimated with a Coulter Counter, model TA II, after a 1 : 20 dilution in filtered seawater. Chlorophylls *a* and *c* were determined spectrophotometrically on an Aminco DW 2a spectrophotometer. Thirty ml of cells were filtered on Gelman AE glass fiber filters and pigments were extracted by grinding in 90 % acetone. Chlorophyll *a* and *c* concentrations were calculated according to Jeffrey & Humphrey (1975). The spectrally averaged optical absorption cross-section normalized to Chl *a*, $\bar{k}_c$ (Atlas & Bannister 1980, Dubinsky et al. 1984), was derived from *in vivo* absorption spectra, as described by Dubinsky et al. (1985).

Proteins were determined on 20 ml aliquots after washing and 5-fold concentration using the Bradford procedure (Bradford 1976). Protein concentrations were measured spectrophotometrically at 595 nm in 1 cm cuvettes using serum albumin standards. For the determination of total carbohydrates 10 ml aliquots were washed and concentrated 10-fold. Carbohydrate concentrations were measured at 625 nm in 1 cm cuvettes against a D-glucose standard using anthrone reagent in 96 % H_2SO_4 according to Herbert et al. (1971).

Photosynthetic activities were measured with a Clark type electrode (YSI 5331) and a multigain amplifier as described by Dubinsky et al. (1985). The initial slope of the P *vs* I plots, α, and the photosynthetic capacity, P_{max}, were derived from a hyperbolic tangent fit (Jassby & Platt 1976) through 12 or more data points. Oxygen flash yields (PSU_{O_2}) were measured with repetitive short (< 3 μs) xenon flashes (GenRad Stroboslave 1539A) with a Rank Brothers electrode as described by Falkowski et al. (1981). The minimal turnover time for O_2 evolution at light saturation, τ, was calculated from PSU_{O_2} and steady-state P_{max} normalized to chlorophyll *a* (Myers & Graham 1971, Falkowski et al. 1981).

For electron microscopy, cells were harvested by centrifugation and fixed in 3 % glutaraldehyde buffered with 50 mM Na-cacodylate, pH 7.4, dehydrated in an acetone/water series and embedded in Epon. The cells were sectioned with a diamond knife and examined with a Phillips 300 transmission electron microscope.

Abbreviations used in this paper are listed in Table 1.

RESULTS

The P *vs* I curves at steady state HL and LL conditions expressed on a per cell and per Chl *a* basis are presented in Fig. 1. On a cellular basis photosynthetic activities of *Thalassiosira weisflogii* were higher over the whole range of light intensities for LL cells (Fig. 1a). When photosynthesis was normalized to Chl *a*, however, the reverse was true (Fig. 1b). After a 1-step change in light irradiance both cell division rates and Chl *a* $cell^{-1}$ changed gradually over time. Cell division rates were maximal (2.3 div d^{-1}; μ = 1.59 d^{-1}) at 593 μE $m^{-2} s^{-1}$ (HL), while growth was light limited (0.7 div d^{-1}; μ = 0.49 d^{-1}) at 72 μE $m^{-2} s^{-1}$ (LL). At LL Chl *a* $cell^{-1}$ was 9.2 pg $cell^{-1}$ and under HL conditions it decreased to 4.3 pg $cell^{-1}$ (Table 2) (see Post et al. 1984 for detailed discussion). Lower Chl *a* contents at HL were accompanied by a slightly higher Chl *a/c* ratio: 8.7 ± 0.2 (mole/mole) *versus* 7.5 ± 0.4 at LL. Photosynthetic activity per cell at culture light intensity (i.e. the photosynthetic performance) was higher for HL cells than LL cells.

PSU_{O_2} sizes, the so-called Emerson and Arnold numbers, were 1,840 ± 84 Chl a/O_2 for LL and 2,115 ± 153

