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1 **An explanation for the inter-species variability of the photoprotective non-**  
2 **photochemical chlorophyll fluorescence quenching in diatoms**

3

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18

19 **Abstract**

20 Diatoms are a major group of microalgae whose photosynthetic productivity supports a  
21 substantial part of the aquatic primary production. In their natural environment they have  
22 to cope with strong fluctuations of the light climate which can be harmful for  
23 photosynthesis. In order to prevent the damage of their photosynthetic machinery,  
24 diatoms use fast regulatory processes among which the non-photochemical quenching of  
25 chlorophyll *a* fluorescence (NPQ) is one of the most important. In a previous work, we  
26 highlighted differences in the kinetics and extent of NPQ between diatom species/strains  
27 originating from different aquatic habitats. We proposed that the NPQ differences  
28 observed between strains/species could potentially participate to their ecophysiological  
29 adaptation to the light environment of their respective natural habitat. In order to better  
30 understand the molecular bases of such differences, we compared the NPQ features of  
31 four strains/species of diatoms known for their NPQ discrepancy. We could identify new  
32 spectroscopic fingerprints concomitant to NPQ and the related xanthophyll cycle. These  
33 fingerprints helped us propose a molecular explanation for the NPQ differences observed  
34 between the diatom species/strains examined. The present work further strengthens the  
35 potential role of NPQ in the ecophysiology of diatoms.

36

37 *Key words:* diatoms; diatoxanthin; ecophysiology; photoprotection; non-photochemical  
38 fluorescence quenching; xanthophyll cycle.

39

40 *Abbreviations:* Chl *a*, Chlorophyll *a*; Chl *a* FIKs, Chl *a* fluorescence induction kinetics;  
41 Chl *a*<sub>711</sub>, Chl *a* fluorescence emission band at 711 nm; DD, diadinoxanthin; DES, de-

42 epoxidation state; DT, diatoxanthin; FCP, fucoxanthin-chlorophyll protein; LHC, light-  
43 harvesting complex; NPQ, non-photochemical Chl *a* fluorescence quenching; PAM,  
44 Pulse-amplified modulation; PS II, Photosystem II; PS II CET, PS II cyclic electron  
45 cycle;  $\Delta\text{pH}$ , transthylakoidal proton gradient;  $\Delta 522$  nm, absorption change at 522 nm.  
46

## 47 **1. Introduction**

48           Diatoms are a major group of microalgae ubiquitous in all marine and freshwater  
49 ecosystems. They are among the most significant photosynthetic organisms contributing  
50 to about 40% of the aquatic primary production, especially in coastal ecosystems [1-2].  
51 Their biological characteristics have largely shaped the structure of contemporary aquatic  
52 ecosystems [1, 3]. As for most microalgae, the photosynthetic productivity of diatoms  
53 strongly depends on the aquatic light climate [4-6]. Planktonic as well as benthic diatoms  
54 tend to dominate ecosystems (coasts and estuaries) where they have to cope with a light  
55 climate with high-frequency irradiance fluctuations coupled with large amplitudes.  
56 Depending on the rate of water mixing, on the tidal cycle, on the daily/seasonal changes  
57 of solar irradiation, diatoms can be exposed to punctual or chronic excess light, possibly  
58 generating stressful conditions that impair their photosynthetic efficiency and their  
59 productivity (i.e. photoinhibition/photoinactivation) [7-10]. In order to prevent such  
60 situation, diatoms [11-12], and to a larger extent microalgae and other photosynthetic  
61 organisms [13-17], have evolved fast regulatory physiological processes which compose  
62 a 'photoprotective network'. They help to safely dissipate the excess of absorbed light  
63 energy as heat and/or to balance the excitation energy within the photosynthetic apparatus  
64 thus preventing/lowering the potential oxidative damages to the photosynthetic  
65 machinery. Additional mechanisms help repairing the unavoidable photodamages [10,  
66 18]. In diatoms, two fast processes are believed to be essential for the photoprotection:  
67 the electron cycle of the photosystem II reaction center (PS II CET) and the non-  
68 photochemical quenching of chlorophyll *a* (Chl *a*) fluorescence (NPQ) [11-12, 14, 19].  
69 NPQ is composed of three components, namely the high-energy state (qE), the state-

70 transition (qT) and the ‘photoinhibitory’ (qI) quenchings [13, 17]. In diatoms, qT does  
71 not exist and the origin of qI remains unclear [11-12]. Therefore, the diatom NPQ mainly  
72 relies on qE, a quenching mechanism which is controlled by i) the build-up of the  
73 transthylakoidal proton gradient ( $\Delta pH$ ), ii) the conversion of the xanthophylls  
74 diadinoxanthin (DD) into diatoxanthin (DT) called ‘xanthophyll cycle’ (XC), and iii) the  
75 presence of specific polypeptides of the light-harvesting complex (LHC) antenna named  
76 Lhcx [11-12, 14, 20-23]. Additionally, there exists a PS II reaction center quenching  
77 which might be related to the PS II CET [24-25]. These fast regulatory mechanisms  
78 proved to be important in field situation for maintaining the photosynthetic efficiency and  
79 productivity of diatoms [15, 21]. NPQ was shown to significantly participate in the  
80 cellular response under fluctuating light conditions [26-28]. Recently, an increasing  
81 number of reports suggested that the diatom/microalgae inter-species differences in NPQ  
82 are involved in the differential colonization of aquatic habitats depending on the rate of  
83 light fluctuations of the light climate [9, 27-33].

84         While the most recent advances in our knowledge of the physiological regulation  
85 of diatom photosynthesis, and especially of NPQ and the XC, were generated by the use  
86 of genetically modified cells of the model diatom *Phaeodactylum tricornutum* [20, 23,  
87 34-35], the comparison of different species/strains [9, 18, 20, 27, 30, 36-37] together with  
88 the use of different growth conditions [18, 30, 37-38] has also proven to be useful.  
89 Indeed, the XC and NPQ extent and kinetics can differ with regards to the species [9, 18,  
90 27, 30], and to the light [30, 38-39] and the temperature [18] acclimations. In a previous  
91 study, we compared the photoprotective ability of *P. tricornutum* and *Skeletonema*  
92 *costatum* [27]. One of the major differences concerned NPQ: its extent in *S. costatum* was

93 approximately half the one in *P. tricornutum*. It was not due to a lower de-epoxidation  
94 rate of DD to DT, as reported before for diatoms and other related organisms [18, 40-41].  
95 We suggested two explanations for such discrepancy [27]: 1) a different organization and  
96 FCP (fucoxanthin-chlorophyll protein) composition of the LHC antenna of PS II,  
97 including both Lhcf and Lhcx proteins, 2) a lower amount of DT molecules involved in  
98 the NPQ process; the two hypotheses not being exclusive from each other [18]. The  
99 present work aimed at going further deep in the understanding of NPQ differences  
100 between diatom species/strains. For that purpose, we examined four species/strains  
101 currently used as models for NPQ investigations and known to show different NPQ  
102 amplitude and regulation [20, 27, 36, 42-44]: a low and a high NPQ strains of *P.*  
103 *tricornutum* (P.t.2 and P.t.4, respectively), *S. costatum* and *Cyclotella meneghiniana*. The  
104 three species are also representative of the two major groups of diatoms, the pennates (*P.*  
105 *tricornutum*) and the centrics (*S. costatum* and *C. meneghiniana*); the genome of *P.*  
106 *tricornutum* is available and *C. meneghiniana* is a close relative of *Thalassiosira*  
107 *pseudonana* whose genome is also published [45]. Finally, the ecological niches where P.t.2,  
108 P.t.4 and *S. costatum* were isolated (temperate estuary, high latitude shore, Mediterranean  
109 semi-enclosed bay, respectively) show very different underwater light climates [20, 27].

