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Review

Parameterization of photosystem II photoinactivation and repair ☆

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ABSTRACT

The photoinactivation (also termed photoinhibition or photodamage) of Photosystem II (PSII) and the counteracting repair reactions are fundamental elements of the metabolism and ecophysiology of oxygenic photoautotrophs. Differences in the quantification, parameterization and terminology of Photosystem II photoinactivation and repair can erect barriers to understanding, and particular parameterizations are sometimes incorrectly associated with particular mechanistic models. These issues lead to problems for ecophysiologists seeking robust methods to include photoinhibition in ecological models. We present a comparative analysis of terms and parameterizations applied to photoinactivation and repair of Photosystem II. In particular, we show that the target size and quantum yield approaches are interconvertible generalizations of the rate constant of photoinactivation across a range of incident light levels. Our particular emphasis is on phytoplankton, although we draw upon the literature from vascular plants. This article is part of a Special Issue entitled: Photosystem II.

1. Introduction

The structure and function of PSII are highly conserved [1] and all known PSII centers share a susceptibility to photoinactivation. PSII photoinactivation (also termed photoinhibition or photodamage) [2–8], the counteracting repair reactions [8–14], and interacting photoprotection mechanisms [15–25] are thus fundamental elements of the metabolism, physiology and ecophysiology of all oxygenic photoautotrophs. Although the key molecular structures and many of the underlying processes have been defined for model species under some growth conditions, the field remains active and contentious, with differing opinions on the relative importance of mechanisms of photoinactivation [7,8,22,26–33]. More generally, models of biological productivity seek to incorporate photoinhibition [34–36], and thus need robust parameterizations applicable to natural light regimes.

The long history of photoinhibition research has led to different traditions in terminology and in the methods used to measure and then parameterize photoinactivation. We aim to bring light to these issues through an overview of terms and comparative analysis of the parameterizations that have been applied to photoinactivation and repair of PSII, with an emphasis on phytoplankton.

1.1. Photoinactivation and net photoinactivation

Research on photoinactivation of photosynthesis can be traced back to Bessel Kok whose work with *Chlorella* [37] led to identification of a damaging reaction which he called photoinhibition and a concurrent restoration reaction. Kyle et al. [38] demonstrated that a protein of PSII had a crucial role in photoinhibition of PSII, and the concurrent repair reaction was soon connected to chloroplast protein synthesis [39,40]. Since then, molecular physiological research has elucidated mechanisms for PSII specific processes of photoinactivation and repair. In parallel the plant ecology and biological oceanography fields have sought to account for the observation that strong light can cause decreases in the photosynthetic quantum yield and sometimes in the maximum photosynthetic rate [41–46].

The word "photoinhibition" may encompass all light-induced decrease in the quantum yield of photosynthesis [43]. In the plant physiological tradition, "photoinhibition" has been used to refer to the reaction(s) that lower PSII activity in the light. Plant ecology literature uses the word "photoinhibition" primarily to describe conditions in which the activity of PSII is below maximum, and uses other terms like photodamage or photoinactivation to describe the reactions that cause photoinhibition. Differences in definitions may lead to conceptual misunderstandings. For example, non-photochemical quenching of excitation energy (NPQ) lowers the quantum yield of PSII electron transport [47] and therefore causes photoinhibition in the plant

Abbreviations: $[A]_0$ and $[A_\infty]$, initial and steady-state concentration of active PSII centers; Chl, chlorophyll; F_M, maximum fluorescence; F_0, initial fluorescence; F_V, variable fluorescence; Φ_{PI}, quantum yield of photoinactivation; J_i, flux of incident quanta; J_a, flux of absorbed quanta; k_{DARK}, rate constant of dark inactivation; k_{DEG}, rate constant of the degradation of the D1 protein; k_{PI}, rate constant of photoinactivation; k_{REC}, rate constant of recovery of PSII activity; k_{SYNTH}, rate constant of the synthesis of the D1 protein; NPQ, non-photochemical quenching; N_t, number of quanta incident on a sample in time t; PSII, Photosystem II; σ_i, target size of photoinactivation; σ_{PSII}, functional absorbance cross section serving PSII photochemistry; σ_{PSII}', functional absorbance cross section serving PSII photochemistry measured in the light

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ecology sense. However, NPQ slows down photoinactivation of PSII [17] and is thus a photoprotection mechanism counteracting photoinhibition in the mechanistic sense [25].

