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CELL CYCLE IMPLICATION ON NITROGEN ACQUISITION AND  
SYNCHRONIZATION IN *THALASSIOSIRA WEISSFLOGII* (BACYLARIOPHYCEAE).<sup>1</sup>

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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 <i>a</i>
CL	constant light
<i>CL</i>	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
<i>Llim</i>	experiment with reduced light supply
<i>ML</i>	experiment simulating mixed layer illumination
Msignal	mitosis signal (see table 2)
N	nitrogen
NH <sub>4</sub> <sup>+</sup>	ammonium
<i>Nlim</i>	experiment with reduced nitrogen supply
NO <sub>2</sub> <sup>-</sup>	nitrite
NO <sub>3</sub> <sup>-</sup>	nitrate
PAR	photosynthetically available radiation
PFD	photon flux density
pG1	percentage of cells in G1

<i>pré-ML</i>	acclimation period prior to <i>ML</i> experiment
S	cell cycle stage "S"
$\Delta G1$	daily amplitude of variation in pG1 (in percentage points)

## **Introduction**

1           The fact that N can limit primary production over large oceanic areas triggered  
2 numerous experimental and field studies. Pioneering works of Caperon (1965, 1967), Dugdale  
3 (1967) and Eppley et al. (1969) established that N acquisition generally displays a  
4 monophasic saturating function of external nutrient concentration. Known as the Michaelis-  
5 Menten equation (Caperon and Meyer 1972, MacIsaac and Dugdale 1972, Sarthou et al.  
6 2005), this function can represent the acquisition of the major forms of inorganic (i.e. nitrate –  
7  $\text{NO}_3^-$ , ammonium –  $\text{NH}_4^+$ ) and organic N (urea, amino acids) by phytoplankton. The  
8 physiological understanding is that the rate of nutrient acquisition increases with the substrate  
9 concentration until saturation of active transporters. Nevertheless, further studies showed that  
10 N acquisition is a much more complicated process. When substrate is supplied at high  
11 concentrations (Collos et al. 2005), or when multiple forms of inorganic nitrogen are available  
12 simultaneously (MacIsaac and Dugdale 1972, Lomas and Glibert 1999), N acquisition cannot  
13 be reduced to a single saturating relationship of the external nutrient concentration. In  
14 addition, N acquisition is also dependent of light. Indeed, energy and reducing power  
15 provided by photosynthesis are necessary for active transport of  $\text{NO}_3^-$  and its subsequent  
16 reduction to  $\text{NH}_4^+$  (Turpin et al. 1988, 1991, Lim et al. 2006). It remains that N acquisition can  
17 be temporally decoupled from light (Eppley and Coatsworth 1966, Laws and Wong 1978,  
18 Needoba and Harrison 2004) when reducing power can be supplied by the catabolism of  
19 neutral lipids or carbohydrates accumulated during photosynthesis (Vanlerberghe et al. 1992,  
20 Cuhel et al. 1984).

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

25 “G2” stage. Mitosis completes the cycle, with the formation of two daughter cells. Transitions  
26 between stages are controlled by nutritional and energy status checkpoints (Chisholm et al  
27 1986), ensuring a cell proceeds to division only when it is mature (Vaulot 1994). At a  
28 population scale, these checkpoints can synchronize cells on a periodic signal of light or  
29 nutrient supply, and consequently extend the impact of the cell cycle at the macroscopic level  
30 (Vaulot 1994, Vaulot and Marie 1999, Binder and Durand 2002).

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

50 transcription of the gene encoding the  $\text{NO}_3^-$  transporters, the latter being related to the cell  
51 cycle. Yet, very few studies have been carried out on the relationship between the cell cycle  
52 and N acquisition.

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

## 58 **Methods**

### 59 **Experimental design**

60 The device - Laboratory experiments were conducted with the marine diatom  
61 *Thalassiosira weissflogii* (Grunow) obtained from the CCMP (Bigelow Laboratory for Ocean  
62 Science, USA). The culture vessels consisted of water jacketed 2-liter cylinders connected to  
63 a circulating water bath that maintained a constant temperature ( $22 \pm 0.05^\circ\text{C}$ ). The growth  
64 medium was prepared using 0.22- $\mu\text{m}$  Millipore filtered and autoclaved ( $105^\circ\text{C}$  for half an  
65 hour) seawater and nutrient enrichments according to f/2 formulation (Guillard and Ryther  
66 1962), except for  $\text{NO}_3^-$  which was added separately to achieve a final concentration of 200  
67  $\mu\text{mol.L}^{-1}$ . The same stock of seawater, collected off the coast of Villefranche-sur-mer  
68 (France), was used over the whole experiment. After cooling and sterile addition of nutrients,  
69 the medium was transferred with independent peristaltic pumps (Gilson III) to each culture  
70 vessel, through a 0.22- $\mu\text{m}$  sterile filter (MediaKap-5, Microgon). The continuous cultures  
71 were operated either in turbidostat (N-replete) or chemostat mode (N-limited). The cultures  
72 were subjected to gentle continuous stirring and bubbling with sterile-filtered air, which