110

## 111 **2. Material and Methods**

### 112 *2.1. Cultures*

113 *Phaeodactylum tricornutum* Böhlin CCAP1052-CCAP1055/1 ('P.t.2 strain', Culture  
114 Collection of Algae and Protozoa, Oban, Scotland) and CCAP 1052/6 ('P.t.4 strain',  
115 Sammlung Algenkulturen Göttingen (SAG), Germany), *Skeletonema costatum* (Greville)  
116 Cleve (Laboratoire Arago algal collection, Banyuls-sur-Mer, France) and *Cyclotella*  
117 *meneghiniana* Kützing SAG 1020\_1a (SAG, Germany) cells were grown  
118 photoautotrophically in sterile natural seawater F/2 medium [46]. Non-axenic cultures of  
119 300 mL were incubated at 18°C in airlifts continuously flushed with sterile air. They were  
120 illuminated at a light intensity of 40  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  with white fluorescent tubes  
121 (Claude, Blanc Industrie, France) with a 16 h light/8 h dark photoperiod ('continuous light',  
122 CL cells, see [47]). Intermittent light (IL) cells of *P. tricornutum* were grown in the same  
123 conditions except the light regime which was 5 min light/55 min dark cycle (see [47]). Cells  
124 were harvested during the exponential or stationary phase of growth as specified in Table 1,  
125 centrifuged at 3000 g for 10 min and resuspended in their culture medium to a final  
126 concentration of 10  $\mu\text{g chlorophyll } a \text{ (Chl } a) \text{ mL}^{-1}$ . The concentrated suspension was then  
127 continuously stirred at 18°C under low light before use.

128

### 129 *2.2. Pigment content*

130 Pigment analyses were performed by HPLC as previously described [47]. Cells  
131 collected from the Clark electrode adapted to the PAM-fluorometer (see below) were  
132 deposited on a filter and frozen in liquid nitrogen. Pigments were extracted with a  
133 methanol:acetone (70:30, v/v) solution. The xanthophyll de-epoxidation state (%) was

134 calculated as  $(DT/(DD+DT)) \times 100$  where DD is the diadinoxanthin, the epoxidized form,  
135 and DT is the diatoxanthin, the de-epoxidized form.

136

### 137 2.3. Spectroscopy

138 Ambient temperature absorption spectra were performed with a DW-2 Aminco  
139 spectrophotometer. For measuring the 522 nm absorption change ( $\Delta 522$  nm) which reflects  
140 the ‘activation of DT’ (see [48]), spectra were performed on dark-acclimated cells (for 20  
141 min) and cells exposed to light (5 min,  $2000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) as previously described  
142 [48]; the half-bandwidth was 0.5 nm. For partial inhibition of NPQ, cells were incubated  
143 prior to illumination (20 min, dark) with dithiothreitol (DTT) or  $\text{NH}_4\text{Cl}$  with a final  
144 concentration of 25  $\mu\text{M}$  and 2 mM, respectively (see [49]). 77K fluorescence emission  
145 spectra were measured with an F-3000 and F-4500 Hitachi spectrophotometer. Half-  
146 bandwidth for emission was 2.5 (F-4500) and 3 (F-3000) nm, respectively. Samples were  
147 dark-acclimated (20 min) and then concentrated at 1  $\mu\text{g Chl } a$  on a Millipore AP-20  
148 prefilter that was immediately frozen in liquid nitrogen before measurement. The same  
149 procedure was used for illuminated cells ( $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  for 5 min) albeit a Chl *a*  
150 amount of 3  $\mu\text{g}$  was immediately frozen after illumination. The angle between the filter  
151 and the light source/detector was  $45^\circ$ . For complete inhibition of NPQ, cells were  
152 incubated prior to illumination (20 min, dark) with 0.2 mM DTT or 5 mM  $\text{NH}_4\text{Cl}$  [49].

153

154 2.4. Chlorophyll fluorescence yield and the non-photochemical fluorescence quenching  
155 (NPQ).

156 Chlorophyll fluorescence yield was monitored with a modified PAM-101  
157 fluorometer (Walz, Effeltrich, Germany) as described previously [9] (see Figure 1A for a  
158 typical recording). Fluorescence was excited by a very weak (non-actinic) modulated  
159 650 nm light beam. After 20 min of dark-acclimation, continuous actinic light of adjustable  
160 intensity was applied. 600 ms pulses of white light ( $4000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) were  
161 admitted by an electronic shutter (Uniblitz, Vincent, USA, opening time 2 ms) placed in  
162 front of a KL-1500 quartz iodine lamp continuously switched on in order to monitor the  
163 evolution of NPQ. The average fluorescence (acquisition time 33  $\mu\text{s}$ ) measured during the  
164 last 400 ms of the pulse was taken as  $F_m$  or  $F_m'$ . For each experiment, 2 mL of cell  
165 suspension were used. Sodium bicarbonate was added at a concentration of 4 mM from a  
166 freshly prepared 0.2 M stock solution in distilled water to prevent any limitation of the  
167 photosynthetic rate by carbon supply. Standard fluorescence nomenclature was used [50].  $F_o$   
168 and  $F_m$  are defined as the minimum PS II fluorescence yield of dark-acclimated cells and the  
169 maximum PS II fluorescence yield reached in such cells during a saturating pulse of white  
170 light, respectively. The maximum photosynthetic efficiency of PS II is the ratio  $F_v/F_m$  where  
171  $F_v$  is the variable part of the fluorescence emission and is equal to  $F_m - F_o$ . NPQ (also known  
172 as  $SV_m$ ) was quantified by the 'Stern-Volmer' expression  $F_m/F_m' - 1$ , where  $F_m'$  is the  
173 maximum PS II fluorescence yield of light-acclimated cells [51].

174

175 **3. Results**

176 The general photosynthetic properties of the strains of *Phaeodactylum*  
177 *tricornutum* (P.t.2 strain) and *Skeletonema costatum* used here have been described  
178 earlier [27]. In our growth conditions, both species have comparable light harvesting  
179 efficiency and photosynthetic capacity. As previously shown, the extent of NPQ in *P.*  
180 *tricornutum* was about twice the one in *S. costatum*; it holds true under saturating and  
181 over-saturating irradiances [27] and under prolonged exposure to over-saturating  
182 intensities [9].

183

184 *3.1. Differences in the relationship between the XC and NPQ between Phaeodactylum*  
185 *tricornutum and Skeletonema costatum cells*

186 The de-epoxidation of DD into DT was measured for the two species under the same light  
187 conditions as for NPQ (Fig. 1 and Fig. S1). While in *P. tricornutum* (P.t.2 strain) the de-  
188 epoxidation started for a light intensity of  $150 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , it was already active  
189 at  $50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in *S. costatum*. For the two species, the de-epoxidation state  
190 (DES) reached a near maximum around  $750 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . As the size of the total  
191 pool of DD+DT remained stable along the range of irradiances, no *de novo* DT synthesis  
192 (i.e. DT synthesis independent from the xanthophyll cycle, see [8-9]) occurred. In these  
193 conditions, the maximal synthesis of DT was lower in *P. tricornutum* ( $2.5 \pm 0.01 \text{ mol.}$   
194  $100 \text{ mol Chl } a^{-1}$ ) than in *S. costatum* ( $6.8 \pm 0.54 \text{ mol. } 100 \text{ mol Chl } a^{-1}$ ) corresponding to a  
195 maximal DES of 27% and 40%, respectively (Fig. 1A, Table 1). For comparison, *P.*  
196 *tricornutum* ‘high NPQ’ cells (see Table 1 for their characteristics) showed a maximal  
197 DES of 46% (Fig. 1A, open circles). Under these light conditions, there was a tight  
198 correlation between the amount of DT and NPQ as previously reported for several diatom

199 species [9, 38] (Fig. 1B). However, in contrast to *P. tricornutum*, for *S. costatum* the  
200 relationship depended on the illumination pattern. When it was fixed to 5 min and the  
201 irradiance increased from 0 to 2000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Fig. S1), the NPQ vs. DT  
202 relationship showed an exponential feature (Fig. 1B). NPQ barely developed (0.2) until  
203 the DT amount reached a value close to 2 mol DT. 100 mol Chl  $a^{-1}$ . Interestingly, the  
204 inflexion point of NPQ development corresponded to an amount of DT synthesized for a  
205 light intensity of about 400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Fig. S1), which was just below the light  
206 intensity for reaching the maximum photosynthetic capacity (Fig. S2) [27]. When the  
207 irradiance was fixed to a maximum of 2000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and the illumination  
208 time increased from 0 to 30 min, the NPQ vs. DT relationship was linear with a slope  
209 twice lower than the one observed in *P. tricornutum* (as previously reported in [9]).

