GENERAL FEATURES OF PHOTOPROTECTION BY ENERGY DISSIPATION IN PLANKTONIC DIATOMS (BACILLARIOPHYCEAE)

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GENERAL FEATURES OF PHOTOPROTECTION BY ENERGY DISSIPATION IN PLANKTONIC DIATOMS (BACILLARIOPHYCEAE) [1]

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ABSTRACT

Planktonic diatoms (Bacillariophyceae) have to cope with large fluctuations of light intensity and periodic exposure to high light. After a shift to high light, photoprotective dissipation of excess energy characterized by the non-photochemical quenching of fluorescence (NPQ) and the concomitant de-epoxidation of diadinoxanthin to diatoxanthin (DT) were measured in four different planktonic marine diatoms (Bacillariophyceae): *Skeletonema costatum* (Greville) Cleve, *Cylindrotheca fusiformis* Reimann et Lewin, *Thalassiosira weissflogii* (Grunow) Fryxell et Hasle and *Ditylum brightwellii* (West) Grunow in comparison to the model organism *Phaeodactylum tricornutum* Böhlin. Upon a sudden increase of light intensity, de-epoxidation was rapid and *de novo* synthesis of DT also occurred. In all species, NPQ was linearly related to the amount of DT formed during high light. In this report, we focused on the role of DT in the dissipation of energy which takes place in the LHC. In *S. costatum* for the same amount of DT, less NPQ was formed than in *P. tricornutum* and as a consequence the photoprotection of PS II was less efficient. The general features of photoprotection by harmless dissipation of excess energy in planktonic diatoms described here partly explain why diatoms are well adapted to light intensity fluctuations.

Key Index Words: diadinoxanthin; diatoxanthin; non-photochemical fluorescence quenching; photoinhibition; photoprotection; planktonic diatoms; xanthophyll cycle

Abbreviations: Chl *a*, chlorophyll *a*; DD, diadinoxanthin; DT, diatoxanthin; LHC, light-harvesting complex; NPQ, non-photochemical fluorescence quenching; PS II, Photosystem II

INTRODUCTION

Diatoms are ubiquitous primary producers accounting for approximately 40% of the primary carbon production in the oceans (Tréguer et al. 1995, Field et al. 1998). Planktonic diatoms (Bacillariophyceae) are the dominant group of phytoplankton. They have to cope with large fluctuations in light intensity and periodic exposures to high light (Harris 1986, Fogg 1991, Long et al. 1994). To survive a sudden increase in light intensity, photoprotective mechanisms are essential (Niyogi et al. 1998, Niyogi 2000). The non-radiative dissipation of excess energy is an important short-term process for the photoprotection of photosystem (PS) II against light-induced damage (Müller et al. 2001). Photoprotective dissipation is attributed to rapid modifications within the light-harvesting complex (LHC) of PS II leading to a non-photochemical chlorophyll (Chl) *a* fluorescence quenching (NPQ). NPQ is induced by the formation of a proton gradient across the thylakoid membrane (ΔpH) and is associated with the operation of a xanthophyll cycle, which converts epoxidized to de-epoxidized forms of xanthophylls. It has been largely documented in higher plants and green migroalgae (for reviews see Pfündel and Bilger 1994, Horton et al. 1996, Eskling et al. 1997, Gilmore 1997, Bassi and Caffarri 2000, Müller et al. 2001) but much less so in diatoms.

The photosynthetic apparatus of diatoms differs in many respects from that of green algae and higher plants. Thylakoid membranes are loosely appressed and organized in extended layers of three without grana stacking; PS I and PS II are randomly distributed (Pyszniak and Gibbs 1992). Diatoms have a specific set of pigments with Chl *c*, fucoxanthin and diadinoxanthin (DD) (which can be de-epoxidized to diatoxanthin, DT), in addition to the Chl *a* and β-carotene found in all photosynthetic organisms (Brown 1988). The xanthophyll concentration relative to Chl can be two to four times more than in higher plant LHC (Wilhelm 1990, Passaquet et al. 1991). These pigments are bound to the LHC subunits that are composed of several highly homologous proteins encoded by a multigene family

(fucoxanthin chlorophyll proteins, FCP, Bhaya and Grossman 1993). The exact organization of LHC subunits in diatoms, and especially the location of DD and its de-epoxidation within these complexes, is currently being studied (Lavaud et al. 2003). No obvious orthologs of some of the important components of LHC of higher plant PS II (e. g. the minor antennae CP 26, CP 29 and CP 22 (PsbS protein)) have been found in *Phaeodactylum tricornutum* Böhlin or within the genome sequence of *Thalassiosira pseudonana* Hustedt (Hasle and Heimdal) (Müller et al. 2001, C. Bowler, personal com.).

