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1 **Response of the sea-ice diatom *Fragilariopsis cylindrus* to simulated polar**
2 **night darkness and return to light**

3

4 Morin, Philippe-Israël¹; Lacour, Thomas²; Grondin, Pierre-Luc¹; Bruyant, Flavienne¹;
5 Ferland, Joannie¹; Forget, Marie-Hélène¹; Massicotte, Philippe¹; Donaher, Natalie³;
6 Campbell, Douglas A³; Lavaud Johann¹ & Babin, Marcel¹

7

8 ¹UMI 3376 Takuvik, CNRS/Université Laval, Département de Biologie-Pavillon Alexandre Vachon,
9 Québec, QC, G1V 0A6, Canada

10 philippe-israel.morin.1@ulaval.ca

11 pierre-luc.grondin.1@ulaval.ca

12 flavienne.bruyant@takuvik.ulaval.ca

13 Joannie.Ferland@takuvik.ulaval.ca

14 Marie-Helene.Forget@takuvik.ulaval.ca

15 philippe.massicotte@takuvik.ulaval.ca

16 Johann.Lavaud@bio.ulaval.ca

17 Marcel.Babin@takuvik.ulaval.ca

18

19 ²Ifremer, PBA, Rue de l'Île d'Yeu, BP21105, 44311 Nantes Cedex 03, France

20 Thomas.Lacour@ifremer.fr

21

22 ³Mount Allison University, Sackville, NB, E4L 3M7, Canada

23 dcampbell@mta.ca

24 ndonaher@mta.ca

25

26 Author for correspondence:

27 *Philippe-Israël Morin*

28 *Tel: +1 418 656 5193*

29 *Email: philippe-israel.morin.1@ulaval.ca*

30 Running head: Diatoms polar night darkness survival

31 **Abstract**

32 Arctic photoautotrophic communities must survive through polar night darkness until light
33 returns in spring. We tracked changes in the cellular resource allocations and functional
34 capacities of a polar sea-ice diatom, *Fragilariopsis cylindrus*, to understand acclimation
35 processes in both darkness and during the subsequent return to light. We measured
36 parameters at specific time-points over 3 months of darkness, and then over 6 days after a
37 return to light. Measured parameters included cell number and size, cellular carbon and
38 nitrogen quotas, lipid and pigment contents, concentration of key proteins of the
39 photosynthetic system, photosynthetic parameters based on both variable fluorescence and
40 carbon assimilation, and the level of non-photochemical quenching.

41 A stable functional state was reached within a few days after the transition to dark and was
42 then maintained throughout three months of darkness. The dark period resulted in a
43 decrease of lipid droplet cell quota (-6%), chlorophyll *a* cell quota (-41%) and the
44 maximum carbon fixation rate per cell (-98%). Return to light after 1.5 months of darkness
45 resulted in a strong induction of non-photochemical quenching of excitation and a fast
46 recovery of the maximum carbon fixation rate within 1 day, followed by a rapid increase in
47 the cell number. Return to light after three months of darkness showed an increase of
48 mortality or a profound down-regulation induced over longer periods of darkness.

49

50 Keywords: *Fragilariopsis cylindrus*, sea-ice diatom, diatoms, acclimation, dark survival,
51 polar night, light return, darkness

52

53

54 Introduction

55 Diatoms experience a wide range of environmental conditions across the oceans, with some
56 imposing extreme stresses upon the cells. Light spans one of the largest ranges of
57 environmental variation as diatoms may transition from high light exposure in the sunlit
58 surface layer to darkness due to ocean mixing or during the night. Beyond diel cycles,
59 diatoms may survive weeks in total darkness during deep ocean mixing events (Cullen &
60 Lewis 1988; Marshall & Schott 1999), and possibly up to centuries during sedimentation
61 events (McQuoid et al. 2002; Godhe & Hårnström 2010; Hårnström et al. 2011). At high
62 latitudes, darkness sometimes lasts as long as ca. six months as a consequence of the sea ice
63 covered with snow and low or even negative sun elevation during the polar night. Given the
64 photoautotrophic nature of diatoms, their survival of a lack of sunlight for up to 6 months is
65 remarkable and has motivated many studies in the past decades to understand the related
66 acclimation processes.

67

68 So far, in experiments studying the response to prolonged darkness, microalgal or diatom
69 cell growth recovered after the imposed dark period (Table 1). Spore production might
70 explain diatom survival during prolonged darkness (Doucette & Fryxell 1983), but spores
71 have only rarely been observed in experiments (Peters & Thomas 1996; Zhang et al. 1998).
72 A “vegetative” or physiological resting state could be more prevalent for overwintering as
73 resting cells have the ability to rapidly recover to their active state (Anderson 1975; Sicko-
74 Goad et al. 1986). Heterotrophic nutrition has also been considered as a means for dark
75 survival (Lewin 1953; White 1974; Hellebust & Lewin 1977), but the extent of its
76 contribution remains uncertain, as it is not always detected (Horner & Alexander 1972;
77 Popels et al. 2002; McMinn & Martin 2013).

78

79 In several experiments on microalgae, not all on polar diatoms, a physiological resting state
80 during prolonged darkness has been characterized by a low rate of metabolic activities. The
81 metabolic activity of chlorophytes was greatly lowered after 10 days in the dark (Jochem
82 1999). The particulate organic carbon and nitrogen cell quotas in three Antarctic diatoms
83 remained stable over 80 days in the dark, also suggesting a lowered metabolism with

84 limited consumption of reserves (Peters & Thomas 1996). Despite low rates of metabolic
85 activity, consumption of energy reserves likely fuels basal metabolic needs shortly after
86 transition to total darkness (Palmisano & Sullivan 1982). Mock et al. (2017) found that
87 60% of all genes were down-regulated in the *F. cylindrus* transcriptome after 7 days of
88 darkness, but genes involved in starch, sucrose and lipid metabolism were up-regulated.
89 Schaub et al. (2017) also found patterns of lipid consumption in a benthic Arctic diatom to
90 be faster in the first two weeks of a two-month long dark experiment. In other experiments
91 with either a temperate diatom (Handa 1969), a *Chlorophyceae* (Dehning & Tilzer 1989) or
92 a *Pelagophyceae* (Popels et al. 2007), similar patterns of rapid consumption early during
93 the dark period occurred with preferential catabolism of proteins and carbohydrate reserves.

94

95 Photophysiology also appears to be strongly downregulated in prolonged darkness. Among
96 *Chlorophyceae* (Hellebust & Terborgh 1967; Dehning & Tilzer 1989), *Pelagophyceae*
97 (Popels et al. 2007) and temperate (Griffiths 1973) or polar (Peters & Thomas 1996)
98 diatoms, the maximum rate of carbon fixation (P_{\max}) per chlorophyll *a* (Hellebust &
99 Terborgh 1967; Griffiths 1973; Dehning & Tilzer 1989; Popels et al. 2007) or per cell
100 (Peters & Thomas 1996) strongly decreased within the first weeks under prolonged
101 darkness. In the experiment of Popels et al. (2007), a drop in the absolute concentration of
102 carbon fixation enzyme RuBisCO was also detected. Lacour et al. (2019), however,
103 recently measured in a polar diatom a rather stable level of RuBisCO-to-carbon ratio,
104 despite a strong decrease in P_{\max} per carbon in prolonged darkness. In other studies, the
105 maximum quantum yield of photochemistry (Φ_M) and the maximum relative electron
106 transport rate (rETR_{max}) also decreased within several weeks of darkness whether
107 studying polar algal communities (Martin et al. 2012), cultures of polar diatoms (Reeves et
108 al. 2011; Lacour et al. 2019), benthic diatom communities (Wulff et al. 2008) or even
109 *Rhodophyta* thalli (Luder et al. 2002).

110

111 At the structural level of the photosynthetic apparatus, the molecular components of the
112 light-harvesting antennae (pigments, proteins) of a green algae (Baldisserotto *et al.*, 2005a;
113 Ferroni *et al.*, 2007) and a *Xanthophyceae* (Baldisserotto *et al.*, 2005b) were partially

114 dismantled or degraded after 2-3 months of darkness. However, in these experiments, the
115 light-harvesting antennae appeared to keep a certain level of organization to re-use light as
116 soon as it became available once again. Generally, a decrease in the chlorophyll *a* cell
117 quota or absolute concentration occurred in previous dark experiments (Table 1), though
118 not always, particularly for dark experiments shorter than 1 month. Chlorophyll *a* remained
119 stable in these shorter term experiments whether expressed as cell quota (Hellebust &
120 Terborgh 1967; Doucette & Fryxell 1983), absolute concentration (Griffiths 1973; Popels
121 et al. 2007; Reeves et al. 2011) or chl-to-carbon ratio (Lacour et al. 2019). In benthic
122 diatoms, Veuger & van Oevelen (2011) also measured a decrease in the dry weight
123 concentration of other pigments, including the photoprotective diadinoxanthin/diatoxanthin
124 pigments, with the largest decrease attributable to the photosynthetic ones (chlorophyll *a*,
125 chlorophyll *c*, Fucoxanthin).

126

127 In polar regions, when the polar night ends, incident irradiance increases, the snow and sea-
128 ice covers then melt, and spring blooms of ice algae and phytoplankton take place
129 (Wassmann & Reigstad 2011). Much of the annual production, and most of the new
130 production in the Arctic Ocean, occur at that time of the year (Sakshaug 2004; Perrette et
131 al. 2011; Ardyna et al. 2013). Sea-ice algae dominated by pennate diatoms are the first to
132 exploit the return of light in spring, before the phytoplankton bloom develops (Mundy et al.
133 2005; Leu et al. 2015; Wassmann 2011). At least a few diatom cells from all species
134 present at any time in polar oceans must survive overwintering in the full darkness to
135 inoculate the populations that grow during summer. The stress imposed by the return of
136 light in spring may further compromise the survival of overwintering populations after such
137 a long period of darkness. Thus, their ability to recover is of crucial importance as regard to
138 their fate.

139

140 Despite the numerous studies on microalgae dark survival, only a few have measured
141 physiological parameters during the recovery upon light return (Table 1). In general, the
142 low photosynthetic performances observed during the dark period, whether measured at the
143 photochemistry level (Luder et al. 2002; Wulff et al. 2008; Martin et al. 2012), the carbon

144 fixation level (Griffiths 1973; Peters & Thomas 1996; Popels et al. 2007) or both (Kvernvik
145 et al. 2018; Lacour et al. 2019), recover within the first days of re-illumination. Peters &
146 Thomas (1996) and Popels et al. (2007) also measured a recovery in particulate nitrogen
147 and carbon levels which requires energy to be available rapidly after re-illumination. Recent
148 studies on polar phytoplankton communities (Kvernvik et al. 2018) and a polar diatom
149 culture (Lacour et al. 2019) focused on the ability to restore growth with different
150 irradiance intensities. Regardless of the light intensity, polar microalgae appeared to
151 recover their photophysiological capacity within 48 hours.

152

153 Although dark survival of microalgae has received considerable attention in the past
154 decades, our understanding of more specifically polar night darkness survival in diatoms
155 remains limited for several reasons. Some of the former studies have shown dark survival
156 for numerous microalgal species over periods representative of the polar night and even
157 beyond (rows 3,5,9,10,13-15,18-20,23,26-28, Table 1). They however documented a
158 limited suite of physiological and biochemical parameters, which did not allow to fully
159 understand the involved cellular mechanisms. Some other experiments did measure several
160 parameters and provided a complete transcriptional profiling of sequenced genes, but for
161 only a short dark period (< 20 days) (rows 4,6, Table 1); 3) Other experiments studied non-
162 polar species (or not diatoms) with detailed characterization and are to be interpreted with
163 caution relative to diatoms in the polar environment (rows 12,21, Table 1); 4). A
164 combination of these limitations is not uncommon (rows 7,8,11,16,17,22,24,25,29-31,
165 Table 1).

166 Our study (row 1, Table 1) aimed at overcoming the limitations described above with an
167 integrative characterization of a sea ice diatom, *Fragilariopsis cylindrus*, tracking
168 physiological and metabolic acclimation over a darkness period representative of the polar
169 night, and over its resumption of growth upon return to light. Our results are largely
170 consistent with earlier findings on parameters measured in common across the studies, but
171 we significantly expand previous knowledge by parallel monitoring of multiple
172 physiological and metabolic features.

173

174 **Table 1** Chronological list of dark survival experiments for microalgal species with the present study highlighted.

Species	Length of experiment (days)	Parameters				T(°C)	References	
		Cell	Metabolism and Reserves	Photosynthetic apparatus				
				Molecular components	Photophysiology			
1	<i>Fragilariopsis cylindrus</i>	D: 90 L: 6*	Number Volume	POC & PON Lipids	Pigments RbcL, PsbA	¹⁴ C P. vs E. curves Fluo	0	This study
2	<i>Chaetoceros neogracile</i>	D: 30 L: 8,14	Number Volume	POC & PON	Pigments RbcL	¹⁴ C P. vs E. curves Fluo	0	Lacour <i>et al.</i> , 2019
3	Arctic phytoplankton community of polar night	D: <i>in situ</i> L: 2	N/A	N/A	Chla	¹⁴ C uptake Fluo	1.5, 2	Kvernvik <i>et al.</i> , 2018
4	<i>Fragilariopsis cylindrus</i>	D: 7	N/A	Gene expression	Gene expression	N/A	-2, 11	Mock <i>et al.</i> , 2017
5	<i>Navicula cf. perminuta</i>	D: 56	N/A	Lipids, Prots, Carbs	N/A	N/A	0, 7	Schaub <i>et al.</i> , 2017
6	<i>Phaeodactylum tricornutum</i>	D: 2 L: 1	Number Morphology	N/A	Pigments Gene expression	Fluo	15	Nymark <i>et al.</i> , 2013
7	Polar algal communities	D: 22-35 L: 1	N/A	Carbs	Chla	Fluo	-2, 4, 10, 20	Martin <i>et al.</i> , 2012
8	<i>Fragilariopsis cylindrus</i> <i>Thalassiosira antarctica</i> ...	D: 30-60 L: growth**	N/A	Carbs	Chla	Fluo	-2, 4, 10	Reeves <i>et al.</i> , 2011
9	Diatom sediment samples	D: 371 L: 1	N/A	N/A	Pigment content (Dark only)	¹³ C uptake (Light only)	17	Veuger & Van Oevelen 2011
10	Sediment sample / isolation of <i>Skeletonema marinoi</i>	D: >100 y L: N/A	Growth (Light only)	N/A	N/A	N/A	10	Harnstrom <i>et al.</i> , 2011
11	Diatom sediment samples	D: 15-64 L: 1-4 h	N/A	N/A	N/A	Fluo	4-6	Wulff <i>et al.</i> , 2008
12	<i>Aureococcus anophagefferens</i>	D: 14 L: 4-5*	Number Bacteries	POC & PON, Lipids, Prots, Carbs	Chlorophyll <i>a</i> RbcL	¹⁴ C P. vs E. curves Fluo	6	Popels <i>et al.</i> , 2007
13	<i>Koliella antarctica</i>	D:60	Morphology	N/A	Chla,b PSII assembly	N/A	5	Ferroni <i>et al.</i> , 2007
14	<i>Xanthonema sp.</i> <i>Koliella antarctica</i>	D: 60-90	Number Morphology	N/A	Chla,b, carotenoid PSII assembly	N/A	4, 5	Baldisserotto <i>et al.</i> , 2005
15	Diatom sediment samples	D: > 55 y L: 30-40	Growth (Light only)	N/A	N/A	N/A	3, 10, 18	McQuoid <i>et al.</i> , 2002
16	<i>Palmaria decipiens</i>	D: 180 L: 28	N/A	N/A	N/A	Fluo	0	Luder <i>et al.</i> , 2002