210 By illuminating the cells with harsher conditions (2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for over 30  
211 min and up to 1 h), the NPQ vs. DT relationship remained true only for *P. tricornutum*  
212 (continuous line). Indeed, for illumination times longer than 30-45 min, the slope of the  
213 relationship NPQ/DT decreased to 0.09 in *S. costatum* (Fig. 1B, closed triangles, dashed  
214 line). During such high and prolonged light exposure *de novo* synthesis of DT occurs  
215 with a similar rate in *P. tricornutum* and *S. costatum* [9]. It can be therefore concluded  
216 that in *S. costatum* part of the newly synthesized molecules of DT were less or not  
217 involved in the development of subsequent NPQ. This feature was confirmed by the  
218 recording of the epoxidation kinetics of DT back to DD after short (5-15 min) and long (1  
219 h) exposures to 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Fig. 2). While after 5 and 15 min exposure the  
220 epoxidation kinetics were similar in both species and well in concordance with the  
221 relaxation of NPQ, it was drastically different after 1 h exposure (Fig. S3). In *S. costatum*,

222 the epoxidation kinetics and NPQ relaxation showed disconnected patterns (Fig. 2B, Fig.  
223 S3). During the first 30 min of recovery, there was a fast DT epoxidation similar as in *P.*  
224 *tricornutum* (Fig. 2A). The amount of DT involved ( $2.4 \pm 1.2$  mol. 100 mol Chl  $a^{-1}$ ) was  
225 similar to the amount of DT synthesized *de novo* ( $2.4 \pm 1.6$  mol. 100 mol Chl  $a^{-1}$ ). In  
226 contrast to *P. tricornutum*, it was accompanied by only a small change in NPQ (13%).  
227 After 1 h of recovery, nearly all DT molecules were converted back to DD in *P.*  
228 *tricornutum* while a large DT pool ( $3.2 \pm 0.9$  mol. 100 mol Chl  $a^{-1}$ ) persisted in *S.*  
229 *costatum*. NPQ remained high too (2/3s of the value after the light extinction). Both  
230 sustained DT and NPQ could not be recovered by the addition of the uncoupler  $\text{NH}_4\text{Cl}$   
231 after 15 or 25 min of recovery (data not shown) [49], hence the  $\Delta\text{pH}$  was most probably  
232 already broken. Additionally, when the light (same intensity) was again switched on after  
233 30 min of recovery, the development of NPQ was faster but not higher (x 2, data not  
234 shown) as previously observed for *P. tricornutum* [52]. These features fit well with the  
235 proposed regulatory features of NPQ in diatoms (see [14, 27]). Lastly, the dark recovery  
236 of the  $F_v/F_m$  was higher in *P. tricornutum* whatever the illumination time (15 or 60 min)  
237 (Fig. S4).

238

### 239 3.2. Light-dependent absorption changes related to the XC and NPQ

240 It has been shown earlier that the absorption increase at 522 nm ( $\Delta 522$  nm)  
241 accounts for the so-called ‘activation’ of the DT molecules, an essential step in the  
242 switching of the PS II LHC antenna to the quenching state (see [48, 52]). Fig. 3A shows  
243 the  $\Delta 522$  nm in *S. costatum* and in *P. tricornutum* cells with different extents of NPQ (see  
244 Table 1 for their characteristics) which were obtained by growing the cells under different

245 conditions: 1) a classical low light regime (16h L:8h D) with a cell harvest during the  
246 exponential phase of growth ('CL exp. '), 2) the same light regime with a cell harvest  
247 during the stationary phase of growth ('CL stat. '), 3) an intermittent light of the same  
248 intensity (5 min light: 55 min dark) ('IL exp. '). In order to complete the range of NPQ,  
249 we included a second strain of *P. tricornutum* (P.t.4), which is known for its lower NPQ  
250 than the strain P.t.2 [20] and *C. meneghiniana* whose NPQ mechanism is more complex  
251 than the one of *P. tricornutum* [36, 42]. In these conditions, the four strains reacted rather  
252 similarly. The maximum photosynthetic efficiency of PS II ( $F_v/F_m$ , Table 1) of 'CL exp. '  
253 cells was very similar (Table 1).  $F_v/F_m$  slightly decreased in 'CL stat. ' cells while it was  
254 maintained in 'IL exp. ' cells. The growth rate of 'CL exp. ' cells was similar (*P.*  
255 *tricornutum* P.t.2,  $2.08 \pm 0.08 \text{ d}^{-1}$ ; P.t.4,  $2.54 \pm 0.2 \text{ d}^{-1}$ ; *S. costatum*,  $2.01 \pm 0.09 \text{ d}^{-1}$ ; *C.*  
256 *meneghiniana*,  $2.11 \pm 0.10 \text{ d}^{-1}$ ) while it dramatically decreased to about 0 in 'CL stat. '  
257 conditions (*P. tricornutum* P.t.2,  $0.05 \pm 0.02 \text{ d}^{-1}$ ; P.t.4,  $0.07 \pm 0.03 \text{ d}^{-1}$ ; *S. costatum*,  $0.02 \pm$   
258  $0.01 \text{ d}^{-1}$ ; *C. meneghiniana*,  $0.03 \pm 0.02 \text{ d}^{-1}$ ) (part of the data compiled from [9, 27, 34]).  
259 Although the growth rate was not measured for all species, it decreases 10-fold in *P.*  
260 *tricornutum* P.t.2 'IL exp. ' cells due to the 8-fold lower daily amount of light supplied by  
261 the IL light regime [38]. Under a fluctuating light regime not as harsh as the IL regime  
262 used here, the growth rate of *P. tricornutum* P.t.4, *S. costatum* and *C. meneghiniana* was  
263 decreased by a factor of 2 to 4.5 (data compiled from [20, 27, 53]).