Studies of the photoprotective dissipation of excess energy in diatoms have mostly been performed using *P. tricornutum* as a model organism (Ting and Owens 1993, Arsalane et al. 1994, Olaizola et al. 1994, Casper-Lindley and Bjorkman 1998, Goss et al. 1999, Mewes and Richter 2002). This choice results in part from the fact that this species is easy to grow and maintain in artificial conditions in dense cultures. In *P. tricornutum*, we have previously shown that NPQ indeed has a photoprotective effect for the PS II reaction centers under an excess of light (Lavaud et al. 2002a). NPQ depends on the size of the DD pool and can reach much larger values than in higher plants (Lavaud et al. 2002a, Li et al. 2002c). NPQ and DT were linearly related and if DT is not present, NPQ cannot be formed (Lavaud et al. 2002a, Lavaud et al. 2002b). Nevertheless, the ecological relevance of *P. tricornutum* is weak compared to other diatom species. In order to define some general features of photoprotection in diatoms, we compared the effects of high light illumination on four species of planktonic marine diatoms: *Cylindrotheca fusiformis* Reimann et Lewin, *Ditylum brightwellii* (West) Grunow and two ecologically relevant species *Skeletonema costatum* (Greville) Cleve, *Thalassiosira weissflogii* (Grunow) Fryxell et Hasle. The photoprotective dissipation of excess energy (kinetics of DD de-epoxidation and of NPQ formation) was characterized in each species as well as in *P. tricornutum* as a control. For the species with the smallest and the

largest NPQ (*S. costatum* and *P. tricornutum*, respectively), the kinetics of PS II photoinhibition were compared.

MATERIALS AND METHODS

Cultures

Phaeodactylum tricornutum Böhlin (Laboratoire Arago algal collection, Banyuls-sur-Mer, France), *Skeletonema costatum* UTEX-LB2308 (Greville) Cleve (University of Texas Collection (UTEX), Austin), *Cylindrotheca fusiformis* CCMP343 Reimann et Lewin (Provasoli-Guillard National Center for Culture of marine Phytoplankton (CCMP), West Boothbay Harbor, Maine), *Thalassiosira weissflogii* CCMP1051 (Grunow) Fryxell et Hasle, and *Ditylum brightwellii* CCMP359 (West) Grunow cells were grown photoautotrophically in sterile natural seawater F/2 medium (Guillard and Ryther 1962). Non-axenic cultures of 300 mL were incubated at 18°C in airlifts continuously flushed with sterile air. They were illuminated at a light intensity of 40 µmol photons.m^{-2}.s^{-1} with white fluorescent tubes (Claude, Blanc Industrie, France) with a 16 h light/8 h dark photoperiod. Cells were harvested during the exponential phase of growth, centrifuged at 3000 g for 10 min and resuspended in their culture medium to a final concentration of 10 µg Chl *a*.mL^{-1}. The concentrated suspension was then continuously stirred at 18°C under low light before use.

Pigment content.

Pigment analyses were performed by HPLC as previously described (Arsalane et al. 1994, Lavaud et al. 2003). Cells collected from the Clark electrode adapted to the PAM-fluorometer (see below) were deposited on a filter and frozen in liquid nitrogen. Pigments were extracted with a methanol:acetone (70:30, v/v) solution. Published extinction coefficients for Chl

(Berkaloff et al. 1990) and for diadinoxanthin (DD) and diatoxanthin (DT) (Johansen et al. 1974) were used. Cell counts were performed with a Thoma hematocymeter, using the public domain NIH Image program (US National Institute of Health).

Chl fluorescence yield and non-photochemical fluorescence quenching (NPQ).

Chl fluorescence yield was monitored with a modified PAM-101 fluorometer (Walz, Effeltrich, Germany) as described previously (Ritz et al. 1999). A Clark electrode was adapted to the PAM light guides in order to record simultaneously oxygen and fluorescence. Fluorescence was excited by a very weak (non-actinic) modulated 650 nm light. After 20 min dark-adaptation, continuous actinic light of adjustable intensity was applied. 600 ms pulses of white light (4000 µmol photons.m^{-2}.s^{-1}) were admitted by an electronic shutter (Uniblitz, Vincent, USA, opening time 2 ms) placed in front of a KL-1500 quartz iodine lamp continuously switched on in order to monitor NPQ evolution. The average fluorescence (acquisition time 33 µs) measured during the last 400 ms of the pulse was taken as F_m or F_m'. Data were recorded with a computer through a 12 bit analogue-digital interface and the system was driven by home-made software (Arsalane et al. 1993). For each experiment, 2 mL of cell suspension were used. Sodium bicarbonate was added at a concentration of 4 mM from a freshly prepared 0.2 M stock solution in distilled water to prevent any limitation of the photosynthetic rate by carbon supply.