17	<i>Brachiomonas submarina</i> <i>Pavlova lutheri</i> ...	D: 10-12 L: 5	Number	Metabolic activity Heterotrophy	N/A	N/A	10	Jochem 1999
18	Polar algal communities	D: 161 L: 30	Number	N/A	N/A	N/A	1	Zhang 1998
19	<i>Thalassiosira antarctica</i> <i>Thalassiosira tumida</i> ...	D: 72-302 L: 5-30*	Number	POC & PON	<i>Chla</i>	¹⁴ C uptake	0	Peters & Thomas 1996
20	<i>Thalassiosira punctigera</i> <i>Rhizosolenia setigera</i> ...	D: 30-70 L: 8-20*	Number	POC & PON	<i>Chla</i>	¹⁴ C uptake	8, 15	Peters 1996
21	<i>Scenedesmus acuminatus</i>	D: 90 L: growth**	Number Volume	Lipids, Prots, Carbs, Dry weight, Heterotrophy	<i>Chla</i> Phaeopigments	¹⁴ C P. vs E. curves	7, 22	Dehning & Tilzer 1989
22	<i>Thalassiosira antarctica</i> var. arctica	D: 10	Number Spores	POC & PON	<i>Chla</i>	N/A	4	Doucette & Fryxell 1983
23	<i>Nitzschia cylindrus</i> Araphid pennate diatom specie	L-D: 30 D: 150	Number Morphology	N/A	N/A	N/A	0,-2	Palmisano & Sullivan 1983
24	<i>Nitzschia cylindrus</i> Araphid pennate diatom specie	L-D: 30	Number	Respiration, Heterotrophy Lipids, Prots, Carbs, ATP	N/A	¹⁴ C uptake	0,-2	Palmisano & Sullivan 1982
25	<i>Nitzschia angularis</i> var. <i>Affinis</i> <i>Cyclotella cryptica</i> ...	D:10-20	N/A	Heterotrophy	N/A	N/A	20	Hellebust & Lewin 1977
26	<i>Thalassiosira pseudonana</i> <i>Phaeodactylum tricornutum</i> ...	D: < 365 L: < 64	Number	N/A	N/A	N/A	2,10, 20	Antia 1976
27	<i>Cyclotella cryptica</i> <i>Coscinodiscus</i> sp.	D: 1 year L: growth**	Number Volume	POC & PON Heterotrophy	<i>Chla</i> ,c	¹⁴ C uptake	18,20	White 1974
28	<i>Thalassiosira gravida</i> <i>Ditylum brightwellii</i> ...	D: 90 L: growth**	Number	N/A	N/A	N/A	15	Smayda & Mitchell 1974
29	<i>Phaeodactylum tricornutum</i>	D: 7-16 L: 7	Number	Prots	<i>Chla</i>	¹⁴ C uptake	18,28	Griffiths 1973
30	<i>Skeletonema costatum</i>	D:10	N/A	POC & PON, Lipids, Prots, Carbs	<i>Chla</i>	¹⁴ C uptake	18	Handa 1969
31	<i>Dunaliella tertiolecta</i>	D: 7	Number	POC	<i>Chla</i>	¹⁴ C P. vs E. curves RuDP activities	18	Hellebust & Terborgh 1967

175 The length of each experiment is shown in days, hours-h or years-y when specified for Dark (D) and Light (L) return experiments. The measured parameters are
176 separated into four categories (Cell, Metabolism, Molecular components and Photophysiology of the Photosynthetic apparatus). T is the temperature in Celsius
177 degrees. Abbreviations: L = Light return, D =Dark, L-D= Light-Dark transition, POC & PON = Particular Organic Carbon & Nitrogen, Prots = Proteins, Carbs =
178 Carbohydrate, ATP = Adenosine triphosphate, ¹⁴C & ¹³C = Radiocarbon, ¹⁴C P. vs E. curves = Photosynthesis versus Irradiance curves of ¹⁴Carbon fixation, Fluo
179 = Fluorescence determinations (PSII variable fluorescence and/or spectrofluorimetry), *Chla-b-c* = Chlorophyll *a-b-c*, PSII = Photosystem II, PsbA = PSII protein
180 D1, RbcL = RuBisCO large subunit.

181 * indicates two or more light return experiments.

182 ** indicates only growth potential was verified upon a light return.

183 ... indicates more species were studied.

184 Materials & Methods

185 **Cell culturing**

186 Axenic cultures of *Fragilariopsis cylindrus* (Grunow) Krieger (strain NCMA3323) were
187 freshly obtained from the National Center for Marine Algae and Microbiota. *F. cylindrus* is
188 the only polar diatom with a sequenced and published genome (see Mock et al. 2017). It
189 was grown in semi-continuous cultures in pre-filtered L1 medium (Guillard & Hargraves
190 1993). Cultures in triplicate were started in 50 ml borosilicate tubes and then sequentially
191 transferred to larger vessels several times until a final transfer of 5 l of culture to 20-l
192 polycarbonate round vessels (Fig. S1). Thereafter, further additions of L1 media served to
193 increase culture volume while matching growth rate so that the cell density remained steady
194 (Wood et al. 2005). Light was provided continuously with DURIS® E3 LED bands (GW
195 JCLMS1.EC, 4000 K) at a scalar irradiance of approximately $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ as
196 measured with a QSL-100 quantum sensor (Biospherical instruments, San Diego, CA,
197 USA) placed in the centre of the vessel. Scalar irradiance ranged from 29.5 to 33.5 μmol
198 $\text{photons m}^{-2} \text{s}^{-1}$ depending on the culture vessel position in the growth chamber (Fig. S1).
199 This irradiance was chosen based on the irradiance at which the growth rate saturated (K_E):
200 $0.244 \pm 0.041 \text{ d}^{-1}$. Each culture was gently mixed with a 12.5 cm magnetic stirrer and
201 bubbled with air filtered through a $0.3 \mu\text{m}$ capsule filter (Carbon CAP, Whatman™ 6704-
202 7500). Temperature of the growth chamber (CARON, model 7901-33-2) was kept at 0°C
203 for the duration of the experiment.

204 **Sampling design**

205 The first two samplings took place once the growth rate, cell diameter and Chlorophyll *a*
206 (Chl*a*) were steady for a minimum of 10 cell generations (MacIntyre & Cullen 2005a), one
207 day before the transition from light to dark (referred to as the -1-day sampling), and on the
208 day of the transition just before turning off the light (referred to as the 0-day sampling).
209 Both are collectively referred to as light-acclimation sampling days. In order to avoid light
210 limitation of growth, the cultures were kept optically thin during the acclimation period
211 (between 4×10^4 to $6 \times 10^5 \text{ cell mL}^{-1}$). The cell suspension density was $\sim 5 \times 10^5 \text{ cell mL}^{-1}$

212 before the dark transition. The light system was then switched off and each vessel was
213 carefully covered with opaque material. Sampling in the dark began 24 hours following the
214 transition and subsequent dark samplings followed after 5, 14, 28, 63 and 83 days of
215 darkness as shown with the timeline in Fig. 1. Syringes used for sampling culture volume
216 were completely opaque, as well as the tubes connecting to the cultures vessels, and all sub-
217 samples were contained in opaque tubes until their respective measurements. Every
218 immediate manipulation (e.g. filtrations, fluorescence determinations and ^{14}C incubations)
219 was completed under very low green light levels in order to avoid excitation of the
220 photosystems. The Light return 1 experiment took place after 1.5 months (48 days) of
221 darkness. Culture volume was carefully transferred to gently aerated 3-l vessels cooled to
222 0°C and illuminated at $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with a slightly different light spectrum (Fig.
223 S2). This second light system was provided by a customized LED system comprising 8
224 colours independently variable in intensity and mounted on 6 LED panels around the 3-l
225 vessels (Fig. S3). The cultures were sampled at 30 minutes, 2 hours, 5 hours, 1 day, 3 days
226 and 6 days following re-illumination (Fig. 1). The Light return 2 experiment took place
227 after 3 months (90 days) in the dark following the same procedure (Fig. 1). All
228 manipulations were completed under very low green light levels for both light return
229 experiments (See background light in Fig. S3)

230 At sampling time-points culture samples were harvested to measure the parameters
231 described below with a few exceptions. The relative electron transport rate (rETR) and non-
232 photochemical quenching (NPQ) were measured for the light return experiments and for
233 several time-points during the dark period (14, 28, 47 and 83 days). Carbon fixation rates
234 were measured at all points except after 3 months of darkness. Lipid droplets were
235 measured from 5 hours to 6 days following both light return experiments and for every
236 time-point during the dark period. The maximum quantum yield of PSII (Photosystem II)
237 photochemistry (Φ_{M}) and the effective absorption cross-section (σ_{PSII}) were also measured 2
238 days after the transition to dark. Table 1 summarizes the measurements made for every
239 sampling time-point.

Table 2 : Sampling time-points of the parameters measured during the dark and light experiments

Time	¹ Cell number & volume	² Carbon & nitrogen	³ Lipid droplets	⁴ Pigment	⁵ Photosynthetic proteins	⁶ Variable fluorescence (FIRe)	⁷ Variable fluorescence (PAM)	⁸ incubations (¹⁴ C)
D : -1 day	X	X	X	X	X	X		X
D : 0 days	X	X	X	X	X	X		X
D : 1 day	X	X	X	X	X	X		X
D : 2 days						X		
D : 5 days	X	X	X	X	X	X		X
D : 14 days	X	X	X	X	X	X	X	X
D : 28 days	X	X	X	X	X	X	X	X
D : 47 days							X	
D : 63 days	X	X	X	X	X	X		
D : 83 days	X	X	X	X	X	X	X	X
L1 : 30 min	X	X		X	X	X	X	X
L1 : 2 hours	X	X		X	X	X	X	X
L1 : 5 hours	X	X	X	X	X	X	X	X
L1 : 1 day	X	X	X	X	X	X	X	X
L1 : 3 days	X	X	X	X	X	X	X	X
L1 : 6 days	X	X	X	X	X	X	X	X
L2 : 30 min	X	X		X	X	X	X	X
L2 : 2 hours	X	X		X	X	X	X	X
L2 : 5 hours	X	X	X	X	X	X	X	X
L2 : 1 day	X	X	X	X	X	X	X	X
L2 : 3 days	X	X	X	X	X	X	X	X
L2 : 6 days	X	X	X	X	X	X	X	X

240 D=Dark experiment, L1 = Light return 1 experiment, L2 = Light return 2 experiment, **1:** Cell number per mL and cell volume (μm^3),
241 **2:** Carbon & Nitrogen cell quotas, **3:** Lipid droplets cell quota, **4:** Pigment (Chlorophyll *a*, Chlorophyll *c*, Fucoxantin, Diadinoxanthin,
242 Diatoxanthin) cell quotas, **5:** PsbA (PSII protein D1) & RbcL (RuBisCO large subunit) cell quotas, **6:** Maximum quantum yield (Φ_M)
243 and the effective absorption cross-section for PSII photochemistry (σ_{PSII}), **7:** Relative electron transport rate (rETR) and non-
244 photochemical quenching (NPQ), **8:** Carbon fixation rate ($\mu\text{g C m}^{-3} \text{h}^{-1}$)

245 **Cell number and volume and culture axenicity**

246 Cells were counted and sized using a Beckman Multisizer 4 Coulter Counter. Three
247 consecutive countings were recorded for each culture sampling point. Total cell counts did
248 not differentiate cell viability; hence mortality could not be assessed through this method.
249 However, mortality can be suspected when looking at the flow cytometry data (see Lipid
250 droplets section and Fig. S4). The flow cytometry data indicated that the number of debris,
251 likely the result of dying cells, appeared to increase with the duration of the experiment,
252 especially during the Light return 2 experiment. The cell volume was calculated using the
253 sphere-equivalent diameter. The biovolume was calculated as cell volume x cell number for
254 each culture. Axenicity of each culture was verified with petri dishes prepared as described
255 in MacIntyre & Cullen (2005b). Axenicity was confirmed once before the acclimation
256 period.

257 **Carbon and nitrogen cell content**

258 Three technical replicates were harvested per algal culture; aliquots of 20 ml were filtered
259 onto binder-free glass-fiber filters (GF/F) (0.7 μm , 25 mm) pre-combusted at 500°C for 24
260 hours. Filters were then dried at 60°C for at least 12 hours and kept desiccated before
261 elemental analysis with a CHN analyzer (2400 Series II CHNS/O; Perkin Elmer, Norwalk,
262 CT, USA).

263 **Lipid droplets**

264 Cells were assessed for their lipid droplets content using the molecular probe BODIPY®
265 505/515 (www.lifetechnologies.com) according to Brennan et al. (2012) using a flow
266 cytometer (488 nm excitation, 520 nm emission, Millipore Guava easycyte flow cytometer)
267 in a 96-well plate. BODIPY fluorescence emitted from the lipid droplets was quantified for
268 each cell as relative fluorescence units (RFU). For samples analyzed at each time-point, a
269 total of 9 wells were prepared (three technical replicates per culture) and BODIPY
270 fluorescence was measured on 5000 cells for each well. Before fluorescence measurements,
271 samples were incubated for 1 h on ice in the dark. Each well contained 300 μl of algal
272 culture marked with 4 μl of a BODIPY solution (final concentration 0.33 μM / 1.32%

273 DMSO). For each time-point sampling, technical replicates were pooled together for each
274 culture and averaged for their mean RFU according to a target function (Fig. S4).

275 **Pigment content**

276 For each culture, 10 ml were filtered onto glass-fiber filters (GF/F) (0.7 μm , 25 mm,
277 Millipore). Filters were immediately flash-frozen in liquid nitrogen and stored at -80°C
278 until analysis. Pigment separation was performed with high performance liquid
279 chromatography (HPLC) according to Zapata et al. (2000). Before HPLC analyses,
280 pigments were extracted in 95% methanol and sonicated for 20 seconds three times.
281 Samples were then centrifuged (4500 rpm) 15 minutes at 4°C and filtered on
282 polytetrafluoroethylene (PTFE) membranes (0.2 μm). Data were analysed using the
283 ChromQuest 5.0 software.