264 In contrast, the effect of the light regimes on the pool size of DD+DT, the de-  
265 epoxidation of DD to DT (DES and DT amount) and on NPQ in the four species/strains  
266 was different. In general, 'CL stat. ' cells showed a higher NPQ than 'CL exp. ' cells due  
267 to the larger DD pool and the higher subsequent DT synthesis [54]. While *P. tricornutum*

268 P.t.2 'IL exp.' cells exhibited a higher NPQ [38, 48], the IL treatment had an adverse  
269 effect on *S. costatum* xanthophyll synthesis and NPQ (Table 1). Consequently, the  $\Delta 522$   
270 nm band was lower in *S. costatum* compared to *P. tricornutum* (P.t.2 strain) whatever the  
271 types of cells considered (see Table 1). NPQ in P.t.4 reacted the same way as in P.t.2 to  
272 growth conditions while for *C. meneghiniana* it was closer to *S. costatum* (Table 1). The  
273 extent of NPQ exponentially correlated with the amplitude of the  $\Delta 522$  nm band (Fig.  
274 3B). In contrast, and as expected, NPQ linearly correlated with the total amount of  
275 synthesized DT with a decreasing slope in the order *P. tricornutum* > *S. costatum* > *C.*  
276 *meneghiniana* (Fig. S5). The NPQ/ $\Delta 522$  nm relationship also held true for cells of *P.*  
277 *tricornutum* (P.t.2, 'low NPQ') in which NPQ was more than half abolished (-60%) by  
278 the use of moderate concentrations of an uncoupler ( $\text{NH}_4\text{Cl}$ ) of the  $\Delta\text{pH}$  and of an  
279 inhibitor of the DD de-epoxidation (DTT, dithiotreitol) [49]. Although *S. costatum* and *C.*  
280 *meneghiniana* showed a larger xanthophyll pool size and a higher DT synthesis than *P.*  
281 *tricornutum* (see also Fig. 1A), NPQ was lower (see also Fig. 1B) in direct correlation  
282 with the lower capacity for DT 'activation' in the NPQ process (i.e. lower  $\Delta 522$  nm)  
283 (Table 1). This situation was especially extreme with 'high NPQ' cells: the huge amount  
284 of synthesized DT (about 16 and 37 mol. 100 DT Chl  $a^{-1}$  for *S. costatum* and *C.*  
285 *meneghiniana*, respectively) did not lead to the expected large increase of NPQ which  
286 instead followed the slight increase of the 522 nm band.

287

### 288 3.3. NPQ capacity and chlorophyll fluorescence emission properties

289 In order to gain more insight into the fluorescence emission properties of the  
290 species examined, we performed 77K fluorescence emission spectra on dark-acclimated

291 cells (Fig. 4). The majority of the Chl *a* fluorescence arose from the 687 nm peak while a  
292 second emission band peaking at 711 nm in *P. tricornutum* was showing a slight shoulder  
293 in *S. costatum* and *C. meneghiniana* (not shown). In *P. tricornutum* (P.t.2 and P.t.4  
294 strains) the amplitude of the 711 nm band increased with the NPQ capacity of the cells,  
295 following a linear relationship whose slope was dependent on the strain (Fig. 5, Table 1).  
296 Probably the most illustrative situation concerns the P.t.2 ‘medium and high NPQ’ cells:  
297 the synthesis of 1.7 fold more DT molecules led to a much higher NPQ (2.7 fold) than  
298 expected regarding the moderate increase of DT activation ( $\Delta 522$  nm); instead the 2.7  
299 fold NPQ increase correlated well with the one of the fluorescence emission at 711 nm.  
300 Although slight variations in the 711/687 nm fluorescence ratio (F711/F687) were  
301 observed in *S. costatum* (Fig. 4B, Table 1), no clear relationship could be established  
302 (Fig. 5). No changes were observed in *C. meneghiniana*.

303         It has been described earlier in *P. tricornutum* how the 711 nm fluorescence band  
304 can arise from the PS II at both room temperature and 77K [55], and how it could be  
305 related to the capacity for excess energy dissipation [43, 55]. When measured after  
306 illumination (5 min,  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in *P. tricornutum* (P.t.2 strain), the 77K  
307 fluorescence signal was decreased by a factor of about 3 and F711/F687 increased by  
308 31% (Table 2) (Fig. S6). When NPQ was inhibited by the addition of  $\text{NH}_4\text{Cl}$  or DTT, the  
309 change of F711/F687 was abolished (Table 2, Fig. S6). By illuminating the cells with far-  
310 red light ( $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), we did not observe any change of F711/F687 (data  
311 not shown).

312

313 **4. Discussion**

314 4.1. The inter-species differences of the involvement of DT in NPQ

315 The DT molecules are distributed among several pools which show a spatial and  
316 functional heterogeneity [37]. It is believed that DT can be : 1) free in the lipid matrix  
317 [37], 2) in the lipid matrix in the close vicinity to the PS II LHC antenna [37], 3) bound to  
318 the LHC antenna system for the harvesting of light [37, 56]; 4) bound to specific FCP  
319 trimers where they participate to NPQ [44]. A change of balance between these pools can  
320 occur during light acclimation [39, 42]. We show that it is also true between species.  
321 Obviously the addressing of DT to the four pools is different in *S. costatum* (and *C.*  
322 *meneghiniana*) compared to *P. tricornutum*. First, the fact that the slope of the NPQ vs.  
323 DT relationship is twice lower in *S. costatum* indicates that twice less DT molecules are  
324 involved in NPQ, i.e. there are 50% less DT in pool 4. Second, while DT *de novo*  
325 synthesis significantly occurs in both species, it is mainly inefficient for NPQ in *S.*  
326 *costatum* (and *C. meneghiniana*, [37]) in contrast to *P. tricornutum* [9]. Hence, in the  
327 latter the *de novo* synthesized DT appears to be mainly part of the pool 4. Interestingly,  
328 *de novo* synthesized DT was recently proposed to be bound by Lhcx proteins and to  
329 participate to the sustained part of NPQ (i.e. qI) [18, 22], thus enhancing the dissipative  
330 capacity of the PS II LHC antenna under prolonged and harsh illumination conditions. In  
331 contrast, it is probable that DT is addressed to the pools 1 and/or 2 in *S. costatum* as also  
332 shown for *C. meneghiniana* [37]. This is supported by their fast dark epoxidation  
333 illustrating their easy access to the DT epoxidase in the lipid matrix of the thylakoid  
334 membrane [37, 57]. Here, they likely participate to the prevention of lipid peroxidation  
335 [8-9, 37]. Hence, in *S. costatum* and *P. tricornutum*, DT would have different main  
336 physiological roles with a higher involvement in NPQ in *P. tricornutum*. Consequently,

337 from a species to another, NPQ often does not correlate with the amount of DT and with  
338 the DES [11, 14]. This discrepancy between the two species was especially true when the  
339 light exposure was short and of moderate intensity. In these conditions, *S. costatum*  
340 synthesizes significant amounts of DT with only low or even no development of NPQ. A  
341 similar relationship was documented before in cells where the  $\Delta\text{pH}$  build-up was  
342 chemically manipulated, the lowest  $\Delta\text{pH}$  generating a significant amount of DT with  
343 barely any NPQ [49]. Such discrepancy was explained before thanks to *P. tricornutum*  
344 mutants showing a DT synthesis divided by half by genetic means [35]. It implies the so-  
345 called ‘activation’ of DT which necessitates the protonation of some FCP binding sites  
346 during the  $\Delta\text{pH}$  build-up [48, 52, 57]. The entering of DT into an ‘activated’ state triggers  
347 the switch of the PS II LHC antenna to the quenching state. We propose that the  
348 discrepancy between DT synthesis and NPQ in *S. costatum* is due to a weak/slow  
349 ‘activation’ of DT in conditions of low  $\Delta\text{pH}$  due to a limited protonation of the PS II  
350 LHC antenna. It is supported by the fact that the NPQ vs. DT vs. light intensity  
351 relationship followed two clear phases in *S. costatum* with a breakpoint corresponding to  
352 the intensity for saturating photosynthesis; this is in total contrast with *P. tricornutum*.

