Modelling xanthophyll photoprotective activity in phytoplankton

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A numerical model describing xanthophyll dynamics in phytoplankton has been developed and used to investigate cellular photoprotective response. The model assumes that, under the transition from limiting to supra-saturating light, the xanthophyll cycling pigments (PX) synthesis implies first (on a time scale of tens of minutes) a stoichiometric conversion of the already existing fucoxanthin (FUCO) and then (on time scales of an hour onwards) an up-regulation of the investment of newly synthesized carbon to PX. The latter is concomitant with a reduction in the new carbon invested in FUCO production, which down-regulates the light-harvesting apparatus. We hypothesize that these dynamics play a major role in those phytoplankton species adapted to live in highly dynamic environments requiring rapid photoprotective response. In fact, under high light-induced stress, the conversion between photosynthetic and photoprotective compounds may be a metabolically efficient photoprotective mechanism not requiring the use of newly assimilated (and then energetically expensive) carbon.

KEYWORDS: phytoplankton models; xanthophylls; photoprotection; photo-acclimation

INTRODUCTION

In the marine environment irradiance can exhibit strong fluctuations in terms of both frequency (few seconds to seasons) and amplitude (from light to darkness) (Lavaud et al., 2007) implying that phytoplankton can pass very quickly from a limited to a supra-saturated light regime. A rapid increase in light intensity can lead to a reduction in the photosynthetic rate referred to as photo-inhibition (Falkowski and Raven, 2007), which can compromise the survival of the cell. To prevent photo-inhibition, phytoplankton have developed several mechanisms to be protected from and acclimate to increasing light conditions.

Photoprotection generally refers to fast processes (time scale of minutes) that allow the cell to rapidly react to changing light conditions. Among these, non-photochemical quenching (NPQ) is one of the fastest and more widely observed in plants (Demmig-Adams and Adams, 1996). NPQ has different origins contributing to its development, and the most important one (called Qe) is related to the inter-conversion of xanthophylls (oxygenated carotenoids) triggered by the build-up of a pH gradient across the thylakoid membrane generated by a high light environment (Goss and Jakob, 2010). This latter process is known as the xanthophyll cycle (XC). Two kinds of XC are observed in phytoplankton depending on the xanthophyll species involved: the violaxanthin cycle (XC) and the diatoxanthin (DT) cycle (observed in Bacillariophyceae, Haptophyta and most of the...
Xanthophyll-cycling pigments (PX) are equally involved in the long-term photosensory responses, accumulating in high light-acclimated cells (Lavaud et al., 2002; Dimier et al., 2009a). As a consequence, the amount of PX relative to photosynthetic pigments (PSPs) increases with the increase in the growth irradiance (Stolte et al., 2000; MacIntyre et al., 2002; Harris et al., 2005, 2009).

Fucoxanthin (FUCO) is the main photosynthetic accessory pigment in Bacillariophyceae (Wilhelm et al., 2006) and some Prymesiophyceae (Harris et al., 2005, 2009), and a reversible inter-conversion between FUCO and DD has been recently proposed by Harris et al. (Harris et al., 2009). These authors observed symmetry between an increase in PX (the combined DD and DT pool) and a decrease in FUCO during the shift from low to high light, while they observed the opposite trend during the shift from high to low light. In both cases, the pigment adjustment started quickly after the light shift and tended to stabilize after 24–36 h. A conversion of DD to FUCO has already been hypothesized by Lohr and Wilhelm (Lohr and Wilhelm, 1999) who studied pigment dynamics in response to the light condition in the diatom *Phaeodactylum tricornutum*. These authors also noted that this kind of conversion would happen with almost no additional metabolic cost. Harris et al. (Harris et al., 2009) expanded the Lohr and Wilhelm hypothesis (Lohr and Wilhelm, 1999) suggesting that the conversion from PX to FUCO could be reversible depending on the light regime. In this way, FUCO would act as an efficient antenna pigment under light-limiting condition and as a pool from which to synthesize PX under supra-optimal light conditions. Furthermore, studying the pigment composition in *Emiliania huxleyi* in relation to light, Stolte et al. (Stolte et al., 2000) found a strong correlation between the growth irradiance and both the chlorophyll to FUCO ratio, suggesting the use of these ratios as markers of the phytoplankton physiological status with respect to irradiance.

