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UNCOUPLING OF SILICON COMPARED WITH CARBON AND NITROGEN METABOLISMS AND THE ROLE OF THE CELL CYCLE IN CONTINUOUS CULTURES OF *THALASSIOSIRA PSEUDONANA* (BACILLARIOPHYCEAE) UNDER LIGHT, NITROGEN, AND PHOSPHORUS CONTROL[1]

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The elemental composition and the cell cycle stages of the marine diatom *Thalassiosira pseudonana* Hasle and Heimdal were studied in continuous cultures over a range of different light- (E), nitrogen- (N), and phosphorus- (P) limited growth rates. In all growth conditions investigated, the decrease in the growth rate was linked with a higher relative contribution of the G2+M phase. The other phases of the cell cycle, G1 and S, showed different patterns, depending on the type of limitation. All experiments showed a highly significant increase in the amount of biogenic silica per cell and per cell surface with decreasing growth rates. At low growth rates, the G2+M elongation allowed an increase of the silicification of the cells. This pattern could be explained by the major uptake of silicon during the G2+M phase and by the independence of this process on the requirements of the other elements. This was illustrated by the elemental ratios Si/C and Si/N that increased from 2- to 6-fold, depending of the type of limitation, whereas the C/N ratio decreased by 10% (E limitation) or increased by 50% (P limitation). The variations of the ratios clearly demonstrate the uncoupling of the Si metabolism compared with the C and N metabolisms. This uncoupling enabled us to explain that in any of the growth condition investigated, the silicification of the cells increased at low growth rates, whereas carbon and nitrogen cellular content are differently regulated, depending of the growth conditions.

Key index words: **carbon; cell cycle; diatom; elemental ratio; limitation; nitrogen; silicon; *Thalassiosira pseudonana***

Abbreviations: **BSi, biogenic silica; G1, gap phase; G2, gap phase; M, mitosis; S, replication of DNA**

The biochemical composition and the cell cycle regulation of microalgae are strongly influenced by the growth environment. The relationship between the elemental composition of algae and their growth rate has been studied extensively with a special emphasis on carbon, nitrogen, and phosphorus content (Droop 1983). The elemental composition of a cell is a function of a number of variables, including nutrient supply, irradiance, day length, and temperature. In the diatom another element, silicon, has an important role in metabolism, because vegetative cell division cannot take place without valve formation of the daughter cells and cell growth cannot occur without girdle band formation (Crawford 1981, Volcani 1981, Pickett-Heaps et al. 1990). Several studies suggest that carbon and nitrogen metabolism are connected. The diel variation in nitrogen metabolism is coordinated with photosynthesis or with some product of photosynthesis (Turpin et al. 1988, Turpin 1991, Vergera et al. 1998), whereas Si metabolism is linked to the cell cycle and not directly to photosynthesis (Brzezinski 1992, Brzezinski and Conley 1994, Martin-Jézéquel et al. 2000). The metabolism of carbon and nitrogen in diatoms and the C and N quotas under various limitations and for different growth rates were described (Perry 1976, Darley 1977, Laws and Bannister 1980, Terry et al. 1985, Harrison et al. 1990, La Roche et al. 1993, Lynn et al. 2000, Guerrini et al. 2000), but none of these investigation included parallel studies of silicon content and regulation of the cell cycle.

The cell cycle is classically divided into four phases, G1, S, G2, and M. The DNA is replicated during the S phase, M corresponds to the period of mitosis and cell division, and G1 and G2 refer to "gaps" in the cycle during which most of the cell growth takes place (Mitchison 1971). Silicon uptake and deposition appear to be associated with the formation of the new siliceous valves just before cell division and thus seems to be mainly confined to the G2 and M period between cytokinesis and daughter cell separation (Sullivan and Volcani 1981, Sullivan 1986, Hildebrand 2000). However, part of nitrogen metabolism is also regulated dur-

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ing the course of the cell cycle (Hildebrand and Dahlin 2000). Studies have shown arrest points in G1 and G2 under nitrogen, silicate, and light starvation (Vaulot et al. 1986, 1987, Brzezinski et al. 1990), and one study for the diatom *Thalassiosira weissflogii* described the effect of nitrogen and light limitation on the cell cycle stages (Olson et al. 1986). The effect of phosphorus on the cell cycle in microalgae remains poorly known, and there is no study of cell cycle regulation in diatoms under phosphorus limitation.

Thus, a complex relationship links diatom elemental composition and cell cycle to growth conditions. The objective of this study was to study the effect of the light (E), nitrogen (N), and phosphorus (P) limitation on cell cycle regulation and elemental composition, with special emphasis on silicon, carbon, and nitrogen cellular quotas of the marine diatom *Thalassiosira pseudonana* grown in continuous cultures at different growth rates.

MATERIALS AND METHODS

Culture conditions. *Thalassiosira pseudonana* Hasle and Heimdal (clone CCMP 1015) was obtained from the culture collection of Dr. R. R. L Guillard (Bigelow Laboratory, Booth Bay Harbor, Maine, USA). The cells were grown in continuous cultures in sterilized 1-L glass vessels at 19° C in artificial seawater (Harrison et al. 1980) modified with selenium addition at a final concentration of 10 nM and enriched as f/2 medium (Guillard and Ryther 1962, Guillard 1975). Cultures were constantly aerated with water-saturated sterile air to prevent limitation in CO_2 and cell sedimentation.

To obtain N and P limitation, initial culture medium contained 88 μM of nitrate or 4 μM of phosphate, respectively. The cultures were illuminated continuously with 150 μmol photons·m^{-2}·s^{-1} (400-W Philips HPIT E40, T. Philips Lighting, Eindhoven, The Netherlands). E-limited cultures were grown at 40 (0.12 d^{-1}), 70 (0.18 d^{-1}), 200 (0.36 d^{-1}), or 400 (0.60 d^{-1}) μmol photons·m^{-2}·s^{-1}. A constant cell density was maintained in cultures to ensure control of the growth rate by irradiance.

Samplings and analyses. The cultures were assumed to be in steady state after a minimum of five generation times at constant cell density. Sampling was carried out in triplicate.

Cell counts. Cell number and cell size were determined with a Coulter counter (model ZM) connected to a Coulter multisizer. Cell integrity was checked microscopically.

