
Decoupling light harvesting, electron transport and carbon fixation during prolonged darkness supports rapid recovery upon re-illumination in the Arctic diatom *Chaetoceros neogracilis*

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Abstract :

During winter in the Arctic marine ecosystem, diatoms have to survive long periods of darkness caused by low sun elevations and the presence of sea ice covered by snow. To better understand how diatoms survive in the dark, we subjected cultures of the Arctic diatom *Chaetoceros neogracilis* to a prolonged period of darkness (1 month) and to light resupply. *Chaetoceros neogracilis* was not able to grow in the dark but cell biovolume remained constant after 1 month in darkness. Rapid resumption of photosynthesis and growth recovery was also found when the cells were transferred back to light at four different light levels ranging from 5 to 154 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. This demonstrates the remarkable ability of this species to re-initiate growth over a wide range of irradiances even after a prolonged period in the dark with no apparent lag period or impact on survival. Such recovery was possible because *C. neogracilis* cells preserved their Chl a content and their light absorption capabilities. Carbon fixation capacity was down-regulated (ninefold dark decrease in PCm) much more than was the photochemistry in PSII (2.3-fold dark decrease in ETRm). Rubisco content, which remained unchanged after one month in the dark, was not responsible for the decrease in PCm. The decrease in PSII activity was partially related to the induction of sustained non-photochemical quenching (NPQ) as we observed an increase in diatoxanthin content after one month in the dark.

Keywords : Arctic microalgae, Polar night, Diatom, Darkness, Photosynthesis, Growth rate, Temperature

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30

50 **Introduction**

51 Diatoms are ubiquitous in the surface ocean, including at very high latitudes in the Arctic where
52 they are the most abundant microalgae in ice and in the phytoplankton community (Poulin et al.
53 2011). During winter, light in the surface ocean is very low due to low sun elevations and the
54 presence of sea ice generally covered by snow (McMinn et al. 1999, Mundy et al. 2009, Leu et al.
55 2015). Several studies have reported on the ability of polar diatoms to survive long periods of
56 darkness and resume fast growth as soon as light becomes available (Smayda and Mitchell-Innes
57 1974, Palmisano and Sullivan 1982, 1983, Peters and Thomas 1996, McMinn et al. 1999, Wulff et
58 al. 2008, McMinn and Martin 2013, Fang and Sommer 2017). Our study provides new insights on
59 the physiological mechanisms of dark survival and recovery.

60
61 Various strategies may allow microalgae to cope with darkness. Several microalgae taxa develop
62 resting stages, including cysts and spores that remain viable for up to a hundred years in the case
63 of dinoflagellates (Lundholm et al. 2011). Nutritional versatility is another strategy whereby
64 microalgae can use both photoautotrophy and heterotrophy to obtain energy (mixotrophy). The
65 heterotrophic capacity of polar diatoms increases as cells go into a simulated polar night (Palmisano
66 and Sullivan 1982). Polar diatoms can assimilate amino acids and glucose both in the light and in
67 the dark (Rivkin and Putt 1987). Some diatoms are even capable of net heterotrophic growth in the
68 presence of glucose (White 1974). However, to our knowledge, net heterotrophic growth of a polar
69 diatom during a prolonged period of darkness has not been observed and the addition of organic
70 substrates does not appear to aid their dark survival capabilities (Dehning and Tilzer 1989, Popels
71 and Hutchins 2002).

72 Our current knowledge of the physiology of dark survival derives mostly from studies using non-
73 Arctic diatoms, or from other algal groups. For instance, green algae seem to partially dismantle
74 their photosynthetic apparatus during long periods in the dark but keep it loosely assembled to
75 rapidly resume photosynthesis when exposed to light (Baldisserotto et al. 2005, Morgan-Kiss et al.
76 2006, Ferroni et al. 2007, Nymark et al. 2013). In natural communities, photosynthetic performance
77 falls to minimal levels after several weeks of darkness (Reeves et al. 2011, Martin et al. 2012) while
78 generally going back to a normal state nearly immediately upon the return of light (Kvernvik et al.
79 2018). In diatoms, dark survival is also characterized by low metabolic rates and the consumption
80 of energy reserves (Peters 1996, Peters and Thomas 1996, Schaub et al. 2017). Diatoms can store

81 large quantities of lipids to buffer energy shortage (Smith and Morris 1980, Palmisano and Sullivan
82 1982), so a balance between energy storage and the rate of utilization may be tuned to increase
83 survival during long periods of darkness.

84
85 In the Arctic marine environment, changes in snow optical properties was shown to be the primary
86 driver for allowing sufficient light to penetrate through the thick snow and initiate algae growth
87 below the sea ice (Hancke et al. 2018). Light increase can also be abrupt because of sea ice breakup
88 and can take place at different moments of the spring and summer. Polar diatoms must thus cope
89 with long period in darkness (or very low irradiance) and sudden light bursts of unpredictably
90 variable intensity even after prolonged darkness. The physiological basis of such flexibility is
91 mostly unknown. Several recent studies have examined the physiological response of polar
92 microalgae to changes in growth irradiance (Kropuenske et al. 2009, Arrigo et al. 2010,
93 Kropuenske et al. 2010, Mills et al. 2010, Petrou et al. 2010, Petrou et al. 2011, Petrou and Ralph
94 2011, van de Poll et al. 2011, Lacour et al. 2018). These studies highlighted how non-
95 photochemical quenching (NPQ) is a crucial physiological mechanism for the survival of polar
96 diatoms at low temperature coupled with other stresses such as high light (including UV radiations)
97 (Petrou et al. 2016). Lacour et al. (2018) have shown, in the Arctic diatom *Thalassiosira gravida*
98 acclimated to high irradiance, a strong sustained (hour kinetics relaxation) non photochemical
99 quenching (NPQs). NPQ (and possibly NPQs) may play an important role to prime diatoms for
100 sudden transitions from dark to (variable) light exposure. The purpose of this study was to describe
101 the physiological strategy that allows polar diatoms to survive in the dark while remaining prepared
102 for light return.

103
104 We first studied the growth and photophysiology at four different growth irradiances of
105 *Chaetoceros. neogracilis*, one of the dominant diatoms in the Beaufort Sea (Balzano et al. 2012,
106 Balzano et al. 2017) after one month in darkness. To understand how *C. neogracilis* manage such
107 recovery, we described in detail the physiological state of the cells after one month in the dark from
108 light capture to carbon fixation.

109

110 **Material and methods**

111 *Algal Cultures*

112
113 We performed two independent experiments: a light recovery experiment (Exp 1) and a dark
114 acclimation experiment (Exp 2). In Exp 1, unialgal cultures of *C. neogracilis* (Roscoff Culture
115 Collection RCC 2278), isolated during the Malina cruise (2009) in the Beaufort sea (Balzano et al.
116 2012) were grown in semi-continuous cultures of 2000 mL in pre-filtered f/2 medium (Guillard
117 1975) enriched with silicate. Salinity was 35 PSU. The illumination was provided continuously by
118 white fluorescent tubes at $23 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ as measured using a QSL-100 quantum sensor
119 (Biospherical Instruments, San Diego, CA, USA) placed in the culture vessel. Culture conditions
120 were maintained semi-continuously by diluting cultures once a day in order to maintain biomass
121 semi-constant (MacIntyre and Cullen 2005) and gently aerated through $0.3 \mu\text{m}$ -pore-filters.
122 Cultures were grown in a growth chamber (Percival Scientific Inc., Perry, IA, USA) that allowed
123 temperature maintenance at 0°C ($\pm 1^\circ\text{C}$). Triplicate cultures were then incubated in complete
124 darkness for 30 days and then illuminated by white fluorescent tubes (Phillips®, 54W/840) at 4
125 different light levels (L/D, 12h/12h) with mean daily irradiances of 5, 27, 41, and $154 \mu\text{mol photon}$
126 $\text{m}^{-2} \text{s}^{-1}$ ($\pm 5\%$) and maximum irradiances (at noon) of 531, 141, 93 and $17 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ (see
127 Online resource 1) controlled by a specific software (IntellusUltraConnect, Percival Scientific Inc.,
128 Perry, IA, USA). Those irradiances roughly correspond to the mean daily irradiances encountered
129 between 70 and 80°N in March, April, May and June respectively (seasonal change in day length
130 was not mimicked due to technical considerations). We monitored cell number and photochemical
131 characteristics over 8 to 14 days following re-illumination (Experiment 1: light recovery).
132 Triplicate culture were sampled at the first sunrise (t_0) and several time during the first light period.
133 Then, the cultures were sampled each day at noon.

134
135 In Exp 2, triplicate cultures were also acclimated to $23 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ (continuous light) or
136 to complete darkness for 30 days before sampling for comparisons of cell number, pigments,
137 particulate carbon and nitrogen, carbon fixation, Photosystem II (PSII) photochemical activity and
138 Rubisco content (RbcL) (Experiment 2: dark acclimation).

139
140 *Cell number, C and N, pigments*

141

142 *Chaetoceros neogracilis* cells were counted and sized (equivalent spherical diameter) before and
143 after culture dilution using a Beckman Multisizer 4 Coulter Counter. The concentrations of
144 particulate C and N were determined daily on triplicate samples. For particulate carbon and
145 nitrogen, an aliquot of 10 mL of algal culture was filtered onto glass-fiber filters (0.7 μm , 25 mm)
146 pre-combusted at 500°C for 12 h. Filters were kept desiccated before elemental analysis with a
147 CHN analyzer (2400 Series II CHNS/O; Perkin Elmer, Norwalk, CT, USA). For pigment analysis,
148 an aliquot of algal culture (5 mL) was filtered onto a GF/F glass-fiber filter, immediately flash-
149 frozen in liquid nitrogen and stored at -80°C until analysis by HPLC using the protocol described
150 in Zapata et al. (2000). Sample filtration were done as fast as possible (< 2 min) under very low
151 green light. The xanthophyll de-epoxidation state (DES in %) was calculated as $Dt/(Dd + Dt)*100$,
152 where Dd is the concentration of Diadinoxanthin, the epoxidized form and Dt is that of
153 Diatoxanthin, the de-epoxidized form (Lavaud et al. 2007).