Standard fluorescence nomenclature was used (van Kooten and Snel 1990). F_o and F_m are defined as the minimum PS II fluorescence yield of dark-adapted cells and the maximum PS II fluorescence yield reached in such cells during a saturating pulse of white light, respectively. The quantum yield of excitation trapping by PS II is the ratio F_v/F_m where F_v is the variable part of the fluorescence emission and is equal to F_m- F_o. Non-photochemical fluorescence quenching (NPQ or SV_m) is quantified by the 'Stern-Volmer' expression F_m/F_m'-1, where F_m' is the maximum PS II fluorescence yield of light-adapted cells (Krause and Weis 1991). NPQ

quantified by the 'Stern-Volmer' expression is proportional to the concentration of the de-epoxidized xanthophyll zeaxanthin or DT (Gilmore and Yamamoto 1991, Lavaud et al. 2002a). Non-photochemical quenching also occurs on the minimal fluorescence level F_o and is also proportional to the concentration of DT (Lavaud et al. 2002a). It is expressed as $SV_o = F_o/F_o'-1$ where F_o' is the PS II fluorescence yield of light-adapted cells. It is always smaller than NPQ (or SV_m) (Lavaud et al. 2002a). While NPQ is frequently used because it is the most easy to determine, F_o' is also very useful to determine the quantum yield of photochemistry in the presence and absence of DT with the following relationship: $\Phi_{Q\,[DT=0]} / \Phi_{Q\,[DT]} = F_o / F_o'$ (Lavaud et al. 2002a).

O_2 yield per flash

The relative O_2 yield produced per flash during a sequence of single-turnover saturating flashes at a frequency of 2 Hz was measured at 18°C with a rate electrode as described by Lemasson and Etienne 1975. Short (5 µs) saturating flashes were produced by a Strobotac (General Radio Co., Concord, MA). For the control sequences, cells were first dark-adapted for 20 min and then deposited on the electrode. Both control and illuminated samples taken from the PAM-fluorimeter were allowed to settle on the electrode for 7 min in darkness before measurement. The steady-state O_2 yield per flash (Y_{SS}) was attained for the last flashes of a sequence of twenty flashes when the classical four-step oscillations due to the S-states cycle (Kok et al. 1970) were fully damped. Y_{SS} was used to evaluate the relative number of oxygen producing PS II reaction centers (see Lavaud et al. 2002a).

RESULTS

Characteristics of the five species of diatoms.

Under the same growth conditions, growth rate, cell size and Chl *a* content per cell of the five species were different but, as can be seen in Table 1, the relative pigment concentrations were rather similar, with a large amount of fucoxanthin, and 5 to 10 times less DD and Chl *c*. The amount of DD ranged between 7 and 9 mol/100 mol Chl a and at the light intensities used for culturing (40 µmol photons.m^{-2}.s^{-1}) no DT was present. β-carotene, known to be bound to the core complexes of both photosystems, was also present in similar quantities. The quantum yield of PS II estimated by the ratio F_v/F_m was similar for all species.

Characteristics of the xanthophyll cycle.

After the light shift, de-epoxidation of DD to DT occurred rapidly and after a few minutes (at most), DD reached a minimal value (Fig. 1). The rate of de-epoxidation can be estimated by fitting the decrease of DD with an exponential ($[DD_t = (DD_{initial} - DD_{minimal}) \times e^{-kt}]$). The values found for the rate constant are shown in Table 2. They vary between 2.2 and 1.2 min^{-1}, the fastest de-epoxidation being in *P. tricornutum*. The maximum de-epoxidation observed was around 50 %. It was smaller for *T. weissflogii* and *D. brightwellii* (Table 2). If the duration of illumination exceeded 15 min at the highest intensity used (2000 µmol photons.m^{-2}.s^{-1}), which is equivalent to full sunlight (Long et al. 1994), then a slow synthesis of DT independent of DD de-epoxidation (*de novo* DT synthesis, Olaizola et al. 1994) could be observed. The rate of *de novo* DT synthesis (estimated from the slope of the slow DT increase independent of DD de-epoxidation) varied by a factor of two between all species (Table 2). The three parameters shown in Table 2 were all light dependent, as illustrated for *P. tricornutum* using a 1 h illumination duration and two different light intensities of 450 and 2000 µmol photons.m^{-2}.s^{-1}. The light dependency observed for all species. The de-epoxidation rate, degree of de-epoxidation and rate of *de novo* DT synthesis were larger at higher light intensities (Table 3).

Correlation between DT accumulation and NPQ.

