Cell cycle implication on nitrogen acquisition and synchronization in ıt Thalassiosira weissflogii (Bacillariophyta)

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CELL CYCLE IMPLICATION ON NITROGEN ACQUISITION AND SYNCHRONIZATION IN *THALASSIOSIRA WEISSFLOGII* (BACYLLARIOPHYCEAE).[1]

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Abstract:

The Michaelis-Menten model of nitrogen acquisition, originally used to represent the effect of nutrient concentration on the phytoplankton uptake rate, is inadequate when other factors show temporal variations. Literature generally links the diurnal oscillations of nitrogen acquisition to a response of the physiological status of microalgae to the photon flux density and to the substrate availability. This work aims at analyzing how the cell cycle also constitutes a significant determinant of nitrogen acquisition and, where appropriate, at assessing the impact of this property at the macroscopic level. For this purpose, we carried out continuous culture experiments of the diatom *Thalassiosira weissflogii* (Grunow) Fryxell & Hasle exposed to various conditions of light and nitrogen supply.

Results revealed a decrease in nitrogen acquisition when a significant proportion of the population is in mitosis. This observation suggests that the nitrogen acquisition is incompatible with mitosis and thus is not continuous during the cell cycle. In addition, environmental conditions, such as the light signal and the nutrient supply, interfere with the cell cycle at the individual level, and finally affect the synchrony of the population.

Keywords: cell cycle, microalgae, mitosis, nitrogen acquisition, physiological status, phytoplankton, synchronization, *Thalassiosira weissflogii*

List of abbreviations:

C	carbon
CH	carbohydrates
Chla	chlorophyll *a*
CL	constant light
CL	experiment under constant illumination
DIC	dissolved inorganic carbon
DMSO	dimethylsulfoxide
G1	cell cycle stage "G1"
G2	cell cycle stage "G2"
G2/M	undissociated period of the cell cycle between S and division
LD	light/dark signal
Llim	experiment with reduced light supply
ML	experiment simulating mixed layer illumination
Msignal	mitosis signal (see table 2)
N	nitrogen
NH_4^+	ammonium
Nlim	experiment with reduced nitrogen supply
NO_2^-	nitrite
NO_3^-	nitrate
PAR	photosynthetically available radiation
PFD	photon flux density
pG1	percentage of cells in G1

pré-ML	acclimation period prior to *ML* experiment
S	cell cycle stage "S"
ΔG1	daily amplitude of variation in pG1 (in percentage points)

Introduction

The fact that N can limit primary production over large oceanic areas triggered numerous experimental and field studies. Pioneering works of Caperon (1965, 1967), Dugdale (1967) and Eppley et al. (1969) established that N acquisition generally displays a monophasic saturating function of external nutrient concentration. Known as the Michaelis-Menten equation (Caperon and Meyer 1972, MacIsaac and Dugdale 1972, Sarthou et al. 2005), this function can represent the acquisition of the major forms of inorganic (i.e. nitrate – NO_3^-, ammonium – NH_4^+) and organic N (urea, amino acids) by phytoplankton. The physiological understanding is that the rate of nutrient acquisition increases with the substrate concentration until saturation of active transporters. Nevertheless, further studies showed that N acquisition is a much more complicated process. When substrate is supplied at high concentrations (Collos et al. 2005), or when multiple forms of inorganic nitrogen are available simultaneously (MacIsaac and Dugdale 1972, Lomas and Glibert 1999), N acquisition cannot be reduced to a single saturating relationship of the external nutrient concentration. In addition, N acquisition is also dependent of light. Indeed, energy and reducing power provided by photosynthesis are necessary for active transport of NO_3^- and its subsequent reduction to NH_4^- (Turpin et al. 1988, 1991, Lim et al. 2006). It remains that N acquisition can be temporally decoupled from light (Eppley and Coatsworth 1966, Laws and Wong 1978, Needoba and Harrison 2004) when reducing power can be supplied by the catabolism of neutral lipids or carbohydrates accumulated during photosynthesis (Vanlerberghe et al. 1992, Cuhel et al. 1984).