73 passed through a 0.1- $\mu\text{m}$  Whatman filter and activated charcoal at a rate of 0.15 liter per  
74 minute. To prevent possible growth limitation by DIC consumption, pH was regulated online  
75 at a constant value of  $8.0 \pm 0.02$ , using an automatic computer-controlled device that  
76 monitored the bubbling of very small amounts of pure  $\text{CO}_2$  into the cultures (for details see  
77 Sciandra et al. 2003). Illumination was supplied by 12 fluorescent lamps (Osram Dulux 12-  
78 950 55W, see Bruyant et al. 2001 for details) and the photon flux density (PFD) was  
79 electronically computer-controlled, allowing different patterns of light regime (see Table 1).  
80 Photosynthetically available radiation (PAR) was measured with a quantum scalar irradiance  
81 meter (QSL-100, Biospherical Instruments).

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

98 density combined with the  $\text{NO}_3^-$  concentration in the renewal medium ( $200\mu\text{mol.L}^{-1}$ ) led to a  
99 residual  $\text{NO}_3^-$  concentration in the cultures below  $150\mu\text{mol.L}^{-1}$ , for which the colorimetric  
100 measurement of  $\text{NO}_3^-$  was linear (see below). In chemostat mode (N-limited), the growth rate  
101 of the population equilibrated with the dilution rate.

102

### 103 **Culture analyses**

104 Nutrient measurements. Nitrite and nitrate ( $\text{NO}_3^-$ ) concentrations were measured with  
105 a Technicon Auto-analyzer and an automated data acquisition system (Malara and Sciandra  
106 1991). A dilution device added at the entrance of the chain measurement maintained a linear  
107 response of the colorimetric reaction for  $\text{NO}_3^-$  concentrations ranging from 0 to  $150\mu\text{mol.L}^{-1}$ .  
108 Depending on the experiment, analyses were performed once or twice per hour.

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

115 Determination of quotas: cellular carbon (C) & nitrogen (N), chlorophyll a (Chla) and  
116 carbohydrates (CH). For cellular C and N determinations, duplicate samples of 10mL were  
117 filtered through glass fiber filters (Whatman GF/C) precombusted at  $500^\circ\text{C}$ , and analyzed  
118 with a PerkinElmer Series 2 2400 CHN analyzer. The replicates agreed typically to within 3  
119 and 5% for C and N analyses respectively. The same sampling procedure was conducted for  
120 the other quotas, except for the sample volume of 5mL and the storage in liquid nitrogen.  
121 Pigments were extracted in a mixture of acetone and 6% of Dimethylsulfoxide (DMSO), and  
122 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

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

161 Smoothing is a crucial step as it can significantly alter the shape of the derivative,  
162 depending on the chosen numerical scheme method. Savitsky-Golay spline estimation, cubic  
163 spline estimation or Gaussian convolution methods were distinctively applied to each data  
164 series as a function of their noise level and acquisition frequency. To illustrate the smoothing  
165 process, examples are given for the data acquired during the *control* experiment (Fig. 1).  
166 Confidence in the smoothing process has been assessed for each data series, especially  
167 concerning the number and position of the peaks. Confidence assessment involves checking  
168 the possible recurrence of the oscillations over days in the same conditions, the number of  
169 points involved in the peak formation and the comparison between signals. For example, the  
170 cell N data point of day 19.3, which seems abnormally high (Fig. 1E), induces a shouldering  
171 in the N assimilation rate which is not reflected by the N acquisition rate, and also triggers an  
172 unexpected value of the  $(\text{NO}_3^- + \text{N})/\text{Sin}$  ratio (Fig. 1F). In contrast, the particular pattern of

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

175

## 176 **Results**

### 177 ***Control experiment***

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

189 Acquisition of N occurred essentially in the daylight (Fig. 3A), the nocturnal  
190 acquisition represented only 20% of the  $1.7 \text{ pmol N}$  acquired per day. More precisely,  
191 acquisition started early in the daylight, increases until 2h before noon, and finally decreased  
192 down to midnight to reduced but significant rates (Fig. 3A). Both signals of N acquisition  
193 were well correlated (Fig. 1F and 2), and both were significantly correlated to the PFD (Fig.  
194 3A and 7). C acquisition was strictly restricted to the daylight (Fig. 3A), and the night losses  
195 per cell represented 5-7% of the  $13 \text{ pmol C}$  acquired in average per day. A cross-correlation  
196 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  $0.69\text{d}^{-1}$  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 ( $\Delta\text{G1}$ ) 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 occurred 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,  $\Delta\text{G1}$

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  $\Delta G1$  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*)**

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

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

### 258 **Mixed layer simulation (ML)**

259 The daily averaged division rate was equivalent in *pre-ML* (acclimation period)  
260 compared to *control* (Table 3), but dropped by 1/3 under *ML* conditions (Table 3).  $\Delta G1$  was  
261 reduced to 20percentage pointson the second day of *ML* conditions . After that, the signal of  
262 pG1 became too noisy to compute a relevant Msignal.