Cell cycle measurements. One milliliter of culture was fixed with 10 μL of paraformaldehyde (10%). After 15 min at room temperature, the sample was frozen at –80° C until analysis. Cellular DNA was stained with Pico Green (Molecular Probes) as described by Veldhuis et al. (1997). Cellular DNA content was measured on a Coulter Elite flow cytometer equipped with a coherent Inova 90 laser (excitation wavelength 488 nm; emission wavelength 520 nm). A minimum of 10,000 events was collected. Distribution of G1, G2+M, and S phases was analyzed using Mcycle software (Phoenix Flow Systems, San Diego, CA, USA). The percentage of each phase was multiplied by the growth rate to give the duration of each phase.

Biogenic silica (BSi). Ten milliliters of culture was filtered onto a polycarbonate filter (0.6 μm pore size, Millipore) and rinsed twice with Si-free medium. After filtration the filters were dried for 12 h at 60° C and stored at 4° C until further analysis (modified from Ragueneau and Tréguer 1994). BSi was digested during 5 days after adding 0.2 mL 2.9 M hydrofluoric acid to the filters, which were placed in a tightly sealed tube. Seven milliliters of Milli-Q water was added to stop digestion. The samples were subsequently diluted with Milli-Q water to permit silicic acid determination in the usual concentration range (0–20 μM). The hydrofluoric acid concentration in the final solution had to be lower than 0.002 M to avoid interference from F^- (Eggimann and Betzer 1976). Silicic acid concentration in the final solution was analyzed using the manual method of Mullin and Riley (1965) modified by Strickland and Parsons (1972).

C and N intracellular concentrations. Fifteen milliliters of culture was filtered onto glass fiber filters (Whatman GF/C, precombusted at 450° C for 4.5 h) and rinsed with 10 mL of artificial seawater. The filters were then oven dried at 60° C and analyzed for elemental C and N using a Carlo Erba NA-2500 elemental analyzer (Nieuwenhuize et al. 1994).

All functions were fitted in Sigmaplot 5.0 (SPSS Inc.) and tested using analysis of variance. The fitted functions were considered statistically significant at $P < 0.05$.

RESULTS

Cell cycle. For the three limitations investigated, a decrease in the growth rate was accompanied by an increase in the length of G1 and G2+M phases (Fig. 1). In all conditions, this increase of the G2+M phase was significant. The length of the G1 phase increased significantly with slower growing E- or P-limited cells. The same trend was observed for the G1 phase of N-limited cells, but the change was not significant. Under E- and N-limited conditions the length of the S phase was negatively related to the growth rate, and this pattern was only significant in the case of the E limitation. The opposite was found for P-limited cultures.

Under E- or N-limited culture conditions, a decrease in the growth rate caused a decrease in the percentage of the cell in G1 (Fig. 2). The percentage of cells in G1 decreased from 88% (μ = 0.60 d^{-1}) to 64% (μ = 0.12 d^{-1}) for E-limited cells. For N-limited cells the decrease of cells in the G1 phase was not significant due to the scatter in the data. Under P limitation, the percentage of cells in the G1 phase was not significantly affected and remained quite stable at 60%. The percentage of cells in G2+M increased for the three limitations when the growth rate decreased (Fig. 2). The percentage of cells in S phase remained constant under E and N limitation, whereas it decreased strongly in P-limited cells, from 56.0% at μ = 0.65 d^{-1} to 1.1% at μ = 0.08 d^{-1} (Fig. 2).

Our results show that culture conditions affected the cell cycle differently. Nonetheless, for all conditions tested in our study, the decrease in growth rate (thus an increase in the doubling time) led to an increase in the percentage and length of the G2+M phase as the major consequence of the regulation of the cell cycle in *T. pseudonana.*

BSi. Cells grown under E, N, or P limitation all showed an increase in the amount of BSi per cell and per cell surface at low growth rates (Table 1, Fig. 3). Under N limitation the cellular BSi ranged from 0.20 pmol·$cell^{-1}$ (μ = 0.60 d^{-1}) to 0.94 pmol·$cell^{-1}$ (μ = 0.20 d^{-1}) and under P limitation from 0.27 pmol·$cell^{-1}$ (μ = 0.65 d^{-1}) to 0.96 pmol·$cell^{-1}$ (μ = 0.08d^{-1}). The cell diameters did not change with growth rate, and cell volumes were comparable under P and N limitation (Table 1). Under E limitation the cell size was reduced and the amount of BSi per cell increased from 0.08 pmol·$cell^{-1}$ (μ = 0.60 d^{-1}) to 0.18 pmol·$cell^{-1}$