Given the large literature using terms "photoinhibition", "photoinactivation" and "photodamage" in different ways, it is not wise to try to fix the meanings of words. Instead, it is important that each study defines the terms it is using. In the present contribution, we will use the term "photoinactivation" to describe the reaction(s) that lead to loss of active PSII centers, such that degradation and synthesis of the D1 protein are required for recovery. Net inactivation occurs if photoinactivation is faster than repair, and the pool of active PSII centers stabilizes if the rates are equal. Net photoinactivation can be provoked either by an increase in incident light or by a decrease in the counteracting PSII repair rate [48].

1.2. Measurement of photoinhibition

A photoinactivated PSII center has lost the ability to mediate photochemical transfer of electrons from water to the plastoquinone pool. The net loss of active PSII centers can be measured by monitoring the light-saturated rate of oxygen evolution using an artificial electron acceptor. In some organisms like cyanobacteria, artificial electron acceptors can be used in vivo but in higher plants, oxygen evolution measurements of PSII activity must be done by isolating thylakoid membranes.

For most studies of phytoplankton, the preferred method is to monitor changes in the photochemical yield of the PSII pool over time by measuring the ratio of variable chlorophyll (Chl) *a* fluorescence (F_V) to maximum fluorescence (F_M). Under a set of relatively general assumptions, the parameter F_V/F_M is equal to the maximum photochemical yield of PSII [49]; see [50–52] for recent reviews of chlorophyll fluorescence. F_V/F_M works well for plants with Chl *a*/*b* antenna systems in which in vivo fluorescence is dominated by emission from Chl *a* associated with PSII. In phytoplankton, with their diverse antenna systems and thylakoid configurations, F_V/F_M must be interpreted with more caution [36,52]. Taxonomic differences in F_V/F_M can result from contributions to the minimum fluorescence (F_0) from pigments outside PSII (see e.g. [53]). Due to the fluorescence from such pigments, the measured values of F_V/F_M may depend on the excitation and emission wavelengths (S.G.H. Simis, M. Babin, Y. Huot, L. Metsamaa, J. Seppälä, unpublished data).

A common way to measure F_V/F_M is to use a weak, sub-actinic modulated measuring beam to measure F_0 and then superimpose a multiple turnover saturating flash to provoke F_M. The physiological particularities of different taxa may require specific sequences of dark, actinic light and treatments with inhibitors like 3-(3,4-dichlorophenyl)-1,1-dimethylurea (see [53]). Division of F_V/F_M by F_0 yields the parameter $1/F_0$-$1/F_M$ which has also been used to quantify photoinhibition [54–56]. The $1/F_0$-$1/F_M$ parameter has the advantage of being independent of NPQ, as $1/F_0$-$1/F_M$ is only influenced by the rate constants of photochemistry and fluorescence. In bio-oceanography studies of phytoplankton in natural waters, F_V/F_M is commonly measured from a fluorescence rise profile provoked either by a single turn-over flash, or by a cumulative train of sub-saturating flashes [36,57]. Fluorescence rise profiles have the additional benefit of supporting estimation of the functional absorbance cross section for PSII photochemistry, σ_{PSII} [58]. Many other methods of measuring PSII activity, including thermoluminescence [59] and reduction of dichloroindophenol [4,60], can also be used to measure the loss of PSII activity via photoinactivation.

Fluorescence and oxygen evolution measurements produce similar but not identical results (see e.g. [53]). Although fluorescence measured from a plant leaf is emitted by a thin surface layer of chloroplasts, the percent photoinhibitory decrease in F_V/F_M closely resembles the loss of oxygen evolution [61–64]. However, diffuse light like sunlight causes a much larger decrease in F_V/F_M than in oxygen evolution in plant leaves [65]. This limitation does not affect the interpretation of F_V/F_M in optically thin phytoplankton suspensions. A more general issue is that light induces both photoinactivation and NPQ. Multiple mechanisms for NPQ are known in different taxa [18–21,23–25], with diverse patterns of response to light, dark and physiological status. In various taxa, the induction and reversal of NPQ can overlap kinetically with the photoinactivation and repair of PSII, and NPQ can provoke changes in measured PSII fluorescence signals. The diverse NPQ mechanisms, however, share the property of reversibility without direct dependence upon protein synthesis.