We have previously shown that in *P. tricornutum* grown under low light intensities, NPQ and DT were linearly related (Lavaud et al. 2002a, Lavaud et al. 2002b). In the five strains, the values of DT and NPQ were concomitantly determined in two different ways: i) by varying the illumination time from 0.5 to 60 min at 2000 µmol photons.m^{-2}.s^{-1} (Fig. 1 and 2) and ii) by varying the light intensity from 50 to 2000 µmol photons.m^{-2}.s^{-1} for a fixed illumination time of 5 min (not shown). These data were used to define the correlation between NPQ and DT (Fig. 3A and B). The regression coefficient of the linear relationship was close to one for all species except for *S. costatum*, in which it was lower (0.5). In *S. costatum*, for the same amount of DT, two times less NPQ was formed. In *T. weissflogii*, for values above 3 mol DT/100 mol Chl *a*, the regression coefficient between NPQ and DT was smaller (Fig. 3B).

Comparison of PS II photoinhibition and recovery kinetics for P. tricornutum and S. costatum.

During an illumination at 2000 µmol photons.m^{-2}.s^{-1}, the variation in the relative concentration of active PSII reaction centers was determined, as a function of the duration of illumination up to 45 min, by measuring the steady-state oxygen yield per flash for the strains with the largest (*P. tricornutum*) and the lowest (*S. costatum*) NPQ. Under excess light illumination photoinactivation of PS II reaction centers was faster in *S. costatum* than in *P. tricornutum*, as shown in Figure 4. *In vivo*, photoinhibition occurs in at least two steps: 1) inactivation of PS II reaction centers activity which can be reversed in the dark, 2) inhibition irreversible in the dark (Kirilovsky et al. 1990). To estimate the irreversible part of dark photoinhibition, the number of active PS II reaction centers was measured after 30 min of

dark relaxation for the different illumination times at 2000 µmol photons.m^{-2}.s^{-1} (Fig. 4). In both species, a partial relaxation was observed but the difference between *P. tricornutum* and *S. costatum* remained: photoinhibition was more pronounced in *S. costatum* than in *P. tricornutum*.

DISCUSSION

DD de-epoxidation characteristics and two different pathways for DT synthesis.

The rapid kinetics of de-epoxidation of DD into DT which takes place in all the strains may be due to the fact that a weak proton gradient is sufficient to activate the DD de-epoxidase (Jakob et al. 2001). DD de-epoxidation thus begins rapidly after the onset of high light and the rate is light intensity-dependent. Part of DD molecules may be constitutive within the LHC (Lohr and Wilhelm 2001) inaccessible to the de-epoxidase, and thus non-convertible, as observed for violaxanthin in higher plants (Ruban et al. 1999). This might be the main reason for the incomplete de-epoxidation of DD (50-55 % *in vivo*). The pool of the convertible population of DD can vary in size and is most likely weakly bound at the periphery of the pigment-protein complexes (Lavaud et al. 2003) or even in the lipid membrane of the thylakoid, as suggested for violaxanthin in higher plants (Eskling et al. 1997 and citations within).

For all species examined, exposure to excess light beyond the first 15-30 min leads to further DT accumulation not resulting from DD de-epoxidation. Such DT synthesis has already been reported and denoted *de novo* DT synthesis (Olaizola et al. 1994, Olaizola and Yamamoto 1994). It corresponds to a direct conversion of violaxanthin into DT without a DD intermediate (Lohr and Wilhelm 2001) because it is insensitive to dithiothreitol, an inhibitor of DD de-epoxidation (Olaizola et al. 1994). The rate of *de novo* DT synthesis varies between species and with light intensity and duration.

Correlation between the light induced DT and NPQ.

With the accumulation of DT, a concomitant formation of NPQ is observed. The direct linear correlation between NPQ and DT concentration, previously found in *P. tricornutum* (Lavaud et al. 2002a, Lavaud et al. 2002b), exists also in the other species studied. All regressions show a slope of 1, except *S. costatum* for which it is 0.5. For *T. weissflogii*, the slope changes for DT values above 3 mol/100 mol Chl *a*. According to Figure 1, it could mostly correspond to *de novo* synthesized DT molecules but this may not be true at other light intensities. We tentatively propose that the smaller slope observed with *T. weissflogii* for high values of DT is the result of a combination between a slope 1 and a horizontal line, indicating that a fraction of the DT molecules does not bind to the NPQ locus in the LHC because it becomes saturated. In *S. costatum*, the slope is smaller from the start, possibly indicating that the amount of DT involved in NPQ is a smaller fraction of the total pool. It has been shown in higher plants that an increase in the pool of violaxanthin did not influence the amplitude of NPQ (Davidson et al. 2002). Also in *S. costatum* the amount of the specific component(s) of LHC involved in NPQ could be lower than in other species. Li et al. 2002c recently demonstrated that, other parameters being unchanged, especially the degree of de-epoxidation, NPQ was doubled by increasing the expression of the PsbS protein. The molecules of DT which are not directly involved in NPQ could nevertheless play a different photoprotective role by preventing thylakoid membrane lipids from photo-oxidation during prolonged exposure to high light as shown for its analogue zeaxanthin in higher plants (Havaux and Niyogi 1999). In this case, de-epoxidized xanthophylls are located in the lipid matrix of thylakoid membrane (Eskling et al. 1997 and citations within).