The present study aims at demonstrating whether the cell cycle also interferes with N acquisition. The cell cycle is a one-way sequence of scheduled steps that cells must complete before dividing. In a typical cycle, cells enter, after division, in a somatic growth stage called "G1". Then, they synthesize DNA during the "S" stage, and prepare for division during the

"G2" stage. Mitosis completes the cycle, with the formation of two daughter cells. Transitions between stages are controlled by nutritional and energy status checkpoints (Chisholm et al 1986), ensuring a cell proceeds to division only when it is mature (Vaulot 1994). At a population scale, these chekcpoints can synchronize cells on a periodic signal of light or nutrient supply, and consequently extend the impact of the cell cycle at the macroscopic level (Vaulot 1994, Vaulot and Marie 1999, Binder and Durand 2002).

Despite the recognized importance of the cell cycle on microalgae physiology, interactions with photosynthesis and N acquisition are not well understood. The difficulty arises mainly from the fact that a cell cycle synchronized on a natural illumination (light/dark signal, or LD) impacts the metabolism on the same period as the light pattern itself, so it is difficult to distinguish both effects. For example, Gerath and Chisholm (1989) suggested that the diurnal photosynthesis activity in the dinoflagellate *Amphidinium carteri* was only linked to the light signal, whereas Claquin et al. (2004) showed that the diatom *Cylindrotheca fusiformis* exhibited greater photosynthetic capacity in G1. As well, Bruyant et al. (2005) suggested that the diurnal oscillations of the photosynthetic parameters in *Prochlorochoccus* were determined both by photoacclimation and cell cycle. Still, some studies emphasized the role of the cell cycle in the nutrient uptake process. Mary et al (2008) showed that the amino acid uptake by *Prochlorochoccus* increased during the G2/M stage, possibly to boost protein synthesis prior to division. Conversely, Chisholm and Stross (1976) observed a decrease in phosphate affinity in late G2/M stage in *Euglena gracilis*, but in this case the authors related the uptake depression to the light cycle rather than to the cell cycle. Then, the increasing silicification observed in lengthening the G2 stage of *Thalassiosira spp.* (Brzezinski and Conley 1994, Claquin et al. 2002) suggests the involvement of the cell cycle in the silica metabolism. Lastly, Hildebrand and Dahlin (2000) suggested that the diurnal variations of N acquisition observed in different studies could not be due to the LD cycle only, but also to the

transcription of the gene encoding the NO_3^- transporters, the latter being related to the cell cycle. Yet, very few studies have been carried out on the relationship between the cell cycle and N acquisition.

The goal of the present study is to evaluate the impact of the cell cycle on the N acquisition of microalgae. Our main task is to determine, for given conditions of light and nutritional status, to what extent the N acquisition of the diatom *Thalassiosira weissflogii* (Grunow) is related to its cell cycle or to its physiological status (nutritional and energetic storages). The potential interference of mitosis on the N acquisition was a particular focus.

Methods

Experimental design

The device - Laboratory experiments were conducted with the marine diatom *Thalassiosira weissflogii* (Grunow) obtained from the CCMP (Bigelow Laboratory for Ocean Science, USA). The culture vessels consisted of water jacketed 2-liter cylinders connected to a circulating water bath that maintained a constant temperature (22 ± 0.05°C). The growth medium was prepared using 0.22-µm Millipore filtered and autoclaved (105°C for half an hour) seawater and nutrient enrichments according to f/2 formulation (Guillard and Ryther 1962), except for NO_3^- which was added separately to achieve a final concentration of 200 $\mu mol.L^{-1}$. The same stock of seawater, collected off the coast of Villefranche-sur-mer (France), was used over the whole experiment. After cooling and sterile addition of nutrients, the medium was transferred with independent peristaltic pumps (Gilson III) to each culture vessel, through a 0.22-µm sterile filter (MediaKap-5, Microgon). The continuous cultures were operated either in turbidostat (N-replete) or chemostat mode (N-limited). The cultures were subjected to gentle continuous stirring and bubbling with sterile-filtered air, which

passed through a 0.1-µm Whatman filter and activated charcoal at a rate of 0.15 liter per minute. To prevent possible growth limitation by DIC consumption, pH was regulated online at a constant value of 8.0 ± 0.02, using an automatic computer-controlled device that monitored the bubbling of very small amounts of pure CO_2 into the cultures (for details see Sciandra et al. 2003). Illumination was supplied by 12 fluorescent lamps (Osram Dulux 12-950 55W, see Bruyant et al. 2001 for details) and the photon flux density (PFD) was electronically computer-controlled, allowing different patterns of light regime (see Table 1). Photosynthetically available radiation (PAR) was measured with a quantum scalar irradiance meter (QSL-100, Biospherical Instruments).