154

155 *Carbon fixation*

156

157 The relationship between the rate of carbon fixation (P) and irradiance (E) (P vs E curve) was
158 determined according to Lewis and Smith (1983). A 50-mL sample was collected in each replicate
159 culture, and inoculated with inorganic ^{14}C ($\text{NaH}^{14}\text{CO}_3$, 2 $\mu\text{Ci mL}^{-1}$). To determine the total amount
160 of bicarbonate added, three 20- μL aliquots of inoculated culture sample were added to 50 μL of an
161 organic base (ethanolamine) and 6 ml of the scintillation cocktail (Ecolume) into glass scintillation
162 vials. Then 1-mL aliquots of the inoculated culture sample were dispensed into twenty-eight 7-mL
163 glass scintillation vials already cooled in their separate thermo-regulated cavities (0 or 5°C). The
164 vials were exposed to 28 different light levels provided by independent LEDs (LUXEON Rebel,
165 Philips lumileds) from the bottom of each vial. The PAR ($\mu\text{mol photon m}^{-2} \text{s}^{-1}$) in each cavity was
166 measured before incubation with an irradiance meter (Biospherical QSL-100) equipped with a
167 4π spherical quantum sensor. After 20 minutes of incubation, culture aliquots were fixed with 50
168 μL of buffered formalin then acidified (250 μL of HCl 50%) under the fume hood for 3 hours in
169 order to remove the excess inorganic carbon (JGOFS protocol, UNESCO 1994). Finally, 6 mL of
170 scintillation cocktail were added to each vial prior to counting in the liquid scintillation counter
171 (Tri-Card, PerkinElmer). The chlorophyll-specific carbon fixation rate was finally computed
172 according to Parsons et al. (1984).

173
 174 *Fluorescence measurements*
 175
 176 Photochemical properties of PSII were determined by variable fluorescence using a Fluorescence
 177 Induction and Relaxation (FIRE) fluorometer (Satlantic, Halifax, NS, Canada) that applies a
 178 saturating, single turnover flash (STF, 100µs) of blue light (455 nm, 60-nm bandwidth) to the
 179 incubated sample to generate a fluorescence induction curve (detected at 680 nm) that can be used
 180 to estimate the minimum fluorescence (F_0 for dark-adapted and F_s for light-adapted samples), the
 181 maximum fluorescence (F_m if dark-adapted and F_m' if light-adapted) and the effective absorption
 182 cross section of PSII (σ_{PSII} if dark-adapted and σ'_{PSII} if light-adapted) using the FIREWORX
 183 algorithm (Pers. Comm. Audrey Barnett, www.sourceforge.net) and the flash lamp calibration
 184 provided by the instrument manufacturer (Thomas and Campbell 2013). We found that 20 min in
 185 darkness was sufficient to fully relax non-photochemical quenching of F_0 and F_m . F_s , F_m' and σ'_{PSII}
 186 were measured repeatedly on the same culture subsample after 2 min exposures under an increasing
 187 range of actinic light levels.

188
 189 We estimated the maximum quantum yield of PSII (F_v/F_m) and the optical absorption cross section
 190 ($\sigma^{\text{OPT}}_{\text{PSII}}$) from 20 min dark acclimated cells (Huot and Babin 2010) and the realized quantum yield
 191 of charge separation at the PSII (Φ_{PSII} , Genty et al. (1989)) as:

$$192 \quad 193 \quad F_v/F_m = \frac{F_m - F_0}{F_m} \quad \text{Equation 1}$$

$$194 \quad 195 \quad \sigma^{\text{OPT}}_{\text{PSII}} = \frac{\sigma_{\text{PSII}}}{F_v/F_m} \quad \text{Equation 2}$$

$$196 \quad 197 \quad \Phi_{\text{PSII}} = \frac{F_m' - F_s}{F_m'} \quad \text{Equation 3}$$

198
 199 To quantify the partitioning of excitation energy between photochemistry, fluorescence and
 200 thermal dissipation, we used the approach of Hendrickson et al. (2004). Φ_{PSII} corresponds to the
 201 fraction of absorbed irradiance used for photochemistry, $\Phi_{\text{f,D}}$ is the sum of the fractions that are

202 lost by either thermal dissipation or fluorescence and Φ_{NPQ} is the fraction that is thermally
 203 dissipated via ΔpH and/or xanthophyll-regulated processes:

$$204 \quad \Phi_{f,D} = \frac{F_s}{F_m} \quad \text{Equation 4}$$

205 and

$$206 \quad \Phi_{\text{NPQ}} = \frac{F_s}{F_{m'}} - \frac{F_s}{F_m} \quad \text{Equation 5}$$

207
 208 The PSII specific electron transport rate (ETR, $\text{e}^- \text{PSII}^{-1} \text{s}^{-1}$) was calculated as (Suggett et al. 2010):

$$209 \quad \text{ETR} = \sigma_{\text{PSII}} \cdot \frac{\Phi_{\text{PSII}}}{F_v/F_m} \cdot E \cdot 6.022 \cdot 10^{-3} \quad \text{Equation 6}$$

210 where E is the actinic irradiance ($\mu\text{mol photon m}^{-2} \text{s}^{-1}$), σ_{PSII} is the effective absorption cross-section
 211 of PSII ($\text{A}^2 \text{PSII}^{-1}$) measured from dark acclimated samples and $6.022 \cdot 10^{-3}$ is a constant to convert
 212 σ_{PSII} to $\text{m}^2 \mu\text{mol photon}^{-1}$.

213
 214 A proxy for the amount of active PSII was estimated as (Oxborough et al. 2012, Silsbe et al. 2015,
 215 Murphy et al. 2017):

$$216 \quad \text{PSII chl a}^{-1} \sim k \times \frac{F_0}{\sigma_{\text{PSII}} \times [\text{Chl a}]} \quad \text{Equation 7}$$

217 where k is an unknown constant.

218
 219 *Data analysis.* The initial slope (α , $\text{g C g}^{-1} \text{Chl a h}^{-1} (\mu\text{mol photon m}^{-2} \text{s}^{-1})^{-1}$ and α^{ETR} , $\text{e}^- \text{PSII}^{-1}$
 220 $(\mu\text{mol photon m}^{-2})^{-1}$) and the maximum value of the rate versus E curves (P_m , d^{-1} and ETR_m $\text{e}^- \text{PSII}^{-1}$
 221 s^{-1}) were estimated by fitting the equation of Platt et al. (1980) (with the photoinhibition parameter
 222 β) to the experimental rate and PAR values as:

$$223 \quad P = P_m \left(1 - e^{-\frac{\alpha E}{P_m}}\right) e^{-\frac{\beta E}{P_m}} \quad \text{Equation 8}$$

$$224 \quad \text{ETR} = \text{ETR}_m \left(1 - e^{-\frac{\alpha^{\text{ETR}} E}{\text{ETR}_m}}\right) e^{-\frac{\beta^{\text{ETR}} E}{\text{ETR}_m}} \quad \text{Equation 9}$$

225
 226 The light-saturation parameters for carbon fixation (E_K , $\mu\text{mol photon m}^{-2} \text{s}^{-1}$) and for electron
 227 production at PSII (E_K^{ETR} , $\mu\text{mol photon m}^{-2} \text{s}^{-1}$) were obtained as:

$$228 \quad E_K = \frac{P_m}{\alpha} \quad \text{Equation 10}$$

$$229 \quad E_K^{\text{ETR}} = \frac{\text{ETR}_m}{\alpha^{\text{ETR}}} \quad \text{Equation 11}$$

230

231 *Protein analyses*

232

233 For RbcL quantitation, 30 mL of each culture was harvested onto GF/F filters (0.7 μm pore size,
 234 Whatman). Filters were flash-frozen in liquid nitrogen and stored at -80°C . Protein extractions
 235 were performed using the FastPrep-24 and bead lysing “matrix D” (MP Biomedicals), using 4
 236 cycles of 60 s at 6.5 m/s in 750 μL of 1X extraction buffer (Agrisera, AS08 300). The supernatant
 237 was assayed using a detergent compatible (DC) assay kit against BGG standard (Biorad), then
 238 equalized volumes containing 0.25 μg of denatured total protein containing 1x sample buffer
 239 (Invitrogen) and 50 mM DTT were loaded onto a 4-12% Bis Tris SDS-PAGE gel (Invitrogen).
 240 Each gel was also loaded with a 5-point quantitation curve using RbcL molar standard
 241 (www.agrisera.se, AS01 017S).

242

243 Proteins were separated via electrophoresis at 200 V then transferred to polyvinylidene difluoride
 244 (PVDF) membranes at 30 V. Membranes were blocked for 1 h in 2% $_{\text{w/v}}$ ECL blocking agent (GE
 245 Healthcare) dissolved in TBS-T (Tris, 20 mM; NaCl, 137 mM; Tween-20, 0.1% $_{\text{v/v}}$), then incubated
 246 in 1:20,000 rabbit polyclonal anti-RbcL antibody for 1 h (Agrisera, AS03 037) and finally in
 247 1:20,000 goat anti-rabbit IgG HRP conjugated antibody (Agrisera, AS10 668) for 1 h. Membranes
 248 were rinsed with TBS-T solution five times after each antibody incubation. Chemiluminescent
 249 images were obtained using ECL Ultra reagent (Lumigen, TMA-100) and a VersaDoc CCD imager
 250 (Bio-Rad). Band densities for samples were determined against the standard curve using the
 251 ImageLab software (v 4.0, Biorad).