353

#### 354 4.2. The efficiency of DT in the NPQ process: the $\Delta 522$ nm absorption change

355 The above mentioned ‘activation’ of DT (section 4.1.) can be followed by an  
356 absorption change at 522 nm ( $\Delta 522$  nm) similar to the qE-related  $\Delta 525/535$  nm in higher  
357 plants [17, 48]. It is supposed to illustrate the effective involvement of DT as an allosteric  
358 regulator of a conformational change in the PS II LHC antenna, a crucial step in the NPQ  
359 process [11, 14]. The conformational change is believed to be an aggregation of part of

360 the PS II LHC antenna generated by the protonation of FCP binding sites [14]. The  
361 inhibition of  $\Delta 522$  nm by  $\text{NH}_4\text{Cl}$  and DTT confirms this view. The effect of DTT most  
362 likely excludes a relationship to an electrochromic shift signal [58].  $\Delta 522$  nm thus  
363 provides a very useful tool to measure the efficiency of DT in the NPQ process. Indeed,  
364 the difference between the relationships NPQ vs. DT (Fig. S5) and NPQ vs.  $\Delta 522$  nm  
365 (Fig. 3B) demonstrates that the  $\Delta 522$  nm fingerprint is different from the total DT amount  
366 in the way it correlates with NPQ for all the species, i.e. the total amount of DT was far  
367 from accounting for the DT which was effectively involved in NPQ ('activated' DT)  
368 especially in *S. costatum* and *C. meneghiniana*. This discrepancy explains the above  
369 mentioned lack of correlation between the DT amount and the DES, and NPQ (section  
370 4.1). We could additionally broaden the validity of the  $\Delta 522$  nm fingerprint to the centric  
371 diatom representatives *S. costatum* and *C. meneghiniana*. The correlation of  $\Delta 522$  nm  
372 with NPQ validates the crucial importance of the amount of 'activated' DT to determine  
373 the extent of NPQ in that order: *P. tricornutum* > *C. meneghiniana*  $\cong$  *S. costatum*. We  
374 could partially manipulate this general trend by modifying the cellular amount of DD+DT  
375 thanks to different growth conditions.

376         Apart from the amount of DT and its binding to the PS II LHC antenna (section  
377 4.1.), two reasons could explain the  $\Delta 522$  nm inter-species differences: 1) a fewer number  
378 of proton binding sites in the FCP proteins (Lhcf and Lhcx) involved in NPQ, 2) a lower  
379 protonation of these sites, their number being stable, as illustrated by the DT-independent  
380 modification of NPQ by the extent of the  $\Delta\text{pH}$ /lumen acidification [42, 52]. Nevertheless  
381 and although we did not measure the  $\Delta\text{pH}$ , it is reasonable to propose that at least in *P.*  
382 *tricornutum* and *S. costatum* the extent of FCP protonation was similar since they showed

383 a similar electron transport rate (see [27]). The first reason, although more plausible, is at  
384 this stage impossible to verify as we do not know which of the LHC proteins might bind  
385 protons in diatoms although both Lhcf and Lhcx proteins have been proposed [44]. The  
386 exponential pattern of the NPQ vs.  $\Delta 522$  nm relationship illustrates an essential feature of  
387 the diatom NPQ regulation: above a certain value (NPQ of about 1.5), NPQ increases  
388 much faster than the  $\Delta 522$  nm fingerprint, i.e. the increase of DT 'activation' cannot fully  
389 account for the huge increase of NPQ, especially in 'high NPQ' cells. Hence, there is  
390 another partner amplifying the dissipative process in the LHC antenna system of PS II.

391

#### 392 *4.3. Amplification of the NPQ process: the chlorophyll 711 nm fluorescence emission* 393 *band (Chl $a_{711}$ )*

394 The presence of a Chl  $a_{711}$  emission band at 77K (or in the range 710-717 nm  
395 depending on the studies and the species) has been reported before in diatoms. It is  
396 species-dependent [55, 59-61]. Similarly, we showed that while Chl  $a_{711}$  can be high in *P.*  
397 *tricornutum*, its amplitude is strongly limited in *S. costatum* and *C. meneghiniana*  
398 whatever the growth conditions. The present work additionally depicts the following  
399 characteristics for Chl  $a_{711}$ : 1) it is positively correlated to the inter-species (*P.*  
400 *tricornutum* > *S. costatum*  $\cong$  *C. meneghiniana*) and intra-species (P.t.2 strain > P.t.4  
401 strain) differences of the NPQ capacity; 2) in *P. tricornutum* and to a much lower extent  
402 in *S. costatum* (but not in *C. meneghiniana*), its amplitude is increased together with the  
403 pool size of DD/DT by changing the growth conditions (see also [55, 59-60]); 3) it is  
404 quenched under high light exposure but not as much as PS II Chl  $a_{687}$ , and this change is

405 abolished by  $\text{NH}_4\text{Cl}$  and DTT; 4) it is insensitive to red light ruling out any involvement  
406 of a state-transitions (i.e. qT) in the quenching process.

407         Some studies, using 77K spectroscopy, classically linked Chl  $a_{711}$  to the core of  
408 PS I and its specific FCP antenna (see [62-63]) while others, using both room temperature  
409 and 77K spectroscopy, demonstrated, it can also originate from PS II by using DCMU or  
410 a preferential excitation of fucoxanthin pigments [43, 55, 64]. In that framework, and  
411 although we did not measure it for all strains/species under all growth conditions, it is  
412 noteworthy that the stoichiometry PS II:PS I was similar in *P. tricornutum* P.t.2 'low'  
413 and 'high' NPQ cells [38]. The PS II:PS I difference was higher (+23%) between *P.*  
414 *tricornutum* P.t.2 and *S. costatum* [27] with more PS I vs. PS II in *P. tricornutum*. In  
415 diatoms, a large change of PS II:PS I stoichiometry is usually generated by an increase in  
416 the light intensity and/or by nutrient deficiency [63, 65]. Hence, the Chl  $a_{711}$  differences  
417 observed here between at least these three types of cells cannot be fully explained by a  
418 difference in PS II:PS I stoichiometry. Although it amounts to few percents of total  
419 chlorophyll, Chl  $a_{711}$  was shown to drain an appreciable fraction of excitation energy  
420 from the PS II [59-61] and was proposed to be responsible for dissipating excess energy  
421 [55]. The Chl  $a_{711}$  emission band was earlier suggested to arise from aggregated protein-  
422 pigment complexes close but spatially separated from the PS II [59-60, 66]. Owens [66]  
423 further demonstrated that Chl  $a_{711}$  is sensitive to the energization of the thylakoid  
424 membrane which explains its  $\text{NH}_4\text{Cl}$  sensitivity. Recently, a time-resolved fluorescence  
425 analysis of the diatom LHC system brought evidence for a 710 nm fluorescence emission  
426 band which resembles our Chl  $a_{711}$  [43]. It is present in *P. tricornutum* but much less in  
427 *C. meneghiniana* and it exists in both unquenched (darkness) and quenched (high light)

428 states. The 710 nm band was assigned to a FCP complex functionally disconnected from  
429 both PS I and PS II upon high light exposure [12, 43]. It was additionally proposed that  
430 this part of the LHC antenna is in an aggregated state similar to the oligomerized LHCII  
431 involved in NPQ in higher plants [67]. Interestingly, the Chl  $a_{710}$  associated to PS I was  
432 shown to arise from FCP complexes weakly bound to the PS I core where they act as a  
433 ‘shallow sink’ of excitation energy [68]. These observations indicate that Chl  $a_{711}$  (or Chl  
434  $a_{710}$ ) probably plays a role in NPQ. Because the quenching was more important on Chl  
435  $a_{687}$  than on Chl  $a_{711}$ , we propose that Chl  $a_{711}$  serves at amplifying the part of the  
436 quenching which directly relies on DT (i.e. related to Chl  $a_{687}$ ). The amplitude of Chl  $a_{711}$   
437 (or F711/F687 nm) measured in dark-acclimated cells is a good fingerprint of the level of  
438 NPQ amplification at light. It explains in part the inter-species differences in NPQ; i.e. in  
439 contrast to *P. tricornutum*, Chl  $a_{711}$  had only a minor role in the extent of NPQ in *S.*  
440 *costatum* and *C. meneghiniana*; it was also reported before that Chl  $a_{710-711}$  is lower in *C.*  
441 *meneghiniana* [43].