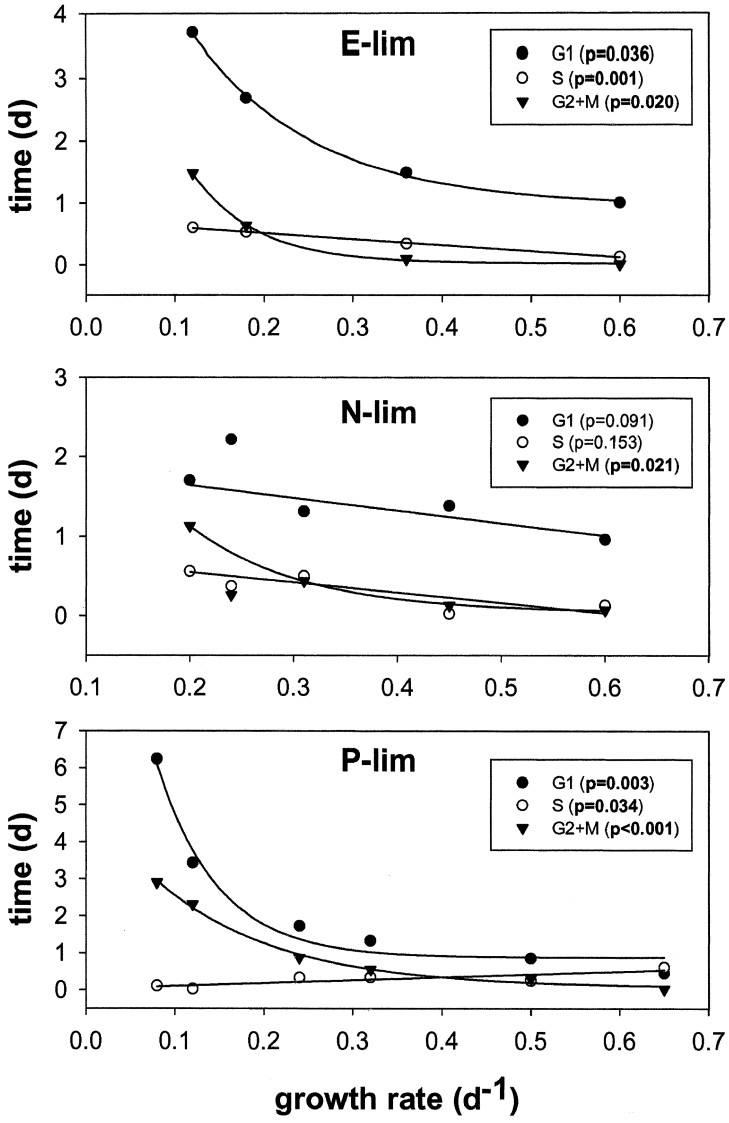

FIG. 1. Length of the different phases of the cell cycle as function of the growth rate under light (E-lim), nitrogen (N-lim), and phosphorus (P-lim) limitation. G1, interphase; S, DNA replication phase; G2, interphase; M: mitosis. Bold values are significant.

(μ = 0.18 d^{-1}). However, the quantity of BSi per cell surface unit followed the same pattern for the three culture conditions, ranging from 0.63 fmol·μm^{-2} for the highest growth rate to 2.96 fmol·μm^{-2} for the lowest. The BSi per cell surface increased linearly and significantly with slower growing cells (Fig. 3).

Carbon and nitrogen content. The carbon per cell was almost constant for E- and N-limited cultures (approximately 1.2 and 9.4 pmol·$cell^{-1}$, respectively), whereas it decreased significantly ($P = 0.005$) with growth rate under phosphorus limitation, from 25.3 pmol·$cell^{-1}$ at μ = 0.08 d^{-1} to 13.5 pmol·$cell^{-1}$ at μ = 0.65 d^{-1} (Table 1). The nitrogen cell quota showed a different pattern than the carbon quota (Table 1). It was relatively constant for E- and P-limited cultures (around 0.12 and 1.92 pmol·$cell^{-1}$, respectively), but it decreased significantly ($P = 0.028$) with growth rate under N limitation, from 0.71 pmol·$cell^{-1}$ (μ = 0.60 d^{-1}) to 0.50 pmol·$cell^{-1}$ (μ = 0.20 d^{-1}). The lower cellular concentrations of C and N, found under E-controlled conditions, were due to the smaller size of the cell compared with N and P limitation. For all growth conditions, the C and N content per unit cell volume followed the same trend as the cellular quotas (Table 1).

Redfield ratios. Under P and N limitation, the C/N ratio increased when the growth rate decreased, that is, when the intensity of the P or N limitation increased (Fig. 4a). The variations were larger for N limitation than for P limitation, from 11.5 (μ = 0.60 d^{-1}) to 20.4 (μ = 0.20 d^{-1}) and from 8.0 (μ = 0.50 d^{-1}) to 12.9 (μ = 0.08 d^{-1}), respectively (Fig. 4a). The C/N ratio decreased marginally (but significantly) under E limitation, from 11.6 (μ = 0.60 d^{-1}) to 9.8 (μ = 0.12 d^{-1}) (Fig. 4a). In all cases the C/N ratio was higher than the Redfield ratio of 6.6, and the C/N ratio obtained in N-limited cells was always higher than in E- or P-limited cells.

The Si/C ratio increased as growth rate decreased for all the culture conditions tested (Fig. 4b). This ra-

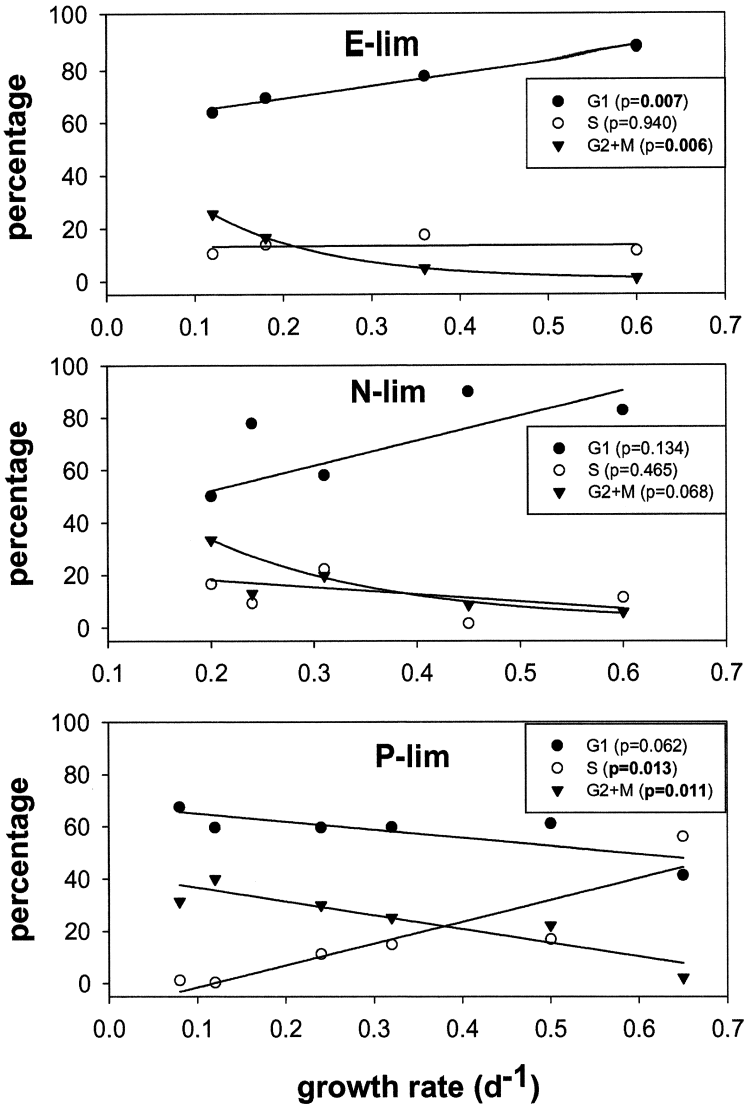

FIG. 2. Percentage of the different phases of the cell cycle as function of the growth rate under light (E-lim), nitrogen (N-lim), and phosphorus (P-lim) limitation. G1, interphase; S, DNA replication phase; G2, interphase; M, mitosis. Bold values are significant.

tio was highest under E limitation and increased from 0.07 (μ = 0.60 d^{-1}) to 0.16 (μ = 0.12 d^{-1}). Under N limitation it increased from 0.02 (μ = 0.60 d^{-1}) to 0.09 (μ = 0.20 d^{-1}), and for the P limitation the increase was limited, between 0.02 (μ = 0.65 d^{-1}) and 0.04 (μ = 0.08 d^{-1}).