Xanthophyll dynamics is a key feature of phytoplankton growth in the mixed layer because of the high variability of light in this dynamic environment. Xanthophyll activity against high light-induced damage is ecologically-trait-dependent and may affect the competitive ability of cells and hence algal succession in the mixed layer (Meyer et al., 2000; Lavaud et al., 2007). Furthermore, the PX cellular quota, which quickly increases in response to fluctuating light, it is also possible to extrapolate useful information on the physical environment in which the algae live, such as the mixing rate the cells have been exposed to (Brunet et al., 2008; Brunet and Lavaud, 2010).

Despite the relevant role of XC-pigments in algae and its important implications, a model explicitly describing xanthophyll dynamics in phytoplankton is lacking. In the present paper, we propose a numerical model describing the xanthophyll-mediated photoprotection applied for the DD–DT-containing algae. The model offers a mathematical description of photoprotection and photo-acclimation mediated by xanthophylls, providing a feedback between PX cellular quota and photosynthesis.

In this study, we aim to test the hypothesis of the FUCO to PX conversion put forward by Harris et al. (Harris et al., 2009). FUCO is assumed to be co-regulated with chlorophyll under light-limited growth, but can be reversibly converted to PX when irradiance exceeds the growth-saturating level. This latter process ensures a fast and metabolically economic pathway to activate the Qe component of NPQ and counteract photo-inhibition on a short-time scale (tens of minutes). On a longer time scale (from hours to days), the model assumes that newly synthesized carbon is needed in order to maintain the high PX cellular quota allowing the cell to photo-acclimate when the high light condition is prolonged.

Three model experiments are presented here. The first reproduces the experiment of Harris et al. (Harris et al., 2009) describing the pigment dynamics in *E. huxleyi* under a low to high and a high to low light shift in a continuous culture; this experiment is designed to describe the cellular response to a sudden change in light conditions. The second experiment aims to simulate the xanthophyll dynamics under increasing light as described in Harris et al. (Harris et al., 2005); in this case the goal is to simulate pigment dynamics in acclimated cells as a function of incoming light. The third experiment aims to reproduce the PX to chlorophyll ratio under gradual light increase observed by Dimier et al. (Dimier et al., 2009a). In this case, the scope is to assess the ability of the model to simulate PX dynamics in different species adapted to different light conditions. In all the experiments, we assume nutrient-replete conditions and constant temperature.

**MODEL THEORY**

The model simulates biomass (P), chlorophyll-α (Chl), FUCO and PX as a function of light in a phytoplankton cell. PX corresponds to the sum of DD and DT, as we mainly focus on the longer term photoprotective...
response (>20–30 min). The first very fast response (<20–30 min), in fact, is the de-epoxidation of the already present DD into DT, consequently without any PX increase. Indeed, the potential involvement of FUCO into PX production occurs after the first fast response.

When grown under light-limiting conditions, phytoplankton are assumed to have a constant low production of PX (more specifically its DD component), as observed in low light-acclimated cells (Harris et al., 2005, 2009). Under light regimes that provide excess energy to the photosynthetic apparatus, we assume a reversible increase in PX at the expense of the FUCO pool. This conceptual model framework is displayed in Fig. 1.

Model state variables and relative units are displayed in Table I while model parameters are listed in Table II. PX and FUCO are modelled through their carbon content in order to facilitate a mass conservative conversion between them. However, in order to directly compare model outputs with literature data, PX, FUCO and associated parameters are converted to the total weight (mg). To do this, modelled FUCO and PX (mg C m$^{-2}$) have been divided by 0.76 [mg C (mg FUCO)$^{-1}$] and 0.82 [mg C (mg DD)$^{-1}$], which are the carbon fraction of the total FUCO and DD mass, respectively. Note that, although composed of the sum of DD and DT, PX is treated as a single state variable having the carbon content of DD. This implies that the difference in the carbon fraction between DD and DT (3%) is assumed to be negligible.