252

253 Apparent Rubisco catalytic turnover rate ($\text{C RbcL}^{-1} \text{ s}^{-1}$) was computed as Wu et al. (2014):

$$254 \text{ RUBISCO catalytic turnover rate} = k_{\text{CAT}}^{\text{C}} = \frac{P_m^{\text{C}}}{\text{RbcL C}^{-1}} \quad \text{Equation 12}$$

255 where P_m^{C} is the carbon-specific maximum fixation rate ($\text{mol C g C}^{-1} \text{ s}^{-1}$) and RbcL C^{-1} (mol RbcL
 256 g C^{-1}) was estimated from immunoquantitation data normalized to carbon.

257

258 *Light absorption*

259

260 A dual beam spectrophotometer (Perkin Elmer, Lambda 850) equipped with an integrating sphere
261 was used to determine the spectral values of the optical density (OD (λ)) of the cultures. Filtered
262 culture medium was used as reference. The chlorophyll *a*-specific absorption coefficient (a^* (λ) in
263 $\text{m}^2 \text{mg Chl}a^{-1}$) was calculated as follow:

$$265 \quad a^*(\lambda) = \frac{2.3 \cdot \text{OD}(\lambda)}{l \cdot [\text{Chl}a]} \quad \text{Equation 13}$$

266
267 where l is the path length of the cuvette (0.01 m) and [Chl*a*] the chlorophyll *a* concentration (mg
268 m^{-3}).

269
270 *Statistical tests*

271 To test for differences between light and dark with regard to physiological characteristics we used
272 t-test. Normality was tested by a Shapiro-Wilk test. To test differences between mean growth rates
273 in Experiment 1 performed an ANOVA. Data analyses were performed using the Sigma Plot 12.5.
274 In the figures, asterisks indicate significant differences (one asterisk : $P < 0.05$, two: $P < 0.001$)
275 between light and dark acclimated cells. In the results section, statistics are described in detail (t, F
276 df, P). Results of Figure 5 statistics are presented in Online resource 5.

277
278 **Results**

279
280 **Experiment 1: From darkness to light.** We monitored the growth and photochemistry of cultures
281 incubated 1 month in the dark and then exposed to 4 different light cycles (different light level but
282 same duration of the photoperiod, see Online resource 1 and Material and methods section,). The
283 light cycles roughly mimicked a natural light cycle and allowed a progressive increase in growth
284 irradiance during the first hours after re-illumination. Cells were able to restart growth during the
285 first day after light recovery (Figure 1). At day 7 and later, growth rates began to decrease due to
286 unknown limitations in the 3 highest of the 4 light conditions. We computed the mean growth rates
287 between days 1 and 6 (i.e when growth was not yet limited). The mean growth rates increased with
288 mean growth irradiance between 5 and 41 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ and then remained constant under
289 higher irradiance (Anova test, $F_3=134.954$, $P < 0.001$ and see the Pairwise multiple comparison
290 procedure with Holm-Sidak method in Online resource 5). The irradiance-saturated growth rate

291 $(0.43 \pm 0.02 \text{ d}^{-1}$ at 41 and $154 \mu\text{mol photon m}^{-2} \text{ s}^{-1})$ is in the range of light saturated growth rates
292 found in polar species grown at 0°C (Sakshaug 2004, Lacour et al. 2017), and slightly lower than
293 *C. neogracilis* growth rates measured under continuous illumination in semi-continuous culture
294 (0.6 d^{-1} , unpublished results). Culture growth rates did not seem affected by the dark period after
295 illumination was resumed (Figure 1B). Between 0 and 8 hours, the instantaneous growth rate
296 remained almost null in all the treatments. Growth restarted between 8 and 32 hours after re-
297 illumination at high rate ($0.58 \pm 0.03 \text{ d}^{-1}$) and was not affected by growth irradiance at this early
298 stage. After 32 hours, the instantaneous growth rates were similar to the irradiance-specific mean
299 growth rates.

300
301 The maximum PSII electron transport rate (ETR_m) increased during the first hour after re-
302 illumination in all the conditions (Figure 2A, 3A). This increase was even more pronounced at high
303 irradiance with no apparent damage to PSII even at the highest re-illumination irradiances. The
304 light saturation parameter for photochemistry (E_K^{ETR}) also increased after re-illumination,
305 apparently to acclimate to the new growth conditions (Figure 2B, 3B). The increases were
306 particularly high at high irradiance, with a 75 % increase in ETR_m and a 124% increase in E_K at
307 $154 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ during the first hours of exposure to light. The slope of the ETR versus E
308 curves (α^{ETR}) decreased during the first day after re-illumination and then increased and stabilised
309 at a value that was dependent on growth irradiance (Figure 2C, 3C). Our results suggest that after
310 2-3 days, cells were nearly acclimated to growth conditions as photochemical parameters (α^{ETR} ,
311 ETR_m and E_K^{ETR}) stabilized. Mean α^{ETR} values (measured between day 3 and 5) decreased with
312 growth irradiance (Figure 2C, D) and mean ETR_m and E_K^{ETR} increased with growth irradiance
313 (Figure 2A, B, D).

314
315 **Experiment 2: Prolonged darkness.** We compared the physiological characteristics of cells
316 acclimated to $23 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ with cells incubated 1 month in complete darkness.
317 Microscopic observations did not reveal the presence of resting spores. Cells incubated in darkness
318 had a lower cell size (Figure 4A, Paired t test, $t_4 = 19.822$, $p < 0.0001$). A fraction of the cells
319 divided once during the first hours of the dark period, which may explain the decrease in the mean
320 cell size. Indeed, the total biovolume of the culture (cell volume x cell number) remained constant
321 throughout the dark period (data not shown). Cell viability was not directly assayed in this study,

322 so that the proportion of the cells that remained viable at the end of the dark period is unknown.
323 However, the rapid and intense growth after light recovery suggests that most of the cells remained
324 viable.

325
326 We observed slightly higher Chl *a* to C ratio in cells incubated in the dark (Figure 4C, Paired t test,
327 $t_4 = -2.987$, $p=0.04$) and relatively unchanged chlorophyll specific absorption coefficient (a^* ,
328 Online resource 2, Paired t test, $t_{10} = 0.893$, $p=0.393$) and optical absorption cross section of PSII
329 ($\sigma^{\text{OPT}}_{\text{PSII}}$, Online resource 3). Cells thus maintained their ability to capture light throughout the dark
330 period. Pigment contents remained unchanged after the dark period with the exception of
331 xanthophylls (Figure 4D). Total xanthophyll was not changed (Paired t test, $t_4 = -1.386$, $p = 0.238$)
332 but the de-epoxidation ratio was dramatically higher in cells incubated in darkness ($\text{DES} = 58$,
333 Paired t test, $t_4 = -160.045$, $p < 0.0001$). This DES was indeed higher than the DES measured in a
334 previous study on *C. neogracilis* acclimated to very high irradiance (continuous light, $400 \mu\text{mol}$
335 $\text{photon m}^{-2} \text{s}^{-1}$; $\text{DES}=27$). This is an unexpected result because diatoxanthin is generally produced
336 at high irradiances (see discussion). The C/N ratio was lower in cells incubated in the dark (Figure
337 4B, Paired t test, $t_4 = 2.810$, $p = 0.048$). The Rubisco to carbon ratio was not significantly different
338 between light and dark (Figure 4E, Paired t test, $t_3 = -0.218$, $p=0.841$). Unchanged Rubisco content
339 and decreased photosynthetic capacity (see below) led to a decrease of the apparent catalytic
340 turnover rate of Rubisco ($k^{\text{C}}_{\text{cat}}$, C s^{-1} per site) in darkness.

341
342 We used incubations at various irradiances to investigate the potential for photochemistry in cells
343 acclimated to the light and to the dark. We used the approach of Hendrickson et al. (2004) to
344 compare the potential fate of absorbed light energy (Figure 5 and materials and methods section).
345 The fraction of absorbed irradiance consumed via photochemistry (Φ_{PSII}) was higher in cells
346 acclimated to $23 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ across all the incubation irradiances tested (see the results of
347 the statistical tests in Online resource 5). The regulated thermal dissipation (Φ_{NPQ}) was also
348 generally significantly greater in the light than in the dark (see Online resource 5). On the contrary,
349 cells incubated 1 month in the dark showed a much larger fraction of absorbed energy consumed
350 by non-regulated thermal dissipation and fluorescence ($\Phi_{\text{f,D}}$). $\Phi_{\text{f,D}}$ is largely dominated by thermal
351 dissipation since fluorescence accounts for only a small fraction of absorbed excitation
352 (Hendrickson et al. 2004). The high de-epoxidation ratio found in the dark may be responsible for

353 a constitutive NPQ that is measured as non-regulated thermal dissipation since short-term changes
354 in light intensity do not alter its efficiency.