The experiments - Six experiments differing by the light regime and/or N status were conducted in duplicate parallel continuous cultures (Table 1). In the *control* experiment, algae were grown in N-replete conditions (turbidostat) under 12:12 light:dark (LD) cycles. The *Nlim* experiment was conducted with the same light conditions, but with a lower dilution rate to obtain N-limited cells (chemostat). *Llim1 and Llim2* experiments were conducted with the same nutrient conditions as in *control* but with a lower light dose. In the *CL* experiment, cultures previously acclimated to LD conditions were transferred to continuous illumination. Finally, conditions for the *ML* experiment were set to simulate theoretical variations of light received by a lagrangian cell moving in a mixed layer, as illustrated in Table 1. All experiments were first run for several days with the *control* conditions. All experiments, except *Nlim*, were conducted under N-replete conditions. To keep the same photon dose in the *ML* experiments as in the LD experiment while preventing photodamage in the former, cells were previously acclimated (pre-*ML*) with a lower PFD than in the *control* (Table 1) similar to Mara (1978, 1980). In the turbidostat cultures (N-replete conditions), the dilution rate was first set to zero to let the cell density reach approximately 1.10^8cells.L^{-1}, then the dilution rate was regularly adjusted to the previous 24 hour averaged growth rate. This level of algal

density combined with the NO_3^- concentration in the renewal medium (200µmol.L^{-1}) led to a residual NO_3^- concentration in the cultures below 150µmol.L^{-1}, for which the colorimetric measurement of NO_3^- was linear (see below). In chemostat mode (N-limited), the growth rate of the population equilibrated with the dilution rate.

Culture analyses

Nutrient measurements. Nitrite and nitrate (NO_3^-) concentrations were measured with a Technicon Auto-analyzer and an automated data acquisition system (Malara and Sciandra 1991). A dilution device added at the entrance of the chain measurement maintained a linear response of the colorimetric reaction for NO_3^- concentrations ranging from 0 to 150µmol.L^{-1}. Depending on the experiment, analyses were performed once or twice per hour.

Particle counting. The concentration of cells was measured with an optical particle counter using the principle of light attenuation (Hiac/Royco, Pacific Scientific Instruments). Before counting, cultures were diluted with an automated, computer-controlled system (Bernard et al. 1996). The cultures were analyzed 12 to 24 times a day, and each analysis consisted of triplicate measurements taken in rapid succession, whose coefficient of variation was less than 3%.

Determination of quotas: cellular carbon (C) & nitrogen (N), chlorophyll a *(Chl*a*) and carbohydrates (CH).* For cellular C and N determinations, duplicate samples of 10mL were filtered through glass fiber filters (Whatman GF/C) precombusted at 500°C, and analyzed with a PerkinElmer Series 2 2400 CHN analyzer. The replicates agreed typically to within 3 and 5% for C and N analyses respectively. The same sampling procedure was conducted for the other quotas, except for the sample volume of 5mL and the storage in liquid nitrogen. Pigments were extracted in a mixture of acetone and 6% of Dimethylsulfoxide (DMSO), and natural fluorescence of the Chla was measured by a Turner fluorometer. Concentration of