Protection against PS II photoinhibition by NPQ.

NPQ has been shown to account for the efficiency of photoprotection of PS II against photoinactivation in *P. tricornutum* cultures with different NPQ capabilities (Arsalane et al. 1994, Lavaud et al. 2002a). Such a correlation had not been tested for different diatoms with different NPQ characteristics. We have shown previously in *P. tricornutum* that energy dissipation involving DT equally decreases the quantum yield of oxygen evolution and the quantum yield of fluorescence emission. We have now shown in this report that for the same light treatment and for the same DD/DT pool size, there is two times less NPQ (or SV_m) formed in *S. costatum* than in *P. tricornutum*. As excepted, SV_o is also smaller in *S. costatum* than in *P. tricornutum* for the same DT concentration (data not shown) and therefore the decrease of the quantum yield of photochemistry is smaller for *S. costatum* than for *P. tricornutum*. As a consequence, kinetics of PS II photoinhibition are faster in *S. costatum* than in *P. tricornutum* and the irreversible part of photoinhibition is larger in *S. costatum* than in *P. tricornutum*.

In summary, in the present report, we have shown that what has been found in the model organism *P. tricornutum* is also valid for other marine planktonic diatoms. They react rapidly to a sudden increase in light intensity protecting their PS II reaction centers (the main target of photoinhibition) by dissipation of excess energy in the antenna complex of PS II. DT plays a crucial role in this mechanism. A second role for DT which could be prevention of lipid peroxidation needs to be verified. Even though the amplitude and regulation of energy dissipation are species dependent, the following general features can be drawn: (1) a fast DD de-epoxidation and concomitant formation of NPQ (within seconds); (2) a direct linear relationship between DT accumulation and NPQ development; (3) a *de novo* synthesis of DT that accounts for supplementary photoprotection under prolonged illumination; and (4) all

parameters (especially those concerning the xanthophyll cycle) are regulated by the light regime. Because recent studies in higher plants show clearly that PsbS plays a major role for energy dissipation and photoprotection (Li et al. 2002a, Li et al. 2002b, Aspinall-O'Dea et al. 2002, Li et al. 2002c), it is intriguing that an ortholog has not been found in diatoms. The possibility that another type of protein plays a similar role has not yet been explored.

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FIGURE LEGENDS

Figure 1. DD de-epoxidation and DT accumulation kinetics for the five species of diatoms. DD (open symbols, dotted line), DT (solid symbols) and DD+DT (open symbols, dashed line) as a function of illumination duration at 2000 µmol photons.m^{-2}.s^{-1}: (A) *P. tricornutum* (circles), (B) *S. costatum* (triangles), (C) *C. fusiformis* (squares), (D) *T. weissflogii* (diamonds), (E) *D. brightwellii* (inverted triangles). Data (± SD) are the average of two to four measurements.

Figure 2. Non-photochemical quenching development during 60 min illumination at 2000 µmol photons.m^{-2}.s^{-1}; (insert) enlargement of the first 5 min. *P. tricornutum* (circles), *S. costatum* (triangles), *C. fusiformis* (squares), *T. weissflogii* (diamonds), *D. brightwellii* (inverted triangles). Data (± SD) are the average of three to six measurements.

Figure 3. Correlation of NPQ and DT concentration. (A) *P. tricornutum* (circles), *C. fusiformis* (squares) and *D. brightwellii* (inverted triangles); and for (B) *P. tricornutum* (circles), *S. costatum* (triangles) and *T. weissflogii* (diamonds). NPQ and DT were formed by varying the illumination time from 0.5 to 60 min at 2000 µmol photons.m^{-2}.s^{-1} (data from Fig. 1 and 2) and by varying the light intensity from 50 to 2000 µmol photons.m^{-2}.s^{-1} for a fixed illumination duration of 5 min (not shown). Linear regressions: NPQ(*Pt*) = 0.95 [DT], NPQ(*Cf*) = 0.96 [DT], NPQ(*Db*) = 1.04 [DT]; NPQ(*Sc*) = 0.49 [DT], NPQ(*Tw*) = 0.99 [DT≤3], NPQ(*Tw*) = 0.34 [DT>3], with $0.91<R^2<0.99$.