284 **Photosynthetic proteins**

285 For PsbA (PSII protein D1) and RbcL (RuBisCO large subunit) quantification, 30 ml of
286 each culture was harvested onto GF/F filters, flash-frozen and stored at -80°C . Protein
287 extraction was performed using the FastPrep-24 and bead lysing ‘matrix D’ (MP
288 Biomedicals), using 3 cycles of 60 seconds at 6.5 m s^{-1} in $300\ \mu\text{L}$ of 1X extraction buffer
289 (Agrisera AS08_300 with 0.4 M of the protease inhibitor AEBSF added), then spun at 16
290 000 g for 5 minutes (Li & Campbell, 2013). For each protein extract supernatant we then
291 estimated the content of total nitrogen derived from the original sample using the
292 parallel determinations of total N content per mL of sample. We then loaded each well of
293 gels with a volume of protein extract sufficient to deliver equivalent total nitrogen across
294 wells. We chose this approach for loading because total nitrogen determinations are more
295 reliable than total protein determinations. Separation of proteins was done in a Bolt 4-12%
296 Bis Tris SDS-PAGE gel (Invitrogen). Proteins were quantified by western-blotting with
297 anti-PsbA (AS05 084) or anti-RbcL (AS01 017) antibodies (www.agrisera.se) (Li et al.
298 2016). Chemiluminescent images were obtained using ECL Ultra reagent (Lumigen, TMA-
299 100) and a VersaDoc CCD imager (Bio-Rad). Band densities for samples were determined
300 against the standard curve using the ImageLab software (v 4.0, Bio-Rad).

301 **Variable fluorescence**

302 Variable in vivo Chla fluorescence at 680 nm was measured using a Fluorescence
303 Induction and Relaxation (FIRE) fluorometer (Satlantic, Halifax, NS, Canada) that applies a
304 saturating, single turnover flash (STF, 100 μ s) of blue light (455 nm, 60-nm bandwidth) to
305 the sample. Based on the fluorescence induction curve, the FIREWORX algorithm (Audrey
306 Barnett, www.sourceforge.net) estimates the effective absorption cross-section for PSII
307 photochemistry (σ_{PSII} , $\text{\AA}^2 \text{ quanta}^{-1}$), the minimum flux of fluorescence (F_0) and the
308 maximum flux of fluorescence of dark acclimated cells (F_m) in relative units (Kolber et al.
309 1998). σ_{PSII} , F_0 and F_m were measured on culture sub-samples shortly after harvesting along
310 the dark acclimation and during the Light return 1 and Light return 2 experiments, after 30
311 minutes of dark acclimation. The maximum quantum yield of PSII (Φ_M) was computed as:

312
$$\Phi_M = \frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \quad (1)$$

313 A Phyto-PAM fluorometer (Phyto-ML, Heinz Walz GmbH, Germany) was also used to
314 assess complementary fluorescence parameters. Using a different fluorometer did not
315 compromise the interpretation of the data altogether, as the trends in Φ_M were similar for
316 both FIRE and Phyto-PAM determinations (Fig S5). Phyto-PAM determinations are
317 typically higher than FIRE determinations, but their relative variations are equivalent and
318 comparable (Röttgers 2007). For the Phyto-PAM fluorometer, cells were dark-acclimated
319 for 30 minutes when applicable (Light return 1 and Light return 2 experiments) and
320 subsequently exposed to a rapid light curve (RLC) protocol using 8 step-wise increasing
321 irradiances from 1 to 111 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and for 10 or 30 seconds each (Lefebvre et
322 al. 2011). After each irradiance-step, F_m' was probed with an actinic flash (500 ms), while a
323 detecting modulated light source measured F_s . Although the instrument allows excitation of
324 fluorescence at four different wavelengths, actinic light was only provided by the actinic
325 LEDs peaking at 655 nm (Fig. S2). To calculate the PSII-specific rETR, the achieved
326 quantum yield of charge separation in PSII (Φ_{PSII}) at each irradiance step was multiplied by
327 the corresponding irradiance (E):

328
$$\Phi_{PSII} = \frac{F_m' - F_s}{F_m'} \quad (2a)$$

329
$$rETR = \Phi_{PSII} \cdot E \quad (2b)$$

330 where F_m' and F_s are the maximum and steady-state fluorescence of light acclimated cells,
 331 respectively. To calculate the maximum relative electron transport rate ($rETR_{max}$), a
 332 function was fitted to the data by least-square fit according to Eilers & Peeters (1988):

$$333 \quad rETR(E) = \frac{E}{aE^2 + bE + c} \quad (3)$$

334 where a, b and c are expressed as:

$$335 \quad a = \frac{1}{s \cdot I_m} \quad (4a)$$

$$336 \quad b = \frac{1}{P_m} - \frac{2}{s \cdot I_m} \quad (4b)$$

$$337 \quad c = \frac{1}{s} \quad (4c)$$

338 and where s is the initial slope, I_m is the optimal irradiance and P_m is the maximal
 339 production rate of the fit. The dynamic non-photochemical quenching (NPQd, also referred
 340 as NPQ in the text) was calculated for each irradiance-step as:

$$341 \quad NPQd = \frac{F_m - F_m'}{F_m'} \quad (5)$$

342 and the sustained and total non-photochemical quenching (NPQs, NPQt) were calculated
 343 as:

$$344 \quad NPQs = \frac{F_{m24h} - F_m}{F_m} \quad (6a)$$

$$345 \quad NPQt = NPQd + NPQs \quad (6b)$$

346 where F_m and F_m' are the maximum fluorescence of dark and light (incubation-irradiance)
 347 acclimated cells, respectively, and F_{m24h} is the maximum fluorescence of dark-acclimated
 348 cells for 24 hours to allow complete relaxation of NPQs. NPQ calculations were computed
 349 with the 30 seconds RLC protocol. To calculate the maximum non-photochemical
 350 quenching (NPQ_{max}) and developed NPQ at $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (NPQ_{30}), a function
 351 was fitted to the data by least-square fit according to Serôdio & Lavaud (2011):

$$352 \quad NPQ(E) = NPQ_{max} \cdot \frac{E^n}{E_{50}^n + E^n} \quad (7)$$

353 where NPQ_{max} is the maximum NPQ value, E_{50} is the irradiance at which 50% of
 354 NPQ_{max} is reached and n is the Hill coefficient of the fit (the sigmoidicity of the curve).

355

356 **¹⁴C incubations**

357 A sample was first collected for each culture, and inoculated with inorganic ¹⁴C
358 (NaH¹⁴CO₃, 2 μCi ml⁻¹). Samples were then processed as described in Bruyant et al.
359 (2005). Inoculated culture aliquots of 1 ml were dispensed into 24 glass scintillation vials
360 of 7 ml cooled in separate thermo-regulated alveoli (0°C). The vials were exposed to 24
361 different light levels provided by separate LEDs (LUXEON Rebel, Philips lumileds) from
362 the bottom of each alveolus. The PAR (E, μmol photons m⁻² s⁻¹) in each alveolus was
363 measured before incubation with a quantum sensor (Heinz Walz GmbH, US-SQL)
364 equipped with a 4π collector. After 20 min of incubation, culture aliquots were fixed with
365 50 μL of buffered formalin then placed under the fume hood and acidified (250 μL of HCl
366 50%) for 3 hours to remove the excess inorganic carbon (JGOFS protocol, UNESCO
367 1994). Finally, 6 mL of scintillation cocktail (Ecolume, MP Biomedicals) were added to
368 each vial prior to counting using a liquid scintillation counter (Perkin Elmer® Tri-Carb
369 2910TR). This step allowed determining the amount of radiolabeled carbon assimilated by
370 the cells from the number of disintegration per minute (DPM). To determine the total
371 amount (total activity) of bicarbonate added, three 20 μl aliquots of radioactive sample
372 were added to 50 μl of an organic base (ethanolamine) and 6 ml of the scintillation cocktail
373 into glass scintillation vials. The carbon fixation rate was finally computed according to
374 Parsons et al. (1984)

375
$$P = \frac{(R_s - R_b) \cdot W}{R \cdot N} \quad (8)$$

376 where P is the rate of carbon fixation [mg C m⁻³ h⁻¹], R is the total activity (DPM), N is the
377 number of hours of incubation, R_s is the sample count (DPM) corrected for quenching, R_B
378 is the blank (or dark sample) count (DPM) corrected for quenching and W is the total
379 weight of carbon dioxide available. The relationship between the rate of carbon fixation (P)
380 and irradiance (E) was fitted to the equation determined by Platt et al. (1980) to obtain the
381 photosynthetic coefficients:

382
$$P = P_S \left[1 - \exp\left(-\frac{\alpha E}{P_S}\right) \right] \exp\left(-\frac{\beta E}{P_S}\right) + P_0 \quad (9)$$

383 where P_S is the maximum carbon fixation rate in absence of photoinhibition [μg C m⁻³ h⁻¹],
384 α is the initial slope of the carbon fixation vs. irradiance curve [μg C m⁻³ h⁻¹ (μmol photons

385 $\text{m}^{-2} \text{s}^{-1})^{-1}$], E is the incubation irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$), β is the photoinhibition
386 coefficient [$\mu\text{g C m}^{-3} \text{h}^{-1} (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$] and P_0 is the intercept of the curve [$\mu\text{g C}$
387 $\text{m}^{-3} \text{h}^{-1}$]. The maximum carbon fixation rate at saturating irradiance (P_{\max}) was calculated as
388 follow:

$$389 \quad P_{\max} = P_S \left(\frac{\alpha}{\alpha + \beta} \right) \left(\frac{\beta}{\alpha + \beta} \right)^{\frac{\beta}{\alpha}} \quad (10)$$

390 **Statistical analysis: mean comparison of parameters between sampling periods**

391 Different time windows were considered to follow the evolution of the measured
392 parameters. The time windows considered are those with large changes in tracked
393 parameter levels. Generally, the full duration of a given phase of the experiment, e.g. 83
394 days of darkness (or 63 days for carbon fixation parameters), is the period considered for
395 statistical comparison. In some cases, within a given phase, there was an apparent change in
396 the direction of the variation, e.g. see the number of cell per ml that increases from 0 to 28
397 days of darkness and decreases after from 28 to 83 days of darkness (Fig. 2a). For a given
398 parameter, the time at which this directional change occurred splits a given phase of the
399 experiment into two periods for statistical comparison, e.g. D_{0-28} and D_{28-83} for the number
400 of cell per ml in the dark. For the dark period (D), there were four main periods considered:
401 up to the first month (D_{0-28}), up to the second month (D_{0-63}), up to the third month (D_{0-83})
402 and between the first and the third months (D_{28-83}). For the light return periods (L1, L2), the
403 periods used for comparisons are specified in the text relatively to time ranges of interest,
404 e.g. from 30 minutes to three days of illumination ($L_{130\text{min}-3\text{d}}$). To determine whether there
405 was a significant variation in the measured parameters during the dark experiment, we used
406 linear mixed models that included an error term (or random effect) on the culture to account
407 for the pseudo-replication of the data. For the light return periods, the mixed effect models
408 also included a fixed effect on the light experiments (whether it was the first or the second
409 light return experiment) and an interaction term between the two fixed effects (time and
410 experiment). To test for temporal autocorrelation for each measured parameter, models
411 were compared including a first order autocorrelation structure or not. ΔAIC was computed
412 between each paired model to determine if the two models showed equivalent power or not.
413 The models were considered equivalent if $\Delta\text{AIC} < 5$ and the model without a first

414 autocorrelation structure was then chosen. For $\Delta AIC > 5$, the model with the lowest AIC
415 was chosen (supplementary files, model_significance_dark & model_significance_light).
416 Temporal autocorrelation was found to be present only rarely and was accounted for when
417 necessary. The linear mixed-effects models were fitted in R using the nlme package v3.1-
418 137 (Pinheiro et al. 2018). All statistical analyze were performed in R 3.5.2 (R Core Team
419 2018). The complete and detailed statistical results are provided in supplementary files. The
420 p-values reported in the text are those for the parameters relative change for the considered
421 periods (supplementary files, posthoc_comparisons_dark & posthoc_comparisons_light) or
422 those for the interaction term between time and experiment to determine if a parameter's
423 variations for both light return periods are similar to each other or not
424 (model_significance_light). The figures presented in the Results and Discussion section
425 show Dark, Light return 1 and Light return 2 data altogether to provide a visual comparison
426 between the three experiments. Light return 1 and Light return 2 data are enlarged in
427 supplementary figures to allow easier comparison between each other. The lines between
428 sampling periods are strictly shown for visual clarity and do not imply interpolated levels
429 of any parameters. The mean values and standard deviations plotted in these figures are
430 available for the main sampling periods in Table S1, S2 and S3.
431

432 Results & Discussion

433 **Prolonged darkness exposure to mimic the polar night**

434 Light acclimated cultures of *F. cylindrus* were exposed to complete darkness over a period
435 of three months to mimic the polar night. Multiple physiological processes were monitored
436 for the first time in a dark experiment to such an extent on the model polar diatom *F.*
437 *cylindrus*. The results are discussed with respect to earlier findings on polar and non-polar
438 species to understand diatom dark survival in polar environments.

439 *Cell and reserves*

440 Cell number per ml of algal culture (Fig. 2a) increased slightly but significantly during the
441 first month of the dark period by 27% (D_{0-28} , $P\text{-value} = 4.26 \times 10^{-3}$), which is likely due to a
442 final cell division for a subset of the cell population at the beginning of the dark period. For
443 the same period, the culture biovolume (Fig. 2a) did not increase significantly (D_{0-28} , $P\text{-}$
444 $\text{value} = 1.05 \times 10^{-1}$) because the average cell volume significantly decreased by 8% (D_{0-28} ,
445 $P\text{-value} = 1.08 \times 10^{-2}$) as a consequence of cell division (Fig. 2b). The cell number and the
446 biovolume then decreased until the end of the third month of darkness, although not
447 significantly (D_{28-83} , $P\text{-value}_{\text{cell number}} = 1.06 \times 10^{-1}$, $P\text{-value}_{\text{biovolume}} = 1.38 \times 10^{-1}$), to end up
448 at values slightly above the t_0 (values just before dark transition). During the first month of
449 darkness, the carbon cell quota significantly decreased by 34% (D_{0-28} , $P\text{-value} = 3.57 \times 10^{-}$
450 3) (Fig. 2c). The Carbon cell quota then significantly increased until the end of the third
451 month (D_{28-83} , $P\text{-value} = 5.19 \times 10^{-4}$) to values slightly above the t_0 . The variations in the
452 nitrogen cell quota were similar to those of cellular carbon but none of the comparisons
453 were significant (D_{0-28} , $P\text{-value} = 1.11 \times 10^{-1}$; D_{28-83} , $P\text{-value} = 9.02 \times 10^{-2}$).