Table 1. List of abbreviations

LL	Low-light growth irradiance	$\mu E\ m^{-2}\ s^{-1}$
HL	High-light growth irradiance	$\mu E\ m^{-2}\ s^{-1}$
L→H	Transition from low to high light	–
H→L	Transition from high to low light	–
Chl *a*	Chlorophyll *a*	–
Chl protein	Chlorophyll protein complex	–
PS I	Photosystem I	
PS II	Photosystem II	
$\bar{k}_c$	The Chl *a* specific, spectrally averaged, light attenuation coefficient	m^2 (mg Chl *a*)$^{-1}$
α	Light utilization efficiency on a per cell basis	$\mu mol\ O_2\ cell^{-1}\ min^{-1}/\mu E\ m^{-2}\ s^{-1}$
α′	Light utilization efficiency on a per Chl *a* basis	$\mu mol\ O_2$ (mg Chl *a*)$^{-1}\ min^{-1}/\mu E\ m^{-2}\ s^{-1}$
P_{max}	Photosynthetic capacity measured at saturating light irradiances	$\mu mol\ O_2$ (mg Chl *a*)$^{-1}\ min^{-1}$
PSU_{O_2}	Photosynthetic unit based on oxygen flash yields (Chl a/O_2)	mole/mole
ATP	Adenosine triphosphate	–
NADPH	Reduced nicotinamide adenine dinucleotide phosphate	–
mRNA	Messenger Ribonucleic acid	–
RUBPcase	Ribulose 1,5-bisphosphate carboxylase	–
τ	The minimum time for 4 electrons to pass through a $PSU\,O_2$, from H_2O to PS I, measured at light saturation	ms
μ	Specific growth rate	d^{-1}

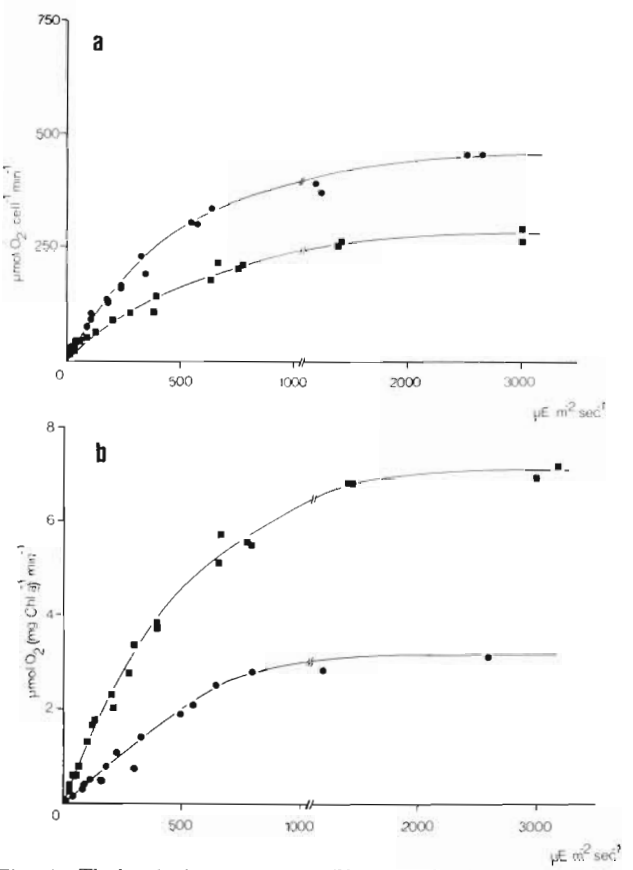

Fig. 1. *Thalassiosira weisflogii*. Photosynthesis-light curves when grown at 72 $\mu E\ m^{-2}\ s^{-1}$ (●) and 593 $\mu E\ m^{-2}\ s^{-1}$ (■) expressed on a per cell basis (a) and on a per Chl *a* basis (b)

Table 2. *Thalassiosira weisflogii*. Steady-state values and first-order rate constants for various parameters before and after a change in growth irradiance level at 17 °C

Process variable	Steady-state HL	Steady-state LL	Transition rate (h^{-1}) L→H	Transition rate (h^{-1}) H→L
Carbohydrates	137[a]	24[a]	+ 0.687	− 0.309
Chl *a*	4.3[a]	9.2[a]	− 0.081	+ 0.005
Protein	120[a]	119[a]	–	–
$\bar{k}_c$	0.0058[b]	0.0038[b]	+ 0.067	− 0.039
μ	1.59[c]	0.49[c]	+ 0.081	− 0.510
α′	0.0095[d]	0.0048[d]	–	–
α	0.41[e]	0.59[e]	− 0.275	+ 0.166
τ	4.5[f]	9.8[f]	− 0.170	+ 0.090
PSU_{O_2}	2115[g]	1840[g]	–	–

[a] pg $cell^{-1}$;
[b] m^2 (mg Chl *a*)$^{-1}$;
[c] d^{-1};
[d] $\mu mol\ O_2$ (mg Chl *a*)$^{-1}\ min^{-1}/\mu E\ m^{-2}\ s^{-1}$;
[e] $\mu mol\ O_2\ cell^{-1}\ min^{-1}/\mu E\ m^{-2}\ s^{-1}$
[f] ms
[g] Chl a/O_2

Chl a/O_2 for HL cells, respectively. Since PSU_{O_2} sizes were not markedly different for HL and LL, we concluded that *Thalassiosira weisflogii* adapts to changes in growth irradiance primarily by changing numbers of PSU_{O_2}, and by extension, the number of PS II reaction centers $cell^{-1}$ (see Falkowski et al. 1981 for discussion).