**Photosynthesis and biomass equations**

The carbon-specific net photosynthesis rate [$P_c^c$, (d$^{-1}$)] is obtained by adding to the standard formulation of Geider et al. (Geider et al., 1997) a dimensionless term taking into account the stress resulting from excess light:

$$P_c^c = P_m^c \cdot \left[1 - \exp \left( -\frac{\alpha \cdot E \cdot \theta}{P_m^c} \right) \right] \cdot E_{lim},$$

(1)

where $E$ is the incoming light (μmol photons m$^{-2}$ s$^{-1}$), $P_m^c$ the potential net photosynthetic rate, $\alpha$ the Chl-specific initial slope of the photosynthesis–irradiance curve (P–I curve) and $\theta$ the Chl to carbon ratio. The photoinhibition term $E_{lim}$ is assumed to be the ratio between two dimensionless terms representing the potential photo-inhibition (PhI) and the photoprotection (PhP), respectively:

$$E_{lim} = \min \left( 1, \frac{PhP}{PhI} \right).$$

(2)

When PhI is greater than PhP, a linear decrease in photosynthesis is predicted by the model. When PhP is greater than PhI, the photoprotective activity prevents photo-inhibition as $E_{lim}$ is limited to 1. We define as PhI the photo-damage due to the excess of light which depends linearly on the incoming light (Marshall et al., 2000). PhI is given by the ratio between the incoming light $E$ and the optimal light for photosynthesis $E_{opt}$:

$$PhI = \frac{E}{E_{opt}}.$$

(3)

Table I: Model state variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symbol</th>
<th>Units</th>
</tr>
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<tbody>
<tr>
<td>Biomass</td>
<td>P</td>
<td>mg C m$^{-2}$</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>Chl</td>
<td>mg m$^{-2}$</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>FUCO</td>
<td>mg m$^{-2}$</td>
</tr>
<tr>
<td>Xanthophyll-cycling pigments</td>
<td>PX</td>
<td>mg m$^{-2}$</td>
</tr>
<tr>
<td>Light</td>
<td>E</td>
<td>μmol photons m$^{-2}$ s$^{-1}$</td>
</tr>
</tbody>
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light and the light-saturating parameter for acclimated photosynthesis ($K_e$):

$$\text{PhI} = \frac{E}{K_e}.$$  

($3$)

$K_e$ is a fixed parameter as defined in Geider et al. (Geider et al., 1997, Table II) and should not be confused with $E_k$, the saturation parameter for instantaneous photosynthesis which, on the contrary, is highly variable depending on the acclimation status of the cell (Falkowski et al., 1994).

$\text{PhP}$ represents both the photoprotection and photo-acclimation activity due to the modulation of the pigment pool and is assumed to be the ratio between xanthophyll cycle (PX) and photochemical pigments ($\text{PSP} = \text{Chl} + \text{FUCO}$) divided by the same ratio in dark acclimated cells ($\text{PPR}_{\text{min}}$):

$$\text{PhP} = \frac{\text{PX}:\text{PSP}}{\text{PPR}_{\text{min}}}.$$  

($4$)

Note that under light-limited growth, equation (1) is equal to the Geider et al. (Geider et al., 1997) formulation. This means that, for a given $E$ and in the absence of photo-oxidative stress, photosynthesis is only dependent on the chlorophyll level ($\theta$) assumed to be a proxy for all the PSPs. It should be noted at this point that the function of FUCO as an antenna pigment is not explicitly modelled. Under supra-optimal light conditions instead, photosynthesis becomes dependent on the PX:PSP ratio.

The equation for the biomass ($P$) can then be written as:

$$\frac{dP}{dt} = PC \cdot P - R \cdot P,$$  

($5$)

where $R$ is a loss term accounting for rest respiration and mortality.