454
455 A potential mechanism for long-term dark survival is to lower metabolism and to fine-tune
456 the utilization rate of stored energy products (Handa 1969; Palmisano & Sullivan 1982;
457 Dehning & Tilzer 1989; Jochem 1999; Popels et al. 2007; Schaub et al. 2017). In our
458 experiment, the carbon cell quota did decrease for the first month of the dark period.
459 However, this decrease was likely the consequence of cell division on cell volume. Indeed,
460 the particulate organic carbon concentration per ml of culture did not decrease significantly

461 during the first month (D_{0-28} , $P\text{-value} = 1.58 \times 10^{-1}$) (Fig. S7). The lipid droplets cell quota
462 probed with BODIPY (RFU) remained mostly constant within the first month with a slight
463 but significant increase of 5% (D_{0-28} , $P\text{-value} = 1.18 \times 10^{-2}$) (Fig. 2b). Lipid droplets then
464 slowly and significantly decreased until the end of the third month in the dark by 11% (D_{28-}
465 $_{83}$, $P\text{-value} = 1.14 \times 10^{-5}$). Hence, this suggests very low dark metabolic rates and low
466 energy reserve consumption as a survival process in *F. cylindrus*. The study by Mock et al.
467 (2017) on the *F. cylindrus* transcriptome response to 7 days of darkness showed that
468 metabolic activities were largely suppressed with approximately 60% of all genes down-
469 regulated. However, genes involved in starch, sucrose and lipid metabolism were up-
470 regulated, which likely fuelled the remaining metabolic needs within the cell for this short
471 period of darkness. It is common that the rate of energy consumption is high in the first
472 weeks of darkness, but decreases as the cells age in darkness (Handa 1969; Dehning &
473 Tilzer 1989; Popels et al. 2007; Schaub et al. 2017). The results of the present study show a
474 more stable pattern in the carbon, nitrogen and lipid droplets quotas, which is in agreement
475 with global metabolism suppression over a long period of darkness. This global metabolism
476 suppression could also be due, to some extent, to the colder temperature at which polar
477 species grow. The rate of energy reserve depletion is likely to be lower for polar species,
478 despite adaptations that compensate for lower kinetics at low temperature (Lyon & Mock
479 2014), and may contribute to longer darkness survival for polar species than for temperate
480 species as observed by Peters (1996).

481

482 The minor increase of the carbon quota after 1 month was unexpected. Note that if bacterial
483 presence and growth were to account for this increase, a sufficient amount of dissolved
484 organic carbon (DOC) would have had to be initially present in the culture medium to
485 sustain heterotrophic bacterial growth (Rivkin & Anderson 1997). The cultures were
486 axenically handled and the fresh culture medium was initially free of DOC. It is unlikely
487 that DOC was released from broken cells by the magnetic stirrer because the cell number
488 remained quite steady through the full length of the experiment. Despite the minor increase
489 in cellular carbon toward the end of the dark experiment, the general trend of the cell
490 reserves data supports the interpretation of a suppression of metabolic activity in the dark.

491 *Photosynthetic apparatus dismantlement*

492 When cells are exposed to a long period of darkness, the photosynthetic machinery is not
493 operating to convert light to chemical energy. A metabolic cost is associated with
494 sustaining the molecular components of the photosynthetic apparatus (Geider & Osborne
495 1989; Quigg & Beardall 2003; Li et al. 2015). Based on the assumption of a lower
496 metabolism and slower protein turnover in darkness (Li et al. 2016), renewal of degraded
497 photosynthetic components should be limited. Hence, Chl*a* and the main photosynthetic
498 accessory pigment Fucoxanthin (Fuco) cell quotas decreased as in past experiments (Peters
499 & Thomas 1996; Baldisserotto et al. 2005a; Veuger & van Oevelen 2011). Chl*a* and Fuco
500 decreased significantly after 3 months of darkness by 41% and 48% respectively (D_{0-83} , P-
501 $\text{value}_{\text{Chl}a} = 6.88 \times 10^{-3}$, $\text{P-value}_{\text{Fuco}} = 1.27 \times 10^{-3}$) (Fig. 3a). For the same period, the
502 photoprotective pigments (Diadinoxanthin (DD) + Diatoxanthin (DT)) cell quotas
503 decreased, although not significantly, by 20% (D_{0-83} , P-value = 4.51×10^{-1}). The pool
504 decrease was attributable to the DD form since DT fully returned to its epoxidized form
505 (DD) after 1 day of darkness (Fig. 3b). Thus, the photosynthetic (Chl*a* + Chl*c* + Fuco) to
506 photoprotective pigments ratio decreased significantly by 31% (D_{0-83} , P-value = 4.31×10^{-6})
507 (Fig. 3c). As a likely consequence of this specific pigment degradation, σ_{PSII} decreased
508 significantly by 27% (D_{0-83} , P-value = 1.41×10^{-4}) (Fig. 3c).

509

510 Photosynthetic proteins cell quotas (PsbA and RbcL) decreased significantly after 3 months
511 of darkness (D_{0-83}) by 85% and 72% respectively (D_{0-83} , P-value $_{\text{PsbA}} = 2.03 \times 10^{-4}$, P-
512 $\text{value}_{\text{RbcL}} = 1.44 \times 10^{-4}$). They reached a much lower detected level relative to t_0 than did
513 the photosynthetic pigments (Fig. 4a). The massive decrease for PsbA is indicative of a
514 PSII core complex degradation as seen in other experiments with a green chlorophyte and a
515 snow xanthophycean algae (Baldisserotto et al. 2005a; Baldisserotto et al. 2005b; Ferroni et
516 al. 2007) under prolonged darkness. Based on the photosynthetic proteins results, one
517 would expect that carbon fixation capacity was largely suppressed in absence of these key
518 proteins, particularly RbcL. Indeed, the carbon fixation *vs.* irradiance curves showed a
519 major significant decrease within 2 months in α and in P_{max} per cell by 92% and 98%
520 respectively (D_{0-63} , P-value $_{\alpha} = 1.16 \times 10^{-3}$, P-value $_{P_{\text{max}}} = 4.58 \times 10^{-6}$) (Figs. 4b,c) (Fig. 5a),

521 as reported before (Hellebust & Terborgh 1967; Dehning & Tilzer 1989; Peters & Thomas
522 1996; Popels et al. 2007; Kvernvik et al. 2018; Lacour et al. 2019). P_{\max} per cell decreased
523 faster than the RbcL cell quota, possibly because of an early inactivation of the carbon
524 fixation enzyme (Hellebust & Terborgh 1967; MacIntyre et al. 1997; Lacour et al. 2019).
525 Interestingly, in the study of Lacour et al. (2019), the RuBisCO-to-carbon ratio did not
526 change for cultures of *Chaetoceros neogracile* exposed to 1 month of darkness, despite a
527 strong decrease in P_{\max} per carbon. Note that the RbcL cell quota in our study also did not
528 decrease significantly during the first month in darkness (D_{0-28} , P-value = 5.41×10^{-1})
529 before it began to decrease, which suggest that an initial decrease in RuBisCO activity,
530 rather than its pool, explains the initial decrease in P_{\max} . Modification of the carbon fixation
531 enzyme to an inactive state may be triggered by dark transition (Parry et al. 2008). P_{\max} also
532 decreased faster relative to α , which significantly lowered the light saturation parameter E_k
533 by 72% (D_{0-63} , P-value = 5.38×10^{-4}) (P_{\max}/α , Fig. S10). This decrease of E_k during
534 prolonged darkness increased the risk of photoinhibition upon subsequent re-illumination,
535 because a given re-illumination level would rise farther above E_k , resulting in excess
536 excitation. The photoprotective NPQ (Eqn 5) was indeed induced even for the lowest RLC
537 irradiances, most likely because of impaired electron sink capacities such as carbon fixation
538 (Huner et al. 1998; Joliot & Alric 2013) (Fig. S11).

539 While the carbon fixation curve parameters decreased, Φ_M (Eqn 1) showed no significant
540 variation after three months of darkness (D_{0-83} , P-value = 9.96×10^{-1}) even though a
541 decrease has previously been observed in prolonged darkness in diatoms (Reeves et al.
542 2011; Martin et al. 2012; Lacour et al. 2019) (Fig. 4b). The internally normalized
543 fluorescence ratio Φ_M reflects the photochemical activity of the remaining PSII capable of
544 at least a single turnover within the remaining pool of viable cells. But the PsbA
545 determinations (Fig. 4a) show that the content of PSII decreased significantly. Analyses of
546 rETRmax (Fig. 4c) also that electron transport away from the remaining PSII was also
547 suppressed. Note that the drop in rETRmax is not available in Fig. 4c because no
548 measurements were made during the first two weeks of the dark period (see Materials &
549 Methods, sampling design). Nevertheless, together with P_{\max} , Fig. 4c strongly suggests that
550 rETRmax was high before the dark transition. Thus, the combination of the drop in PSII

551 content and the drop in rETRmax can together explain the drop in carbon fixation curve
552 parameters. Furthermore, the ¹⁴C incubations lasted 20 minutes. Viable cells taken out of an
553 extended period of darkness and exposed to ¹⁴C incubations may suffer more from impaired
554 electron sink capacities than during a nearly instantaneous measurement of photochemical
555 activity using a single saturating flash.

556 **Light exposure after darkness**

557 The dark acclimated *F. cylindrus* cultures were re-exposed to light after 1.5 months and
558 after 3 months of darkness and monitored for the same physiological parameters. Previous
559 experiments that studied the light transition from prolonged darkness in polar diatoms are
560 scarce. However, Kvernik et al. (2018) and Lacour et al. (2019) recently studied the ability
561 of dark acclimated polar phytoplankton communities and *Chaetoceros neogracile* culture,
562 respectively, to resume photophysiological activity and cell growth over a wide range of
563 irradiance. We found results consistent with their studies, with the addition of other
564 physiological and metabolic features that complement our understanding of the acclimation
565 processes at stake for polar night survival and return to light.

566

567 *Photosensitivity and photosynthetic apparatus reassembly*

568 *F. cylindrus* cells acclimated to darkness largely dismantled key catalytic complexes of
569 their photosynthetic apparatus, while retaining much of their pigment bed. To limit photo-
570 damage, diatoms mainly rely on NPQ mediated by the xanthophyll cycle (Lavaud & Goss
571 2014), especially for *F. cylindrus* growing at low temperatures, which limits other
572 physiological responses (Petrou et al. 2010, Petrou et al. 2011). Given the low
573 photosynthetic capacities reached during the dark period, a rapid NPQ response was
574 expected to occur immediately upon light return to dissipate excessive excitation. For the
575 Light return 1 experiment, the highest NPQ30 was indeed observed immediately upon re-
576 illumination (Fig. 6a). This level of NPQ significantly decreased by 69% to ‘a steady state’
577 within 3 days of re-illumination (L1_{30min-3d}, P-value = 5.43 x 10⁻¹¹). The de-epoxidation
578 state (DES: DT/(DD+DT)) showed consistent variations (64% significant decrease, L1_{30min-}
579 _{3d}, P-value = 2.35 x 10⁻³) indicating that NPQ was mostly related to DT (Lavaud & Goss

580 2014) (Fig. 6a). The NPQ30 was close to the NPQmax induced during the RLC (at 111
581 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, Fig. S11).

582 In the Light return 2 experiment, the NPQ induction showed a different pattern. Despite the
583 presence of DT (Fig. 3b), NPQ remained low (Fig. 6b). This particular inconsistency
584 between DT synthesis and NPQ has previously been observed with *Phaeodactylum*
585 *tricornutum* (Lavaud & Kroth 2006; Lavaud & Lepetit 2013) and *F. cylindrus* (Kropuenske
586 et al. 2009). According to Kropuenske *et al.* (2009), 30 minutes of dark-acclimation before
587 the NPQ measurements are not sufficient to achieve complete re-epoxidation of DT for
588 highly light-stressed cells, so that NPQ remains ‘locked-in’ and relaxes only over several
589 hours (Lavaud & Goss 2014). To allow this sustained part of NPQ to relax, extra samples
590 were dark acclimated for 24 hours to calculate NPQt (Eqn 6a, 6b). NPQt developed at 30
591 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ was higher after 2 hours, 5 hours and 1 day, but not after 30 minutes
592 of re-illumination (Fig. S11). Possibly, for this short timing, the PSII light-harvesting
593 system was still too dismantled to activate a fully dynamic NPQ response immediately
594 upon re-illumination.

595

596 In the Light return 1 experiment, Chla and Fuco cell quotas first decreased over 3 days of
597 re-illumination, although not significantly ($L1_{30\text{min-3d}}$, $P\text{-value}_{\text{Chla}} = 9.96 \times 10^{-1}$, $P\text{-value}_{\text{Fuco}}$
598 $= 7.76 \times 10^{-2}$) (Fig. 3a). The simultaneous decrease of Chla and Fuco and significant
599 increase of DD cell quota ($L1_{30\text{min-3d}}$, $P\text{-value} = 2.05 \times 10^{-4}$) (Fig. 3b) led in turn to a
600 significant 43% decrease of the Photosynthetic/Photoprotective pigment ratio until the third
601 day of the Light return 1 experiment ($L1_{30\text{min-3d}}$, $P\text{-value} = 4.01 \times 10^{-5}$) (Fig. 3c). After 3
602 days, photosynthetic pigments stopped decreasing and started to build up as previously
603 observed in past experiments on polar and non-polar diatoms (Griffiths 1973; Peters &
604 Thomas 1996; Nymark et al. 2013). The late build-up of photosynthetic pigments, although
605 not achieving statistical significance ($L1_{3\text{d-6d}}$, $P\text{-value}_{\text{Chla}} = 8.32 \times 10^{-2}$, $P\text{-value}_{\text{Fuco}} = 5.56 \times$
606 10^{-2}), stabilised the Photosynthetic/Photoprotective pigment ratio until the 6th day of the
607 Light return 1 experiment ($L1_{3\text{d-6d}}$, $P\text{-value} = 5.90 \times 10^{-1}$).

608 In the Light return 2 experiment, variations in Chla and Fuco cell quotas were not
609 significantly different to the Light return 1 experiment according to the interaction terms

610 (P-value_{Chl a} = 8.21×10^{-2} , P-value_{Fuco} = 7.63×10^{-2}) (Fig. 3a), but DD cell quota did not
611 increase similarly as in the Light return 1 experiment and thus was significantly different,
612 (P-value_{DD} = 2.96×10^{-4}) (Fig. 3b). Nevertheless, in both light experiments, the
613 Photosynthetic/Photoprotective pigment ratio significantly decreased until the 6th day of
614 light exposure by 30% and 35% respectively (L1_{30min-6d}, P-value = 5.34×10^{-3} / L2_{30min-6d},
615 P-value = 2.19×10^{-3}) (Fig. 3c). Thus, the pigment composition of the light harvesting
616 antennae shifted gradually to a higher proportion of xanthophylls (DD+DT), typical of high
617 light acclimated algal cells (MacIntyre et al. 2002; Kropuenske et al. 2009; Lepetit et al.
618 2013). The σ_{PSII} variations were similar to Photosynthetic/Photoprotective pigment,
619 although not decreasing significantly (L1_{30min-6d}, P-value = 9.92×10^{-1} / L2_{30min-6d}, P-value =
620 1.39×10^{-1}) (Fig. 3c), and are in agreements with the observations by Kvernvik et al. (2018)
621 on Arctic microalgae communities.