355
356 Cells incubated in the dark showed significantly lower F_v/F_m (Paired t test, $t_3 = 27.626$, $p = 0.0001$),
357 σ_{PSII} (Paired t test, $t_3 = 14.017$, $p = 0.0007$) and $F_0/(\sigma_{PSII} \text{ Chl } a)$ (Paired t test, $t_3 = 18.002$, $p = 0.0004$),
358 a proxy for the content of active PSII (Figure 6A) (Oxborough et al. 2012, Silsbe et al. 2015,
359 Murphy et al. 2017). The PSII ETR versus incubation irradiance curves were highly affected by
360 the dark period (Figure 6B, 6C and **Online resource** 4). ETR_m and α^{ETR} were both 2.3 fold lower
361 after 1 month in the dark, (Paired t test, $t_{10} = 9.320$, $p < 0.0001$ and Paired t test, $t_{10} = 10.336$, $p <$
362 0.0001 respectively), resulting in an unchanged E_K^{ETR} (Paired t test, $t_{10} = -0.411$, $p = 0.690$).

363
364 We obtained carbon fixation rate versus incubation irradiance curves (P vs E curves) for cells
365 incubated 1 month in the dark. We compared the photosynthetic characteristics of cells incubated
366 in the dark with cells acclimated to 3 other continuous growth irradiances (10, 50, 80 $\mu\text{mol photon}$
367 $\text{m}^{-2} \text{s}^{-1}$) (see methods, Figure 7). The carbon-specific maximum fixation rate (P_m^C) was not affected
368 by growth irradiance (Figure 7A) and was ~9 times higher than the P_m^C of cells incubated in the
369 dark for 1 month (Figure 7B, Paired t test, $t_{10} = 31.516$, $p < 0.0001$). The Chl *a* specific initial slope
370 of the PI curve (α^*) was also ~7 times higher in the light than in the dark (Figure 7C, Paired t test,
371 $t_{10} = 3.998$, $p = 0.0025$). The capacity of cells incubated in the dark to fix carbon was thus initially
372 restricted upon re-exposure to both low and high irradiance.

373 **Discussion**

374
375 **Physiology of recovery.** The duration of the total darkness is often longer than one month in the
376 Arctic environment. However, most of the physiological acclimatory changes have been shown to
377 occur during the first days of the dark period and cell physiology after one month seems to be
378 therefore representative of the dark acclimation state (Peters and Thomas 1996).
379 Diatoms, particularly polar species are known for their dark survival capabilities (Antia and Cheng
380 1970, Bunt and Lee 1972, Smayda and Mitchell-Innes 1974, Palmisano and Sullivan 1982, 1983,
381 Murphy and Cowles 1997, Fang and Sommer 2017, Kvernvik et al. 2018). However, how they
382 achieve survival and deal with the return to light is unclear.

383

384 In order to study light recovery from darkness in *C. neogracilis*, we used a range of light from 5 to
385 $154 \mu\text{mol photon}^{-1} \text{m}^{-2} \text{s}^{-1}$. We did not observe any time delay before growth resumed in any of the
386 light regimes (Figure 1). Such rapid recovery is not a ubiquitous response in microalgae. For
387 example, the pelagophyte *Aureococcus anophagefferens* needs more than 20 days to restart growth
388 after 30 days in the dark (Popels and Hutchins 2002). The duration of the lag phase was shown to
389 depend on temperature, on the duration of the dark period and on the growth phase before the dark
390 period and may result from having a significant proportion of the cells being dead (Peters 1996,
391 Peters and Thomas 1996, Popels and Hutchins 2002). In the field, Berge et al. (2015) showed that
392 in Kongsfjorden (Svalbard) during the polar night, primary producers were physiologically active
393 and able to rapidly restart photosynthesis as soon as irradiance reached $0.5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.
394 Our results demonstrate the strong ability of *C. neogracilis* to survive long periods of darkness and
395 a high level of physiological plasticity allowing fast growth recovery upon re-illumination.

396
397 Our results show that the light intensity experienced during re-illumination only slightly influence
398 the growth rate recovery. The initial growth recovery was fast and intense, as instantaneous growth
399 rate was 0.58 d^{-1} between 8 and 32 hours, which corresponds to almost 1 doubling per day. Such a
400 growth rate is similar to the growth rates measured in healthy light-saturated cultures of *C.*
401 *neogracilis* at 0°C (unpublished results) and higher than the mean growth rate of polar species at
402 0°C ($0.46 \pm 0.23 \text{ d}^{-1}$, (Lacour et al. 2017)). It also indicates that most of the cells remained viable
403 throughout the dark period. Moreover, the resumption of growth between 8 and 32 hours was also
404 high under re-illumination with only $5 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, which is a light-limiting level for longer
405 term growth. This suggests that the growth resumption was not fuelled mainly by photosynthesis
406 but likely by carbon reserves. During the following days, growth stabilized at different rates, which
407 were indeed dependent on light intensity. Such rapid recovery relies largely on the rapid
408 acclimation of its photophysiology. The photochemical properties of the cells rapidly acclimated
409 to the new growth conditions (Figure 2, Figure 3). Kvernvik et al. (2018) also showed rapid
410 increases in the efficiency of photosynthetic electron transport of natural phytoplankton
411 communities upon re-illumination. The immediate and intense increase (within 1 hour) of E_K and
412 ETR_m suggests a re-organisation of the existing photosystems rather than *de novo* synthesis of new
413 proteins and pigments (Peters and Thomas 1996, Baldisserotto et al. 2005, Morgan-Kiss et al. 2006,
414 Ferroni et al. 2007, Nymark et al. 2013). It can be explained by light-induced reactivation of

415 enzymes involved in downstream reactions (Maxwell and Johnson 2000). The relaxation of
416 xanthophyll-related NPQ may also account for such rapid recovery of photochemical capacity.
417 Culture growth rates (computed between days 1 and 6) and photophysiological properties (Figure
418 2D) are comparable to those of this microalgae (unpublished results) and other polar microalgae
419 cultured without previous period of darkness under comparable light intensities (reviewed in
420 Lacour et al. (2017)). It suggests that prolonged darkness has no impact on Arctic diatom ability
421 to acclimate to new growth conditions.

422

423 **Dark physiology. How does *C. neogracilis* prepare for rapid growth recovery?**

424

425 *C. neogracilis* cells kept in darkness maintain their capacity to capture light, as shown by the
426 photosynthetic pigments (Figure 4C), the chlorophyll specific absorption coefficient (Online
427 resource 2) and the optical absorption cross section of PSII ($\sigma_{\text{PSII}}^{\text{OPT}}$, Online resource 3) that
428 remained relatively unchanged after one month in the dark, with $a^*(455\text{nm})$ and $\sigma_{\text{PSII}}^{\text{OPT}}(455\text{nm})$
429 are $\approx 0.9X$ of the levels found in cells acclimated to the light (data not shown). At 12°C, Chl *a*
430 per Cell in *Thalassiosira weissflogii* was previously shown to remain constant during 2 months in
431 total darkness (Murphy and Cowles 1997). The benefit to maintaining their light harvesting
432 capacity is probably the ability to resume growth relatively promptly when conditions become
433 favorable upon re-illumination.

434

435 The potential for photochemistry was, however, drastically reduced. We observed decreases in
436 F_v/F_m , σ_{PSII} (the effective, rather than the optical, absorption cross section) and consequently in
437 both the light-limited (α_{ETR}) and light-saturated (ETR_m) activity of PSII. Decreases in the potential
438 for photochemistry were observed in polar diatoms (Wulff et al. 2008, Reeves et al. 2011, Martin
439 et al. 2012) and polar rhodophytes (Lüder et al. 2002) incubated in darkness and were interpreted
440 as a progressive degradation of light-harvesting antennae and/or reaction centres. Nymark et al.
441 (2013) suggested instead that decreases in α_{ETR} were explained by lower resonance energy transfer
442 efficiency from the light-harvesting antenna pigments to the PSII reaction centre, probably
443 resulting from structural changes within the light-harvesting antenna complexes. Our results
444 suggest that in the case of *C. neogracilis*, the decrease of the potential for photochemistry is a
445 regulated response to prolonged darkness rather than actual PSII damage or dismantlement. Indeed,

446 this decrease may be at least partially due to an accumulation of Diatoxanthin (35 fold DES
447 increase) that induces sustained heat dissipation and thereby lowers σ_{PSII} even though pigment
448 content and $\sigma^{\text{OPT}}_{\text{PSII}}$ are maintained. Some observations in the field (Brunet et al. 2006, Brunet et
449 al. 2007) and in culture (Deventer and Heckman 1996, Jakob et al. 1999, Lavaud et al. 2002)
450 showed that microalgae exposed to darkness exhibit significant levels of chlororespiration.
451 Chlororespiratory energization of the thylakoid membrane maintains a proton gradient, an activated
452 xanthophyll cycle and an ATP synthase in an active state during dark periods, which could be
453 advantageous upon re-exposure to light (Goss and Jakob 2010). This respiratory pathway may also
454 provide energy to sustain metabolic activity and/or balance the ATP:reductant levels in the
455 chloroplast under prolonged darkness. The accumulation of Dt probably keeps *C. neogracilis* cells
456 in a photo-protected, highly dissipative state with a low conversion efficiency of absorbed light
457 into photochemistry and thus a low risk of reactive oxygen dependent damage (Oguchi et al. 2011,
458 Murphy et al. 2017). When light returns, *C. neogracilis* can relax xanthophyll-related NPQ to
459 optimize light harvesting.