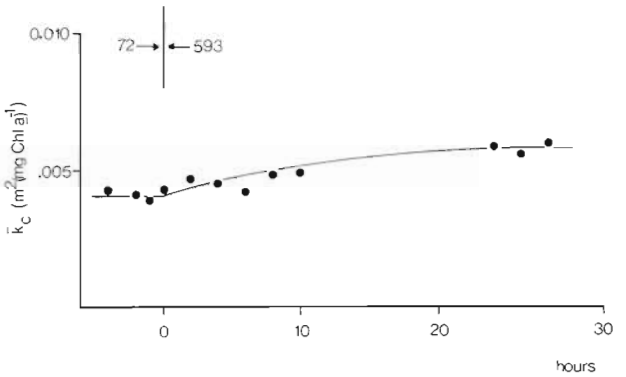

Fig. 2. *Thalassiosira weisflogii.* Changes in the spectrally averaged light attenuation coefficient, $\bar{k}_c$, after an increase in growth irradiance from 72 to 593 μE m^{-2} s^{-1}

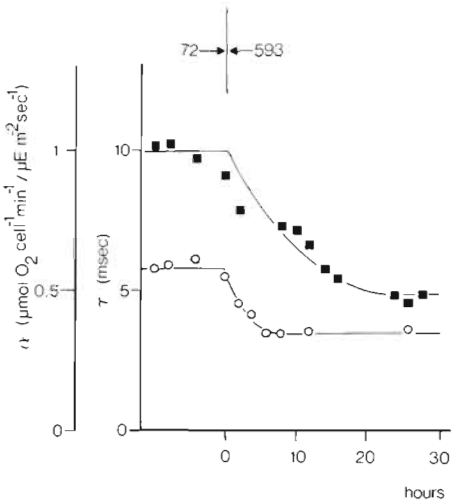

Fig. 3. *Thalassiosira weisflogii.* Changes in the photosynthetic characteristics α (○) and τ (■) after an increase in growth irradiance from 72 to 593 μE m^{-2} s^{-1}

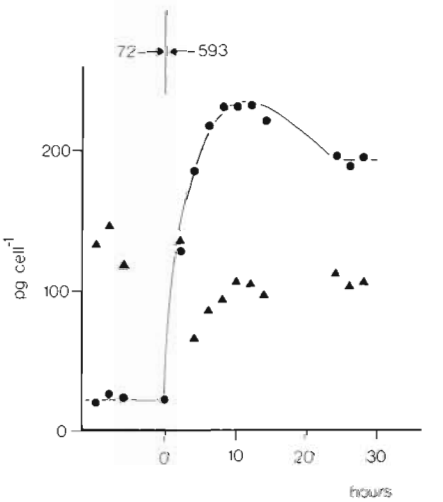

Fig. 4. *Thalassiosira weisflogii.* Changes in the cellular pool sizes of proteins (▲) and carbohydrates (●) after an increase in light intensity from 72 to 593 μE m^{-2} s^{-1}

Low- to high-light transition

Following a 1-step change in growth irradiance, from 72 to 593 μE m^{-2} s^{-1} (L → H), Chl *a* $cell^{-1}$ decreased as growth accelerated (see Post et al. 1984). The Chl *a* specific light attenuation coefficient, $\bar{k}_c$, or absorption cross-section, increased from 0.0038 m^2 (mg Chl *a*)$^{-1}$ at LL to 0.0058 m^2 (mg Chl *a*)$^{-1}$ at HL (Fig. 2). $\bar{k}_c$ changed inversely to Chl *a* $cell^{-1}$, and the time scale of adaptation was essentially the same for both processes (~ 20 h). The product of $\bar{k}_c$ and Chl *a* $cell^{-1}$ provides an estimate of the apparent *in vivo* absorption cross-section of the cells, having units of m^2 $cell^{-1}$. The *in vivo* absorption cross-sections were 0.35×10^{-4} m^2 $cell^{-1}$ and 0.23×10^{-4} m^2 $cell^{-1}$ for LL and HL cells, respectively. The ratio of LL/HL cellular Chl *a* was 2.1. Thus, the ability of the cells to harvest light is 1.5 times higher in LL adapted cells compared with HL adapted cells, however the change in apparent absorption cross-section is not proportional to the increase of the Chl *a* content.