442

#### 443 4.4. The consequence of NPQ inter-species/strains differences on the ecophysiology of 444 diatoms

445 All and all, the differences in the NPQ extent observed between species and  
446 strains of the same species (*P. tricornutum*) can be explained by the extent of  $\Delta 522$  nm  
447 and of Chl  $a_{711}$  together with the distribution of DT within the photosynthetic machinery,  
448 but independently of the total amount of DD+DT and of the DES. We propose that a high  
449 NPQ ability (as observed in the *P. tricornutum* P.t.2 strain) would rely on 1) a high  
450 amount of DT which effectively participate to NPQ ( $\Delta 522$  nm fingerprint); 2) the

451 capacity to form many/large functionally disconnected oligomeric FCP complexes (Chl  
452  $a_{711}$  fingerprint) in order to amplify the DT-dependent quenching process. These features  
453 are in dramatic contrast with the ones measured for *S. costatum*. The most relevant  
454 specific features of NPQ regulation in *S. costatum* are ([9, 27] and this study) the  
455 coupling between i) the early onset of NPQ, i.e. already for a light intensity  
456 corresponding to the one the cells are acclimated to, ii) the slow development of NPQ vs.  
457 DT vs. light for non-saturating irradiances, iii) the apparent slow recovery of NPQ related  
458 to a limited epoxidation of DT (also reported for *C. meneghiniana* and *P. tricornutum*  
459 P.t.4, [57]), iv) the low investment of *de novo* synthesized DT in the enhancement of  
460 NPQ. These features, together with the absence of a PS II CET [27] (which can explain in  
461 part the points i) and ii)) cannot promote a similarly strong and fast irradiance-tuning of  
462 NPQ as in *P. tricornutum*. Ultimately, as shown here and before [27], these differences  
463 lead to a higher susceptibility of *S. costatum* to photoinhibition during exposure to excess  
464 light and/or to a fluctuating light climate negatively inferring on its photosynthetic  
465 productivity and growth. Such record brings a molecular basis for explaining the  
466 adaptation of the regulatory photosynthetic machinery to the aquatic light environment as  
467 proposed before [11, 21, 27, 30]. Indeed, the general characteristics for high NPQ in the  
468 *P. tricornutum* P.t.2 strain, which originates from an estuarine habitat, fit well with an  
469 adaptation to fast and strong light fluctuations while the ones for low NPQ fit better with  
470 the slow and moderate light changes that characterize the habitat the strain of *S. costatum*  
471 used here originates from (a Mediterranean semi-enclosed bay). The recent report that *S.*  
472 *costatum* and *C. meneghiniana* behave similar under fluctuating light conditions [53]  
473 further supports the same impact of their seemingly close regulatory NPQ machinery on

474 their photophysiology. Also supporting and extending conclusions to strains/ecotypes of  
475 the same species, is the fact that the low NPQ *P. tricornutum* P.t.4 strain shows an NPQ  
476 vs. DT relationship which slope is similar to the one of *S. costatum* [35]. Different from  
477 the P.t.2 strain, P.t.4 originates from a high latitude habitat characterized by a low light  
478 environment [20]. As observed here and before [20] in *P. tricornutum* strains/ecotypes,  
479 the amounts of DD/DT, of Chl  $a_{711}$  FCP oligomers and of Lhcx1 are concomitantly  
480 influenced by the light environment the cells are adapted to. The slope of the NPQ vs. DT  
481 relationship [9, 30] therefore could be a good indicator of the adaptation of a  
482 species/strain to an aquatic niche as a function of the light climate. The present work  
483 further confirms and extends the potential role for the light-dependent fast regulation of  
484 photosynthesis, and especially NPQ, in the ecophysiology of diatom phytoplanktonic and  
485 microphytobenthic communities [11, 19, 21, 32]. It could explain in part the ecological  
486 diversity of diatoms [1] which has been participated to their ecological success in  
487 contemporary oceans [1-3].

488

#### 489 **Appendix A. Supplementary data**

490       Supplementary data can be found under the numbering Figure S1 to Figure S6.

491

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500

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692

693

694 **Figure legends**

695

696 Figure 1. A- The de-epoxidation state (calculated as  $DT/(DD+DT) \times 100$ ) in *S. costatum*,  
697 *P. tricornutum* (P.t.2 strain) and *P. tricornutum* ‘high NPQ’ cells. Data ( $\pm$ SD) are the  
698 average of three to five independent measurements (but for *P. tricornutum* ‘high NPQ’  
699 cells, only one experiment). DD, diadinoxanthin; DT, diatoxanthin. B- Relationship  
700 between DT synthesis and NPQ development in *S. costatum* as a function of the  
701 illumination time (from 0.5 to 60 min) at  $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (triangles,  $R^2 = 0.92$ )  
702 and as a function of the light intensity for an illumination time of 5 min (inverted  
703 triangles,  $R^2 = 0.81$ ); hence both curves meet for the cells sampled after 5 min  
704 illumination at  $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (highlighted area). The numbers refer to the X  
705 slope of the relationship  $\text{NPQ} = X \times [\text{DT}]$ ; the times refer to the illumination times. The  
706 same relationship with the same light conditions obtained in *P. tricornutum* (P.t.2 strain)  
707 cells is shown by a continuous line (circles, see also [9, 38]).

708

709 Figure 2. Dark relaxation of NPQ (closed symbols) and DT epoxidation back into DD  
710 (open symbols) after a 5 min (squares), 15 min (triangles) and 60 min (circles)  
711 illumination at  $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Actinic Light, AL) in *P. tricornutum* (P.t.2  
712 strain) (A) and *S. costatum* (B).

713

714 Figure 3. A- Light-treated minus dark-acclimated difference absorption spectra in *P.*  
715 *tricornutum* (P.t.2 strain) and *S. costatum*. For each of the species, the three NPQ types of  
716 cells (‘low’, ‘medium’ and ‘high’, see Table 1) were used. The absorption band at 522

717 nm ( $\Delta 522$  nm) is highlighted. B- Relationship between NPQ and  $\Delta 522$  nm. NPQ was  
718 measured after 5 min illumination at  $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . For each of the species,  
719 *P. tricornutum* (P.t.2 strain, circles), *S. costatum* (triangles) and *Cyclotella meneghiniana*  
720 (squares), the three NPQ types of cells ('low', 'medium' and 'high', see Table 1) were  
721 used. The exponential relationship was  $\text{NPQ} = 0.35 \times e^{(1.24 \Delta 522 \text{ nm})}$  ( $R^2 = 0.93$ ). For *P.*  
722 *tricornutum*, the same measurements were performed on the 'low NPQ' cells in presence  
723 of  $\text{NH}_4\text{Cl}$  (2 mM) and dithiothreitol (DTT,  $25 \mu\text{M}$ ) during the illumination (open circles).  
724 See the text for further details. Data ( $\pm\text{SD}$ ) are the average of three independent  
725 measurements.

726

727 Figure 4. 77K Chl *a* fluorescence emission spectra excited at 440 nm of *P. tricornutum*  
728 (P.t.2 strain) (A) and *S. costatum* (B) dark-acclimated cells showing different NPQ  
729 capacity ('low', 'medium' and 'high', see Table 1). The spectra were normalized at their  
730 687 nm maximum.

731

732 Figure 5. Relationship between NPQ and the ratio of Chl *a* fluorescence emission at 711  
733 nm and 687 nm (as measured from Fig. 4). NPQ values were measured after 5 min  
734 illumination at  $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . For each of the species, *P. tricornutum* (P.t.2  
735 strain, closed circles), *P. tricornutum* (P.t.4 strain, open circles), *S. costatum* (triangles)  
736 and *C. meneghiniana* (squares), the three NPQ types of cells ('low', 'medium' and 'high',  
737 see Table 1) were used. The linear relationship was  $\text{NPQ} = 8.10 \times F_{711}/687 \text{ nm}$  ( $R^2 =$   
738  $0.99$ ) and  $\text{NPQ} = 4.62 \times F_{711}/687 \text{ nm}$  ( $R^2 = 0.98$ ) for the P.t.2 and P.t.4 strains,  
739 respectively. Data ( $\pm\text{SD}$ ) are the average of three independent measurements.