1.3. Measurement of repair of photoinactivated PSII

The biosynthetic and metabolic processes that regenerate a functional PSII center from a photoinactivated one are termed the PSII repair cycle. The D1 subunit appears to be removed from a photoinactivated PSII center primarily through the progressive action of the ATP-dependent FtsH proteases [12,14,66], with the Deg proteases in an auxiliary role [14]. Protein synthesis replaces the D1 protein which is reassembled with the remaining subunits of PSII [8,14,67,68]. Removal and replacement of the D1 protein are coordinated [10,11] but the dynamics of PSII protein turnover are complex [27] and evidence suggests that under various conditions, or in different taxa, either removal [69] or replacement of the D1 protein [70] can be the rate-limiting step of PSII repair.

For modeling and analysis it is important to distinguish between photoinactivation and repair [6,71–74]. Photoinactivation is driven by incident photons, while repair is more influenced by the physiological state of the organism and by environmental factors like temperature [6,68,70,75,76].

Inhibitors of chloroplast protein synthesis like lincomycin can be used to block PSII repair and thereby unmask photoinactivation. Furthermore, protein synthesis inhibitors allow photoinactivation to be distinguished from NPQ, as NPQ relaxes in the presence of the inhibitors, even though the repair of photoinactivated centers is blocked. Lincomycin does not have serious short-term side effects in the concentrations used to inhibit chloroplast protein synthesis (0.46–2.3 mM) for relatively short incubation periods, but extreme concentrations can affect non-photochemical quenching [77], and prolonged incubations on the time scale of a cellular generation ultimately lead to cell death. A specific advantage of lincomycin is that it does not inhibit mitochondrial protein synthesis [78]. In natural, cold, salt water microcosms, Bouchard et al. [79] found evidence that some phytoplankton communities partially escape from the effects of lincomycin after incubations of 1 h or more, possibly through inactivation or sequestration of lincomycin. Chloramphenicol was used extensively in the past but is now avoided because it acts as an efficient electron acceptor of PSI [80].

2. Results and discussion

2.1. Rate constants for photoinactivation

The simplest model of photoinactivation and recovery was formulated by Kok [37] using two opposing reactions as follows:

$$A \underset{k_{REC}}{\overset{k_{PI}}{\rightleftarrows}} B, \tag{1}$$

where A and B represent the active and photoinactivated photosystems, respectively. The additional simplifying assumption that both photoinactivation and recovery are first-order reactions, leads to the differential equation

$$\frac{d[A]_t}{dt} = -k_{PI}[A]_t + k_{REC}([A]_0 - [A]_t), \tag{2}$$

where $[A]_0$ and $[A]_t$ represent the initial and current amount (concentration) of active PSII, respectively, k_{PI} is the rate constant of photoinactivation (or photoinhibition, using Kok's definition), k_{REC} is the rate constant of recovery, and t is time.

Eq. (2) is integrated to yield [37]

$$[A]_t = [A]_0 \frac{k_{REC} + k_{PI} e^{-(k_{PI} + k_{REC})t}}{k_{PI} + k_{REC}}. \quad (3)$$

In this simple model, recovery is treated as one reaction whose rate is proportional to the number of PSII centers that have lost activity and are thus ready to be regenerated [56,81–83]. In fact, the PSII repair cycle involves multiple sub-processes and the inactive PSII centers can be divided to at least two sub-pools; those still containing a D1 protein and those from which the D1 protein has been cleared. The model of Eq. (1) formally assumes that one of the sub-processes is much slower than the others, so that virtually all photoinactivated PSII centers belong to the substrate pool of this rate-limiting step. Furthermore, the Kok model assumes that the total PSII content is constant, which is violated if the total PSII content fluctuates. In particular, rapid changes in PSII pool size can occur in growing or acclimating phytoplankton [84,85; D. A. Campbell, H. Wu, unpublished data], since cell growth can dilute the PSII pool, or net biosynthesis of PSII can increase the cellular content of active PSII with kinetics comparable to those of photoinactivation under moderately high light.