Figure 4. Loss of active PS II reaction centers during illumination at 2000 µmol photons.m^{-2}.s^{-1} (solid symblos) in *P. tricornutum* (circles) and *S. costatum* (triangles), for different duration of illumination up to 45 min, immediately after the cessation of light and after 30

min dark relaxation (open symbols). 30 min is the time sufficient for the relaxation of the dark reversible step of photoinhibition as monitored with the chlorophyll fluorescence emission. The number of active PS II reaction centers was estimated by the steady-state O_2 yield per flash. It was normalized to the activity (100 %) measured in dark-adapted cells. Data (± SD) are the average of three to four measurements.

Table 1. Quantum yield of PS II (F_v/F_m) and pigment content of the five planktonic marine diatoms examined.

Species	F_v/F_m [a] (± 0.03)	Fucoxanthin	Chl *c*	DD	DT	β-carotene
		(mol.100 mol Chl a^{-1})				
P. tricornutum	0.77	67.1 ± 1.3	13.1 ± 0.6	9.2 ± 1.7	0	7.4 ± 0.3
S. costatum	0.77	73.3 ± 1.8	15.3 ± 1.2	8.3 ± 2.1	0	6.4 ± 0.5
C. fusiformis	0.79	71.4 ± 1.9	17.5 ± 0.9	6.6 ± 0.6	0	5.7 ± 0.4
T. weissflogii	0.79	53.6 ± 1.6	11.1 ± 0.5	9.4 ± 0.9	0	8.3 ± 1.5
D. brightwellii	0.82	81.3 ± 1.8	22.7 ± 0.4	7.5 ± 0.4	0	6.9 ± 0.5

([a]) F_v/F_m ($F_v=F_m-F_o$) is the quantum yield of PS II. Data (± SD) are the average of two to four measurements.

Table 2: Characteristics of the DD de-epoxidation and DT *de novo* synthesis measured under 2000 µmol photons.m^{-2}.s^{-1}

Species	k [a] (min^{-1})	Degree of de-epoxidation [b] (%)	Rate of *de novo* DT synthesis (mmol.mol Chl a^{-1}.min^{-1})
P. tricornutum	2.2 ± 0.6	50 ± 1	0.54 ± 0.05
S. costatum	1.7 ± 0.3	50 ± 4	0.59 ± 0.09
C. fusiformis	1.2 ± 0.1	47 ± 5	0.26 ± 0.08
T. weissflogii	1.4 ± 0.1	33 ± 6	0.36 ± 0.04
D. brightwellii	1.2 ± 0.1	40 ± 1	0.24 ± 0.05

([a]) *k* is the rate constant of DD de-epoxidation (see equation in the Results section). ([b]) The degree of DD de-epoxidation is [DT/(DD+DT)x100] measured before DT *de novo* synthesis starts (30 min for *P. tricornutum*, *S. costatum* and *C. fusiformis*, 15 min for *T. weissflogii* and *D. brightwelii*). Data (± SD) are the average of two to three measurements.

Table 3. Effect of light intensity on the DD de-epoxidation and DT *de novo* synthesis in *P. tricornutum* cells.

Light intensity (μmol photons.m^{-2}.s^{-1})	k [a] (min^{-1})	Degree of de-epoxidation [b] (%)	Rate of *de novo* DT synthesis (mmol.mol Chl a^{-1}.min^{-1})
450 [c]	1.4	32	0.27
2000	2.8	51	0.50

([a]) k is the rate constant of DD de-epoxidation (see equation in the Results section). ([b]) The degree of DD de-epoxidation is [DT/(DD+DT)x100] measured before DT *de novo* synthesis starts (from 30 min for the two light intensities). ([c]) 450 µmol photons.m^{-2}.s^{-1} is just saturating for the oxygen emission (as measured with a Clark electrode) while 2000 µmol photons.m^{-2}.s^{-1} corresponds to full sunlight (well above saturation).