460
461 The potential for carbon fixation was particularly affected by darkness. Both the light-limited (α)
462 and the light-saturated carbon-specific fixation rates (P^{C}_{m}) (20 min incubations at various
463 irradiances) were drastically lowered in dark conditions, to an even greater degree than the drop in
464 ETR_{m} . Several authors noticed a drop in photosynthetic capacity after several days in darkness
465 (Palmisano and Sullivan 1982, Dehning and Tilzer 1989, Peters and Thomas 1996). Interestingly,
466 the Rubisco protein content was not significantly affected by darkness. Thus, Rubisco protein pool
467 size (Young et al. 2015) is not in this case responsible for the observed decrease in P^{C}_{m} as illustrated
468 by the reduced apparent catalytic turnover rate of Rubisco in darkness. However, a regulated
469 decrease in Rubisco activity could account for the decline in P^{C}_{m} . In fact, MacIntyre et al. (1997)
470 showed that deactivation of the carbon assimilating machinery (e.g., RuBisCO) occur very rapidly
471 in light–dark transitions in the chlorophyte *Dunaliella tertiolecta* and especially in the diatom
472 *Thalassiosira pseudonana* (timescale: minute). The maintenance of the Rubisco pool size probably
473 contributes to the rapid recovery through re-activation when light returns.

474
475 The discrepancy between the 2.3 fold dark decrease in ETR_{m} versus the 9 fold dark decrease of
476 P^{C}_{m} shows that during prolonged darkness carbon fixation potential is down regulated more than

477 photochemistry potential per PSII. We used a proxy of the amount of active PSII per Chl *a*
478 (Oxborough et al. 2012, Silsbe et al. 2015, Murphy et al. 2016) to understand if the number of
479 active PSII can help to reconcile carbon fixation and photochemistry. The number of active PSII
480 indeed decreased significantly by 1.5 fold in the dark but cannot fully account for the discrepancy
481 between a predicted $2.3 \times 1.5 = 3.5$ fold decrease in the potential for electron transport and the 9
482 fold decrease in the potential for carbon fixation. This suggests that electrons produced at PSII are
483 diverted away from carbon fixation when cells acclimated to prolonged darkness are re-
484 illuminated. Schuback et al. (2017) had similar observations in Arctic field populations,
485 particularly in assemblages exposed to short-term super-saturating irradiances. They also suggested
486 that it could be related to an up-regulation of alternative electron pathways. Laboratory (Wagner et
487 al. 2006, Bailey et al. 2008, Cardol et al. 2008) and field studies (Mackey et al. 2008, Grossman et
488 al. 2010, Schuback et al. 2015, Schuback et al. 2017, Zhu et al. 2017, Hughes et al. 2018b) have
489 examined the processes that uncouple rates of CO₂ assimilation and photosynthetic electron
490 transport. Those pathways are generally active under high irradiance or under severe nutrient
491 limitation, when cells are subjected to metabolic unbalance (see also the review by Hughes et al.
492 (2018a)). After one month under complete darkness, some downstream photosynthetic processes
493 are probably constrained, generating such metabolic unbalance. Alternative electron pathways may
494 create an electron valve on the acceptor side of PSII and thus protect the system from photodamage
495 by lowering the redox pressure until carbon assimilation can be re-activated. Again, upon light
496 recovery, those alternative electron flows can be rapidly redirected to carbon fixation in order to
497 fuel growth.

498

499 **Conclusion**

500

501 Polar microalgae live under extreme environmental conditions: permanently low temperatures,
502 extreme variations in irradiance and above all, long period of complete darkness. The ability to
503 survive such periods of darkness and to re-initiate growth when light returns affects the fitness of
504 a phytoplankton species in this environment. This study illustrates how the Arctic diatom *C.*
505 *neogracilis* is able to withstand long periods of darkness and sudden light bursts of variable
506 intensity. The capacity to recover safely and rapidly relies on the maintenance, through the dark
507 period, of the main components of the photosynthetic machinery (PSII and pigments, Rubisco).

508 The flexibility of *C. neogracilis* probably relies on the induction of xanthophyll-related NPQ and
509 possible induction of alternate electron pathways. The extremely low expenditures of energy during
510 darkness - suggested by undetectable organic carbon consumption during one month in darkness-
511 is one extremely important aspect that was not directly studied in this work and needs further
512 investigation.

513

514 **Compliance with Ethical Standards**

515 The authors declare that they have no conflict of interest.

516

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832

833 **Figure1_Lacour et al.**

834
835 **Figure 1:** Changes in cell density of *Chaetoceros neogracilis* cultures exposed to 4 different light
836 cycles after 1 month in total darkness at 0°C (A). Mean growth rates of triplicate cultures computed
837 between days 1 and 6 are indicated on the graph. Note that mean growth rates were equal in cells
838 exposed to 41 and 154 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. Each data point is the mean \pm SD of the 3 different
839 cultures. Instantaneous growth rates of *C. neogracilis* measured during the first eight hours,
840 between eight and 32 hours and between 32 and 54 hours under different light cycles after 1 month
841 in darkness (B). Each bar is the mean \pm SD of the 3 different cultures.

842
843 **Figure2_Lacour et al.**

844
845 **Figure 2:** Changes in photochemical properties of *Chaetoceros neogracilis* at 4 different light
846 cycles after 1 month in total darkness at 0°C. ETR_m (A), E_K^{ETR} (B), α^{ETR} (C) as a function of time
847 under different light cycles. In A, B, C each data point is the mean \pm SD of triplicate cultures.
848 Relationship between mean growth irradiance and mean ETR_m , E_K^{ETR} and α^{ETR} (D). In D, each data
849 point is the mean \pm SD of 3 consecutive days (day 3, 4 and 5) from triplicate cultures.

850
851 **Figure3_Lacour et al.**

852
853 **Figure 3:** Enlargement of part of Figure 2. ETR_m (A), E_K^{ETR} (B), α^{ETR} (C) as a function of time
854 under different light cycles. In A, B, C each data point is the mean \pm SD of triplicate cultures.

855
856 **Figure4_Lacour et al.**

857
858 **Figure 4:** Biochemical characteristics of cells acclimated to 23 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ or to the dark.
859 Cell diameter (A), C\N (B), Chl *a*/C (C), (Dd + Dt)/Chl *a*, DES (DES =Dt/(Dd+Dt)), Dt/Chl *a* and
860 β -carotene/Chl *a* (D), Rubisco to carbon ratio and apparent Rubisco catalytic turnover rate (s^{-1})(E)
861 in cultures acclimated to 23 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ (white bars) and after 1 month in the dark (black
862 bars). Each bar is the mean of 3 different cultures. Error bars represent standard deviations.
863 Asterisks indicate significant differences (one asterisk : $P < 0.05$, two: $P < 0.001$) between light and
864 dark acclimated cells.

865

866 **Figure5_Lacour et al.**

867

868 **Figure 5:** Estimated fraction of absorbed irradiance consumed via photochemistry (Φ_{PSII}),
869 regulated thermal dissipation (Φ_{NPQ}) and non-regulated thermal dissipation and fluorescence ($\Phi_{\text{f,D}}$)
870 as a function of incubation irradiance for cultures of *Chaetoceros neogracilis* acclimated to 23
871 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ (A) and incubated one month in the dark (B). Those parameters were defined
872 by Hendrickson et al. (2004) to compare the fate of absorbed light. Each point is the mean of 3
873 different cultures. Error bars represent standard deviations.

874

875 **Figure6_Lacour et al.**

876

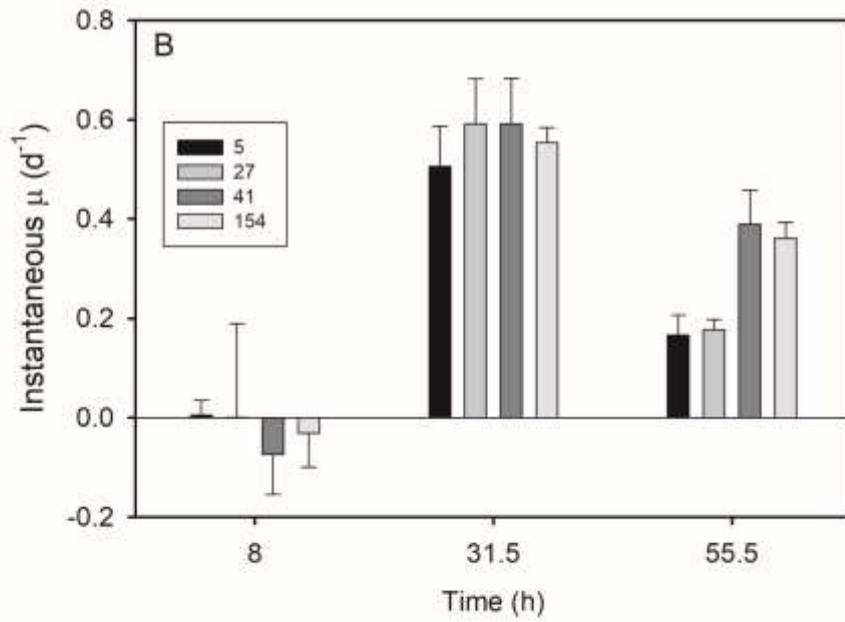
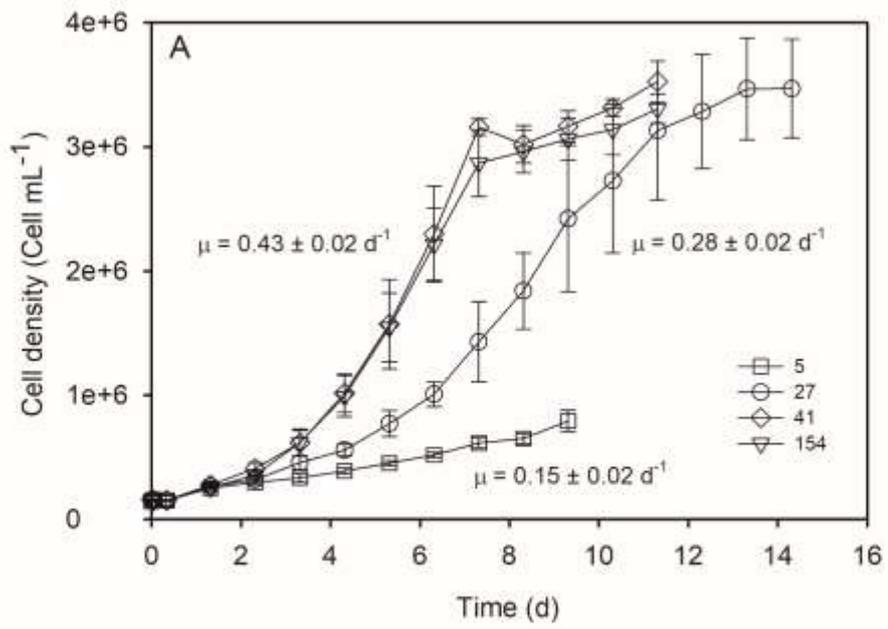
877 **Figure 6:** PSII activity in the light and in the dark. F_v/F_m , σ_{PSII} and proxy for PSII content $F_o/$
878 ($\sigma_{\text{PSII Chl } a}$) (A), ETR_m and E_K^{ETR} (B), α^{ETR} (C) in cultures acclimated to 23 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$
879 (white bars) and after 1 month in the dark (black bars). Each bar is the mean of 3 different cultures.
880 Error bars represent standard deviations. Asterisks indicate significant differences (one asterisk :
881 $P < 0.05$, two: $P < 0.001$) between light and dark acclimated cells.