123 Chla was calculated by the modified method of Yentsch & Menzel (1963). CH were
124 measured by colorimetric reactions after reaction with phenol and sulfuric acid (Sun et al.
125 1984). Mean variation coefficients were less than 2% for Chla and 4% for CH.
126 *Cell cycle measurements*. Using an automatic device, 2mL of culture were fixed every
127 hour with 0.2mL of glutaraldehyde at 10% (final concentration of 1%) at room temperature.
128 Every 8 hours, samples were transferred to liquid nitrogen until analysis. Cellular DNA was
129 stained with SYBR-Green1 (Invitrogen) at 5% during 15mn. Relative cellular DNA content
130 was measured with a BectonDickinson FACSCalibur flow cytometer equipped with an argon
131 laser (Model 163A1202 Spectraphysics, USA; excitation wavelength 488nm, emission
132 wavelength 520nm). A minimum of 10^3 cells were analyzed per sample. A Matlab®
133 (Mathworks®) program has been developed to extract target information from cytometer files
134 and to fit two normal curves on the green fluorescence signal in order to determine the
135 proportions of cells in G1 and G2. Precise differentiation between G2 and M was not
136 possible, but M was assumed to be the final event of the G2+M period.
137
138 **Smoothing, derivative and rate computation**
139 The high frequency acquisitions performed for most of the measurements allow the
140 discrete raw data to be smoothed to obtain continuous time series. This procedure has two
141 main interests. First, the interpolation of the data series makes it continuous; allowing the
142 calculus of rates and the normalization of signals measured using distinct sample timing.
143 Secondly, smoothing the data series allows the identification of the relevant signal amongst
144 noise, but a case-by-case check has to be performed to avoid the creation or deletion of
145 information.
146 Division rate was computed by deriving cell number data series (see Table 2 and Fig.
147 1A). The net C fixation rate (see Table 2 and Fig. 1E and 1G) was computed from the POC

time variation. Instantaneous N acquisition rate were calculated from the temporal change of both concentration and PON in the culture,. In our experiments, these two rates were always well correlated (Fig. 2), so only the second will be shown in the following (see Table 2 and Fig. 1D). We also calculated the "mitosis signal" (Msignal) by deriving the percentage of cells in stage G1 (see Fig. 1B and Table 2). As explained previously, the mitosis stage was not experimentally distinguishable from the G2 phase because of a lack of change in cell DNA content. However, mitosis ends the G2/M phase, so we consider Msignal to be a proxy of the entry rate into G1 or exit rate from G2/M. Therefore, we think that Msignal provides good information on the proportion of cells in mitosis. This proxy can be very useful in transient periods as the trend is not related to the growth conditions, in contrast to the division rate. In the following, Msignal will be preferred to identify the mitosis event when daily averaged division rate vary in response to changing growth conditions. In all our experiments, the cross correlations between division rate and Msignal showed a maximum time lag of 2h.

Smoothing is a crucial step as it can significantly alter the shape of the derivative, depending on the chosen numerical scheme method. Savitsky-Golay spline estimation, cubic spline estimation or Gaussian convolution methods were distinctively applied to each data series as a function of their noise level and acquisition frequency. To illustrate the smoothing process, examples are given for the data acquired during the *control* experiment (Fig. 1). Confidence in the smoothing process has been assessed for each data series, especially concerning the number and position of the peaks. Confidence assessment involves checking the possible recurrence of the oscillations over days in the same conditions, the number of points involved in the peak formation and the comparison between signals. For example, the cell N data point of day 19.3, which seems abnormally high (Fig. 1E), induces a shouldering in the N assimilation rate which is not reflected by the N acquisition rate, and also triggers an unexpected value of the (NO_3^- +N)/Sin ratio (Fig. 1F). In contrast, the particular pattern of

division rate on day 18 (division in two peaks) is caused by only one measurement, but the same pattern is observed on the Msignal, which increases the confidence in this observation.

Results

Control experiment

The division rate of 1.4 d^{-1} reached by *Thalassiosira weissflogii* in non limiting growth conditions is consistent with the value reported by Clark et al. (2002) and Chisholm and Costello (1980). The daily pattern of the division rate – i.e. one major peak just before dusk and one or two others at night (Fig. 3A) – was also previously observed by Chisholm and Costello (1980). At steady state, the daily averaged division rate equaled the dilution rate (Table 3) as well as the specific N & C fixation rates. Cells can thus be considered in a physiological state of balanced growth. Percentage of cells in G1 stage (pG1) varied by 40 points around a mean value of 56% (Fig. 1B). Msignal peaks are globally concomitant with those of the division rate at more or less 2h (Fig. 1C). The facts that division rate was never null and that Msignal variation was less than 100 points mean that the population was not fully synchronized.