Figure 1-Lavaud, Rousseau and Etienne

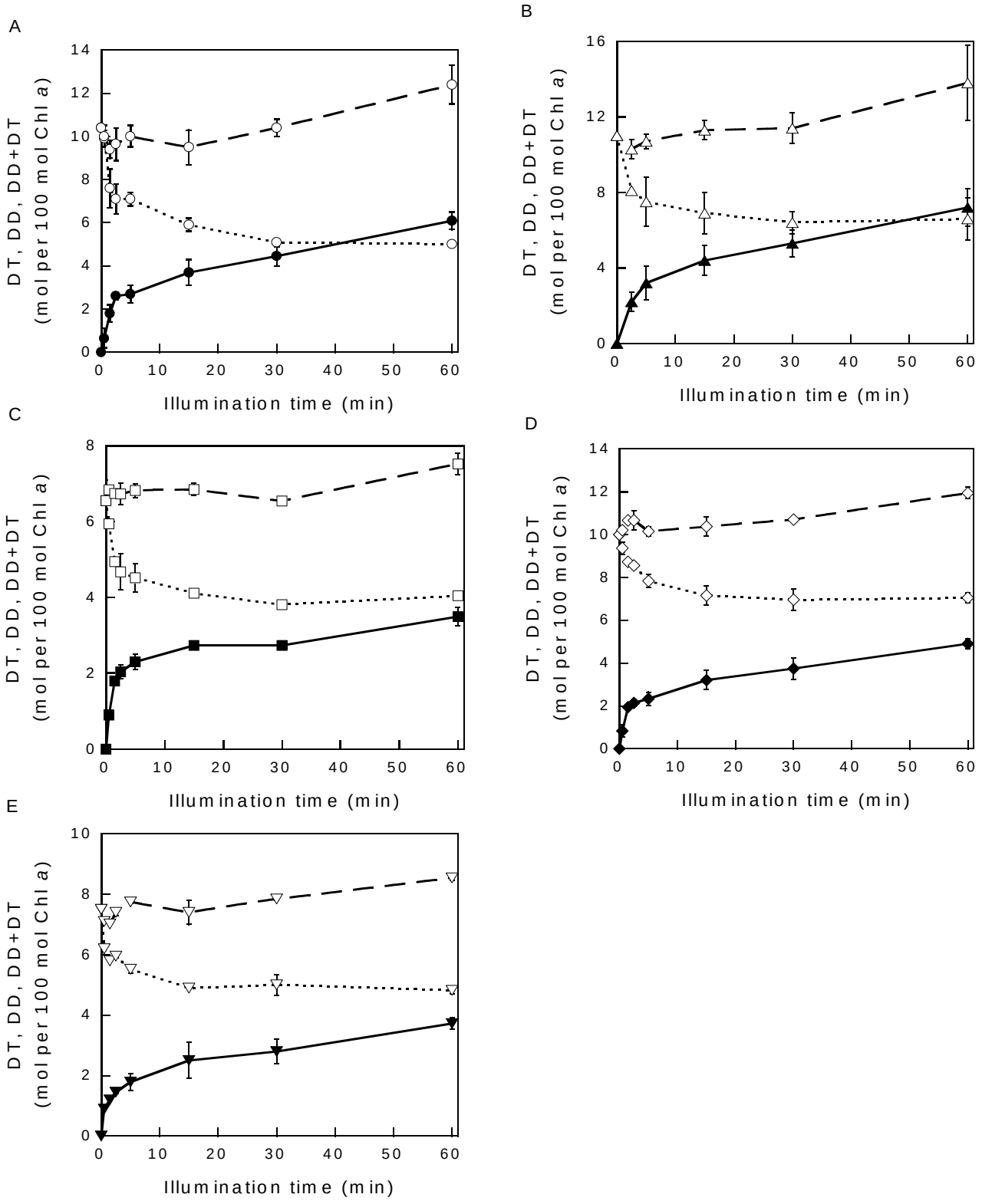

Figure 2-Lavaud, Rousseau and Etienne