**Photosynthetically active pigments: Chlorophyll-a and fucoxanthin**

Chlorophyll production is formulated following the Geider et al. (Geider et al., 1997) model, which describes a monotonic decrease in the Chl content with the increase in the incoming light. FUCO synthesis is assumed to be co-regulated with chlorophyll under light intensity lower than $K_e$. This assumption is based on the observation that, under limiting irradiance, the cellular content of accessory (photosynthetically active) pigments declines in the same way as chlorophyll does (MacIntyre et al., 2002). When the incoming light exceeds $K_e$, FUCO is converted to PX resulting in a decrease in the FUCO:Chl ratio. This process is proportional to the incoming light and is constrained by a lower limit of the FUCO:Chl ratio ($\text{FUCOC}_{\text{min}}$), which is estimated from high light-acclimated growth (Harris et al., 2009). The PX pool is converted back to FUCO when the irradiance drops below $K_e$. This latter reaction is limited by a lower limit of PX given as the PX to carbon ratio ($\text{PX}_{\text{min}}$) estimated from low light-acclimated growth (Harris et al., 2009). The above described dynamics are modelled through the following
The model was run for 10 days at different irradiances to simulate cell (i.e. acclimated to each of the tested irradiances) are also presented in Fig. 2. If we consider a roughly constant proportion of PX observed under unsaturated light condition (Harris et al., 2009):

\[ A = PP \cdot PX_{\text{min}}. \]  

Process B implies that under increasing light regimes, there is a reduction in the FUCO to Chl ratio (FUCOC) which, in turn, down-regulates the amount of primary produced carbon directed to FUCO synthesis [see equation (6)]. The model assumes that this carbon is reversibly re-allocated to PX production, which is given by term C in equation (9):

\[ C = \rho \cdot (\text{fucoX} - \text{FUCOC}), \]  

where fucoX is the FUCO:Chl ratio observed under light-limited growth (Harris et al., 2009). The meaning and the relative importance of these terms are discussed later.

The term \( \zeta \) describes the degradation given by the already described loss term \( R \):

\[ \zeta = R \cdot PX. \]  

**MODEL SIMULATIONS**

**Modelling photo-inhibition and photo-acclimation**

Photo-inhibition and high light photo-acclimation are modelled through the term \( E_{\text{lim}} \). The evolution of \( E_{\text{lim}} \) as function of increasing irradiance is displayed in Fig. 2. The model was run for 10 days at different irradiances in order to reach a stable PX:PSP ratio (implying acclimation to the given irradiance) and then instantaneously exposed to the irradiance levels reported in the figure. The values of \( E_{\text{lim}} \) for a fully acclimated simulated cell (i.e. acclimated to each of the tested irradiances) are also presented in Fig. 2. If we consider dark acclimated cells (PX:PSP~0.01), the model predicts photo-inhibition starting from any irradiance greater than \( K_e \). When the growth irradiance increases, the increase in the PX:PSP ratio allows the model cell to acclimate, delaying photo-inhibition. For a fully
acclimated cell, the model does not predict photo-inhibition till the irradiance level of 2800 \( \mu \text{mol} \ \text{photons m}^{-2} \ \text{s}^{-1} \).

\( E_{\text{lim}} \) is a function of \( \text{PhI} \) and \( \text{PhP} \) displayed in Fig. 3. The interception between \( \text{PhI} \) and \( \text{PhP} \) represents the model photo-inhibition threshold.

**Experiment 1: step changes in irradiance: low to high and high to low**

The model has been set up in order to reproduce the light exposure experiments described in Harris *et al.* (2009). Consistently, the model was run for 10 days under 50 \( \mu \text{mol} \ \text{photons m}^{-2} \ \text{s}^{-1} \), then the irradiance was increased to 800 \( \mu \text{mol} \ \text{photons m}^{-2} \ \text{s}^{-1} \) for 7 days after which it was lowered to the initial value.

The Chl to carbon, PX:Chl and the FUCO:Chl ratios simulated during a shift from low to high and high to low light are displayed in Fig. 4 together with the data (plus standard deviation) reported in Harris *et al.* (2009). The correlation index, the root mean square error (RMSE) and the mean percentage error (MPE) between observations and simulations are shown in Table III.

The observed pattern is captured by the model as shown by the correlation between data and simulations which is high (\( r > 0.85, P < 0.001 \)) for all the variables considered. The quantitative agreement between observed and simulated values, evaluated through the RMSE and the MPE, is particularly good for the PX:Chl ratio simulated under the low to high light shift (with RMSE = 0.01 and MPE = 3.33%), good for the FUCO:Chl ratios simulated under both low to high and high to low light shift (RMSE = 0.01 in both cases, and MPE = 8.2 and 6.2%, respectively), and less...
satisfactory for the remaining variables considered, with an MPE, however, never exceeding 35%. Fig. 5 shows the evolution of the model term $E_{\text{lim}}$ during the shift from low to high light. Values lower than 1 imply photo-inhibition while values equal to 1 mean that photosynthesis is unaffected by the excess of light. The model predicts that photosynthesis is heavily photo-inhibited just after the light switch but fully recovers after a few hours.