740

741 **Table 1**

742 **Photophysiological properties of the species/strains and of the NPQ type of cells**

743 **used in this study**

Species and type of cells	Growth status	F <sub>v</sub> /F <sub>m</sub>	DD+DT	F711/F687	DES	DT	Δ 522 nm x 10 <sup>2</sup>	NPQ
<i>P. tricornutum</i> (P.t.2) 'low NPQ'	'CL', exp.	0.65 ± 0.02	8.8 ± 0.4	0.35 ± 0.06	27	2.38 ± 0.1	1.3 ± 0.10	2.38 ± 0.24
<i>P. tricornutum</i> (P.t.2) 'medium NPQ'	'CL', stat.	0.61 ± 0.03	13.6 ± 0.9	0.50 ± 0.07	36	4.9 ± 0.3	2.1 ± 0.10	3.50 ± 0.25
P.t.2, 'high NPQ'	'IL', exp.	0.68 ± 0.02	17.4 ± 2.3	1.22 ± 0.26	46	8.0 ± 1.1	2.5 ± 0.20	9.40 ± 0.61
<i>P. tricornutum</i> (P.t.4) 'low NPQ'	'CL', exp.	0.67 ± 0.02	12.7 ± 0.2	0.48 ± 0.01	38	4.8 ± 0.1	n.d.	1.58 ± 0.11
P.t.4, 'medium NPQ'	'CL', stat.	0.60 ± 0.03	23.8 ± 0.2	0.62 ± 0.09	43	10.2 ± 0.1	n.d.	2.63 ± 0.19
P.t.4, 'high NPQ'	'IL', exp.	0.64 ± 0.02	30.9 ± 0.7	1.00 ± 0.19	61	18.9 ± 0.4	n.d.	4.08 ± 0.21
<i>S. costatum</i> (S.c.) 'low NPQ'	'IL', exp.	0.64 ± 0.01	8.3 ± 2.1	0.19 ± 0.02	33	2.7 ± 0.7	0.33 ± 0.15	0.51 ± 0.07
S.c. 'medium NPQ'	'CL', exp.	0.59 ± 0.07	18.1 ± 0.1	0.29 ± 0.01	40	7.2 ± 0.05	1.00 ± 0.15	1.35 ± 0.29
S.c.	'CL',	0.69 ±	26.9 ±	0.32 ±	59	15.9 ±	1.73 ±	2.5 ±

'high NPQ'	stat.	0.02	0.7	0.01		0.4	0.17	0.30
<i>C. meneghiniana</i> (C.m.) 'low NPQ'	'CL', exp.	0.63 ± 0.03	24.1 ± 0.3	0.29 ± 0.01	52	12.5 ± 0.2	0.86 ± 0.28	0.77 ± 0.09
C.m. 'medium NPQ'	'IL', exp.	0.58 ± 0.03	23.6 ± 0.2	0.29 ± 0.02	23	5.4 ± 0.05	0.70 ± 0.13	0.90 ± 0.09
C.m. 'high NPQ'	'CL', stat.	0.69 ± 0.02	57.9 ± 1.2	0.25 ± 0.01	64	37.1 ± 0.8	1.44 ± 0.26	1.94 ± 0.22

744

745 'CL', 'continuous light': 16 h:8 h Light:Dark cycle; 'IL', 'intermittent light': 5 min/55  
746 min L:D cycle (see the Material and methods section for details); exp. growth,  
747 exponential phase of growth; stat. growth, stationary phase of growth; the fluorescence  
748 ratios  $F_v/F_m$  (the maximum photosynthetic efficiency of PS II) and  $F_{711}/F_{687}$  were  
749 measured on dark-acclimated cells (see also Fig. 4);  $\Delta 522 \text{ nm} \times 10^2$  (the absorption  
750 change at 522 nm), NPQ and DES (de-epoxidation state of diadinoxanthin (DD) into  
751 diatoxanthin (DT) in %:  $(DT/(DD+DT)) \times 100$ ), DD and DT in mol.  $100 \text{ mol Chl } a^{-1}$ ) were  
752 measured after 5 min at  $2000 \mu\text{mol s}^{-1} \text{ m}^{-2}$  (see also Fig. 1 and Fig. 3).

753

754 **Table 2**  
 755 **Effect of light and inhibitor treatments on the pattern of 77K chlorophyll**  
 756 **fluorescence emission as measured by the ratio F711/F687 nm in *Phaeodactylum***  
 757 ***tricornutum* (P.t.2 strain) ‘medium NPQ’ cells (see Table 1)**  
 758

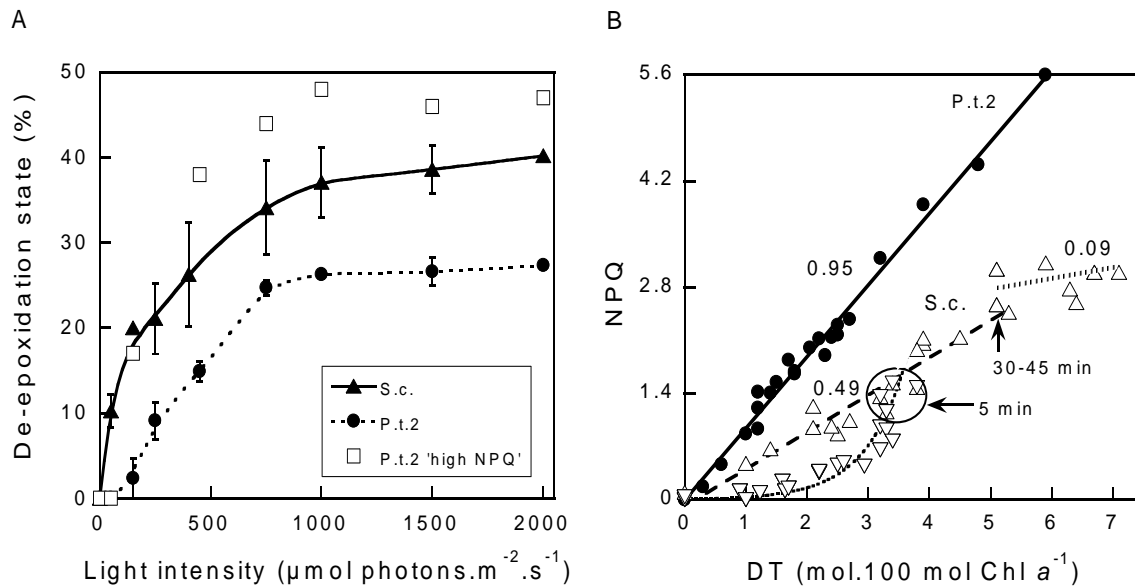
	Dark-acclimated cells	Illuminated cells
No inhibitors	0.78 ± 0.05 *	1.02 ± 0.09
+DTT	0.81 ± 0.06 *	0.77 ± 0.07
+NH <sub>4</sub> Cl	0.70 ± 0.05 *	0.69 ± 0.02

759  
 760 \* There was no significant difference between the dark samples whatever the conditions  
 761 (student test, p>0.05). Treatments: dark-acclimation was for 20 min, light exposure was  
 762 2000 μmol photons m<sup>-2</sup> s<sup>-1</sup> during 5 min, DTT (0.2 mM) and NH<sub>4</sub>Cl (5 mM) were applied  
 763 15 min in the dark before subsequent measurement either in the dark or at light. Values  
 764 are average of three measurements ± SD. Full spectra are shown as Supplemental  
 765 Material (Fig. S6).