Because the changes in Si content are larger than the changes in N content when the growth rate decreased (Table 1), the Si/N ratio also increased in all experiments (Fig. 4c). The largest variation was obtained with N limitation, from 0.28 (μ = 0.60 d^{-1}) to 1.87 (μ = 0.20 d^{-1}). For E- and P-controlled conditions, it increased from 0.87 (μ = 0.60 d^{-1}) to 1.62 (μ = 0.12 d^{-1}) and from 0.17 (μ = 0.65 d^{-1}) to 0.49 (μ = 0.08 d^{-1}), respectively. The Si/N and Si/C ratios were lowest for P-limited cells compared with E and N limitation. Hence, for the three culture conditions the increase in Si/C and Si/N ratios with low growth rates could be explained by the overriding influence of the large increase in silicification at low growth rates (Fig. 4, b and c).

DISCUSSION

Our experiments on *T. pseudonana* under N and E limitation showed a very similar regulation of the cell cycle. G1, S, and G2+M increased in duration at lower growth rates, and the percentage of cells in the G1 phase decreased with growth rate, whereas the percentage of cells in G2+M increased. The effects of E limitation on the duration of cell cycle phases are consistent with the findings of Olson et al. (1986) and Vaulot et al. (1986), who observed light-dependent segments in G1 and in G2 in *T. weissflogii*. The blocking of the cell cycle in G1 and G2 was shown only for diatoms. For all other groups of microalgae only the G1 phase was affected (Spudich and Sager 1980, Vau-

TABLE 1. Chemical compositions per cell and per cell volume for *Thalassiosira pseudonana* growing in continuous culture under light (E), nitrogen (N), and phosphorus (P) limitation.

Growth rate (d^{-1})	Elemental composition (pmol·cell^{-1})			Elemental composition (fmol·μm^{-3})			Cell volume (μm^{-3})
	Carbon	Nitrogen	Silicon	Carbon	Nitrogen	Silicon	
E-limited							
0.12	0.98 ± 0.12	0.10 ± 0.01	0.16 ± 0.02	11.39 ± 1.44	1.16 ± 0.15	1.86 ± 0.26	86.39
0.18	1.82	0.18	0.18 ± 0.01	21.77	2.21	2.22 ± 0.08	83.38
0.36	1.17 ± 0.01	0.11 ± >0.01	0.14 ± 0.03	15.23 ± 0.13	1.43 ± 0.05	1.83 ± 0.38	76.59
0.60	1.08 ± 0.02	0.09 ± >0.01	0.08 ± 0.01	13.84 ± 0.28	1.19 ± 0.04	1.03 ± 0.13	78.06
N-limited							
0.20	10.28 ± 0.12	0.50 ± 0.02	0.94 ± 0.03[a]	19.12 ± 0.22	0.93 ± 0.04	1.75 ± 0.05	537.91
0.24	7.51 ± 0.07	0.51 ± 0.05	0.58 ± 0.02[a]	13.98 ± 0.13	0.95 ± 0.10	1.08 ± 0.04	537.27
0.31	11.17 ± 0.25	0.60 ± 0.03	0.50 ± 0.03[a]	20.57 ± 0.46	1.10 ± 0.05	0.93 ± 0.05	542.83
0.45	9.93 ± 0.17	0.72 ± 0.01	0.27 ± 0.01[a]	19.04 ± 0.33	1.38 ± 0.01	0.52 ± 0.02	521.14
0.60	8.15 ± 0.31	0.71 ± 0.05	0.20 ± 0.10[a]	16.15 ± 0.62	1.41 ± 0.09	0.39 ± 0.18	504.57
P-limited							
0.08	25.32 ± 1.35	1.96 ± 0.08	0.96 ± 0.03[a]	46.02 ± 2.46	3.56 ± 0.11	1.75 ± 0.05	550.19
0.12	22.10 ± 0.21	1.84 ± 0.04	0.95 ± 0.07[a]	34.74 ± 0.33	2.88 ± 0.07	1.51 ± 0.11	636.24
0.24	20.19 ± 0.64	1.98 ± 0.08	0.62 ± 0.03[a]	36.45 ± 1.16	3.58 ± 0.14	1.13 ± 0.05	553.94
0.32	20.94 ± 0.38	1.84 ± 0.04	0.48 ± 0.02[a]	45.68 ± 0.82	4.02 ± 0.09	1.04 ± 0.05	458.44
0.50	18.70 ± 0.03	2.33 ± 0.10	0.39 ± 0.04[a]	35.59 ± 0.05	4.45 ± 1.19	0.74 ± 0.08	525.22
0.65	13.59 ± 0.38	1.61 ± 0.11	0.26 ± 0.06[a]	25.48 ± 0.72	3.01 ± 0.21	0.49 ± 0.11	532.97

[a]Data from Martin-Jézéquel et al. (2000).

lot et al. 1986), but Jacquet et al. (2001) showed very recently that different strains of *Prochlorococcus* are affected by light both in S or G2 phases. In the present study we observed a major nitrogen-dependent segment in the G2+M phase in *T. pseudonana* cultures. Olson et al. (1986) did not observe the same trend for N-limited *T. weissflogii*. They found an almost exclusive lengthening of the G1 phase by N limitation. Vaulot et al. (1987) observed also that nitrogen-starved cells were arrested in the G1 phase. These results imply that the G1 phase is more nitrogen dependent than the other phases in *T. weissflogii*.

However, differences of cell cycle regulation of *T. weissflogii* compared with *T. pseudonana* and other diatoms species were also observed by Brzezinski et al. (1990). In their work, *T. weissflogii* cells spend only a small proportion, 26%, of their cell cycle in G1 under optimal growth conditions, whereas *T. pseudonana* cells spend more than 80% of the cycle in the G1 phase. This aspect can certainly explain differences observed in the gap phases (G1 and G2) of these two species of *Thalassiosira*, in our results and in those of Olson et al. (1986) and Vaulot et al. (1986). The duration of the S phase remained constant in *T. weissflogii* under E-controlled conditions but increased under N limitation (Olson et al. 1986). Our study is in accordance with these results, because under E and N limitation the length of the S phase increased slightly with lower growth rate. Under P limitation, as with the other limitations, the percentage of cells of *T. pseudonana* in the G2+M phase increased at the low growth rates.