**Experiment 2: pigment dynamics under increasing growth irradiance**

The simulated PX:Chl, PX:FUCO and FUCO:Chl ratios under increasing irradiance are displayed in Fig. 6 along with data reported in Harris et al. (Harris et al., 2005). The simulation set-up was based on the experimental design described in the same paper. The model was run at each irradiance for 7 days (assuming constant illumination) in order to achieve acclimation. For this experiment, only the shape of the model curve has been statistically evaluated (through the correlation index $r$, also displayed in Fig. 6) as a reliable assessment of the model bias is obscured by the small size of the data set considered (only four data points for each variable) and the high variability of the values. The observed increase in the PX:Chl and PX:FUCO ratios is qualitatively captured by the model as evident from the high correlation ($r > 0.95$) between observation and simulation which is also displayed. The observed decrease in the FUCO:Chl ratio as a function of increasing light is well reproduced by the simulation which shows an initial steep decrease that stabilizes towards a steady state from 200 μmol photons m$^{-2}$ s$^{-1}$ onwards.

**Experiment 3: pigment dynamics in low, high and variable light-adapted species**

These simulations reproduce the experimental set-up reported in Dimier et al. (Dimier et al., 2009a). Light was gradually increased from 40 to 200 μmol of photons m$^{-2}$ s$^{-1}$ and then (after 200 min) further
increased up to 400 μmol of photons m\(^{-2}\) s\(^{-1}\) as shown in Fig. 7D.

Figure 7 shows the simulated PX:Chl ratio along with the data reported in Dimier et al. (Dimier et al., 2009a) relative to three phytoplankton species: Bolidomonas mediterranea, Pelagomonas calceolata and Phaeocystis cordata, which were classified as high light-adapted, low light-adapted and variable light-adapted species, respectively (Dimier et al., 2009a). The parameters used in each of the simulations that differ from the ones shown in Table II are displayed in Table IV. The model reproduces values of the PX:Chl ratio that are comparable with the observed ones for all the species considered, as evident from the values of the correlation index, the RMSE and the MPE displayed in Table V. Concomitantly with the increase in the PX:Chl ratio, the model also simulates a decrease in the FUCO:Chl ratio (Table VI). The latter is only observed in P. cordata, while, for the other two species, no significant change in the FUCO:Chl ratio was observed throughout the experiment.

**DISCUSSION**

To the best of our knowledge, the model presented is the first attempt to numerically simulate xanthophyll dynamics in relation to high light photo-acclimation and photo-inhibition of phytoplankton.

Photo-inhibition in phytoplankton is thought to be mainly due to the damage of the D1 protein associated with the PSII reaction centre. D1 protein repair implies the degradation of the damaged protein, excision from the PSII and de novo synthesis of a new D1 protein (Aro et al., 1993; Marshall et al., 2000) and can have different

| Table IV: Model parameters used in experiment 3 that have been changed with respect to experiments 1 and 2 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Parameter       | B. mediterranea | P. calceolate   | P. cordata      | Reference       |
| \( \theta_{\text{max}} \) | 0.015           | 0.015           | 0.015           | This study      |
| fucoX           | 0.35            | 0.36            | 0.38            | (Dimier et al., 2009a) |
| PX\(_{\text{min}}\) | 9.7 × 10\(^{-4}\) | 4.4 × 10\(^{-4}\) | 6.3 × 10\(^{-4}\) | (Dimier et al., 2009a) |

| Table V: Correlation index (\( r \)), root mean square error (RMSE) and mean percentage error (MPE) between simulations and observed mean values reported in Fig. 7 |
|-----------------|-----------------|-----------------|-----------------|
| \( r \) (\( P < 0.001 \)) | 0.97 | 0.85 | 0.86 |
| RMSE            | 0.008           | 0.01           | 0.02           |
| MPE             | 1.3%            | 5.5%           | −19.6%          |