Changes both in $\bar{k}_c$ and PSU_{O_2} during the L → H transition were related to changes in the photosynthesis-irradiance relation. We calculated the initial slope of P-I curves, α, and the minimal turnover time, τ, of a PSU_{O_2}. Fig. 3 shows that α and τ decreased during the L → H transition. A new steady state α was reached within 8 h. The change in α occurred faster than in Chl *a* $cell^{-1}$ or $\bar{k}_c$.

The 2 major pools of carbon in *Thalassiosira weisflogii,* carbohydrate and protein, responded quite differently during the L → H transition (Fig. 4). The carbohydrate pool reached a maximum after 8 h and declined thereafter, reaching a new steady-state level after 24 h. Specifically, cellular carbohydrate increased 10-fold in 8 h, overshooting the steady-state level at high light by 15 %. Protein $cell^{-1}$ was relatively constant immediately after the change in light irradiance, however, after 2 h it decreased 50 % and then increased rapidly again to a new steady-state value, close to the initial value. These results suggest that following the L → H transition, most of the carbon fixed by the cells was directed into the carbohydrate pool. As μ reaches a new steady state at HL, the carbohydrate/protein ratio is 5- to 6-fold higher than previously at LL.

High- to low-light transition

Changing growth irradiance from 593 to 72 μE m^{-2} s^{-1} (H → L) was characterized by an immediate sharp drop in growth rate and a slow increase in Chl *a*

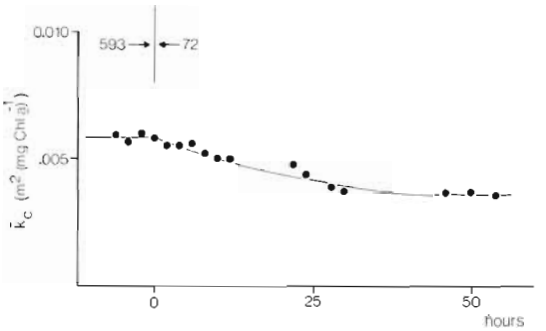

Fig. 5. *Thalassiosira weisflogii*. Changes in the spectral averaged light attenuation coefficient $\bar{k}_c$ after a decrease in growth irradiance from 593 to 72 μE m^{-2} s^{-1}

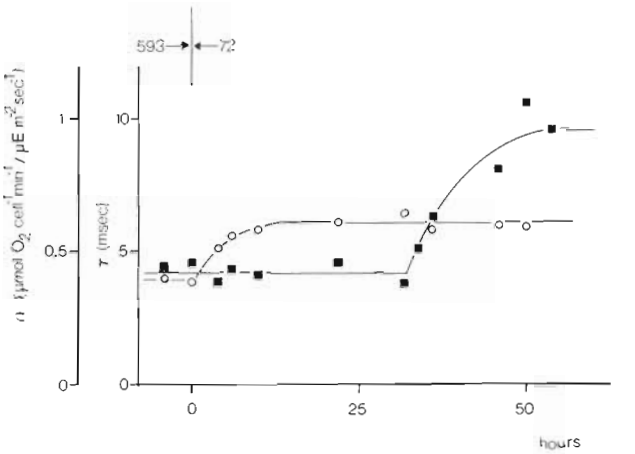

Fig. 6. *Thalassiosira weisflogii*. Changes in the photosynthetic characteristics α (○) and τ (■) after a decrease in growth irradiance from 593 to 72 μE m^{-2} s^{-1}

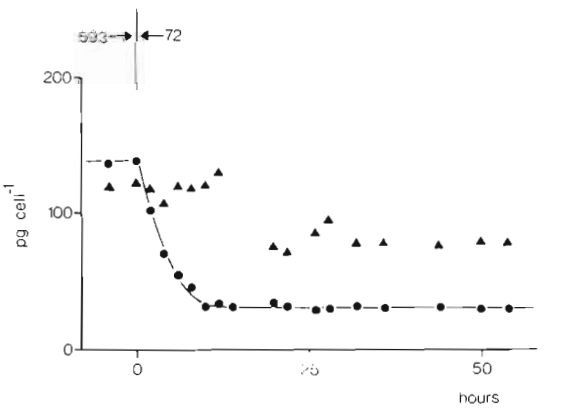

Fig. 7. *Thalassiosira weisflogii*. Changes in the cellular pool sizes of proteins (▲) and carbohydrates (●) after a decrease in growth irradiance from 593 to 72 μE m^{-2} s^{-1}