For more explicit coverage of the general case, more complicated models are required, such as

$$\begin{array}{ccc} & \xrightarrow{k_{PI}} & B \\ A & & \downarrow k_{DEG} \\ & \xleftarrow[k_{SYNTH}]{} & C \end{array} \quad (4)$$

where A represents active PSII, B represents inactive but D1-containing PSII centers, C represents PSII sub-complexes without D1 protein awaiting regeneration, and each reaction is of the first order, with rate constants k_{DEG} for degradation and k_{SYNTH} for synthesis of the D1 protein. Eq. (4) was successfully applied to analysis of singlet oxygen production during photoinhibition in vivo [74]. Analysis of the kinetics of the sub-processes of the repair cycle, or cell populations which grow or acclimate on timescales comparable to photoinactivation rates, may warrant the use of still more complicated models.

If $k_{REC} = 0$, as when repair is blocked, both models described above yield a first-order reaction for loss of active PSII centers

$$[A]_t = [A]_0 e^{-k_{PI} t}. \quad (5)$$

The first-order nature of photoinactivation, when repair is blocked, has been repeatedly verified by research groups using diverse experimental procedures both in vitro and in vivo, across a range of photoautotrophs [60,64,68,69,72,86–88]. Some evidence exists for considerable deviation from first order under prolonged light treatments [89,90]. However, no deviation from first order was seen in similar experiments using oxygen evolution measurements [64].

In low light where repair is active but photoinactivation is negligible (e.g. after a high light treatment), Eq. (2) can be integrated as

$$[A]_t = [A]_0 + ([A]_i - [A]_0) e - k_{REC} t \quad (6)$$

where $[A]_i$ is the concentration of active PSII at the moment when k_{PI} suddenly drops to (approximately) zero. Eq. (3) has been applied to recovery in low light after a photoinhibition treatment, and a good fit with experimental data was obtained for three phytoplankton species [45]. In moderate light where $k_{PI} > 0$, Eq. (3) is appropriate for analyzing recovery after a high-light treatment [56].

Prolonged in vivo photoinhibition treatments in the presence of antibiotics or other chemicals, and especially in vitro treatments of subcellular fractions may cause progressive loss of PSII activity, separate from photoinactivation. This dark inactivation must be measured and taken into account. If dark inactivation follows first-order kinetics, then the two first-order reactions with the same substrate, active PSII, add up as follows:

$$[A]_t = [A]_0 e^{-(k_{PI} + k_{DARK})t}, \quad (7)$$

where k_{DARK} is the rate constant of dark inactivation. In this case, the actual rate constant of photoinactivation is obtained simply by subtracting k_{DARK} from the raw rate constant obtained by fitting the loss of PSII activity to a first-order reaction equation [60]. However, dark inactivation in vitro may follow more complex kinetics [64]. For in vivo experiments over shorter time periods of much less than a cellular generation period, dark inactivation can generally be ignored [69].

Eq. (5) can be linearized as follows:

$$ln\left(\frac{[A]_t}{[A]_0}\right) = -k_{PI} t. \quad (8)$$

Deviations from first order behavior are easy to see in a plot of Eq. (8), and this form also allows the use of simple linear regression for the extraction of k_{PI}. Eq. (5) is preferred if experimental error varies strongly between different time points because individual weighting of data points in curve fitting is simpler if original data are used.

The rate constant of photoinactivation is a convenient measure for comparison of photoinactivation in different samples under the same light level. However, for comparisons across different or changing light levels, it is more convenient to estimate how many PSII centers are inactivated per photon. Two inherently equivalent and interconvertible methods, quantum yield [60,91] and target size [45,69,92–94], have been used for this purpose.