Letters “A” to “C” refer to the corresponding panels of Fig. 7.

| Table VI: Observed (Dimier et al., 2009a) and simulated FUCO:Chl ratio (g g\(^{-1}\)) under 40, 200 and 400 μmol photons m\(^{-2}\) s\(^{-1}\) |
|-----------------|-----------------|-----------------|-----------------|
| Light           | B. mediterranea | P. calceolate   | P. cordata      |
| 40              | 0.35 (0.35 ± 0.02) | 0.36 (0.36 ± 0.006) | 0.38 (0.38 ± 0.006) |
| 200             | 0.32 (0.34 ± 0.01) | 0.33 (0.35 ± 0.01) | 0.35 (0.34 ± 0.01) |
| 400             | 0.28 (0.34 ± 0.02) | 0.29 (0.38 ± 0.02) | 0.31 (0.32 ± 0.02) |

Simulated values are in bold, observed values ± SD are in parenthesis.
levels of efficiency depending on the species considered. Although the D1 protein is damaged under any light intensity (Adir et al., 1990), photo-inhibition only occurs when the damage exceeds the repair capacity, the latter also accounting for the metabolic cost of the repair.

The balance between D1 protein damage and repair is implicitly modelled by means of the light threshold level $K_e$. The model assumes that, for any light higher than $K_e$, photo-inhibition occurs unless a photoprotective strategy is adopted. Conversely, when $E < K_e$, photoinhibition is prevented without invoking any photoprotective mechanism. $K_e$ can be, therefore, considered as the light intensity under which the D1 protein repair capacity reaches its maximum. As a consequence, by setting different values of $K_e$, it is possible to mimic the different capacity of D1 protein repair among phytoplankton species. For $E > K_e$, the model assumes that the rate of repair is constant but the damage can be minimized by photoprotection. The latter, here considered as high light photo-acclimative responses developed on an hourly time scale, is described through the increase in the PX pool and the concomitant decrease in the PSP pool (FUCO + Chl). When $E > K_e$, in fact, photosynthesis is assumed to be dependent on the dynamically varying PX:PSP ratio.

Photoprotection can significantly increase the light level at which photosynthesis starts to be photo-inhibited (see Figs 2 and 3). However, if the light still increases once the cell’s photoprotective capacity has been reached, then photo-inhibition occurs. In the model, this happens at the irradiance corresponding to the intercept between the terms PHI and PhP, as displayed in Fig. 3. The modelled E. huxleyi does not show evidence of photo-inhibition even at very high growth irradiances (up to 2700 $\mu$mol photons m$^{-2}$ s$^{-1}$) and this agrees with observations made with this alga (Paasche, 2002; Ragni et al., 2008). Nevertheless, by further increasing the light to (unrealistically) extreme levels (>2700 $\mu$mol photons m$^{-2}$ s$^{-1}$), the model predicts the occurrence of photo-inhibition.

On the basis of model assumptions and simulation results, we propose the following scenario to describe xanthophyll-mediated high light photo-acclimation: when light increases to supra-optimal levels, a (reversible) pigment conversion leads to an increase in PX at the expenditure of FUCO as suggested by Harris et al. (Harris et al., 2009). This pathway is advantageous for phytoplankton that exhibit it, since FUCO to PX conversion implies the use of no new synthesized organic carbon and hence has a negligible metabolic cost. The concomitant decrease in the PSP pool (FUCO + Chl), on the other hand, lowers the excess of energy reaching the photosystems. The combination of the two processes, allows the cell to counteract photo-inhibition (Fig. 5). After a few hours, however, the FUCO to PX conversion is limited by the amount of available FUCO. The resulting decrease in the FUCO:Chl ratio up-regulates the amount of new carbon to be invested in PX synthesis [see equation (12) and Fig. 1] allowing the cells to reach a condition of balanced growth (after 2–3 days from the light switch) under which they continuously maintain a high quota of PX. The latter could have three functions: (i) to maintain the development of NPQ under high light, (ii) to screen the photosystems from excess of light, also protecting thylakoidal membranes from photo-oxidation and (iii) to ensure a rapid re-conversion to a light-harvesting pigment in case of decreasing light intensity.