To our knowledge there are no other studies on the effect of P limitation on cell cycle regulation in diatoms. In *Prochlorococcus* spp., the arrest of cells in the S phase under P limitation is the opposite of what we observed in our study (Parpais et al. 1996). In general, the deprivation of a nutrient arrests cells in the G1 phase (Murray and Hunt 1993). This general feature does not seem to apply to cell cycle regulation in diatoms (Vaulot et al. 1986, 1987, Brzezinski et al. 1990, this study). All deprivations we investigated led to a strong correlation with the elongation of the G2+M phase and an increase of the percentage of cells in this phase. In contrast, the G1 phase tended to represent a higher percentage only under phosphorus limitation.

In the present study, the quantity of BSi found for the N-and P-limited cultures varied between 0.20 and 0.96 pmol Si·cell^{-1}. These values are higher than the concentrations usually observed for *T. pseudonana* (between 0.06 and 0.21 pmol Si·cell^{-1}; Paasche 1980, Brzezinski 1985). These Si cell quotas are also higher than

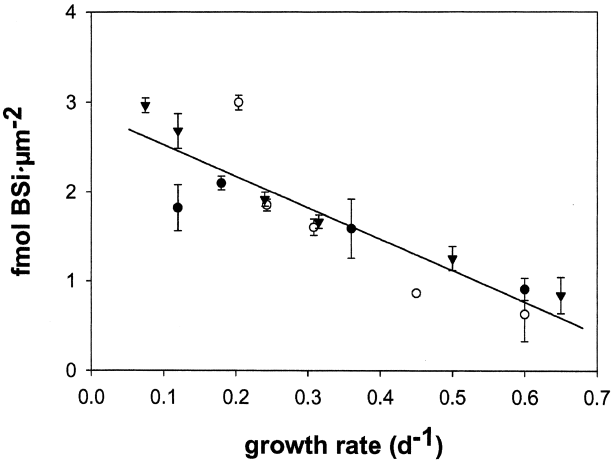

FIG. 3. Silica cell content (Bsi) per cell surface as a function of growth rate under light (●), nitrogen (○), and phosphorus (▼) limitation. Linear regression for the three limitations ($y = 2.87–3.52x$, $r^2 = 0.78$, $P < 0.001$).

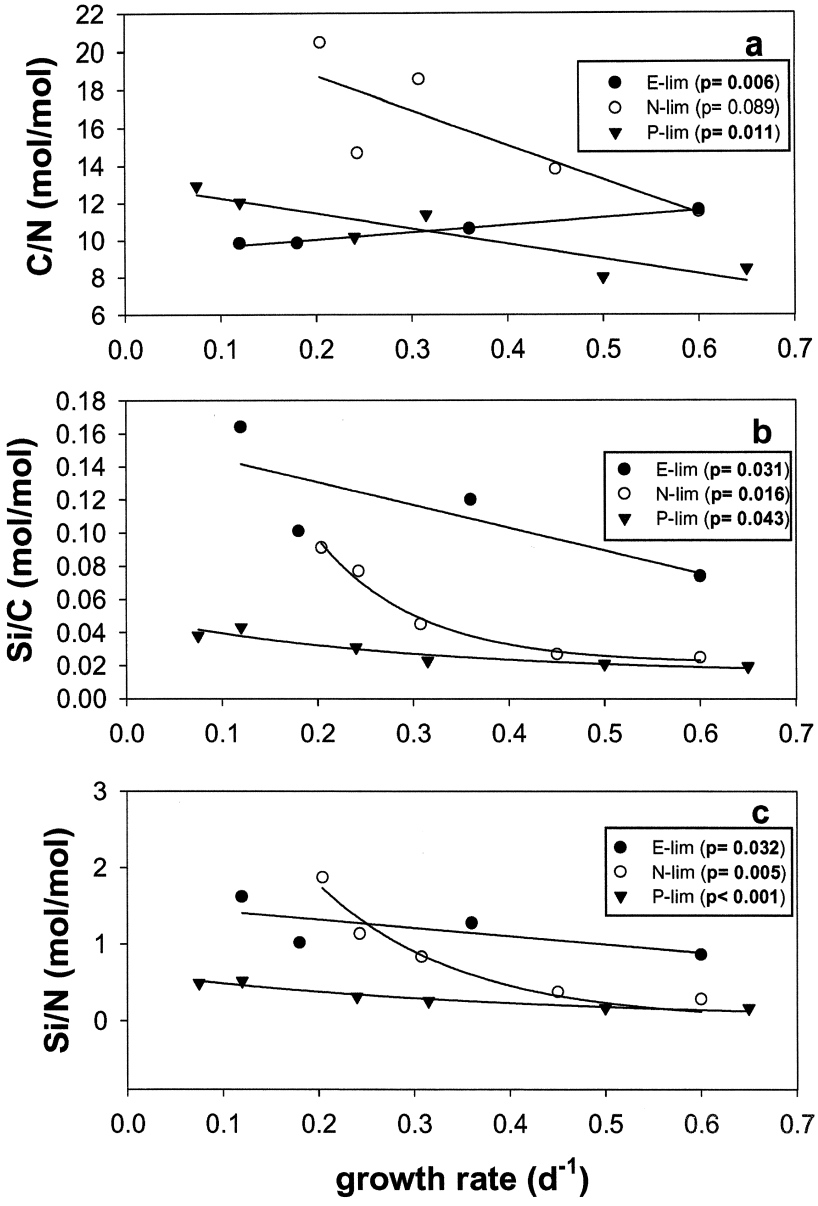

FIG. 4. Elemental ratios as a function of the growth rate under light (E-lim), nitrogen (N-lim), and phosphorus (P-lim) limitation. Bold values are significant.