2.2. Quantum yield of photoinactivation

The quantum yield of photoinactivation (or photoinhibition, following the terminology used by Kok and many other authors before the mid-2000s) (Φ_{PI}) is the initial number of PSII centers inactivated per unit time when repair is blocked, divided by the quanta incident on the sample per unit time (flux) (J_i). The loss of active PSII centers with time is not linear (Eq. (3)) but rather follows an exponential decay, and therefore the number of PSII centers inactivated per unit time must be calculated by multiplying the initial amount of active PSII with the initial rate of photoinactivation (k_{PI}).

$$\Phi_{PI} = [A]_0 \frac{k_{PI}}{J_i}. \quad (9)$$

If $[A]_0$ is expressed on an area basis in μmol (PSII) m^{-2}, k_{PI} is expressed in s^{-1} and J_i is expressed in μmol (quanta) $m^{-2}\,s^{-1}$, then Φ_{PI} is a dimensionless number which indicates the probability that a quantum incident upon the sample causes inactivation of a PSII reaction center. In higher plant leaves and isolated thylakoids Φ_{PI} is usually in the range of 5×10^{-8} to 1×10^{-7} [4,60,91].

For comparison of optically similar samples, a relative quantum yield can be defined simply as

$$\Phi_{PI}(rel.) = \frac{k_{PI}}{J_i}. \quad (10)$$

If J_i is expressed in μmol (quanta) m^{-2} s^{-1} and k_{PI} in s^{-1}, then Φ_{PI} (rel.) has units of m^2 μmol $(quanta)^{-1}$.

Φ_{PI} can also be expressed per quantum absorbed by the sample; this is the preferred form if absorption of incident light differs strongly among the samples:

$$\Phi_{PI}(absorbed) = [A]_0 \frac{k_{PI}}{J_a}, \quad (11)$$

where J_a is the flux light absorbed by the sample, expressed as J_i x absorptance, where absorptance is the dimensionless fraction of incident light absorbed by the sample (as opposed to light reflected or transmitted).

If $[A]_0$ is expressed on a projected area basis in μmol (PSII) m^{-2}, k_{PI} is expressed in s^{-1} and incident light in μmol (quanta) m^{-2} s^{-1}, then Φ_{PI} (absorbed) is a dimensionless number which indicates the probability that a photon absorbed by the sample causes inactivation of a PSII reaction center.

Measurements of Φ_{PI} can be used for spectral analyses aiming at identification of photoreceptors, or aiming at definitions of biological weighting factors for different wavelengths of light [95]. Φ_{PI} can also be expressed per quantum absorbed by a particular photoreceptor driving photoinactivation. For example, if photoinactivation is assumed to be driven by light received through the antenna of PSII, then J_i can be estimated in terms of quanta delivered to PSII:

$$\Phi_{PI}(\sigma_{PSII}) = [A]_0 \frac{k_{PI}}{\sigma_{PSII} J_i}, \quad (12)$$

where σ_{PSII} ($Å^2$ $quanta^{-1}$) is the functional absorption cross-section of PSII [58], a measure of the probability that an incident quantum provokes PSII photochemistry. A similar receptor-specific Φ_{PI} can be calculated by assuming that the manganese ions of PSII [4,5] or both manganese and chlorophyll [5,17,64,74] act as photoreceptors of photoinactivation. Receptor-specific Φ_{PI} values can be used for theoretical comparisons but are not suitable for robust modeling. Measurement of photoinhibition by assuming dependence on light absorbed by PSII antenna may be particularly misleading, as most earlier results from higher plants show little or no dependence of photoinactivation on antenna size in vivo [96] or in vitro [72]; however, see [94] for data suggesting antenna size dependence of photoinactivation. Our data from phytoplankton [69,87,88] show only weak correlations between photoinactivation and PSII effective antenna size. For example, in a panel of picocyanobacteria and *Prochlorococcus* strains, σ_{PSII} for blue light varied from 20 to 290 $Å^2$ $quanta^{-1}$ while k_{PI}, measured under blue light, varied only slightly across the strains [69].