cell^{-1} (see Post et al. 1984). Although the light-harvesting capacity increased per cell, due to the increase in pigment contents, the Chl *a* specific absorption coefficient, $\bar{k}_c$, decreased until a new steady state was reached after 50 h (Fig. 5). As *Thalassiosira weisflogii* adapted to decreased light there was an increase in α, which reached a new steady state long before $\bar{k}_c$ and Chl *a* cell^{-1} (Fig. 6). The light saturated turnover time, τ, was ~ 4 ms for the first 30 h after the change in light, after which it increased to ~ 10 ms (Fig. 6).

Changes in pigment contents and photosynthetic rates following the H → L transition did not prevent a sharp decrease in division rates, especially during the first 15 h following the transition. During this initial period, cellular carbohydrate contents decreased markedly, while protein cell^{-1} remained relatively constant (Fig. 7). Carbohydrate cell^{-1} reached its minimum level after 15 h. Protein cell^{-1} decreased slightly during the next 15 h, after both α and carbohydrate cell^{-1} had reached steady state.

Kinetics of adaptation to growth irradiance

With the exception of cellular protein, the growth-irradiance-dependent changes in all parameters studied during both H → L light and L → H light transitions were analyzed using a first order kinetic model (Falkowski 1984). In this analysis the following equation is used:

$$A_t = (A_o - A_\infty)^{-kt} + A_\infty \quad (1)$$

where A_t = a process-variable at time t; A_0 and A_∞ = initial and final levels of the process-variable A; k = first order rate constant having units of h^{-1}. It should be noted that rate constants have a sign implicitly associated with them. When a process leads to an accumulation of a product (e.g. carbohydrate) or an increase in a rate (e.g. τ), the rate constant is positive. Conversely, when process leads to a depletion of a pool or deceleration of a rate the rate constant is negative. Calculated first order rate constant (k values) for the changes in the various parameters for both transient states are presented in Table 2.

During the L → H transition μ, Chl *a* cell^{-1}, and $\bar{k}_c$ all have similar rates of change. Rate constants for the photosynthetic parameters α and τ are similar to each other but are 3 to 4 times faster than those calculated for μ, $\bar{k}_c$ or Chl *a* cell^{-1}. The highly dynamic nature of the carbohydrate pool is reflected in its rate of change, which is ~ 2.5 times higher than those of α and τ.

During the H → L transition division rate and carbohydrate cell^{-1} changed fastest. The decrease in protein cell^{-1} during the H → L transition was very small and could not be fitted to first order kinetics with any degree of confidence. The slowest rates of measurable change were observed for Chl *a* cell^{-1} and $\bar{k}_c$ in the H → L transition.

DISCUSSION

Our results show that during growth irradiance transitions in *Thalassiosira weisflogii*, (a) the carbohydrate pool is highly dynamic; (b) the time scales of change in photosynthetic parameters and Chl *a* cell^{-1} are different and (c) there may be a delay before a change in a pool or a rate is observed (see also Rivkin et al. 1982a).

Before discussing the relation of these observations to each other and to the overall metabolism of the cell, we would like to briefly discuss the distinctions between pools, rates, and changes in pools or rates. These concepts, which are often clear in the steady-state, may be confusing in analysis of data in transition studies. A pool has dimensions of mass or weight, which may be normalized to cell number or Chl *a* or any other convenient timeless unit (e.g. liter, area, etc.). A rate or flux has a dimension in time. During steady-state balanced growth the pools of all cell constituents are time invariant. Under these conditions the net flux of material into a pool is equal to the growth rate, but the actual flux of the pool may be greater than μ if the pool turns over (e.g. Chl *a*: Riper et al. 1979). During a transition state, however, a pool may change with time. Under these conditions, the rate of change is a net flux. An analogous situation exists with respect to rates or fluxes in the steady-state. In the steady-state a flux (e.g. growth rate) is time invariant, but during a transition state a rate or flux may change, i.e. a rate may accelerate or decelerate.

In steady state it is not normally possible to infer fluxes from pool sizes. During a transition, however, a change in a pool size is (by definition) a flux and a change in a flux is (by definition) an acceleration or deceleration of a rate. In this study we followed the time dependent changes in 4 pools, carbohydrate, protein, chl *a*, and $\bar{k}_c$ (which is not a true 'pool' but an optical property without a time dimension), and 3 fluxes, μ, α, and τ (note that α is actually a ratio of 2 fluxes).