The FUCO to PX conversion is assumed to be relatively fast (tens of minutes) and is the main source of PX (process B in Fig. 8). On the contrary, 12 and 36 h after the light step increase, the fraction of PX produced from FUCO accounts for a small % of the total PX production, while the processes using newly synthesized carbon to produce PX (processes A and C) dominate (Fig. 8).

We would like to stress that the first and very fast photoprotective response (already existing DD to DT), which is not resolved by the model, is insufficient to cope with a very steep light increase and, therefore, unlikely to play a major role in the experimental condition reproduced here. Given the very low amount of DD already existing (because of the low light acclimation before the light shift), an increase in PX is required in order to sustain the development of NPQ. The increase of PX due to the FUCO conversion can be considered a second stage in the overall photoprotective response.
acting as a “bridge” between fast photoprotection and slow photo-acclimation, which implies more structural changes in the PSII (dramatic variations in chlorophyll-a and accessory pigments).

The model, generally, reproduces less well the observed pigment dynamics during the shift from high to low light. While the simulated FUCO:Chl ratio is consistent with observations, the model produces a larger inertia than the real response of the Chl to carbon and the PX:Chl ratios. This behaviour may be for two reasons. The first implies that the model underestimates the rapid onset of the epoxidation from DT to DD as soon as cells experience a low light environment. In this case, the fact that the simulation of the FUCO:Chl ratio is very close to the real data, leads us to hypothesize that the loss in PX does not fuel the FUCO pool, but might be diverted to other pigments, as β-carotene, or other xanthophylls (for instance the violaxanthin-cycle pigments; Dimier et al., 2009b). The second possible reason implies that the Chl recovery under the high to low light transition is underestimated by the model leading to a weaker response of the simulated PX:Chl ratio, which is what we obtained.

The capacity of the model to realistically reproduce the PX long-term acclimation is more specifically highlighted by the second experiment presented (Fig. 6), which simulates pigment dynamics under increasing growth irradiance as described in Harris et al. (Harris et al., 2005). The model reproduces pigment ratios (PX:Chl, PX:FUCO and FUCO:Chl) observed under high growth irradiance (800 μmol photons m−2 s−1), while the quantitative agreement with observed values is less good under lower growth irradiance (0–200 μmol photons m−2 s−1). We can speculate that cells react to moderate excess of light by firstly converting to DT the already synthesized DD, thus reducing the net increase in the PX (DD + DT) pool. As already stated, the model, focusing on the variability of the PX:PSP ratio, does not resolve the DD to DT conversion (XC) and this could account for the discrepancy between model and data under low-growth irradiance. The explicit formulation of XC will be considered in the next development step of the model in order to study the down-regulation of photo-acclimation due to fast photoprotection.

The model qualitatively reproduces the observed trends, as highlighted by the high correlation between simulated and observed values. This means that through the adjustment of the pigment pool (given by the variability of the above-mentioned pigment ratios), the model keeps “its memory” of the cell’s “light history”. This is because each pigment ratio results from a specific light condition under which the cell has grown. This is particularly important as the model predicts different photo-inhibition thresholds depending on the light level at which the algae have grown (Fig. 2). This skill could be particularly useful in future model development, when the formulation presented will be cast into a coupled hydrodynamic-biogeochemical model. At the ecosystem scale, in fact, the relative PX cellular content (and the consequent capacity to photoprotect and acclimate) can be related to physical features such as the mixing velocity the cells are exposed to (Brunet and Lavaud, 2010) and can contribute to ecologically relevant dynamics such as phytoplankton succession and dominance (Lavaud et al., 2007).

The model’s ability to reproduce PX dynamics under a gradual light increase and in different species has been tested in our third experiment, where we attempted to simulate the evolution of the PX:Chl ratio observed by Dimier et al. (Dimier et al., 2009a). The species considered are: (i) a coastal species adapted to variable light conditions, P. cordata, (ii) an open ocean, surface species adapted to high light and (iii) a low-light-adapted species living in the deep chlorophyll maximum layer, P. calceolata. By changing the pigment-related parameters (Table IV), the model simulates the PX:Chl ratio comparable with the observed ones for all the species considered (Fig. 8 and Table V). However, the model also shows limitations in its ability to reproduce observed pigment trends on the time scale of this experiment (minutes), notably with P. cordata (Fig. 7C). Nevertheless, the decrease in the FUCO:Chl ratio which is observed only in P. cordata is, well reproduced (Table VI).