Acquisition of N occured essentially in the daylight (Fig. 3A), the nocturnal acquisition represented only 20% of the 1.7pmolN acquired per day. More precisely, acquisition started early in the daylight, increases until 2h before noon, and finally decreased down to midnight to reduced but significant rates (Fig. 3A). Both signals of N acquisition were well correlated (Fig. 1F and 2), and both were significantly correlated to the PFD (Fig. 3A and 7). C acquisition was strictly restricted to the daylight (Fig. 3A), and the night losses per cell represented 5-7% of the 13pmolC acquired in average per day. A cross-correlation analysis suggested that acquisitions of C and N were quite in phase, as the time lag between

197 the two rates was less than 2h. Following the different patterns observed for N and C
198 acquisitions during the LD cycle, the molar N/C ratio varied from 0.20 at night to 0.11 during
199 the day (Fig. 1H). Chla and CH contents increased in the daylight and decreased at night (Fig.
200 1I), following the pattern observed for C & N contents.

201 **Nitrogen limitation experiment (*Nlim*)**

202 Compared to the *control* experiment, the lower dilution rate of $0.69d^{-1}$ applied in this
203 experiment induced a N limitation reflected by lower values of the N/C ratio, N cell quota,
204 and division rate (Table 3).

205 Two Msignal peaks were observed per day just before dawn and dusk (Fig. 3B). Three
206 days after the onset of N limiting conditions, the amplitude of variation of the percentage of
207 cells in G1 (ΔG1) was 25 points lower than in the *control*. This observation indicated a
208 decrease in the population synchronicity. The daily averaged rate of N acquisition decreased
209 by about 80%, in accordance with the decrease in cell N (Table 3). In contrast to the *control*
210 experiment, N acquisition was not restricted to the daylight period, as diurnal and nocturnal
211 averaged rates did not differ significantly (Fig. 3B). Then, except during the second dusk, N
212 acquisition was low each time Msignal was high (Fig. 3B) and a cross correlation analysis
213 shows a time lag of 13h between the two signals. As expected, the net C fixation was strongly
214 related to the PFD and occured only during the daylight period (Fig. 3B). Finally, C loss rates
215 during the night phase were similar to those observed in the *control* experiment.

216 **Moderate light limitation experiment (*Llim1*)**

217 Following the initiation of light limitation, the daily average of the division rate
218 decreased by 55% compared to *control*, underlining the light limitation. Moreover, ΔG1

219 decreased by 25 points (Table 3), indicating a decrease in the synchronicity. However,
220 variations of Msignal remained strong, with a major peak in the late daylight period (Fig.3C)
221 The specific N acquisition rate was equal to the division rate (Table 3), indicating that
222 a steady state was reached from the second day of light limitation. The daily averaged N and
223 C acquisition rates decreased by 60% compared to *control* (Table 3), but kept the same 24h-
224 period pattern (Fig.3C). N was acquired essentially in the daylight, the dark acquisition being
225 only 1/10 of the diurnal one (Fig. 3C). According to a cross correlation analysis, N acquisition
226 and Msignal were shifted with a time lag of 4h (Fig. 3C).

227 **Severe light limitation experiment (*Llim2*)**

228 From the first day following the onset of new light conditions, the daily averaged
229 division rate dropped to $0.30d^{-1}$ and then stabilizes around $0.05d^{-1}$. At hourly scale, mitosis
230 periods occurred mainly at dusk (Fig. 3D). The ΔG1 decreased to 10pts (Table 3), denoting
231 again a partial desynchronization of the population.
232 During the first two days, the daily averaged N acquisition rate decreases by 60%
233 compared to *control* (Table 3), but daily variations were still clearly visible, and also phased
234 with C fixation (Fig. 3D). Most of the N acquisition occurred during the daylight period, the
235 dark part being only 1/10 of the diurnal one (Fig. 3D). N acquisition and Msignal appeared in
236 opposite phases, as a cross correlation analysis revealed a time lag of 12h between the two
237 signals (Fig. 3D).

238 **Continuous light experiment (*CL*)**

The continuous light (CL) regime and the increase in the light dose triggered an increase of the daily averaged division rate, which stabilized around 2.0d^{-1} (Table 3). Specific N and C acquisition rates were close to the division rate, meaning that steady state had been reached (Table 3). Despite the continuous light regime, significant daily variations of pG1 were still observed during 2 days of CL conditions (Fig. 3E). On the first day, Msignal shows a slightly larger peak than in *control*, centered on the first subjective dusk. Two important Msignal events occur the day following during the same interval. Finally, a major burst of Msignal was observed the 3rd day, during the dark restoration (Fig. 3E).