622

623 The abundance of photosynthetic proteins PsbA and RbcL increased during the Light return
624 1 experiment (Fig. 4a). The PsbA cell quota remained stable within the first 5 hours
625 (L1_{30min-5h}, P-value = 1.00×10^0), consistent with rapidly-induced photoprotection
626 protecting a further degradation of PsbA (Wu et al. 2011). It then significantly increased
627 until the 6th days (L1_{5h-6d}, P-value = 2.86×10^{-8}) to end up near t0 values, supporting a
628 reassembly of the PSII reaction center (RCII) back to the pre-acclimation state. The RbcL
629 cell quota was nearly undetectable within the first day, but then increased significantly into
630 a quantifiable range until the 6th day (L1_{1d-6d}, P-value = 2.38×10^{-14}) (Fig. 4a). However,
631 P_{max} per cell increased significantly within 1 day of light exposure (L1_{dark-1d}, P-value = 1.54
632 $\times 10^{-8}$, much of the increase occurred between the previously measured dark level (D₂₈) and
633 the first 30 minutes of illumination) (Fig. 4c) (Fig. 5b) as also observed by Popels et al.
634 (2007). The discrepancies with the apparent delayed recovery of RbcL content results from
635 RbcL in darkness and early re-illumination falling below a quantifiable range. Indeed, the
636 low residual content of RuBisCO would operate at maximal activity within 1 day of light
637 exposure (MacIntyre et al. 1996; MacIntyre et al. 1997). Φ_M also increased rapidly within 1
638 day of light exposure (L1_{30min-1d}, P-value = 9.55×10^{-6}) (Fig. 4b), supporting a fast recovery
639 of the photophysiology (Luder et al. 2002; Kvernvik et al. 2018; Lacour et al. 2019).

640 Despite the slow synthesis of photosynthetic proteins and the decrease of the
641 Photosynthetic/Photoprotective pigment ratio, the cells achieved efficient coupling from
642 RCII photochemistry to carbon fixation within 1 day in the Light return 1 experiment.
643 In the Light return 2 experiment, PsbA and RbcL cell quotas were initially nearly
644 undetectable. The recovery slopes of P_{\max} per cell and Φ_M appeared lower and are
645 significantly different from those in the Light return 1 experiment according to the
646 interaction terms ($P\text{-value}_{P_{\max}} = 7.32 \times 10^{-6}$, $P\text{-value}_{\Phi_M} = 5.79 \times 10^{-9}$) (Figs. 4b,c). The
647 recovery in the Light return 2 experiment was possibly slowed by a more extensive
648 dismantling of the photosynthetic apparatus and electron sink capacities, and by a likely
649 substantial population of dead cells under longer darkness acclimation (3 months vs. 1.5
650 months). As in the dark experiment, the variations in Φ_M likely reflected the fluorescence
651 ratio recovery within the PSII pool of remaining viable cells, while P_{\max} reflected the whole
652 cell population (including dead cells and empty frustules). Thus, the speed of recovery was
653 indeed lower in the Light return 2 experiment as shown by the variations in Φ_M .

654 *Metabolic recovery*

655 All together the cells rapidly (within 1 day) acclimated to the applied irradiance ($30 \mu\text{mol}$
656 $\text{photons m}^{-2} \text{s}^{-1}$) in the Light return 1 experiment, as illustrated by the rapid significant
657 increase of E_k ($L1_{\text{dark-1d}}$, $P\text{-value} = 2.95 \times 10^{-5}$, much of the increase occurred between the
658 previously measured dark level (D_{28}) and the first 30 minutes of illumination) (Fig. S10).
659 The interaction term is also not significant for E_k , meaning that the increases in E_k for the
660 Light return experiments 1 and 2 are statistically equivalent ($P\text{-value} = 1.44 \times 10^{-1}$). Lacour
661 et al. (2019) also measured a rapid increase in E_k and $rETR_{\max}$ within the first hours of
662 light return for *Chaetoceros neogracile* cultures exposed to 4 different re-illumination
663 levels (5, 27, 41 and $154 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) with a more rapid recovery for higher light
664 levels. Kvernvik et al. (2018) also measured a similar fast recovery in photophysiology on
665 Arctic microalgal communities for low and high re-illumination levels (1 and $50 \mu\text{mol}$
666 $\text{photons m}^{-2} \text{s}^{-1}$). In the present study, the time at which E_k matches the actual irradiance the
667 cells were exposed to during light returns testifies to their full ability to perform
668 photochemistry and fix carbon to support anabolism and ultimately cell growth, particularly
669 during the Light return 1 experiment.

670 Carbon and nitrogen cell quotas increased significantly by 62% and 66%, respectively, over
671 6 days for the Light return 1 experiment ($L_{130\text{min-6d}}$, $P\text{-value}_{\text{carbon}} = 1.12 \times 10^{-2}$, $P\text{-value}_{\text{nitrogen}}$
672 $= 2.10 \times 10^{-6}$) (Fig. 2c). The lipid droplets cell quota also significantly increased by 14% in
673 6 days ($L_{15\text{h-6d}}$, $P\text{-value} = 3.85 \times 10^{-6}$) (Fig. 2b). However, carbohydrates probably
674 accounted for some of the increase in carbon quotas (Myklestad 1974; Chauton et al. 2013)
675 despite the lack of carbohydrates data to support this statement. The cell volume
676 significantly increased by 31% in 6 days ($L_{130\text{min-6d}}$, $P\text{-value} = 1.19 \times 10^{-11}$) (Fig. 2b) which
677 is consistent with a larger content in lipids, carbon and nitrogen. As expected, the cell
678 population entered an exponential growth phase measured between 1 day and 6 days
679 following exposure to light with a mean population growth rate of $0.146 \pm 0.010 \text{ d}^{-1}$ (Fig.
680 2a). This was 40% lower than the mean growth rate measured during the pre-darkness
681 acclimation period ($0.244 \pm 0.041 \text{ d}^{-1}$), possibly as a consequence of the larger size of new
682 cells and of potential mortality before and during light return. For the Light return 2
683 experiment, the increase in the carbon cell quota was statistically equivalent to the Light
684 return 1 experiment as supported by the interaction term ($P\text{-value} = 1.28 \times 10^{-1}$) while
685 nitrogen cell quota, lipid droplets cell quota, cell volume and cell number per ml of algal
686 culture slopes were significantly different from the Light return 1 experiment ($P\text{-value}_{\text{nitrogen}}$
687 $= 7.28 \times 10^{-5}$, $P\text{-value}_{\text{lipid}} = 2.34 \times 10^{-2}$, $P\text{-value}_{\text{cell volume}} = 3.12 \times 10^{-11}$, $P\text{-value}_{\text{cell number}} =$
688 1.38×10^{-7}) (Figs. 2a,b,c). Cell growth was not observed over the Light return 2 tracked re-
689 illumination period. Along with the greater dismantling of the photosynthetic apparatus
690 impacting the speed of photophysiological recovery, mortality may have compromised the
691 population ability to reinitiate detectable growth upon light exposure. Nevertheless, a
692 fraction of the population recovered as the Φ_M data suggest that living cells recovered
693 function of the PSII pool after ~ 3 days in Light return 2 (Fig. 4b). For parameters that
694 showed a lag phase, that lag phase may be in fact only apparent, because those parameters
695 were normalized to the total number of cells (including the dead ones). As healthy cells
696 divide and the relative contribution of dead cells thereby decreases, full population
697 recovery may be observed. The lag phase was previously reported to increase with
698 increasing previous dark period (Dehning & Tilzer 1989; Peters & Thomas 1996). In
699 antarctic diatoms, the lag phase lasted 4 days following 74 days in the dark (Peters &

700 Thomas 1996). The results of the present experiment suggest that *F. cylindrus* had a lag
701 phase longer than 6 days before reaching detectable exponential growth after 3 months of
702 darkness, yet recovery of exponential growth could not be confirmed within the timescale
703 of our measures.

704 Conclusions

705 *F. cylindrus* achieved a physiological resting state a few days following the transition to
706 dark and maintained it throughout until the return to light (Fig. 7). This rather stable state
707 was characterized by very low consumption of energy reserves, a slow decrease of
708 photosynthetic pigments, a faster decrease in key photosynthetic protein complexes, and
709 very low photosynthetic capacities. Subsequent transition back to light after 1.5 months
710 first triggered fast photoprotection followed by the renewal of photosynthetic components.
711 Rapid recovery of photophysiology occurred within a few hours of return to light, followed
712 by resumption of cell growth after 1 day of re-illumination. The re-acclimated light state
713 showed similar characteristics to high light grown cells regarding the changes in pigment
714 composition, at least over the initial 6 days. The results from the transition to light after 3
715 months highlighted an apparent lag phase that increases in length with longer periods of
716 darkness. Mortality in the dark may have delayed the full recovery of the population with
717 an apparent lag phase longer than 6 days.

718

719 The results of this study suggest that the low rate of energy consumption for dark survival
720 and high photoprotective capacity upon light return may be two physiological traits that
721 help *F. cylindrus* to thrive in polar oceans. It remains to be investigated whether mortality
722 or sustained down regulation, or both, are the major factor(s) explaining the stronger
723 physiological drop-down and the delayed recoveries of measured physiological and
724 molecular parameters after prolonged darkness (3 months). Progressive dark and light
725 transitions, rather than sudden shifts as in this experiment, should also be tested and
726 coupled with mortality measurements to determine if a particular light return regime
727 compromises survival more than another one. The role of heterotrophy in dark survival
728 remains to be clarified, because available dissolved organic carbon within and underneath
729 sea-ice (Riedel et al. 2008) could potentially improve diatom survival to the winter polar
730 night. Finally, the expression of genes was not within the scope of this study and should
731 also be addressed in future experiments to uncover the signature of metabolic pathways
732 over a dark period that is significant to the Arctic polar night.

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745

746 Authors Contribution

747 Morin P. I. is the main author of this work with major contributions to designing and
748 running the experiments, analysing the data and writing the paper. All co-authors helped
749 with running the experiments and/or revising the paper. Campbell D. A., J. Lavaud and M.
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1001
1002

1003 Figure legends

1004

1005 **Figure 1. Timeline of the sampling strategy**

1006 Before dark transition, cultures were grown under stable light conditions for 52 days.
1007 Cultures were then transferred to complete darkness (grey arrow) at ‘day 0’ (t₀ in the
1008 following figures). The vertical lines show the times of sampling, and the dashed lines
1009 show transfer of a fraction of the replicate cultures to Light return 1 after 1.5 months (48
1010 days) and Light return 2 after 3 months (90 days) of dark incubation. Upon light return,
1011 dark acclimated subsamples were transferred to the same light conditions as before
1012 darkness.

1013

1014 **Figure 2. Cells and reserves**

1015 **a)** Biovolume (μm^3 , red) and Cell number per ml (blue); **b)** Cell volume (μm^3 , red) and
1016 lipid droplets cell quota (RFU, blue); **c)** μg carbon (red) and μg nitrogen per cell (blue) of
1017 *Fragilariopsis cylindrus* cultures kept in the dark at 0°C for up to 3 months and then re-
1018 exposed to continuous light of $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ after 1.5 months or 3 months of
1019 darkness for the light return experiments 1 and 2, respectively. Values from the light return
1020 experiments are shown enlarged in Fig. S6. Each point is the mean of the three cultures
1021 with the standard deviation as the error bar, except for the carbon points after the first
1022 month of darkness from which a divergent culture replicate was discarded (red dots) from
1023 the mean and standard deviation calculations.

1024

1025 **Figure 3. Photosynthetic and photoprotective pigments**

1026 **a)** μg Chlorophyll *a* (Chl*a*, red) and Fucoxanthin per cell (Fuco, blue); **b)** μg
1027 Diadinoxanthin (DD, red) and Diatoxanthin per cell (DT, blue); **c)** the effective absorption
1028 cross-section for PSII photochemistry (σ_{PSII} , $\text{\AA}^2 \text{ quanta}^{-1}$, red) and photosynthetic /
1029 photoprotective pigments (Chl*a* + Chl*c* + Fuco / DD + DT, blue) of *Fragilariopsis*
1030 *cylindrus* cultures kept in the dark at 0°C for up to 3 months and then re-exposed to
1031 continuous light of $30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ after 1.5 months or 3 months of darkness for the
1032 light return experiments 1 and 2, respectively. Values from the light return experiments are

1033 shown enlarged in Fig. S8. Each point is the mean of the three cultures with the standard
1034 deviation as the error bar.

1035

1036 **Figure 4. Photosynthetic proteins and photosynthesis parameters**

1037 **a)** μg PsbA (PSII protein D1, red) and μg RbcL per cell (RuBisCO large subunit, blue); **b)**
1038 α (initial slope of carbon fixation, [$\mu\text{g C cell}^{-1} \text{h}^{-1} (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$], red) and Φ_M
1039 (maximum quantum yield of PSII, dimensionless, blue); **c)** P_{max} (maximum carbon fixation
1040 rate, $\mu\text{g C cell}^{-1} \text{h}^{-1}$, red) and rETRmax (maximum relative electron transport rate,
1041 dimensionless, blue) of *Fragilariopsis cylindrus* cultures kept in the dark at 0°C for up to 3
1042 months and then re-exposed to continuous light of 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ after 1.5 months
1043 or 3 months of darkness for the light return experiments 1 and 2, respectively. Values from
1044 the light return experiments are shown enlarged in Fig. S9. Each point is the mean of the
1045 three cultures with the standard deviation as the error bar, except for the PsbA Light return
1046 1 points from which a culture replicate was discarded (red dots) of the mean and standard
1047 deviation calculations. Note that other samples for PsbA (after 2 months of darkness and
1048 during Light return 2, red dots) and RbcL (after 2 months of darkness, during Light return 1
1049 until day 1 and during Light return 2, blue dots) are to be treated with caution as most of
1050 them were detectable but did not fall into a quantifiable range.

1051

1052 **Figure 5. ^{14}C incubation curves**

1053 **a)** Carbon fixation vs. irradiance curves ($\mu\text{g C cell}^{-1} \text{h}^{-1}$) of *Fragilariopsis cylindrus* cultures
1054 kept in the dark at 0°C for up to 3 months and then exposed to continuous light of 30 μmol
1055 photons $\text{m}^{-2} \text{s}^{-1}$ after **b)** 1.5 months (Light return 1) or **c)** 3 months of darkness (Light return
1056 2). Each curve is fitted on data points pooled from three cultures for each sampling time.