the quotas obtained in E-limited cultures. These high concentrations can be explained by an increase in cell size, which we observed in P- and N-limited cultures. At low growth rates, the BSi per cell and per cell surface increased for all limiting conditions. The BSi per cell increased by a factor of 2 under E control and by almost a factor of 4 for P and N limitations. These results are comparable with literature data, which show the amount of BSi per cell was higher when cultures were grown at a low growth rates (reviewed by Martin-Jézéquel et al. 2000). The BSi per cell surface is a good indicator of frustule thickness. The highly significant increase in frustule thickness for the three limitations ($P < 0.001$) suggests that frustule thickness is mainly controlled by the growth rate. This result could be explained by the links between the cell cycle and the silicon metabolism (Martin-Jézéquel et al. 2000). Silicate uptake is closely related to the G2+M phase (Brzezinski 1992). During this phase, synthesis of the silicic acid transporters takes place (Hildebrand et al. 1997, Del Amo and Brzezinski 1999, Hildebrand 2000) and new valve formation occurs. Moreover, the processes that control silica deposition are driven by species-specific sets of polyamines and a few silica-precipitating proteins (Kröger et al. 1999, 2000) to which Si is added by an energetically cheap process linked to respiratory metabolism (Raven 1983, Martin-Jézéquel et al. 2000). As a consequence, the energy demand of the uptake and assimilation processes is low. On this basis, we hypothesize that as the cell takes up silicic acid, assimilation into the frustule could occur. The present study shows under every growth condition investigated that an increase of the length of the G2+M phase at low growth rate entails an increase of the silicification,

and this process is emphasized by the increase of the percentage of the G2+M phase. Thus, it appears that cellular silicon content and frustule thickness are regulated by the total amount of Si taken up, which is directly driven by the length of the cell cycle. Hence, the silicon content variations do not seem to be linked to the type of the limitation but to the intensity of the limitation. Obviously, these results cannot be obtained under silicon limitation, and in this case the cellular amount of BSi decreases with lower growth rate (Martin-Jézéquel et al. 2000). This regulation is linked to the complex control of the silicon uptake and assimilation by the internal silicon pools (Conway et al. 1976, Conway and Harrison 1977, Hildebrand 2000) and probably not by the length of the phases during the cell cycle.

In our experiments neither C nor N cellular quota showed a close trend with the cell cycle phases, as the BSi did. Hildebrand and Dahlin (2000) showed in *C. fusiformis* that the nitrate transporter mRNA level is higher in early G1 phase and decreases during the remainder of the G1 phase and after mitoses. However, the uptake of nitrogen is uncoupled from assimilation, which depends on the regulation of different processes (Collos and Slawyk 1976, Cresswell and Syrett 1979, Dortch et al. 1979, Collos 1982, Raimbault and Mingazzini 1987, Boyd and Gradmann 1999). Therefore, even if the nitrate uptake is linked to the cell cycle, the present study shows that the length of phase variations have no effect on the global metabolism of the nitrogen because the cellular N content did not change significantly with growth rate under either E or P limitation. The C and N elemental composition of *T. pseudonana* is mainly dependent on which factor limits growth. As shown in the work of Laws and Bannister (1980) on *T. fluviatilis* (i.e. *T. weissflogii*) and Perry (1976) on *T. pseudonana*, in P-limited conditions we observed that C per cell increased under lower growth rates and that N per cell or per cell volume remained relatively stable and was higher than in all other culture conditions. These results are probably due to the accumulation of storage products (carbohydrates and lipids) and the relative stable protein level (Lynn et al. 2000, Guerrini et al. 2000). In contrast to phosphorus, N limitation entails a decrease of total protein (Darley 1977, Harrison et al. 1990, La Roche et al. 1993, Lynn et al. 2000, Guerrini et al. 2000) that lowered the N content of *T. pseudonana*. Under N limitation, the accumulation of carbohydrate and triglycerides could occur (Harrison et al. 1990). However, in our study C per cell remained almost constant, and the same was observed for *T. fluviatilis* (i.e. *T. weissflogii*) (Laws and Bannister 1980). Under light-controlled conditions, we observed a rather stable cellular N content, as did Harrison et al. (1990) for *T. pseudonana*. In contrast, Laws and Bannister (1980) observed a decrease of C and N per cell with lower light-limited growth rates.

Our results of C/N ratios are in accordance with literature data for diatoms that showed different patterns depending on E, P, or N limitation (Eppley and Renger 1974, Perry 1976, Laws and Wong 1978, Laws and Bannister 1980, Terry et al. 1985) and for microalgae in general (Goldman 1980). Under N limitation, the C/N ratio increased mainly at low growth rates because the N content decreased, whereas the C content remained fairly stable. Under P limitation, the C/N ratio increased slightly with lower growth rates, but in this case it was due to an increase in C per cell and a stable N content per cell. The C/N ratio was weakly affected by the variation of the light intensity. In contrast, Si/C and Si/N ratios followed the same pattern for E, N, and P conditions, with a 2- to 6-fold increase in each case. These patterns are largely due to the changes in BSi content at different growth rates. These results are in accordance with previous studies. Brzezinski (1985) showed in numerous clones of *C. curvisetum* that the Si/C and the Si/N ratios are, respectively, 2 and 1.6 times higher in low irradiance than in high irradiance. In *S. costatum*, Davis (1976) observed a 1.7-fold increase in the Si/N ratio under light limitation.

The ratios of the main elements (C, N, and Si) are representative of the interaction of their metabolisms, and our study shows that the C/N ratio is regulated differently in comparison with the Si/C and Si/N ratios. The relatively small variation of the C/N ratio (10% under E limitation and 50% under P limitation) illustrates the coupling between C and N metabolism, in contrast to the high range of variation of the Si/C and Si/N ratios, which reflect the uncoupling of Si content to the other cellular elements. The physiological patterns observed in the present study clearly demonstrate that the mechanisms regulating Si assimilation are different from those controlling assimilation of N and C. This can be explained by the energetic contribution from photosynthesis and the processes of amino acids and proteins synthesis that link the N and C metabolisms (Syrett 1981, Turpin 1991, Huppe and Turpin 1994), in contrast with the low energetic requirement of Si metabolism and the control of silicon uptake during the cell cycle, although the constitution of the frustule is connected to the protein metabolism for the synthesis of the silicate uptake carriers and the organic matrix (Sullivan and Volcani 1981, Martin-Jézéquel et al. 1997, 2000). These differences between Si metabolism and C and N metabolism enable us to explain that, despite growth limitation, silicification of cells increases at low growth rates. Our main finding is that the pattern of Si cellular content is not dependent on the type of limitation and allows us to infer the growth rate from the frustule thickness (i.e. BSi per cell surface) for *T. pseudonana*. As a secondary finding, this uncoupling of Si relative to C and N could control the Redfield ratio in diatoms.