The higher daily photon dose applied in CL compared to *control* triggered a 40% increase in N acquisition per cell. This was accentuated at the population level by the large increase in cell numbers. At an hourly scale, there were conserved daily variations of N acquisition in CL (Fig. 3E). The periods of low N acquisition were globally associated with positive Msignal periods (Fig. 3E) and a maximum cross correlation between these two signals was obtained with a shift of 6h. Note that as soon as LD regime was applied again, diurnal variations of N acquisition resumed as in previous experiments (Fig. 3E). The C fixation pattern was different as it ceases to display clear daily variations after the first subjective night in CL (Fig. 3E). Finally, it is worth noting that the cell content of Chla continued to show daily variations in the CL conditions (Fig. 4A), whereas CH content per cell remained fairly constant after the first day under CL conditions (Fig. 4A).

Mixed layer simulation (*ML*)

The daily averaged division rate was equivalent in *pre-ML* (acclimation period) compared to *control* (Table 3), but dropped by 1/3 under *ML* conditions (Table 3). ΔG1 was reduced to 20percentage pointson the second day of *ML* conditions . After that, the signal of pG1 became too noisy to compute a relevant Msignal.

C and N acquisition rates varied according to the PFD: each peak of light triggered an enhancement of N and C acquisitions (Fig. 3F), which then stopped during the dark period. The cellular contents of Chla and CH increased during the 12h of daylight and especially during each light peak (Fig. 4B). Conversely, cell Chla and CH decreased at night, and also, though less markedly, during each dark period of the daylight phase.

Discussion

The Michaelis-Menten relationship is commonly used to calculate the uptake of a given nutrient from its concentration in the surrounding medium. However, it appears clearly inappropriate to represent the pattern of N acquisition in our experiments. N acquisition is not simply related to its external concentration (Fig. 5) when cells are grown under a LD regime and within a range of N concentrations well above the half saturation constant. In addition, the N acquisition rate showed a 24h cyclic pattern, with high and low values observed respectively during day and night. Thus, one or several parameters linked to the LD light regime should exert an additional control on the N acquisition. Most studies monitoring nutrient acquisition under LD signals explain the variation observed by the effect of the light dose on the physiological status (Chisholm and Stross 1976, Cochlan et al. 1991, Marsot et al. 1992, Clark et al. 2002), but to the best of our knowledge none examined the potential implication of the cell cycle.

This is a quite difficult task because the physiological status and the cell cycle are not independent properties. As the cell cycle is usually studied on populations synchronized by a diurnal light signal, it is not easy to discern whether the diurnal variation of one specific process results from the oscillation of the PFD or from the cell cycle, or from both factors. We have chosen to manipulate environmental conditions to tackle this issue. Thus, we

induced transient periods in changing growth conditions, which highlighted some phenomena that were unclear under balanced growth conditions.

Does the cell cycle influence nitrogen acquisition?

As expected, our results showed that C fixation rate correlates strongly with instantaneous PFD (Fig. 6) and that N acquisition occurs essentially during the light phase (Fig. 3). This suggests a relation between these two processes and the PFD, which agrees with previous results (Cochlan et al. 1991, Vincent 1992, Jauzein et al. 2008) obtained in culture or in *in situ* experiments. For N replete conditions and regular LD cycles, N acquisition measured during the day is positively related to the PFD (Fig. 7A), whereas the rate of dark acquisition, which is much lower than acquisition during the day, is related to the amount of energy stored as CH in the cell (Fig. 7B). Thus, our data show that N acquisition is clearly related to the energetic status of the cell.

Now, particular attention should be paid to the fact that the dispersion of the points around the relationship between acquisition and irradiance (Fig. 7A) is structured. The dispersion reflects a hysteresis phenomenon where the rates of N acquisition measured during the afternoon are, for the same PFD, systematically lower than those measured in the morning. These results show that the energetic status of the cell is not sufficient to fully explain the variations of the N acquisition at the daily time scale. The key point lies in the fact that low rates of N acquisition are all measured when a significant proportion of the population is in mitosis (see also Fig. 7B). Thus, these two processes – respectively devoted to somatic growth and cell division – seem mutually exclusives. The variation in the gene expression encoding NO_3^- transporter during the cell cycle reported in *Cylindrotheca fusiformis* is consistent with our observations, especially the low expression in the late G2/M phase reported by Hildebrand and Dahlin (2000). They also reported a variable expression of

the NO_3^- transporter gene between G1 and G2, but the expected consequence on N acquisition swa not visible in our results, possibly because of the incomplete synchronization of the population.