2.3. Target size of photoinactivation

Extensive experimental data [60,86,92,97,98] show that k_{PI} is usually directly proportional to J_i. Photoinactivation provoked by nanosecond laser pulses is an exception [59,99]. The direct proportionality can be expressed as reciprocity between exposure time and light intensity by rearranging Eq. (10)

$$k_{PI} = \Phi_{PI}(rel.) \times J_i. \quad (13)$$

Substituting k_{PI} in Eq. (5) with the right side of Eq. (13) yields

$$[A]_t = [A]_0 e^{-\Phi_{PI}(rel.) J_i t}. \quad (14)$$

In this formulation the relative quantum yield Φ_{PI}(rel.) can be called the target size [92], which we term σ_i. Thus, $k_{PI} = \sigma_i \times J_i$. In calculation of σ_i, note that J_i times t is equal to the number of quanta incident on the sample over time t (N_t), and thus

$$[A]_t = [A]_0 e^{-\sigma_i N_t}. \quad (15)$$

σ_i is usually expressed in m^2 or in square Ångströms ($Å^2$) per quantum. In this form, σ_i can be understood as the functional size of a target that a photon must hit to cause photoinactivation. A small σ_i indicates that the probability of a hit is small, and larger σ_i indicates higher susceptibility to photoinactivation per incident photon. However, no actual molecular entities are present in the derivation of σ_i, and therefore the target size is a fully formal entity without any connection to a physical area, similar to σ_{PSII}. σ_i is the functional absorption cross-section of the photoreceptor(s) provoking photoinactivation.

The σ_i concept is particularly useful for ecological and ecophysiological modeling, since the ratio $\sigma_{PSII}/\sigma i$ expresses the number of photons delivered to PSII per photoinactivation event (H. Wu & D. A. Campbell, unpublished data), which varies across taxa and growth conditions. If fluorescence data allowing the measurement of σ_{PSII} in the light (σ_{PSII}') is available, then $(\sigma_{PSII}' \times q_P)/\sigma i$ expresses the ratio of photochemical charge separations per photoinactivation event. Since to maintain PSII function, each round of photoinactivation must be countered through PSII repair, the ratio σ_{PSII}/σ_i gives a baseline estimate of the photochemical return on investment per cycle of PSII repair [9].

Like Φ_{PI}, σ_i could in principle be defined using incident quanta, absorbed quanta, or quanta absorbed by a particular photoreceptor. In practice, only incident quanta are used. Both Φ_{PI} and σ_i vary depending upon the spectral profile of the incident light but for a given spectral quality, σ_i applies for a range of incident light levels. Thus, the key difference between the concepts of Φ_{PI} and σ_i is that σ_i can be measured without measuring the number of PSII reaction centers in the sample, whereas measurement of a true Φ_{PI} requires the knowledge of the number of PSII centers. Furthermore, for Φ_{PI} the most convenient expression of PSII content is on an area basis, which is well suited to flat leaf geometry, but less convenient for phytoplankton suspensions. σ_i and Φ_{PI} (rel.) are interconvertible entities, and therefore always lead to the same conclusions.

In phytoplankton ecology, it may be difficult to use lincomycin to obtain k_{PI}, σ_i or Φ_{PI} values, and trapping cells in bottle treatments introduces errors and distortions compared to freely mixing phytoplankton communities. A mathematical model [45] extends the Kok model of photoinactivation and repair to allow the estimation of σ_i and k_{REC}, even under variable illumination. In the model, a time series of PSII activity (A) is fitted to the equation

$$[A](t) = [A]_1 + \int_{t_1}^{t} \{k_{REC}[A]_0 - (1 + \sigma_i J_i(t))[A](t)\} dt, \quad (16)$$

where t_1 is the time when activity $[A]_1$ was measured and $[A]_0$ is the maximum value of A. Eq. (16) is a solution of a form of Eq. (2) in which the constant k_{PI} is replaced with the variable $\sigma_i J_i$, and thus Eq. (16) integrates changes in [A] over a period of fluctuating light to calculate the combined effects of photoinactivation and repair. In constant light, Eq. (16) is equivalent to Eq. (3), but with k_{PI} replaced by $\sigma_i J_i$, as outlined in Eq. (13). The model was shown to successfully predict changes in F_V/F_M in phytoplankton under various mixing regimes in a lake [45].