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Boyd, C. M. & Gradmann, D. 1999. Electrophysiology of the marine diatom *Coscinodiscus wailesii* III. Uptake of nitrate and ammonium. *J. Exp. Bot.* 50:461–70.

Brzezinski, M. A. 1985. The Si:C:N ratio of the marine diatoms: interspecific variability and the effect of some environmental variables. *J. Phycol.* 21:347–57.

Brzezinski, M. A. 1992. Cell-cycle effects on the kinetics of silicic acid uptake and resource competition among diatoms. *J. Plankton Res.* 14:1511–39.

Brzezinski, M. A. & Conley, D. J. 1994. Silicon deposition during the cell cycle of *Thalassiosira weissflogii* (Bacillariophyceae) determined using rhodamine 123 and propidium iodide staining. *J. Phycol.* 30:45–55.

Brzezinski, M. A., Olson, R. J. & Chisholm, S. W. 1990. Silicon availability and cell-cycle progression in marine diatoms. *Mar. Ecol. Prog. Ser.* 67:83–96.

Collos, Y. & Slawyk, G. 1976. Significance of cellular nitrate content cultures. *Mar. Biol.* 34:27–32.

Collos, Y. 1982. Transient situations in nitrate assimilation by marine diatoms. III. Short-term uncoupling of nitrate uptake and reduction. *J. Exp. Biol. Ecol.* 62:285–95

Conway, H. L. & Harrison, P. J. 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation. IV. Transient response of *Chaetoceros debilis*, *Skeletonema costatum* and *Thalassiosira gravida* to a single addition of the limiting nutrient. *Mar. Biol.* 43:33–43.

Conway, H. L., Harrison, P. J. & Davies, C. O. 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient. *Mar. Biol.* 35:187–199.

Crawford, R. M. 1981. The siliceous components of the diatom cell wall and their morphological variation. *In* Simpson, T. L. & Volcani, B. E. [Eds.] *Silicon and Siliceous Structures in Biological Systems.* Springer-Verlag, New York, pp. 129–56.

Cresswell, R. C. & Syrett, P. J. 1979. Ammonium inhibition of nitrate uptake by the diatom *Phaeodactylum tricornutum. Plant Sci. Lett.* 14:321–25.

Darley, W. M. 1977. Biochemical composition. *In* Werner, D. [Ed.] *The Biology of Diatoms. Botanical Monographs.* Vol 13. University of California Press, Berkeley, pp. 198–223.

Davis, C. O. 1976. Continuous culture of marine diatoms under silicate limitation. II. Effect of light intensity on growth and nutrient uptake of *Skeletonema costatum. J. Phycol.* 12:291–300.

Del Amo, Y. & Brzezinski, M. A. 1999. The chemical form of dissolved Si taken up by marine diatoms. *J. Phycol.* 35:1162–70.

Dortch, Q., Ahmed, S. I. & Packard, T. T. 1979. Nitrate reductase and glutamate dehydrogenase activities in *Skeletonema costatum* as measures of nitrogen assimilation rate. *J. Plankton Res.* 1:169–86.

Droop, M. R. 1983. 25 years of algal growth kinetics: a personal view. *Bot. Mar.* 26:99–112.

Eggimann, D. W. & Betzer, P. R. 1976. Decomposition and analysis of refractory suspended material. *Anal. Chem.* 48:1886–90.

Eppley, R. W. & Renger, E. H. 1974. Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture. *J. Phycol.* 10:15–23.

Goldman, J. C. 1980. Physiological processes, nutrient availability and the concept of relative growth rate in marine phytoplankton ecology. *In* Falkowski, P. G. [Ed] *Primary Productivity in the Sea.* Plenum Press, New York, pp. 179–94.

Guerrini, F., Cangini, M., Boni, L., Trost, P. & Pistocchi, R. 2000. Metabolic responses of the diatom *Achnanthes brevipes* (Bacillariophyceae) to nutrient limitation. *J. Phycol.* 36:882–90.

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. *In* Smith, W. L. & Chanley, M. H. [Eds.] *Culture of Marine Invertebrate Animals.* Plenum Press, New York, pp. 26–60.

Guillard, R. R. L. & Ryther, J. H. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* 8:229–39.

Harrison, P. J., Thompson, P. A. & Calderwood, G. S. 1990. Effects of nutrient and light limitation on the biochemical composition of phytoplankton. *J. Appl. Phycol.* 2:45–56.

Harrison, P. J., Waters, R. E. & Taylor, F. J. R. 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *J. Phycol.* 16:28–35.

Hildebrand, M. 2000. Silicic acid transport and its control during cell wall silicification in diatoms. *In* Bauerlein, E. [Ed.] *Biomineralization of Nano-and Micro-Structures.* Wiley-VCH, Weinheim, Germany, pp. 171–88.

Hildebrand, M. & Dahlin, K. 2000. Nitrate transporter genes from the diatom *Cylindrotheca fusiformis* (Bacillariophyceae): mRNA levels controlled by nitrogen source and by the cell cycle. *J. Phycol.* 36:702–13.

Hildebrand, M., Volcani, B. E., Gassman, W. & Schroeder, J. I. 1997. A gene family of silicon transporters. *Nature* 385:688–9.

Huppe, H. C. & Turpin, D. H. 1994. Integration of carbon and nitrogen metabolism in plant and algal cells. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45:577–607.

Jacquet, S., Partensky, F., Marie, D., Casotti, R. & Vaulot, D. 2001. Cell cycle regulation by light in *Prochlorococcus* strains. *Appl. Env. Microbiol.* 67:782–90.

Kröger, N., Deutzmann, R. & Sumper, M. 1999. Plycationoc peptides from diatom biosilica that direct silica nanosphere formation. *Science* 286:1129–32.

Kröger, N., Deutzmann, R., Bergsdorf, C. & Sumper, M. 2000. Species-specific polyamines from Diatoms control silica morphology. *Proc. Natl. Acad. Sci. USA* 97:14133–8.

La Roche, J., Geider, R. J., Graziano, L. M., Murray, H. & Lewis, K. 1993. Induction of specific protein in eukaryotic algae grown under iron-, phosphorus-, or nitrogen-deficient conditions. *J. Phycol.* 29:767–77.