Our experiments, through the depression of N uptake observed when a significant part of the population is in mitosis stage suggests that these two processes, respectively devoted to somatic growth and cell division, are mutually exclusive, and cannot occur simultaneously during the cell cycle. This statement could become crucial at the population level when cells are synchronized and consequently exhibit the same characteristics on the same time.

Synchronization and growth conditions

Synchronization occurs when cells progress similarly in their cell cycle. Thus, synchronization is the macroscopic manifestation (at population scale) of the existence of a cell cycle (occurring at cellular scale). Therefore, the rate of synchronicity is related to the proportion of individuals that are simultaneously in the same stage, which results from the time control of the cell cycle by checkpoints. Usually, microalgae have a checkpoint responding to light and N conditions in G1 phase, and some diatoms have a supplementary light checkpoint in G2 (Olson and Chisholm 1986, Vaulot et al. 1986). Consequently, an oscillating signal of light (Spudich and Sager 1980, Vincent 1992) or nutrient (Wheeler et al. 1983, Bernard et al. 1996) can trigger the synchronization of cells within their cell cycle.

In the *control* experiment where the nutrient and light conditions were not limiting for growth, the occurrence of one major division peak visible every day suggests that the population was synchronized by the LD signal (Fig. 3A). Actually, two major division peaks should occur per day on a totally synchronized population growing at $1.4d^{-1}$ – which is not the case here. It follows that the population was only partially synchronized by the LD conditions. This is also reflected by the existence of secondary division peaks of lower amplitude

identified at different times of the day. A partial synchronization reflects differences between individuals, notably in their ability to achieve their cell cycle in the same time. Therefore, the level of synchronicity depends also on the degree of individual variability that characterizes the whole population. This feature can explain why the population lost its synchronization when grown under suboptimal conditions. Under N-limited conditions (*Nlim* experiment), the desynchronization can be explained by another checkpoint in the cell cycle. Indeed, under a LD signal and non-limiting nutrients, the cell cycle of *T. weissflogii* is only structured with a light-based checkpoint in G2 (%G2 increases in *Llim* experiments, Table 3). Adding N-limitation leads the cell cycle to be also possibly stopped in G1 (pG1 increases in *Nlim* experiment, Table 3), disturbing the synchronizing effect of the LD signal.

It appears that when growth conditions are optimal, individuals show a rather similar behavior, notably to exploit their energetic and nutritional resources and to achieve their cell cycle. By contrast, when cells have to adapt to suboptimal growth conditions, individual differences are accentuated because cells do not have the same ability for acclimation, due to phenotypic differences. Finally, the individual differences, or intraspecific diversity, increases the discrepancy between the cell cycles, and leads the population to desynchronization. Hence, our results suggest that phenotypic variability and growing conditions are intrinsically linked to the synchronization process of a population.

As a conclusion, our results suggest that the nitrogen acquisition is incompatible with mitosis and thus is not continuous during the cell cycle. We showed that environmental conditions, such as the light signal and the nutrient supply, affect the synchrony of the population, and finally, have macroscopic consequences on the population dynamics. The consequences of cell synchronization on nutrient acquisition should now be taken into

358 account from an ecological point of view and included in mathematical models in order to
359 better account for phytoplankton and nutrient dynamics in the natural environment.

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Figures

#1. Results of control experiment. A. Number of cells and division rate; B. G1 percentage cells and Msignal; C. Division rate and M signal; D. Extracellular NO3 concentration and N acquisition rate; E. Cellular N concentration and N acquisition rate; F. Overlay of the two N acquisition rate signals and (NO3+N)/Sin ratio; G. Cellular C concentration and C fixation rate; H. Cellular C and N content and N/C ratio; I. Chlorophyll a and carbohydrates. Grey line represents the photon flux density ($\mu molquanta.m^{-2}.s^{-1}$).

#2. Correlation between the two signals of N acquisition in *control* experiment.

#3. Rates of nitrogen acquisition (continuous lines), carbon fixation (dotted lines) and division (dashed lines) in the different experiments. Photon flux density in grey. A. *Control* experiment, B. *Nlim* experiment, C. *Llim1* experiment, D. *Llim2* experiment, E. *CL* experiment, F. *ML* experiment.