Our interpretation of this experiment draws on the different ecological behaviour of the phytoplankton considered. Species such as P. cordata are very fast and efficient in reacting to light variations; they mainly convert DD to DT as a first rapid response (Dimier et al., 2009a), then (after few tens of minutes) increase the PX pool. The fact that the model underestimates the PX increase, while still correctly reproducing the FUCO:Chl decrease, suggests that the new PX does not come only from FUCO inter-conversion, but from other pathways implying new carbon investment in PX production. The latter scenario could be explained by the great efficiency of this coastal species in producing photoprotective pigments. We can hypothesize that P. cordata is able to perform the FUCO to PX conversion but that the latter is not the only PX production process when the light increase is mild (and then not very stressful) as in the experimental conditions considered. Under these latter situations and in a highly variable light regime, it may be more efficient to preserve a relatively high amount of FUCO and invest new carbon in
PX production, in order to be able to harvest the major quantity of light as soon as cells will experience low light conditions.

The other two species considered, *B. mediterranea* and *P. calceolata*, may not have the need for a fast and metabolically cheap conversion between photosynthetically active and XC-pigments as they are adapted to live in a more stable environment. On the other hand, we can also speculate that, also in these species, the FUCO to PX conversion is triggered by highly stressful conditions that are not induced under the experimental set-up presented.

Combining all our results, we hypothesize that the FUCO to PX conversion plays a relevant role in algal photoprotection behaviour. However, this process is ecologically trait-dependent and may mainly take place in high and variable light-adapted species. Furthermore, its relative contribution to the PX production is proportional to the intensity of the light-induced stress. As stated by Harris et al. (Harris et al., 2009) the “FUCO to PX conversion” hypothesis needs to be substantiated through further experiments. However, the fact that our model, which is based on that hypothesis, successfully reproduces observed pigment patterns supports it and strengthens the case for further experimentation.

Finally, it is useful to note the following methodological consideration on the way we performed the comparison between data and simulations. Even though the model is not totally independent from the data set used for the comparison, this only partially compromises the effectiveness of the validation presented. We used the data set of Harris et al. (Harris et al., 2009) to estimate three of the model parameters (fucoX, FUCOC<sub>min</sub> and PX<sub>min</sub>, see Table II), parameters that constrain the pigments ratios to the observed minimum and maximum values. The use of these parameters clearly influences the quantitative goodness of fit by setting limits. However, it does not affect the “qualitative behaviour” (i.e. the shape of the curve), which is dependent on the theoretical assumptions and mathematical formulation of the model. The above-mentioned parameters are likely to be highly variable between phytoplankton species and should be properly tuned depending on the phytoplankton species/groups of interest. In experiment number 3, for example, we had to change two of the above-mentioned parameters (fucoX and PX<sub>min</sub>) and θ<sub>max</sub> in order to reproduce values of the PX:Chl ratio comparable with the observed ones in three different species. Model skill in reproducing observed dynamics is further supported by the second experiment, where simulated pigment ratios highly correlate (r > 0.95) with totally independent data reported in Harris et al. (Harris et al., 2005). However, this comparison should be regarded with caution considering the small size of the data set (4 values per variable) and the high variability of the data values.

**Conclusions and future work**

The model describes phytoplankton photoprotection and photo-inhibition through the varying PX to PSP ratio, which is an important diagnostic variable in marine ecosystem. We have shown that it is possible to model observed pigment dynamics under light shifting conditions and under increasing growth irradiance by assuming a fast light-dependent photosynthetic to photoprotective pigment conversion, and that this pathway could protect the cell from photo-inhibition under a sudden and steep increase in light. The next steps are to explicitly resolve the DD de-epoxidation (and the consequent NPQ development at a time scale of minutes) in order to investigate the interactions between fast and long-term photoresponses and to study the possible consequences of the xanthophyll dynamics for competition and succession in phytoplankton communities.

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