Laws, E. A. & Wong, D. C. 1978. Studies of carbon and nitrogen metabolism by three marine phytoplankton species in nitrate limited continuous culture. *J. Phycol.* 14:406–16.

Laws, E. A. & Bannister, T. T. 1980. Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phytoplankton growth in the ocean. *Limnol. Oceanogr.* 25:457–73.

Lynn, S. G., Kilham, S. S., Kreeger, D. A. & Interlandi, S. J. 2000. Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). *J. Phycol.* 36:510–22.

Martin-Jézéquel, V., Daoud, N. & Quéguiner, B. 1997. Coupling silicon, carbon and nitrogen metabolisms in marine diatoms. *In* Dehairs, F., Elskens M. & Goyens, L. [Eds.] *Integrated Marine System Analysis.* Université de Bruxelles, Bruxelles, pp. 65–83.

Martin-Jézéquel, V., Hildebrand, M. & Brzezinski, M. A. 2000. Silicon metabolism in diatoms: implications for growth. *J. Phycol.* 36:821–40.

Mitchison, J. M. 1971. *The Biology of the Cell.* Cambridge University Press, Cambridge, 313 pp.

Mullin, J. B. & Riley, J. P. 1965. The spectrophotometric determination of silicate—silicon in natural waters with special reference to sea water. *Anal. Chim. Acta* 46:491–501.

Murray, A. & Hunt, T. 1993. *The Cell Cycle: An Introduction.* Freeman W.H. & Company (Eds.), NY, 251 pp.

Nieuwenhuize, J., Maas, Y. E. M. & Middelburg, J. J. 1994. Rapid analyses of organic carbon and nitrogen in particulate materials. *Mar. Chem.* 44:217–24.

Olson, R. J., Vaulot, D. & Chisholm, S. W. 1986. Effects of environmental stresses on the cell cycle of two marine phytoplankton species. *Plant Physiol.* 80:918–25.

Paasche, E. 1980. Silicon content of five marine plankton diatom species measured with a rapid filter method. *Limnol. Oceanogr.* 25:474–80.

Parpais, J., Marie, D., Partensky, F., Morin, P. & Vaulot, D. 1996. Effect of phosphorus starvation on the cell cycle of the photosynthetic prokaryote *Prochlorococcus* spp. *Mar. Ecol. Prog. Ser.* 132:265–74.

Perry, M. J. 1976. Phosphate utilization by oceanic diatom in phosphorus-limited chemostat culture and in the oligotrophic waters of the central North Pacific. *Limnol. Oceanogr.* 21:88–107.

Pickett-Heaps, J., Schmid, A. M. M. & Edgar, L. A. 1990. The cell biology of diatom valve formation. *Prog. Phycol. Res.* 7:1–168.

Ragueneau, O. & Tréguer, P. 1994. Determination of biogenic silica in coastal waters: applicability and limits of alkaline digestion method. *Mar. Chem.* 45:43–51.

Raimbault, P. & Mingazzini, M. 1987. Diurnal variations of intracellular nitrate storage by marine diatoms: effects of nutritional state. *J. Exp. Mar. Biol. Ecol.* 112:217–32.

Raven, J. A. 1983. The transport and function of silicon in plants. *Biol. Rev.* 58:179–207.

Spudich, J. L. & Sager, R. 1980. Regulation of the *Chlamydomonas* cell cycle by light and dark. *J. Cell Biol.* 85:136–45.

Strickland, J. D. H. & Parsons, T. R. 1972. A practical handbook of water analysis. *Bull. Fish. Res. Bd. Can.* 167:310 pp.

Sullivan, C. W. 1986. Silicification by diatoms. *In* Evered, D. & O'Connor, M. [Eds.] *Silicon Biochemistry.* Ciba foundation Symposium 121. Wiley Interscience, Chichester, pp. 59–89.

Sullivan, C. W. & Volcani, B. E. 1981. Silicon in the cellular metabolism of diatoms. *In* Simpson, T. L. & Volcani, B. E. [Eds.] *Silicon and Siliceous Structures in Biological Systems.* Springer-Verlag, New York, pp. 15–42.

Syrett, P. J. 1981. Nitrogen metabolism of microalgae. *In* Platt, T. (Ed.). *Physiological Bases of Phytoplankton Ecology. Can. Bull. Fish. Aquat. Sci.* 210:182–210.

Terry, K. L., Hirata, J. & Laws, E. A. 1985. Light-, nitrogen-, and phosphorus-limited growth of *Phaeodactylum tricornutum* Bohlin strain TFX–1: chemical composition, carbon partitioning, and the diel periodicity of physiological processes. *J. Exp. Mar. Biol. Ecol.* 86:85–100.

Turpin, D. H., Elrifi, I. R., Birch, D. G., Werger, H. G. & Holmes, J. J. 1988. Interactions between photosynthesis, respiration, and nitrogen assimilation in microalgae. *Can. J. Bot.* 66:2083–97.

Turpin, D. H. 1991. Effects of inorganic N availability on algal photosynthesis and carbon metabolism. *J. Phycol.* 27:14–20.

Vaulot, D., Olson, R. J. & Chisholm, S. W. 1986. Light and dark control of the cell cycle in two marine phytoplankton species. *Exp. Cell Res.* 167:38–52.

Vaulot, D., Olson, R. J., Merkel, S. & Chisholm, S. W. 1987. Cell-cycle response to nutrient starvation in two phytoplankton species *Thalassiosira weissflogii* and *Hymenomonas carterae. Mar. Biol.* 95:625–30.

Veldhuis, M. J. W., Cucci, T. L. & Sieracki, M. E. 1997. Cellular DNA content of marine phytoplankton using two new fluorochromes: taxonomic and ecological implications. *J. Phycol.* 33:527–41.

Vergera, J. J., Berges, J. A. & Falkowski, P. G. 1998. Diel periodicity of nitrate reductase activity and protein levels in the marine diatom *Thalassiosira weissflogii* (Bacillariophyceae). *J. Phycol.* 34: 952–61.

Volcani, B. E. 1981. Cell wall formation in diatoms: morphogenesis and biochemistry. *In* Simpson, T. L. & Volcani, B. E. [Eds.] *Silicon and Siliceous Structures in Biological Systems.* Springer-Verlag, New York, pp. 157–200.