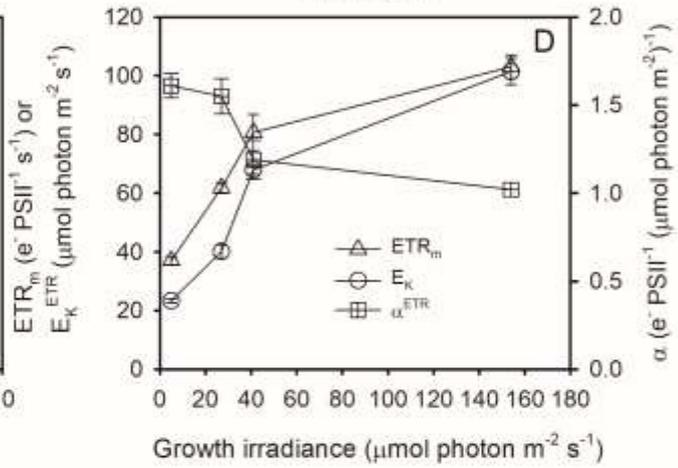
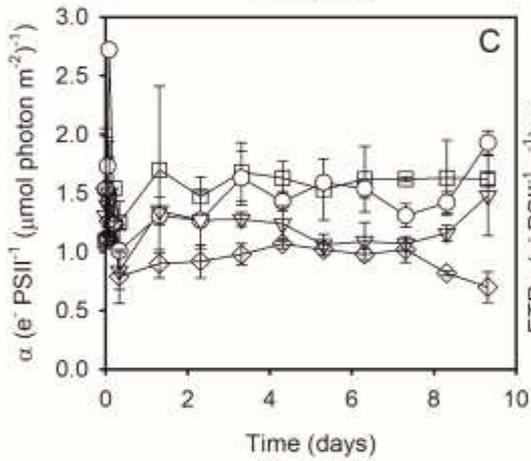
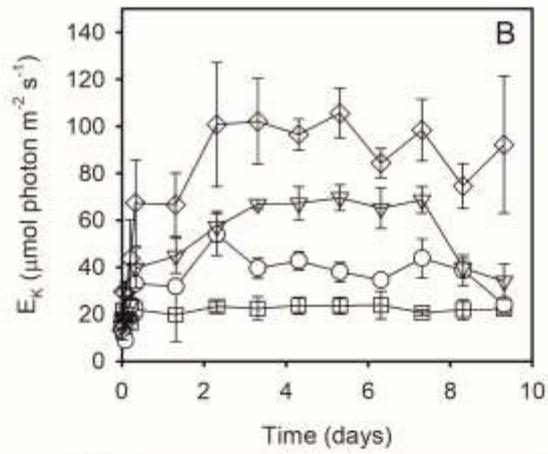
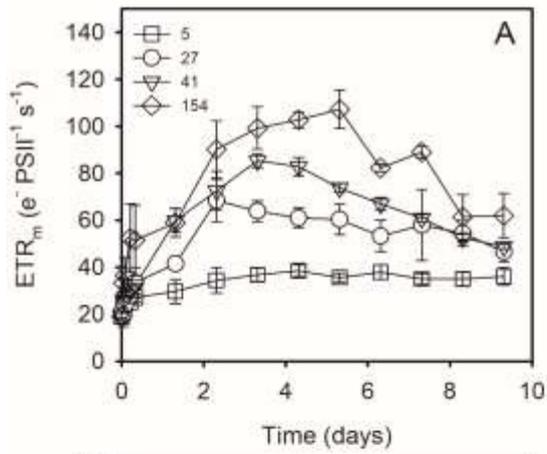
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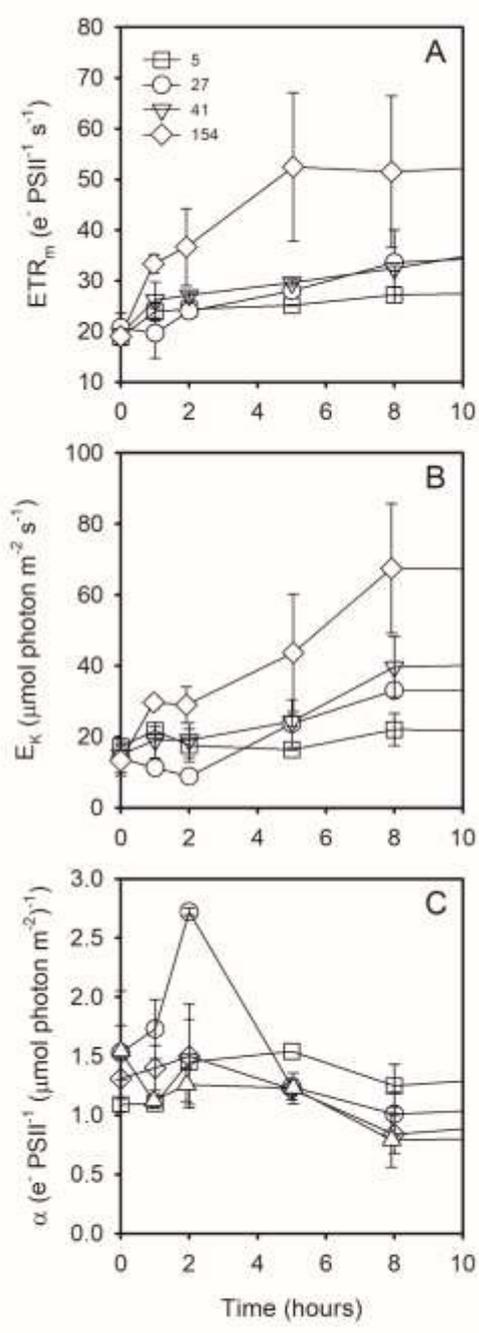
883 **Figure7_Lacour et al.**