Since in diatoms the cell-wall polymers do not consist of carbohydrates, almost all carbohydrate can be considered energy and/or carbon storage. During the L → H transition the carbohydrate pool increased markedly. During this transition period the cell growth rate was accelerating, but not at a rate fast enough to keep pace with the rate of carbohydrate production. In a sense, the cell synthesized 'excess' photosynthate, i.e. it exceeded the rate required for balanced growth. This 'excess' photosynthate was stored in the cells as carbohydrate. During the H → L transition the carbohydrate pool was reduced immediately after the transition to the new light regime while initially protein cell^{-1} remained constant. Protein cell^{-1} started to decrease only after carbohydrate cell^{-1} reached its minimum level. The relation between the carbohydrate and protein pools in the H → L transition suggests that carbohydrates were initially used as an energy source and used for protein synthesis. After carbohydrate cell^{-1} reached a minimum level, both carbohydrate and protein pools depended solely on the flux of photosynthate.

The first order rate constants for carbohydrate cell^{-1} imply that carbohydrate pools underwent the fastest change during the L → H transition. This indicates that carbohydrate accumulation in *Thalassiosira weisflogii* can occur at a much higher rate than respiratory processes can catabolize this pool. The dynamics in the carbohydrate pools can be advantageous for *T. weisflogii* in environments with rapidly changing light conditions, as been suggested by Post et al. (1984). In both H → L and L → H transition states the carbohydrate pool served as an energy reserve or buffer, allowing *T. weisflogii* to store photosynthetically available radiation when abundant and providing an energy source, and thus time to adapt, when growth irradiances were reduced.

Unlike the carbohydrate pool, Chl underwent proportionally much smaller changes and these changes were slower. *In vivo* chlorophylls as well as the other pigments involved in light harvesting are associated with specific proteins and organized in membranes. During a growth-irradiance transition, the assembly of pigment-protein complexes into functional photosynthetic 'units' in thylakoid membranes does not necessarily occur on the same time scale as the synthesis of the pigment-protein complexes *per se*. Our previous analysis of changes in Chl suggested that during a L → H transition the decrease in cellular Chl could be explained solely by dilution due to cell division (i.e. the first order rate constants for changes in Chl *a* cell^{-1} and μ are equal but of opposite signs).

The HL-adapted cells have fewer stacked thylakoids in their chloroplasts than their shade-adapted counterparts (Fig. 8). Our data suggest that a measure of the relative degree of thylakoid stacking is the *in vivo* Chl specific light absorption coefficient, $\bar{k}_c$ (Falkowski et al. 1985). As Chl *a* cell^{-1} decreased, $\bar{k}_c$ increased, and during the L → H transition these processes occurred at similar (but not identical) rates. It is tempting to conclude that the slower process (i.e. changes in $\bar{k}_c$) results from the reorganization of thylakoid membranes within the chloroplast.

In contrast, during a H → L transition, new Chl proteins must be synthesized. In *Thalassiosira weisflogii* this process is relatively slow. Changes in $\bar{k}_c$ occur at a rate almost an order of magnitude faster than the change in Chl *a* cell^{-1} (Table 2). These results suggest that reorganization of thylakoids during a H → L tran-