1057

1058 **Figure 6. Photoprotective capacity**

1059 **a)** NPQ developed at 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (NPQ30, dimensionless, red) and De-
1060 epoxidation state of Diadinoxanthin (DES, blue) of *Fragilariopsis cylindrus* cultures kept
1061 in the dark at 0°C for up to 3 months and then re-exposed to continuous light of 30 μmol

1062 photons $\text{m}^{-2} \text{s}^{-1}$ after 1.5 months (Light return 1) or **b**) 3 months of darkness (Light return 2).
1063 Each point is the mean of the three cultures with the standard deviation as the error bar.

1064

1065 **Figure 7. Scheme of the acclimation processes in *Fragilariopsis cylindrus* to prolonged**
1066 **darkness and the return of light**

1067 Acclimation processes are summarized by their levels, shown in a table with ‘+’ and ‘-’
1068 signs, and as schematic representations of a *F. cylindrus* cell. Note that the 30 minutes
1069 time-points of Light return 1 (L1) and Light return 2 (L2) are not shown in the schematic
1070 representations. **Cell growth** is based on the cell number per ml and volume (μm^3)
1071 parameters. **Reserves** are based on the lipid droplets cell quota (RFU) and the carbon and
1072 nitrogen cell quotas. **Photosynthetic apparatus** is based upon molecular components and
1073 photophysiology, including Photosynthetic/Photoprotective pigments (Chlorophyll *a* +
1074 Chlorophyll *c* + Fucoxanthin / Diadinoxanthin + Diatoxanthin) and PsbA (PSII protein D1)
1075 cell quotas and with rETR_{max} (maximum relative electron transport rate), NPQ (non-
1076 photochemical quenching) and P_{max} (maximum carbon fixation rate per cell). Note that
1077 rETR_{max} and NPQ levels are hypothesized before dark transition (day 0) as there were no
1078 measurements available for this particular sampling time. The number of photosynthetic
1079 and photoprotective pigments (green and orange oval shapes, respectively) aims to
1080 represent the measured levels per cell and their ratio to each other, rather than an exact
1081 view of the photosynthetic apparatus. Legend for levels: +++ (highest), ++ (high), + (moderately high), - (moderately low), -- (low), --- (lowest). Each level is assigned relative
1082 to the observed variation for a given parameter within the entire experiment.

1084

1085 Supporting information legend

1086 **Table S1** Mean±SD values of sampling dark days.

1087 **Table S2** Mean±SD values of sampling Light return 1 days.

1088 **Table S3** Mean±SD values of sampling Light return 2 days.

1089

1090 **Figure S1** Picture of *F. cylindrus* cultures grown during light acclimation.

1091 **Figure S2** Spectrum of the different light sources used during the experiments.

1092 **Figure S3** Picture of *F. cylindrus* cultures grown during the light return experiments.

1093 **Figure S4** Flow cytometry data for emitted BODIPY fluorescence

1094 **Figure S5** Φ_M determinations from PAM and FRe fluorometers

1095 **Figure S6** Figure 2 enlarged for the light return experiments.

1096 **Figure S7** Comparison between carbon and nitrogen per cell and per ml.

1097 **Figure S8** Figure 3 enlarged for the light return experiments.

1098 **Figure S9** Figure 4 enlarged for the light return experiments.

1099 **Figure S10** E_k^{14C} (P_{max}/α) and E_k PAM ($rETR_{max}/\alpha$).

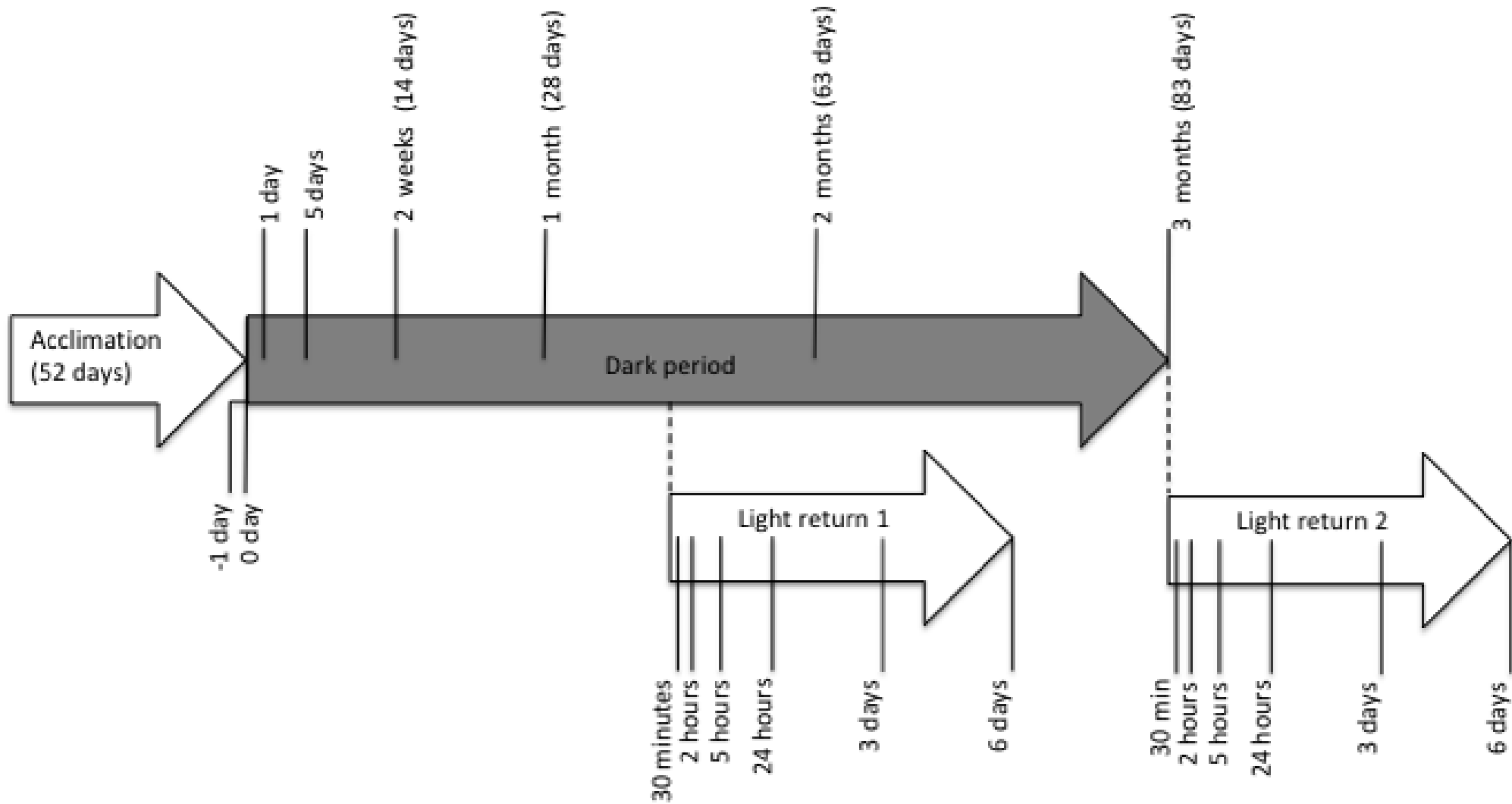
1100 **Figure S11** Curves of dynamic and total non-photochemical quenching.