#4. Chlorophyll *a* (filled circles, continuous lines) and carbohydrates (open circles, dashed lines) in different experiments. Photon flux density in grey. A. *CL* experiment, B. *ML* experiment.

#5. Nitrogen acquisition vs. substrate concentration in *control*. Opened circles correspond to daylight measures, filled ones to measures at night.

#6. Carbon fixation vs. photon flux density ($r^2 = 0.93$) during the daylight in *control*.

#7. Effet of energy status and mitosis on nitrogen acquisition. Symbols in circles report positive Mitosis signal. A. Nitrogen acquisition vs. photon flux density ($r^2 = 0.54$) during the daylight in *control*, B. Nitrogen acquisition vs. carbohydrate content at night ($r^2=0.37$) during *control* and *Nlim*.

Tables

Table 1. Light (photoperiod, light intensity at noon I_{max}, daily averaged light dose $I_{/}$) and nutritional (culture mode, dilution rate D (d^{-1}), number of divisions per day N) conditions during the different experiments.

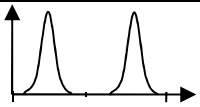
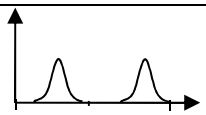
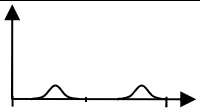
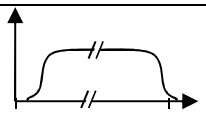
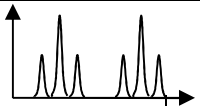
Experiment	Light conditions	Photoperiod L/D $I_{max} - I_{/}$	Culture mode D - N
Control		12/12 800 - 200	Turbidostat 1.4 - 2.0
Nlim		12/12 800 - 200	Chemostat 0.69 - 1.0
Llim1		12/12 100 – 25	Turbidostat 0.80 - 1.1
Llim2		12/12 20 - 5	Turbidostat 0.40 - 0.5
CL		24/00 400 - 400	Turbidostat 2.00 - 2.9
ML		12/12 1350 - 350	Turbidostat 0.90 – 1.2

Table 2. Formula for computation of division rate, NO3 absorption rate, N and C assimilation rate and mitosis signal. For all formula, t represents time.

Division rate	$\mu = \frac{dX}{dt}\frac{1}{X} + D$	X = cell density (.L^{-1}) D = dilution rate (.d^{-1})
N acquisition rate (computed on nitrate concentration)	$\rho_s = -\frac{d(S)}{dt} + D(S_{in} - S)$	S = nitrate concentration in culture (µmol.L^{-1}) S_{in} = nitrate concentration in supplied medium
C and N acquisition rates (computed on cellular biomass)	$\rho_C = \frac{dC}{dt}\frac{1}{C} + D$ $\rho_N = \frac{dN}{dt}\frac{1}{N} + D$	C and N = cellular carbon and nitrogen concentrations (µmol.L^{-1})
Mitosis signal	$\mathrm{M} = \frac{\mathrm{dpG1}}{\mathrm{dt}}\frac{1}{\mathrm{dpG1}/\mathrm{dt}}$	G1 = percentage of cells in the G1 phase

Table 3. Daily average results for all experiments at day 2. Legend: nm: not measured. *at day 3.

	control	Nlim	Llim1	Llim2	CL	pre ML	ML
division rate (/d)	1.4	0.7	0.7	0.1	2.0	1.4	1.1
Cell cycle amplitude of pG1 (pp)	40	15	25	10	10	40	<5*
N acquisition rate (pmol/d/cell)	1.7	0.4	0.9	0.3	2.5	1.8	0.9
Specific (/d)	1.4	0.5	0.7	0.4	2.0	1.6	1.0
C acquisition rate (pmol/d/cell)	13.4	4.4	4.9	2.1	21.7	11.2	8.3
Specific (/d)	1.4	0.8	0.5	0.3	2.0	1.2	1.0
C & N quotas C/cell (pmol/cell)	9.2	2.6	8.9	7.5	10.6	8.9	8.3
N/cell (pmol/cell)	1.22	0.76	1.14	1.00	1.30	1.32	1.14
N/C (molN/molC)	0.14	0.11	0.13	0.14	0.13	0.15	0.14