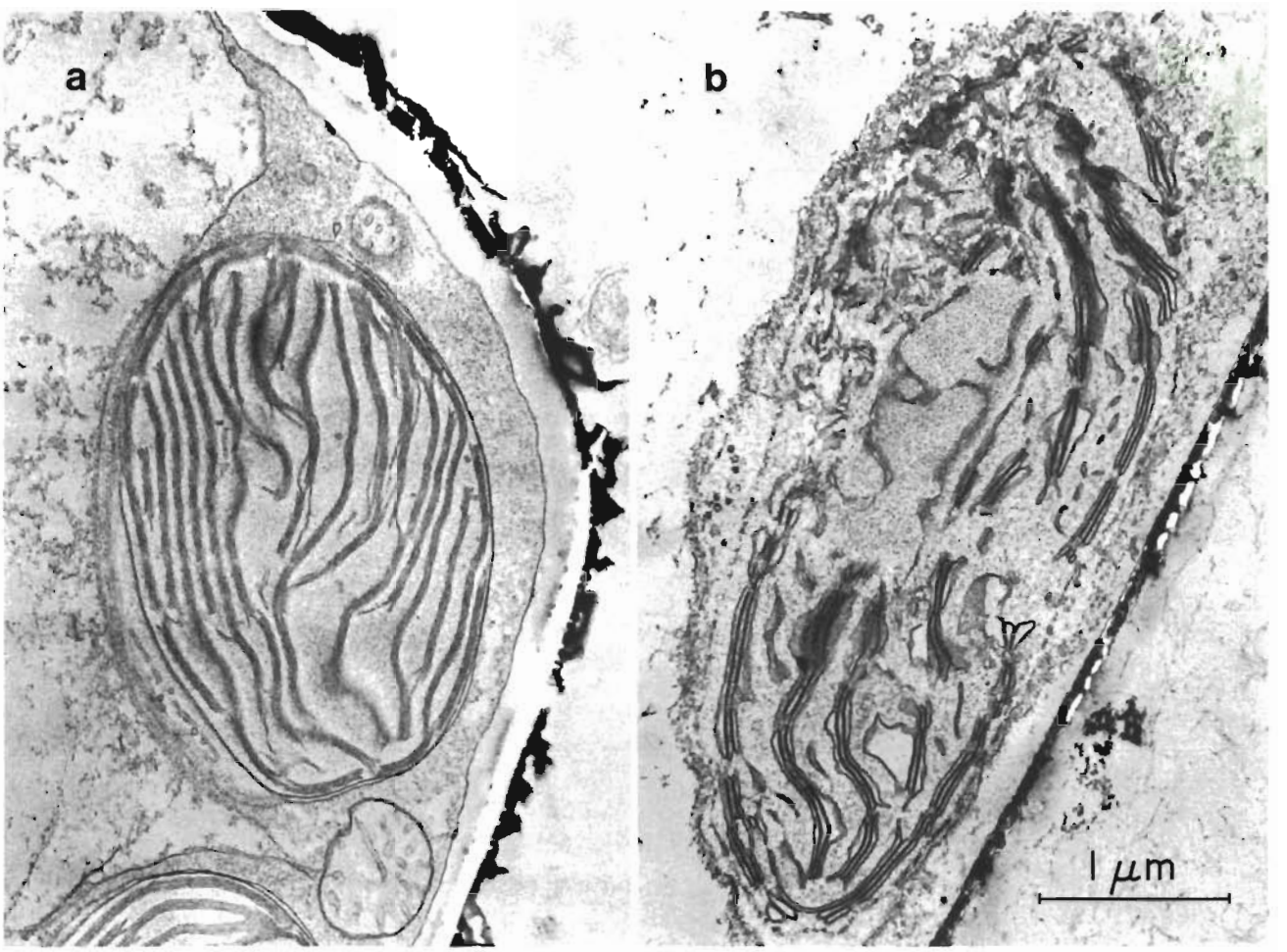

Fig. 8. *Thalassiosira weisflogii*. Transmission electron micrographs of sections through LL (a) and HL (b) adapted chloroplasts. Note that thylakoid stacking is greater in LL chloroplasts than in HL, but chloroplast volume is lower in LL

sition may preceed the synthesis of Chl. These hypotheses, developed from physiological and biochemical data, should be examined in future studies by following the time course of ultrastructional change in cell chloroplasts.

Changes in $\bar{k}_c$ are related to the initial slope of the photosynthesis-irradiance curve by the relation:

$$\alpha' = \Phi \bar{k}_c \quad (2)$$

where α' is expressed on a Chl basis and Φ is a quantum yield. In this study α was measured by following changes in O_2 cell^{-1} as a function of irradiance.

During the H → L transition α increased while during the L → H transition it decreased. The changes in α were accompanied by inverse changes in $\bar{k}_c$, however changes in $\bar{k}_c$ were not sufficient to offset apparent changes in the minimum quantum requirement for O_2. The changes in α were not solely due to changes in $\bar{k}_c$, but also resulted from changes in the minimum quantum yield for O_2. At least 2 hypotheses, not mutually exclusive, can be proposed to account for the changes in α. Firstly, in HL-grown cells, accessory pigments (e.g. β-carotene) are produced which absorb visible light and serve to protect the cell from photoinhibition but which do not contribute to photochemistry. In the H → L transition such accessory pigments are broken down rapidly. Secondly, at HL the proportion of excitation energy directed towards PS I is larger than in LL grown cells (Dubinsky et al. 1985). The fraction of excitation energy directed towards PS II or PS I may be regulated by irradiance, e.g. through movement of antennae Chl proteins within a thylakoid (see Bennett 1980 for review). Whatever the mechanisms, it is clear that the changes in α are not simply due to change in Chl *a* cell^{-1} or $\bar{k}_c$, but reflect more fundamental and subtle changes at the biophysical and molecular levels in the photosynthetic apparatus.