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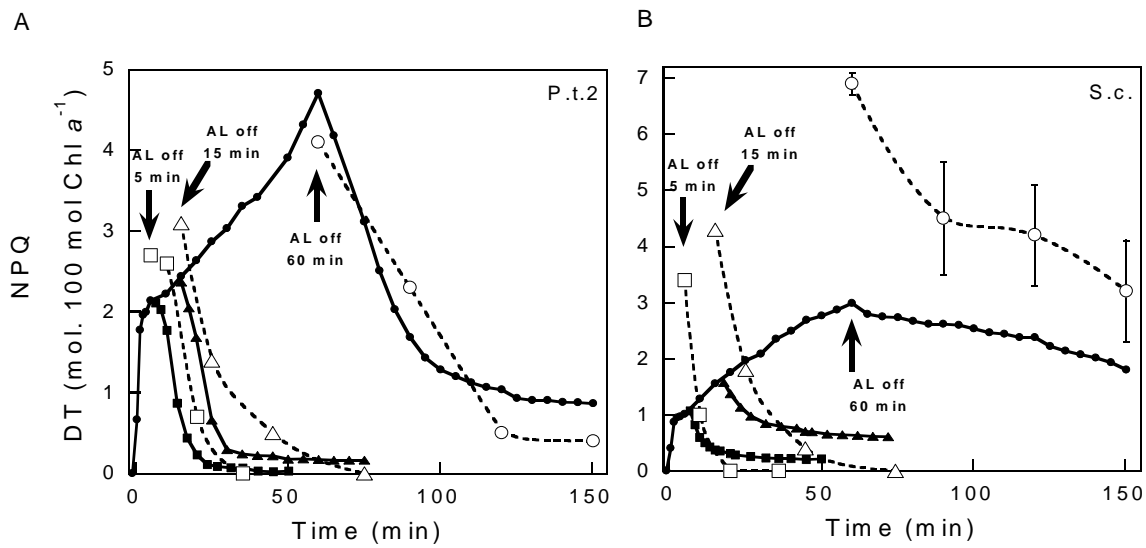
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772 **Figure 2- Lavaud and Lepetit**

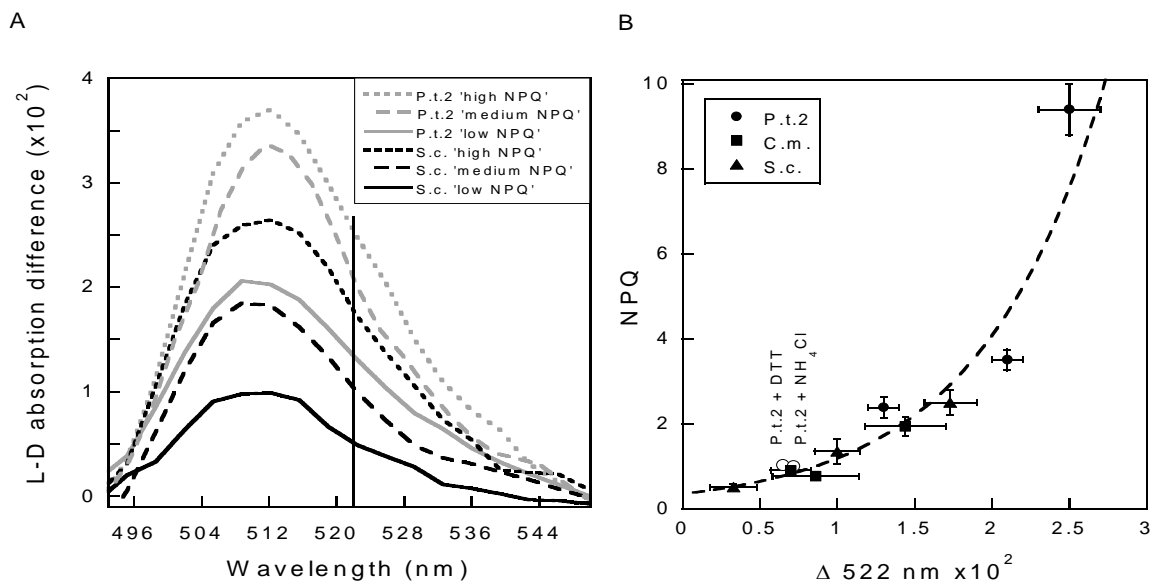


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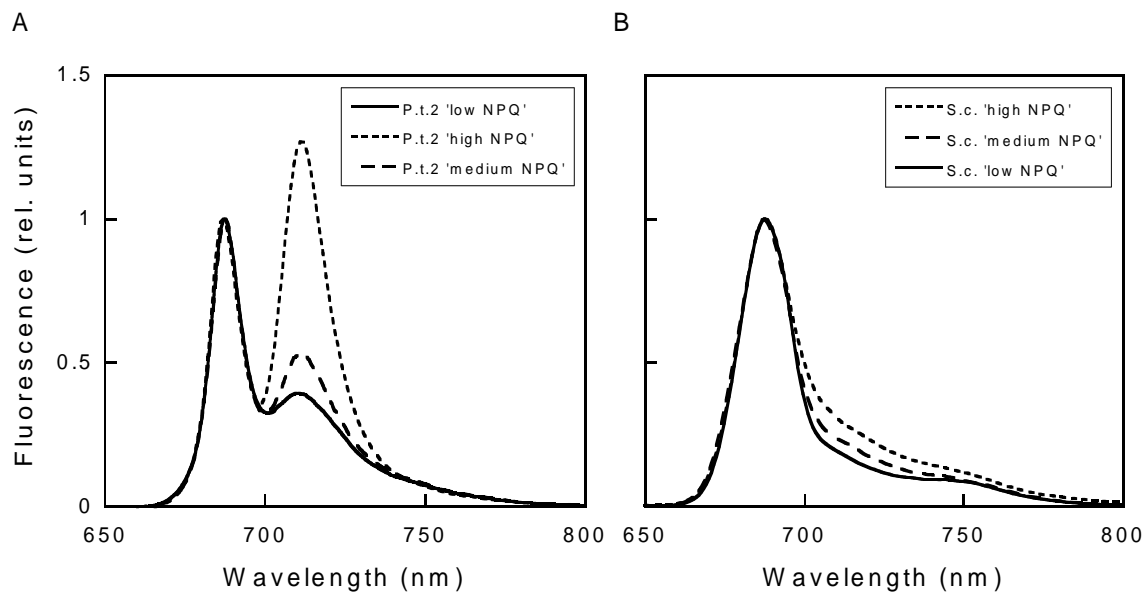


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781 **Figure 4- Lavaud and Lepetit**



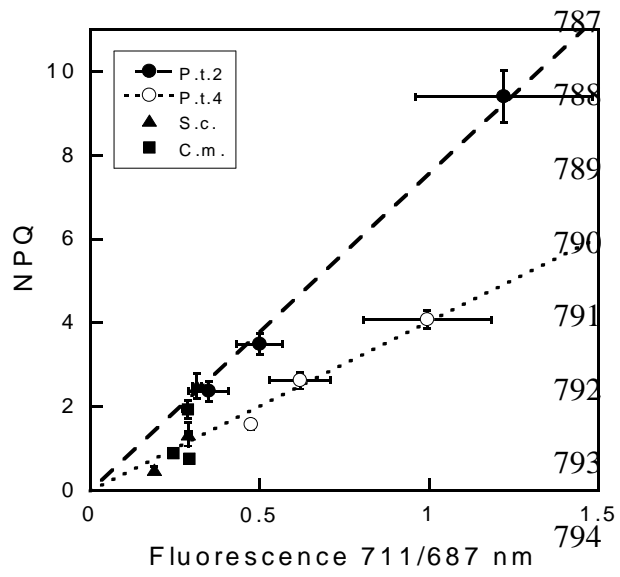
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784 **Figure 5- Lavaud and Lepetit**

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