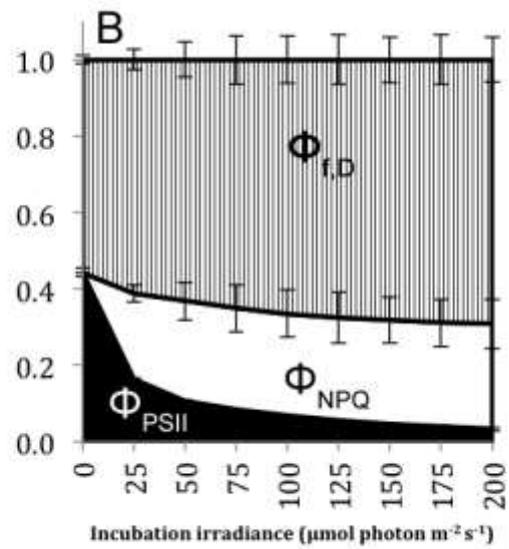
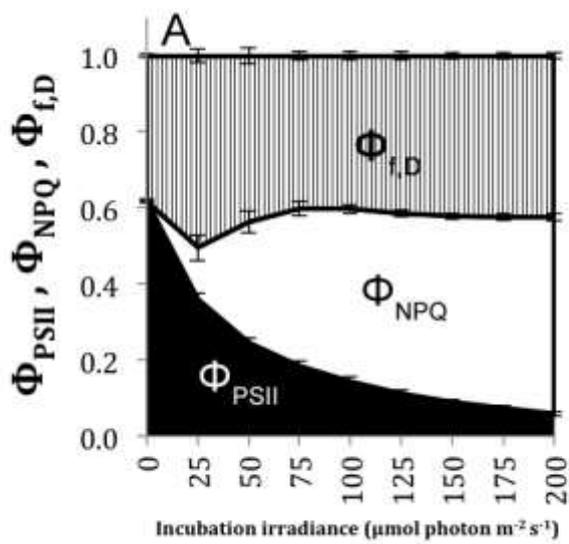
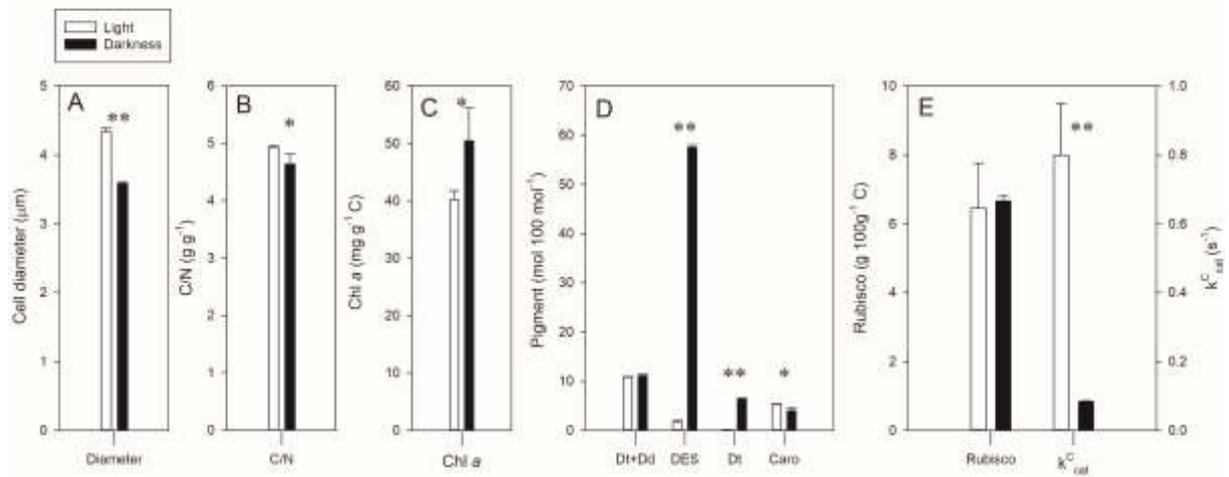
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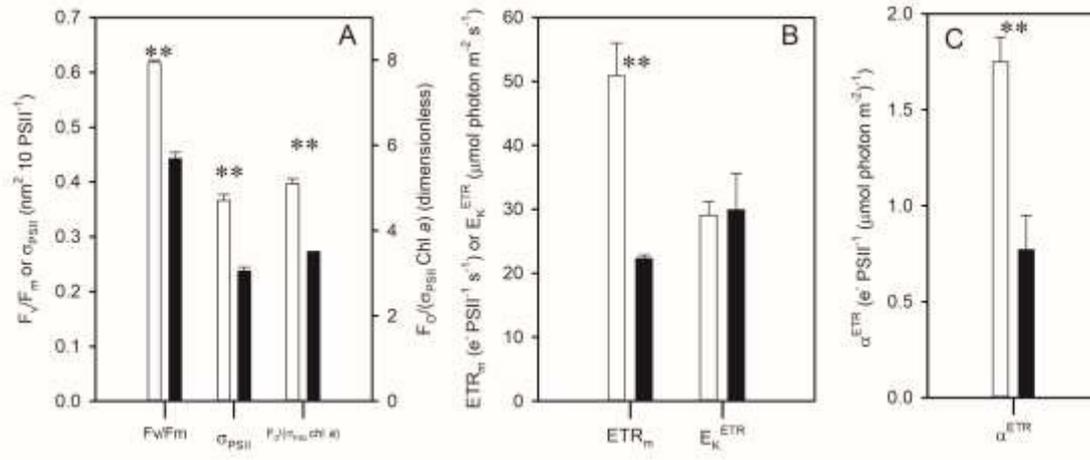
885 **Figure 7:** Carbon specific fixation rate versus incubation irradiance curves of cells incubated in
886 the dark for one month or acclimated to 10, 50 and 80 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ (minimum of 10
887 generations, see methods) (A). Each data point is the mean of measures from 3 different cultures.
888 Error bars represent standard deviations. α^* (B) and P_m^C (C) of cell incubated in the dark (black
889 bars, mean \pm SD of triplicate cultures incubated 1 month in the dark) or pooled determinations for
890 cultures acclimated to light (white bars, mean \pm SD of pooled determinations from 10, 50 and 80
891 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$). Asterisks indicate significant differences (one asterisk: $P < 0.05$, two:
892 $P < 0.001$) between light and dark acclimated cells.

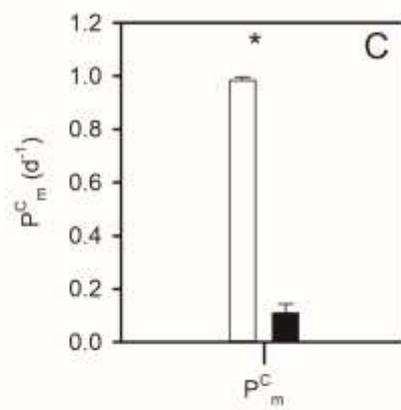
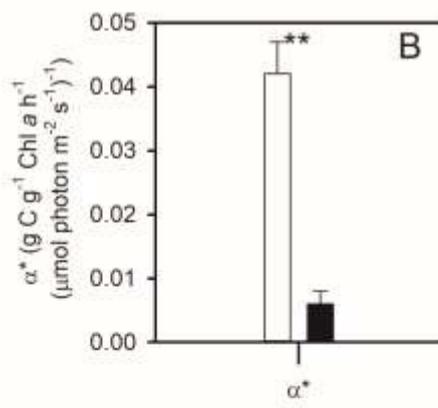
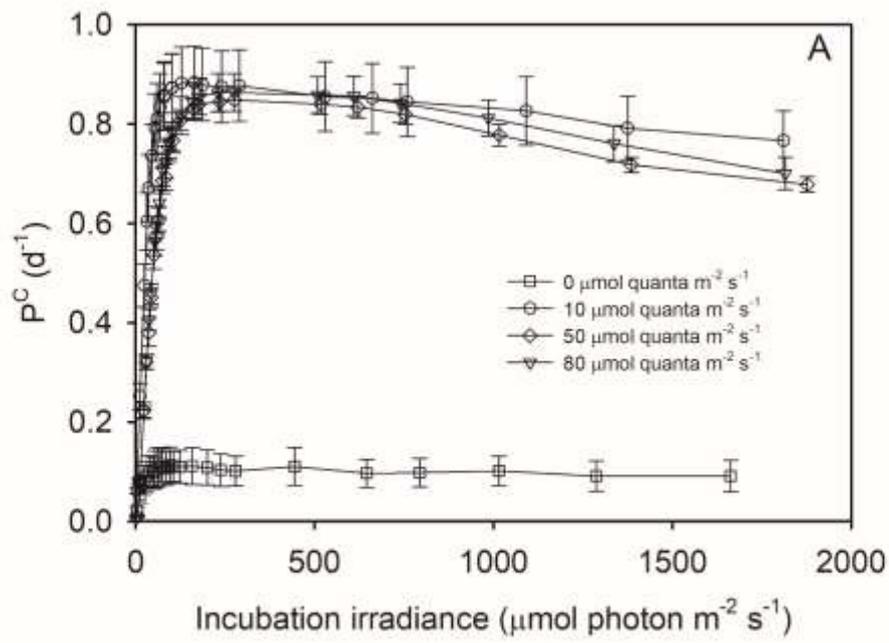














Online resources

Decoupling light harvesting, electron transport and carbon fixation during prolonged darkness supports rapid recovery upon re-illumination in the polar diatom *Chaetoceros neogracilis*

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Online resource 1: Diel light cycle applied during light recovery. Irradiance as a function of the time of the day in cultures previously incubated in the dark for 1 month. Triplicate cultures were illuminated at each light cycle. Mean diel growth irradiances were 154, 41, 27 and 5 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. Maximum growth irradiances (at noon) were 531, 141, 93 and 17 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$.

Online resource 2: Chlorophyll *a* specific absorption coefficient (a^* in $\text{m}^2 \text{mg Chla}^{-1}$) versus the wavelength of light in cultures acclimated to 23 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ and after 1 month in the dark (A). Relative difference (in %) between a^* at 23 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ and after 1 month in the dark (B).

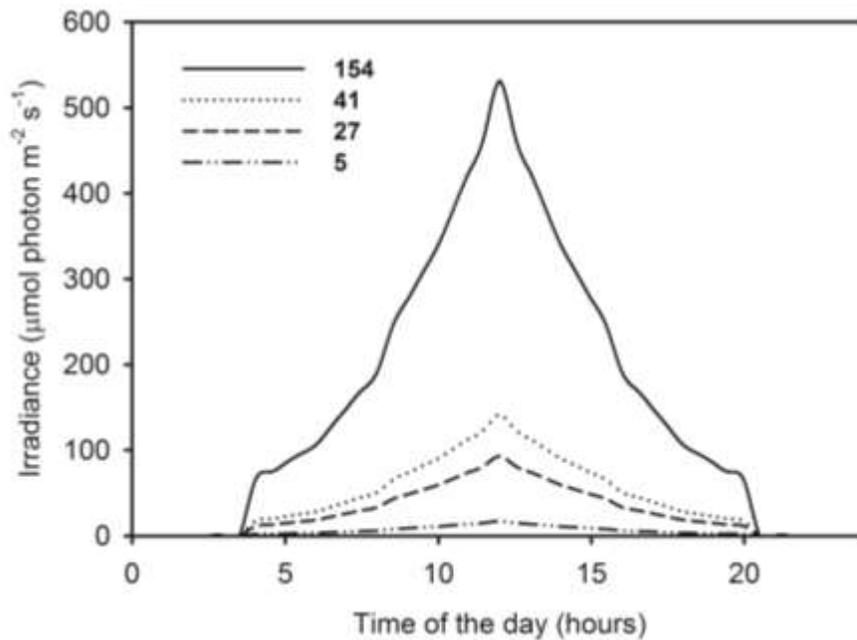
Online resource 3: $\sigma^{\text{OPT}}_{\text{PSII}}$ in cells acclimated to 23 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ (white bars) and after 1 month in the dark (black bars).