From our results we can develop a working model of the events occurring during a L → H or H → L transition in *Thalassiosira weisflogii*. Light provides an electron flux in the photosynthetic apparatus. As a first approximation this flux is simply related to O_2 produc-

tion, i.e. the rate of O_2 evolution gives a measure of the rate at which electrons are moved from PS II to PS I. Inevitably the electrochemical gradient established in a given photon flux is used to generate ATP and reductant, and, as correlary, the rate of formation of ATP and reductant is directly but non-linearly related to the photon flux. The capacity for a cell to store ATP and reductant (e.g. NADPH, ferredoxin) is limited.

When cells are grown at low irradiance levels and brought to high irradiance levels, ATP and NADPH production exceeds the rate required to reduce CO_2 or NO_3^- or form macromolecules to maintain a steady-state growth rate. In the absence of CO_2 or other nutrient limitation, ATP and reductant are used to reduce CO_2 in 'excess', forming carbohydrate. The carbohydrate pool rapidly accumulates. This pheomenon is sometimes called 'overflow' metabolism (see Myers 1980). As carbohydrates accumulate, more NO_3^- may be reduced, leading to the potential synthesis of protein. At this point the mechanisms by which protein synthesis are enhanced are unclear, however because the first order rate constants for μ exceed change in protein cell^{-1}, we hypothesize that translation of mRNA is enhanced by a L → H transition. Growth and division are not necessarily coupled during this transition period. We observed that protein cell^{-1} decreased by 50 % 2 h after a L → H transition, suggesting a sudden, synchronous division. That cell protein returned rapidly to its initial value suggests that the new steady-state growth rate was closely coupled to the rate of protein synthesis. The data also indicate that during a L → H transition cells are not in 'balanced' growth, e.g. the rate of synthesis of carbohydrate exceeds that of protein.

As cell division proceeds in a L → H transition, Chl *a* cell^{-1} is reduced (see Post et al. 1984). The reduction of Chl *a* cell^{-1} is accompanied by a decrease in τ. At HL the cells are at, or close to, light saturation of photosynthesis. Over-excitation of PS II could lead to photoinhibition, especially if the ultimate recipient of reductant (e.g. CO_2 or NO_3^-) is limiting. The cell appears to overcome the problem of balancing photon input with electron flow by decreasing light harvesting potential and increasing the rate at which electrons can be moved from H_2O to CO_2. Again, at present, we do not know exactly how this is achieved. One obvious mechanism is by increasing the synthesis of a rate limiting electron carrier in the photosynthetic apparatus. Preliminary results with polyclonal antibodies indicate that this may be via increased synthesis of RUBPcase (Bennett & Falkowski unpubl.).

When HL-grown cells are transferred to LL, photosynthetic electron flow decreases in proportion to the difference in photon flux densities. The rate of production of ATP and reductant from photosynthetic electron flow is reduced, yet the cell still contains high levels of mRNA and high demand for macromolecules. To offset the energy crisis, carbohydrates are catabolized; nevertheless, cell growth rates decrease markedly. In some species, however, growth appears to be maintained for considerable periods (Rivkin et al. 1982b). As cell growth decreases the Chl *a* pool increases, leading to an increased potential to harvest light. Only as a last resort is protein used for an energy source. It should be pointed out that the types and number of various proteins synthesized in HL and LL are different. At LL the Chl proteins are synthesized to a greater extent than in HL cells, while the situation for RUBPcase is just the opposite (Falkowski unpubl.).

In temperate and tropical latitudes, natural phytoplankton assemblages undergo H → L and L → H light transitions daily (or more frequently if mixing is great) (see Falkowski 1984 for review). Under such circumstances the fluctuation in carbohydrate and protein pools, reported here for a relatively long time scale observation, are also observed on the diel time scale (Cuehel et al. 1984, Post et al. 1985). However, cells appear to distinguish between light/dark cycles and variations in light irradiance (Post et al. 1984); cells do not 'shade' adapt at night. This paradox suggests that light-shade adaptation is not simply a consequence of post-translational control resulting from overflow metabolism, but is controlled at the translational and transcriptional levels, involving light dependent controls on protein synthesis.

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