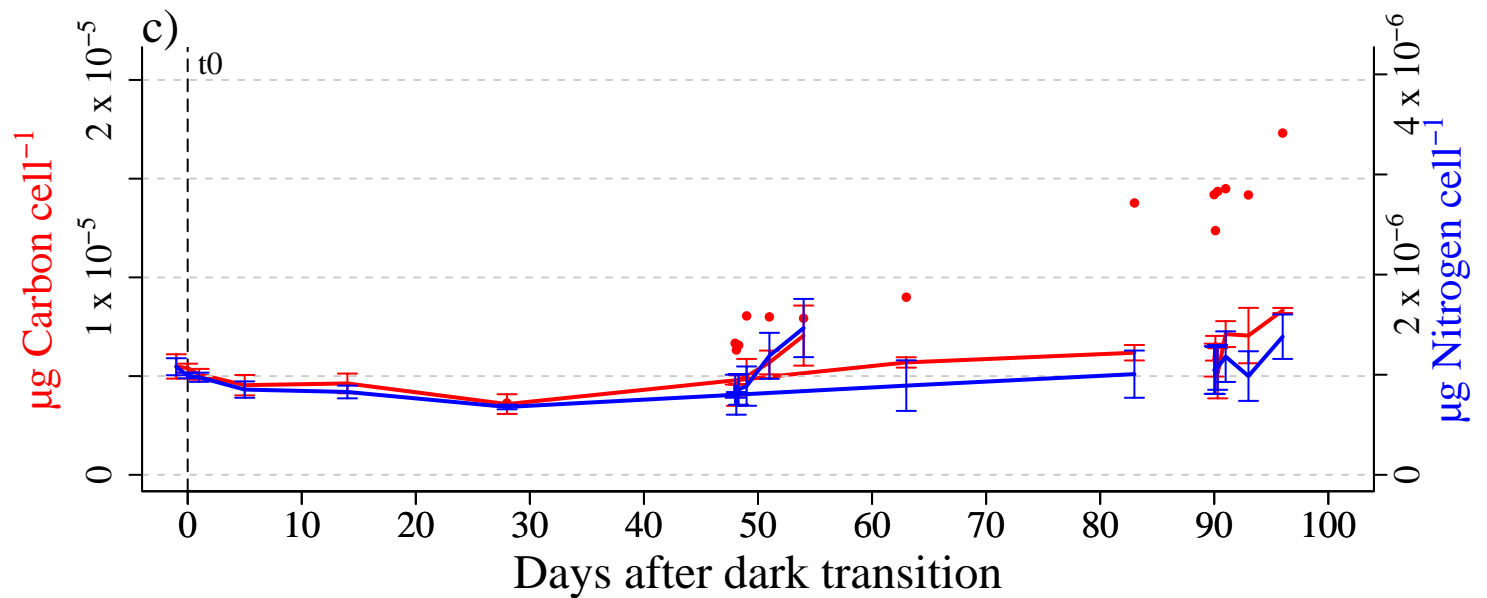
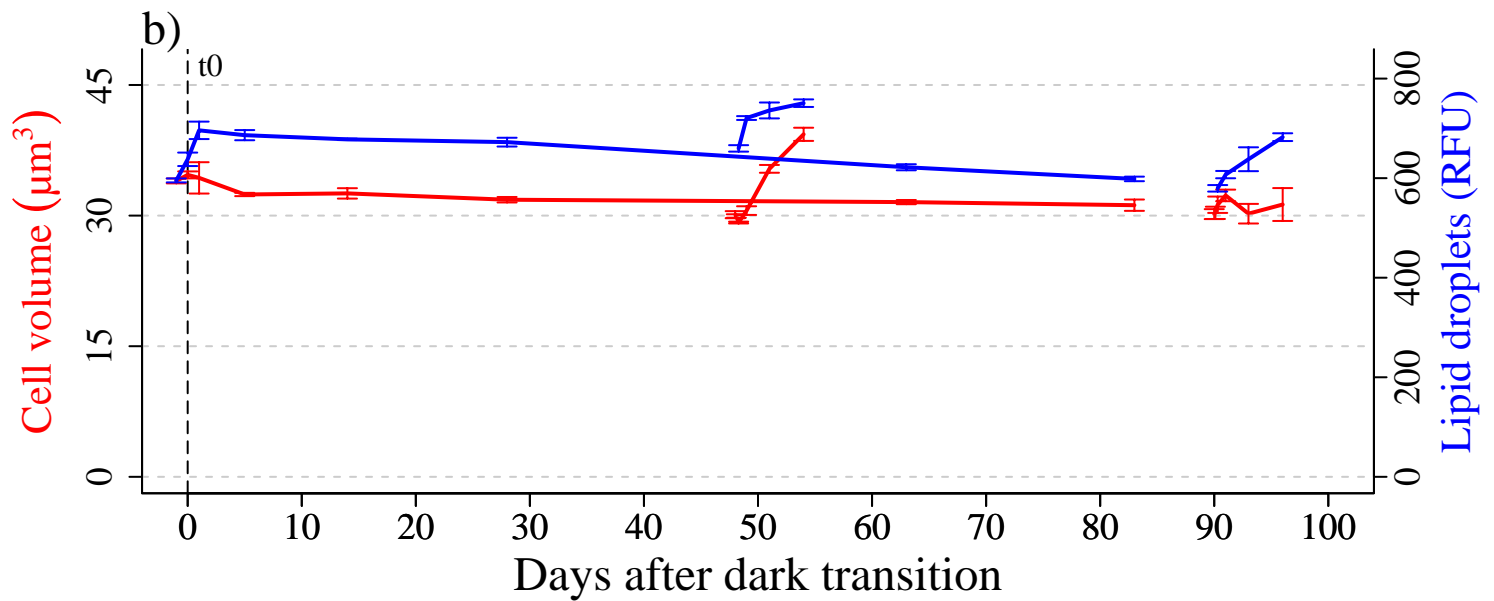
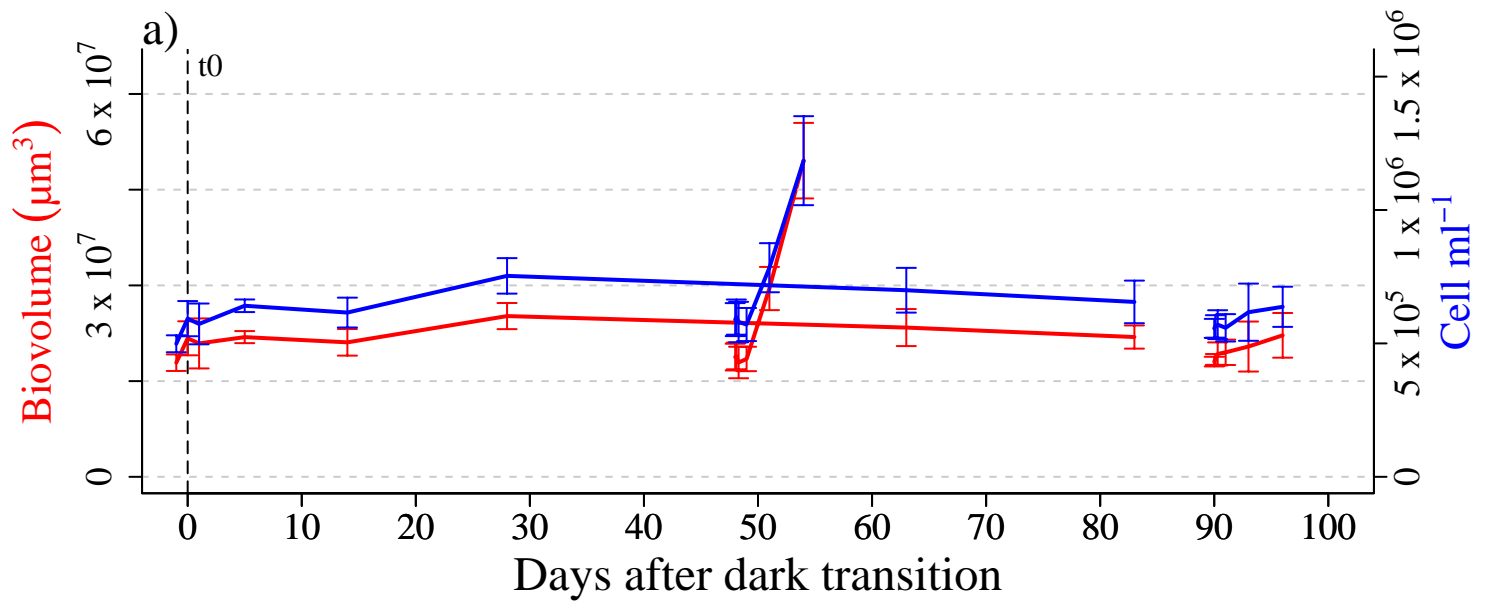
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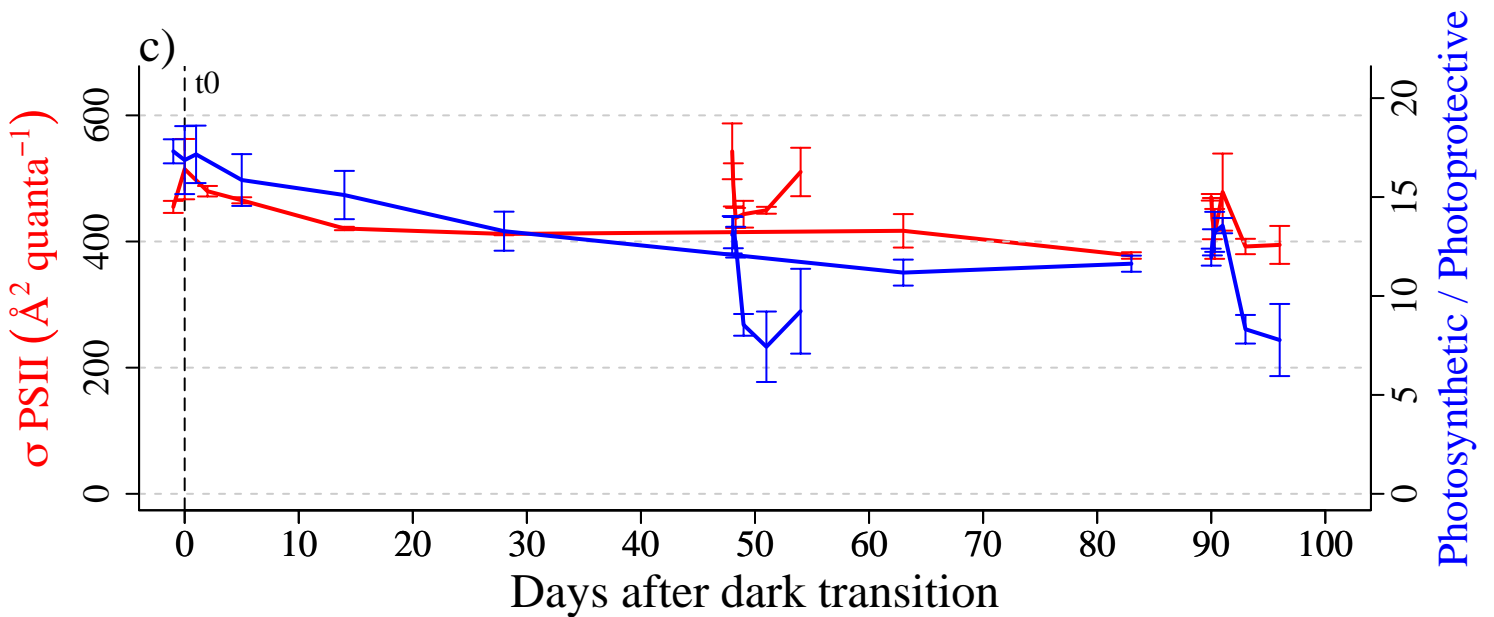
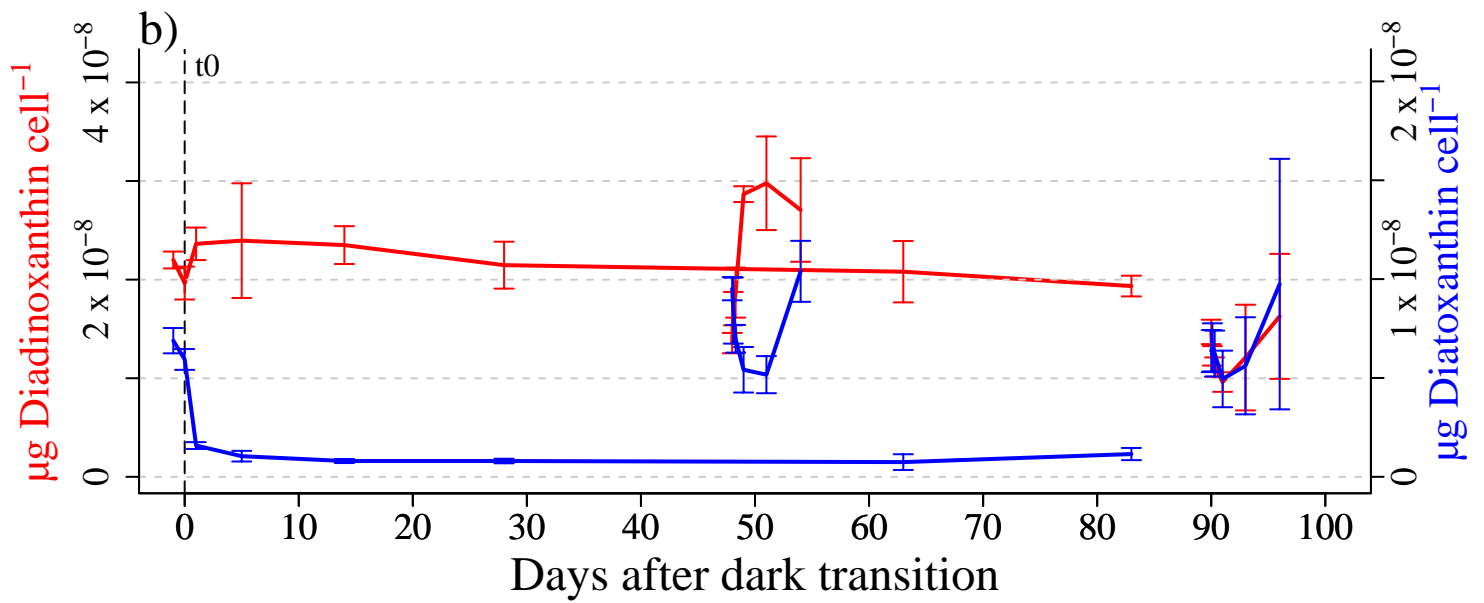
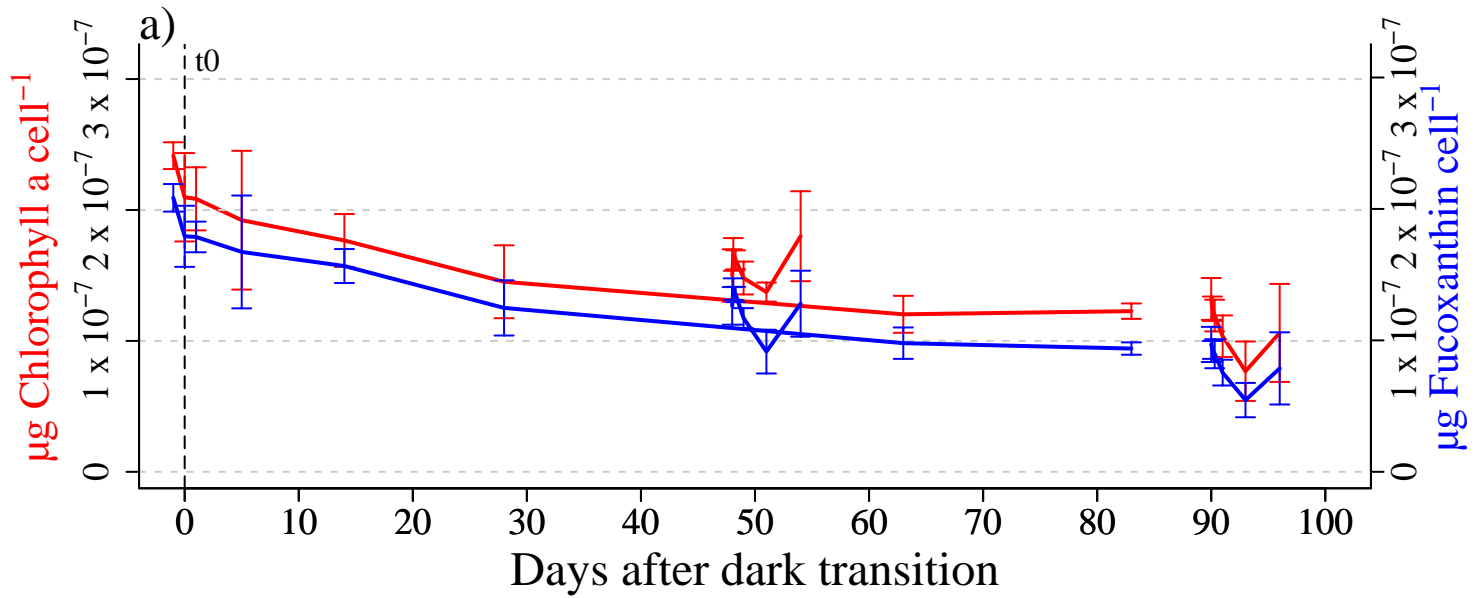
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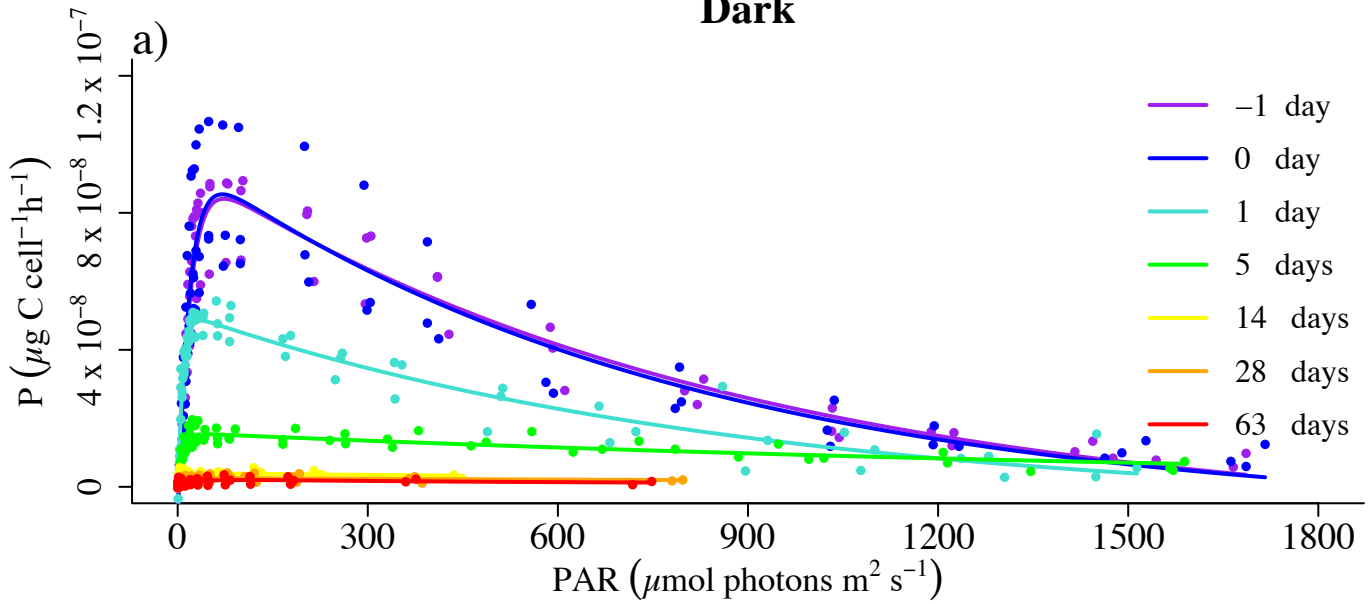
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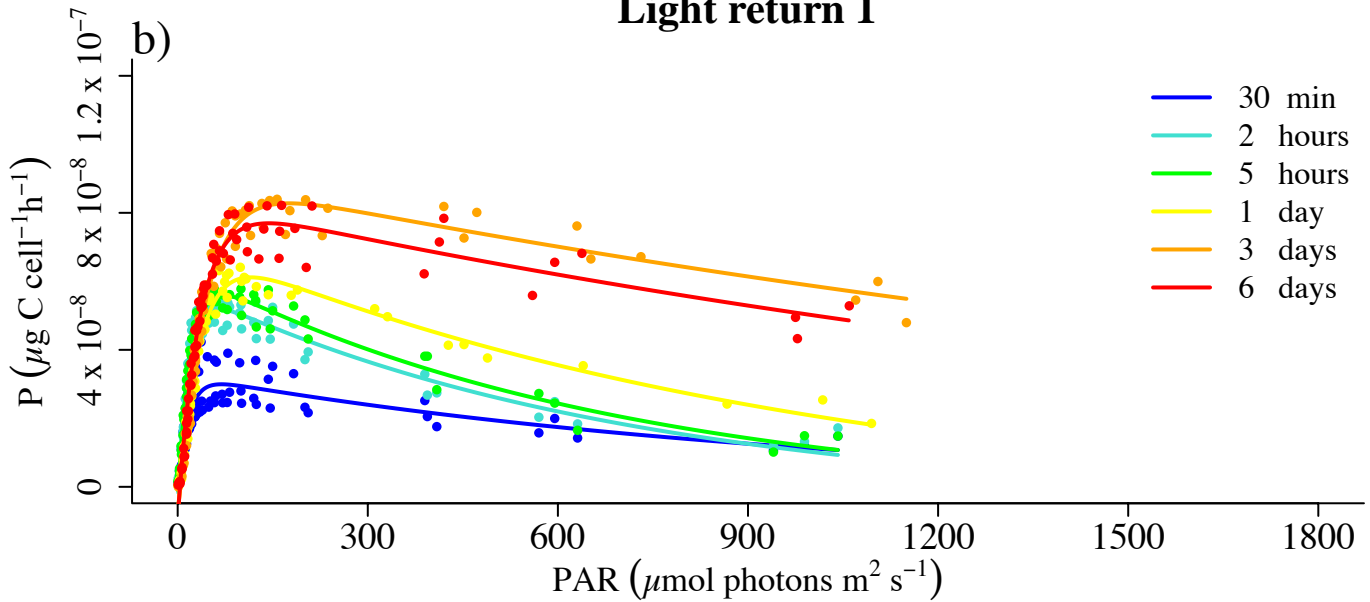