Online resource 4: Electron transport rate versus incubation irradiance curves of cells incubated in the dark for one month or acclimated to 23 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. Each data point is the mean of measures from 3 different cultures. Error bars represent standard deviations. A model (Platt *et al.*, 1980) was fitted to the data to estimate ETR_m , E_K^{ETR} , α^{ETR} and β^{ETR} (see Figure 3, 7 and methods).



Online resource 5: Results of the t-tests comparing photo-physiological parameter at different irradiance of cells acclimated to light and (see statistic section and Figure 4).

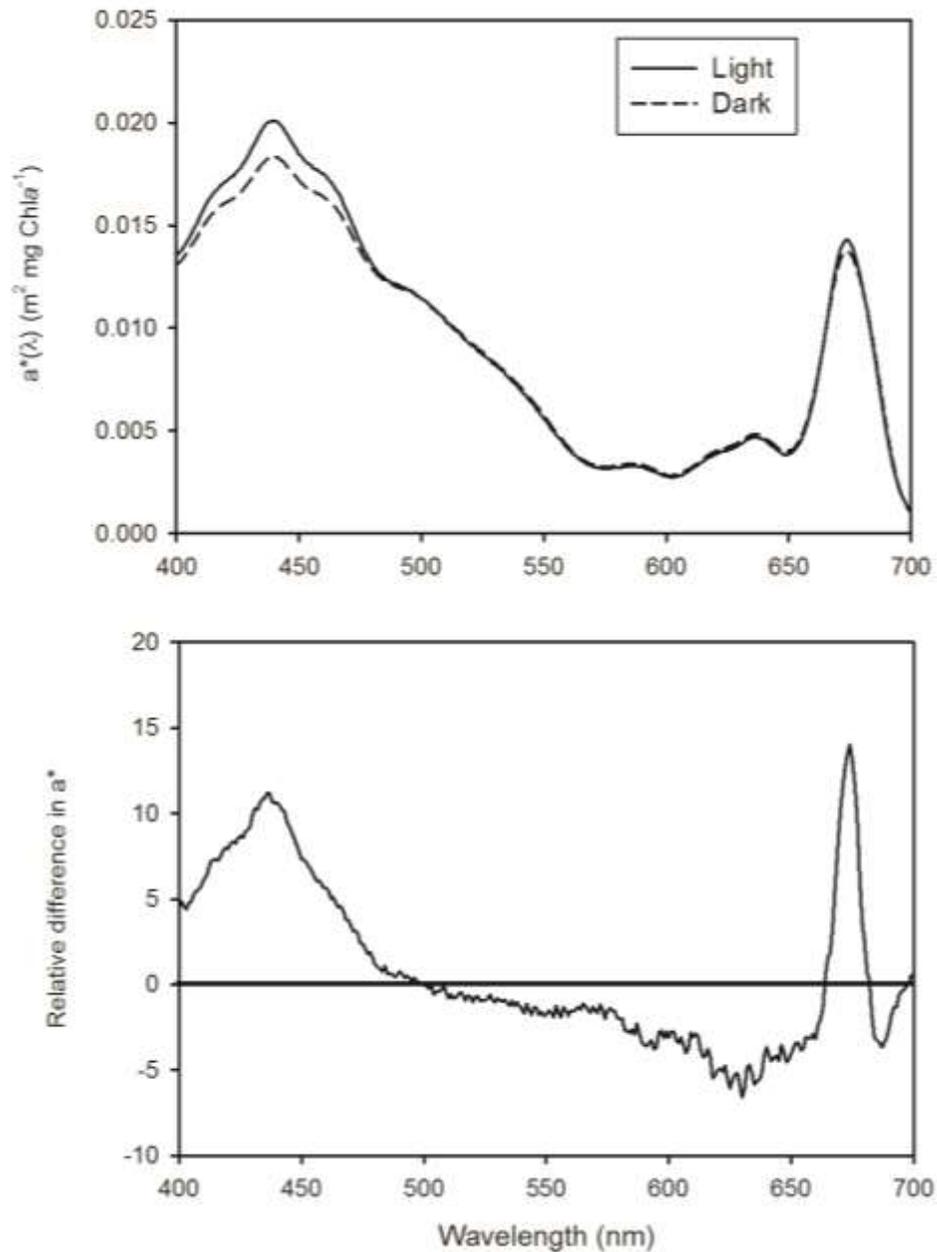
Online_resource_1_Lacour et al.



Online resource 1: Diel light cycle applied during light recovery. Irradiance as a function of the time of the day in cultures previously incubated in the dark for 1 month. Triplicate cultures were illuminated at each light cycle. Mean diel growth irradiances were 154, 41, 27 and 5 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. Maximum growth irradiances (at noon) were 531, 141, 93 and 17 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$.



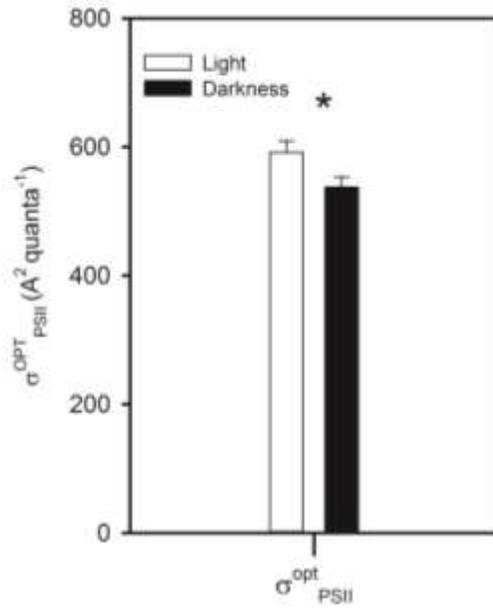
Online_resource_2_Lacour et al.



Online resource 2: Chlorophyll *a* specific absorption coefficient (a^* in $\text{m}^2 \text{mg Chla}^{-1}$) versus the wavelength of light in cultures acclimated to $23 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ and after 1 month in the dark (A). Relative difference (in %) between a^* at $23 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ and after 1 month in the dark (B).

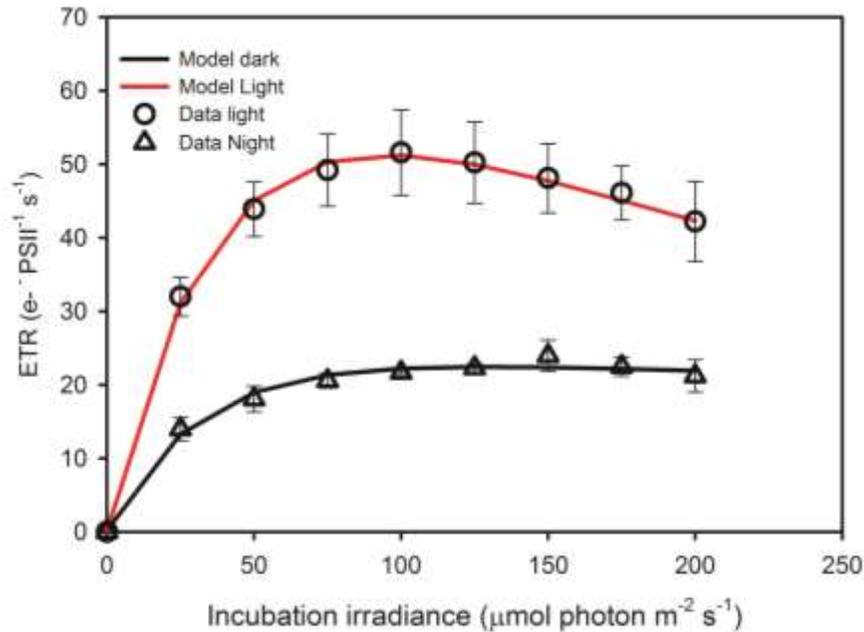


Online_resource_3_Lacour et al.



Online resource 3: $\sigma_{\text{PSII}}^{\text{OPT}}$ in cells acclimated to 23 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ (white bars) and after 1 month in the dark (black bars).

Online_resource_4_Lacour et al.



Online resource 4: Electron transport rate versus incubation irradiance curves of cells incubated in the dark for one month or acclimated to $23 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$. Each data point is the mean of measures from 3 different cultures. Error bars represent standard deviations. A model (Platt *et al.*, 1980) was fitted to the data to estimate ETR_m , E_K^{ETR} , α^{ETR} and β^{ETR} (see Figure 3, 7 and methods).

**Online_resource_5_Lacour et al.**

Results of the t-tests comparing photo-physiological parameter at different irradiance of cells acclimated to light and (see statistic section and Figure 5). Results of each test (df, t, p) at each incubation irradiance are presented.

E	Φ_{PSII}			Φ_{NPQ}			$\Phi_{f,d}$		
	df	t	p	df	t	p	df	t	p
0	9	34.55	<0.0001	NA	NA	NA	9	-34.550	<0.0001
25	9	14.678	<0.0001	9	-3.505	0.0067	9	-7.096	<0.0001
50	9	15.869	<0.0001	9	2.139	0.0611	9	-10.084	<0.0001
75	9	11.148	<0.0001	9	7.132	<0.0001	9	-13.592	<0.0001
100	9	9.259	<0.0001	9	10.201	<0.0001	9	-14.832	<0.0001
125	9	9.165	<0.0001	9	11.112	<0.0001	9	-14.174	<0.0001
150	9	9.254	<0.0001	9	13.057	<0.0001	9	-15.326	<0.0001
175	9	9.665	<0.0001	9	13.313	<0.0001	9	-14.734	<0.0001
200	9	5.379	0.0004	9	13.361	<0.0001	9	-16.100	<0.0001

Platt T, Gallegos CL, and Harrison WG. 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *Journal of Marine Research*. 38:687-701.