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

## 268 **Discussion**

269 The Michaelis-Menten relationship is commonly used to calculate the uptake of a  
270 given nutrient from its concentration in the surrounding medium. However, it appears clearly  
271 inappropriate to represent the pattern of N acquisition in our experiments. N acquisition is not  
272 simply related to its external concentration (Fig. 5) when cells are grown under a LD regime  
273 and within a range of N concentrations well above the half saturation constant. In addition,  
274 the N acquisition rate showed a 24h cyclic pattern, with high and low values observed  
275 respectively during day and night. Thus, one or several parameters linked to the LD light  
276 regime should exert an additional control on the N acquisition. Most studies monitoring  
277 nutrient acquisition under LD signals explain the variation observed by the effect of the light  
278 dose on the physiological status (Chisholm and Stross 1976, Cochlan et al. 1991, Marsot et al.  
279 1992, Clark et al. 2002), but to the best of our knowledge none examined the potential  
280 implication of the cell cycle.

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

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

### 288 **Does the cell cycle influence nitrogen acquisition?**

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

298 Now, particular attention should be paid to the fact that the dispersion of the points  
299 around the relationship between acquisition and irradiance (Fig. 7A) is structured. The  
300 dispersion reflects a hysteresis phenomenon where the rates of N acquisition measured during  
301 the afternoon are, for the same PFD, systematically lower than those measured in the  
302 morning. These results show that the energetic status of the cell is not sufficient to fully  
303 explain the variations of the N acquisition at the daily time scale. The key point lies in the fact  
304 that low rates of N acquisition are all measured when a significant proportion of the  
305 population is in mitosis (see also Fig. 7B). Thus, these two processes – respectively devoted  
306 to somatic growth and cell division – seem mutually exclusives. The variation in the gene  
307 expression encoding  $\text{NO}_3^-$  transporter during the cell cycle reported in *Cylindrotheca*  
308 *fusiformis* is consistent with our observations, especially the low expression in the late G2/M  
309 phase reported by Hildebrand and Dahlin (2000). They also reported a variable expression of

310 the  $\text{NO}_3^-$  transporter gene between G1 and G2, but the expected consequence on N acquisition  
311 was not visible in our results, possibly because of the incomplete synchronization of the  
312 population.

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

318

### 319 **Synchronization and growth conditions**

320 Synchronization occurs when cells progress similarly in their cell cycle. Thus,  
321 synchronization is the macroscopic manifestation (at population scale) of the existence of a  
322 cell cycle (occurring at cellular scale). Therefore, the rate of synchronicity is related to the  
323 proportion of individuals that are simultaneously in the same stage, which results from the  
324 time control of the cell cycle by checkpoints. Usually, microalgae have a checkpoint  
325 responding to light and N conditions in G1 phase, and some diatoms have a supplementary  
326 light checkpoint in G2 (Olson and Chisholm 1986, Vaultot et al. 1986). Consequently, an  
327 oscillating signal of light (Spudich and Sager 1980, Vincent 1992) or nutrient (Wheeler et al.  
328 1983, Bernard et al. 1996) can trigger the synchronization of cells within their cell cycle.

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

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

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

353         As a conclusion, our results suggest that the nitrogen acquisition is incompatible with  
354 mitosis and thus is not continuous during the cell cycle. We showed that environmental  
355 conditions, such as the light signal and the nutrient supply, affect the synchrony of the  
356 population, and finally, have macroscopic consequences on the population dynamics. The  
357 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 NO<sub>3</sub> concentration and N acquisition rate; E. Cellular N concentration and N acquisition rate; F. Overlay of the two N acquisition rate signals and (NO<sub>3</sub>+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 ( $\mu\text{molquanta.m}^{-2}.\text{s}^{-1}$ ).

#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  $I_f$ ) and nutritional (culture mode, dilution rate  $D$  ( $d^{-1}$ ), number of divisions per day  $N$ ) conditions during the different experiments.



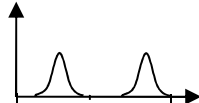
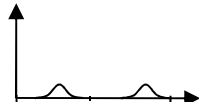
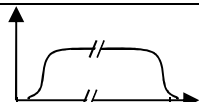
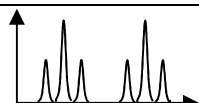
Experiment	Light conditions	Photoperiod L/D $I_{\max} - I_f$	Culture mode D - N
<i>Control</i>		12/12 800 - 200	Turbidostat 1.4 - 2.0
<i>Nlim</i>		12/12 800 - 200	Chemostat 0.69 - 1.0
<i>Llim1</i>		12/12 100 - 25	Turbidostat 0.80 - 1.1
<i>Llim2</i>		12/12 20 - 5	Turbidostat 0.40 - 0.5
<i>CL</i>		24/00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

Table 2. Formula for computation of division rate, NO<sub>3</sub> absorption rate, N and C assimilation rate and mitosis signal. For all formula, t represents time.

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

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
<b>division rate</b> (/d)	1.4	0.7	0.7	0.1	2.0	1.4	1.1
<b>Cell cycle</b> amplitude of pG1 (pp)	40	15	25	10	10	40	<5*
<b>N acquisition rate</b> (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
<b>C acquisition rate</b> (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
<b>C &amp; N quotas</b>							
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