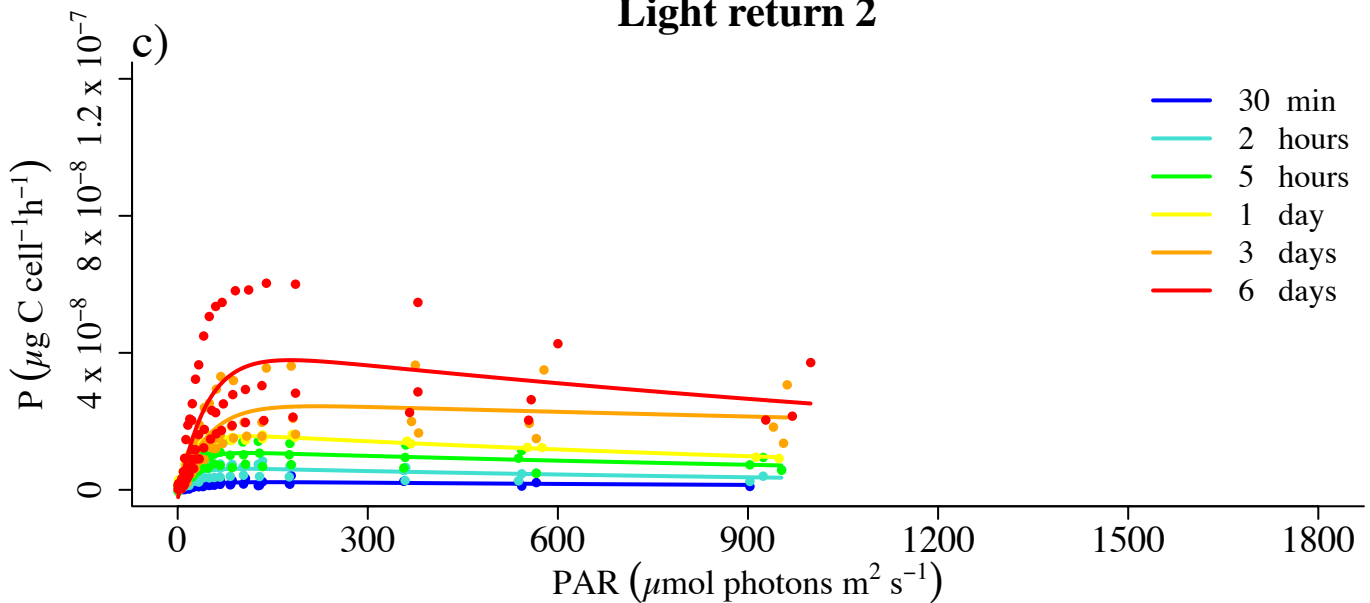
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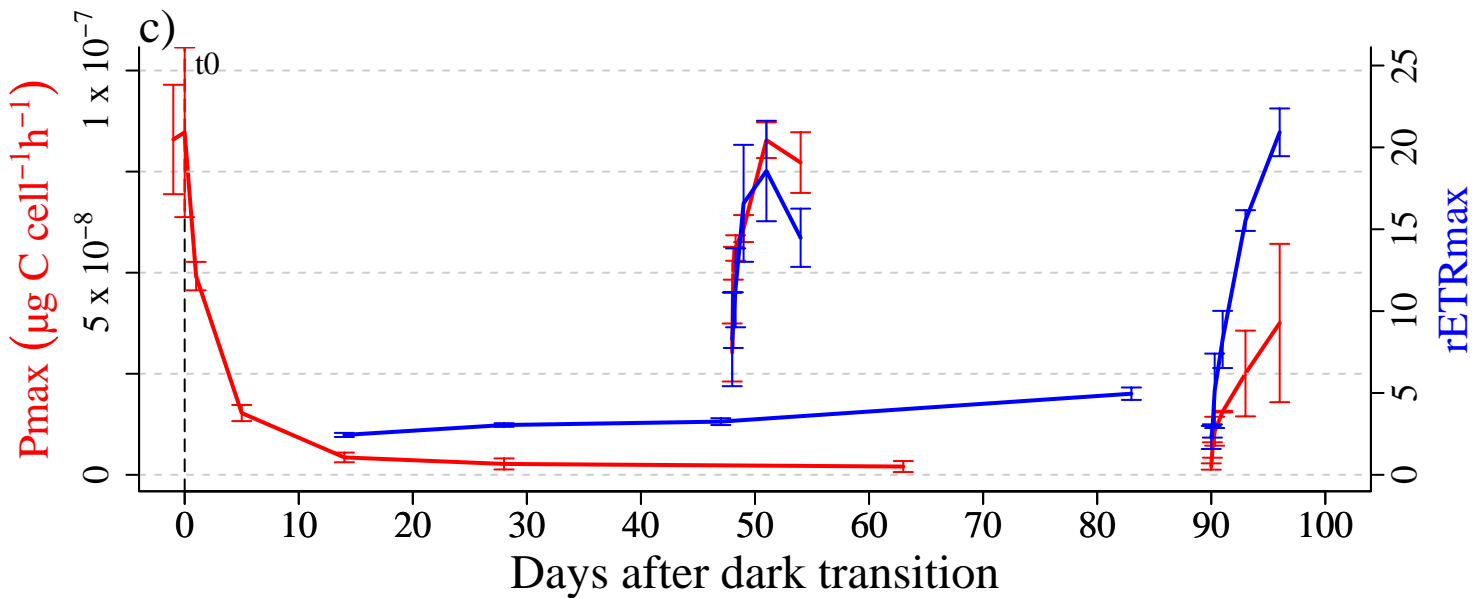
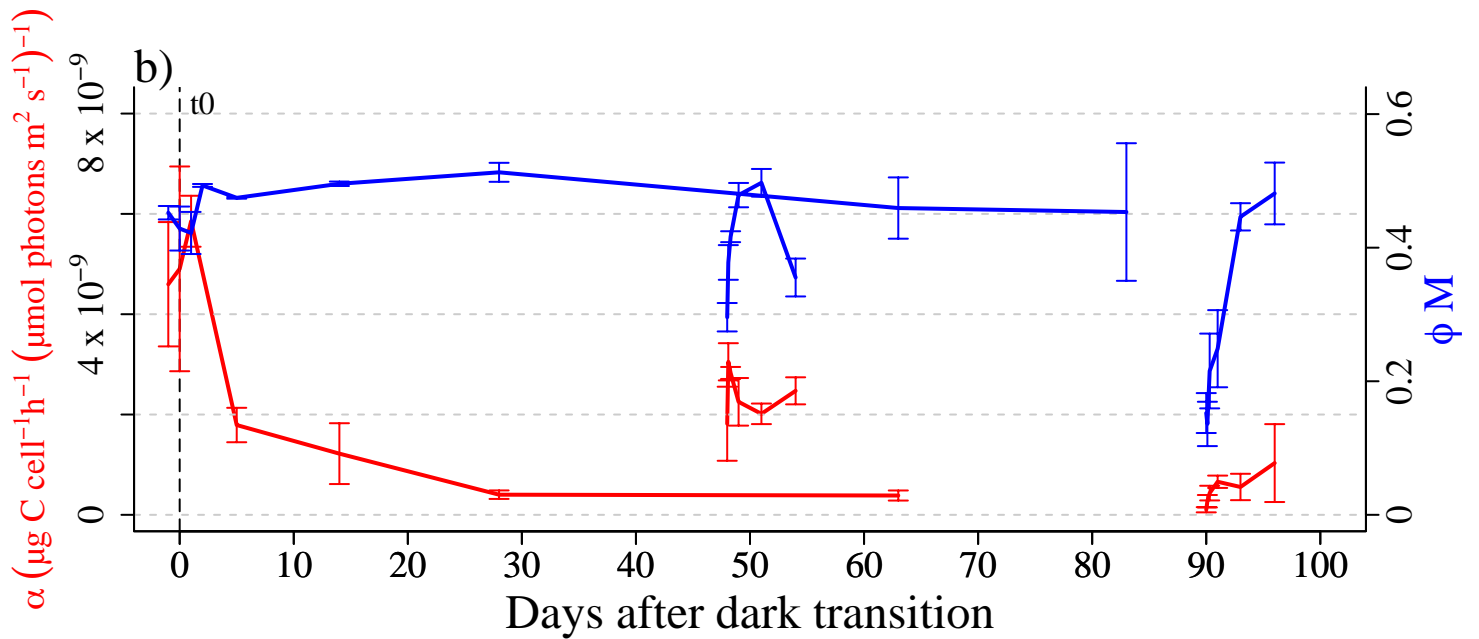
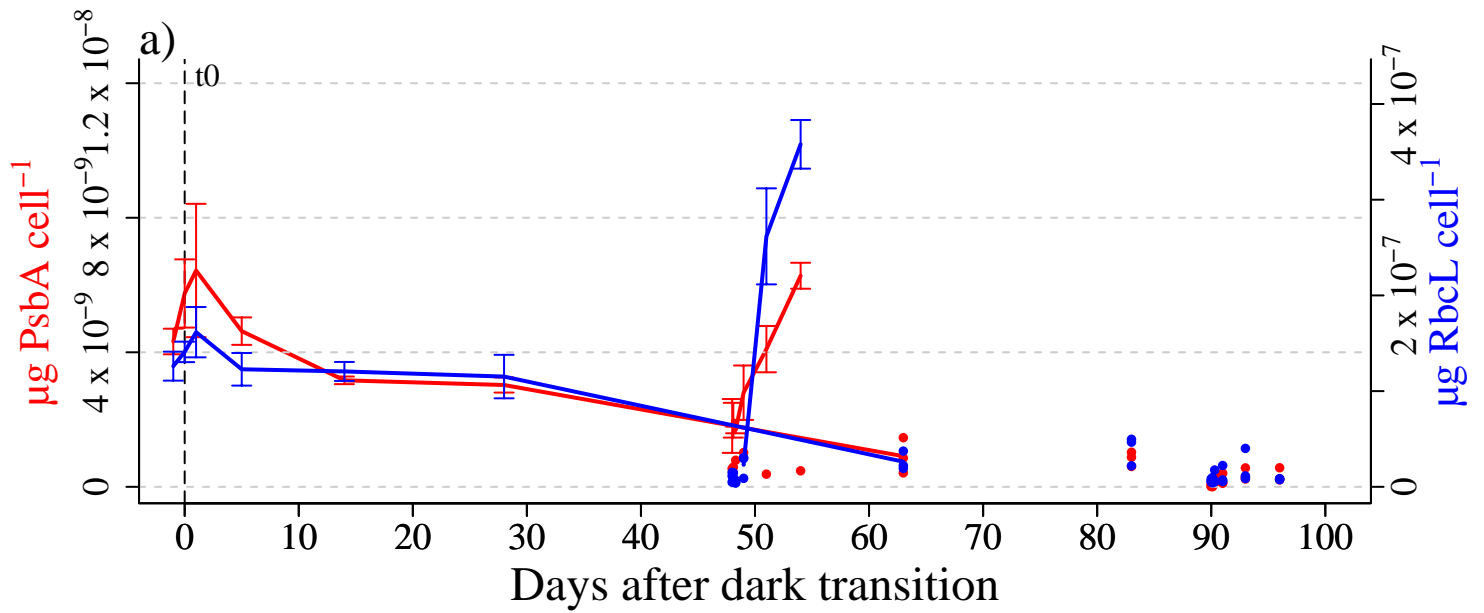


Light return 1

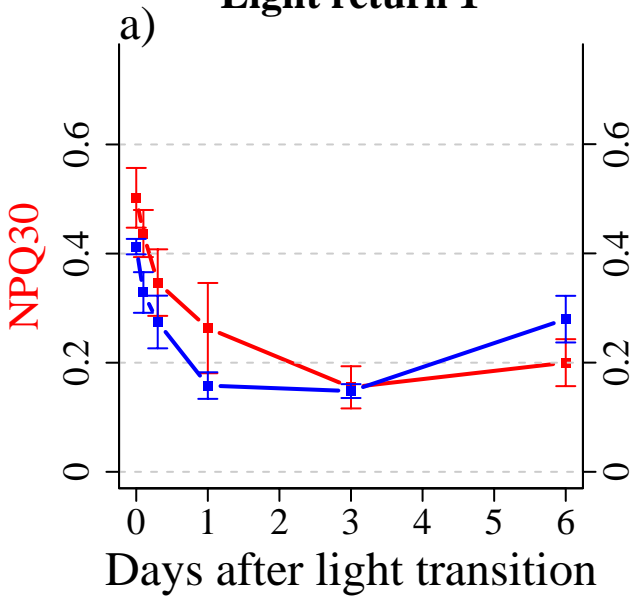


Light return 2

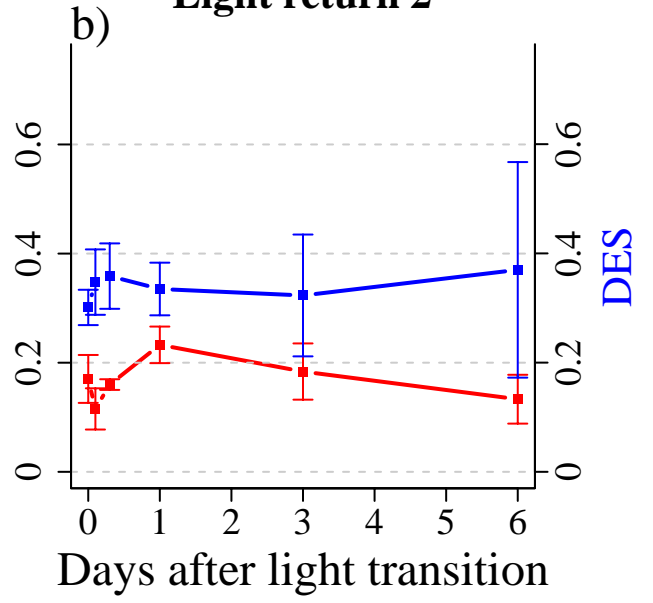




Light return 1



Light return 2



Time	Cell growth	Reserves	Photosynthetic apparatus				
			Molecular components		Photophysiology		
			Photosynthetic/Photoprotective pigments	PsbA	rETRmax	NPQ	Pmax
D: 0 months	+	++	+++	+++	+++	+	+++
D: 1 month	++	++	++	+	---	+++	---
D: 3 months	+	+	+	---	---	+++	---
L1: 30 min	+	++	++	-	+	+++	+
L1: 1 day	+	+++	-	+	+++	++	+++
L1: 6 days	+++	+++	+	+++	+++	+	+++
L2: 30 min	+	+	+	---	+	+	-
L2: 1 day	+	+	+	--	++	+++	+
L2: 6 days	+	++	-	--	+++	++	++