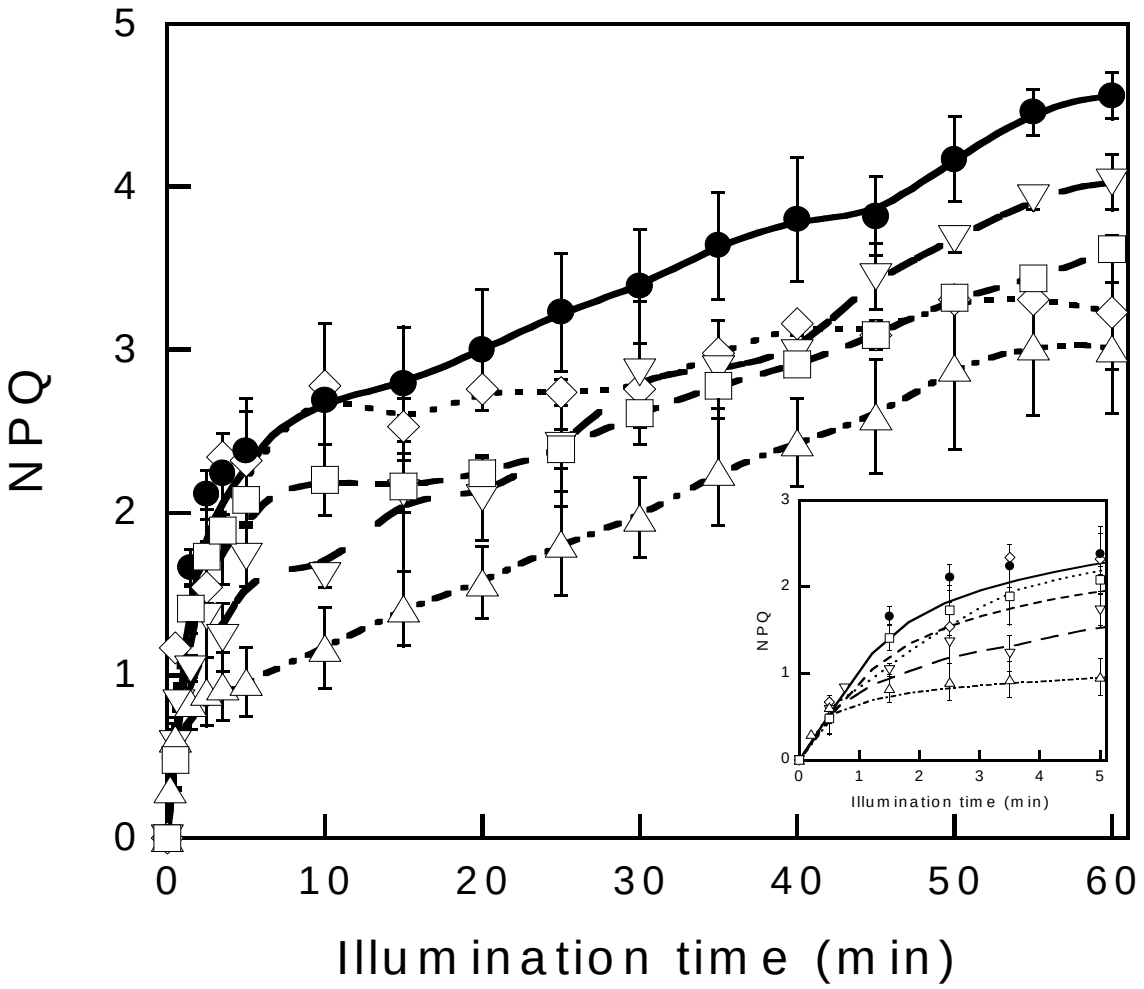

Figure 3-Lavaud, Rousseau and Etienne

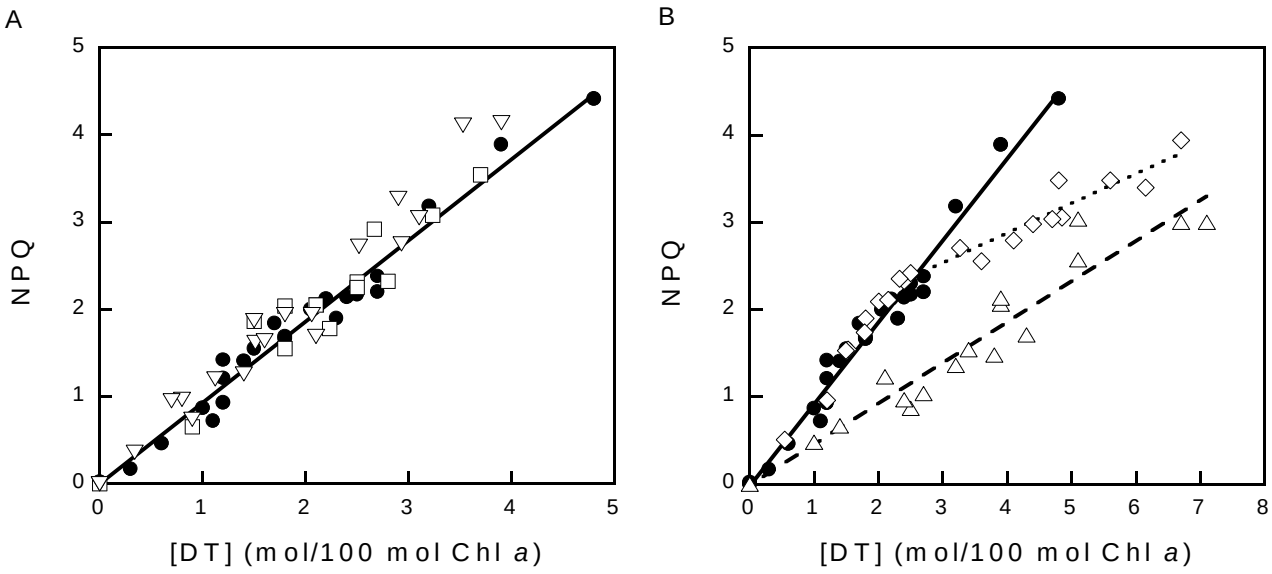

Figure 4-Lavaud, Rousseau and Etienne