2.4. Application to experimental data

2.4.1. Literature values

For unicellular phytoplankton suspension cultures, σ_i parameterization of photoinactivation has been useful for picocyanobacteria [69], for centric diatoms [88], for picoprasinophytes [87] and for pelagophytes, rhodophytes and coccolithophores (Six, McCarthy, Loebl, Campbell, unpublished data). Earlier, Nagy et al. [92] studied *Synechocystis*, a freshwater cyanobacterium, and found that under white light, σ_i is 4.15×10^{-25} m^2 quanta^{-1} (4×10^{-5} Å^2 quanta^{-1}). In comparison, Six et al. [69] measured a σ_i value of 9×10^{-5} Å^2 quanta^{-1} for marine picocyanobacteria and *Prochlorococcus* strains under blue light; this difference is reasonable since blue light, like UV-A radiation, is more efficacious than white light in provoking photoinactivation [4,5,17,86,100].

We now present representative analyses of photoinactivation and repair, and compare parameterizations of these processes. In all experimental data, changes in the pool of functional PSII were monitored using F_V/F_M. Results from *Ostreococcus tauri* [101] a prasinophyte marine picophytoplankter with a Chl *a*/*b* antenna, are compared with *Thalassiosira pseudonana* [102] a small centric marine diatom with a Chl *a*/*c* antenna. Both thrive under conditions of variable light and relatively high nutrients, and both are widely used models for phytoplankton physiology.

2.4.2. Fixed time treatments or time series measurements

To track photoinactivation one can choose between fixed time treatments with endpoint measurements, or repeated measures from a sample over a time series. If the PSII repair cycle is allowed to run, i.e. in the absence of lincomycin, a time series is the only option because fixed-time measurements would not show whether equilibration between photoinactivation and repair has been reached. In the presence of lincomycin, both approaches can be applied. Due to the reciprocity between exposure time and incident light level in photoinactivation of PSII, determination of Φ_{PI} or σ_i can in principle be done either by exposing a sample to a fixed light level across a time course, or by exposing equivalent samples for a fixed time across a range of incident light levels. The most reliable way of measuring Φ_{PI} or σ_i is to use both several time points and several light intensities [60], but this approach is time-consuming. We are inclined to recommend time series measurements because only a time series can show whether the expected kinetic model was realized, and whether confounding effects such as induction of NPQ are present. If samples are growing or acclimating rapidly, or for ship-board measurements, a fixed time period may be a more appropriate experimental design.

2.4.3. A plot of F_V/F_M versus time in the presence or absence of lincomycin

When F_V/F_M is used to measure photoinactivation in the presence of lincomycin (Fig. 1, solid symbols), the formula for curve fitting is Eq. (5), with $[A]_0$ replaced by F_V/F_M taken at time zero and $[A]_t$ with F_V/F_M measured repeatedly along the time course, as follows

$$\frac{F_V}{F_M} = \left(\frac{F_V}{F_M}\right)_{t=0} \times e^{-k_{PI}t}, \text{or} \quad ln\left(\frac{F_V}{F_M}\right) = ln\left(\frac{F_V}{F_M}\right)_{t=0} - k_{PI}t. \qquad (17)$$

In low or moderate light where PSII activity remains at the control level when PSII repair is active (absence of lincomycin), k_{REC} cannot be estimated. When an intact organism with active PSII repair is brought under strong light, PSII activity may first decrease but then stabilize to a light-intensity dependent level $[A]_\infty$ ([82]; Fig. 1B). In this steady state, the rates of inactivation and repair are equal, $k_{PI}\times[A]_\infty = k_{REC}\times(1\text{-}[A]_\infty)$, and if k_{PI} has been measured in the absence of repair, the rate constant of repair can be calculated from Eq. (3) as

$$k_{REC} = \frac{[A]_\infty/[A]_0 \times k_{PI}}{1-[A]_\infty/[A]_0} = \frac{\{F_V/F_M\}_\infty/\{F_V/F_M\}_0 \times k_{PI}}{1-\{F_V/F_M\}_\infty/\{F_V/F_M\}_0}. \qquad (18)$$

In Fig. 1B, note the initial decline in F_V/F_M over the first 900 s, occurring in cells with and without active PSII repair. Thereafter, cells with repair (open symbols) achieve near-stabilization as PSII repair is induced to fully counter photoinactivation, while cells without repair (closed symbols) continue to decline. The value of k_{REC}, obtained for *T. pseudonana* from Eq. (18), was very close to the value obtained by fitting the kinetic data to Eq. (3).

Full stabilization is often not reached but instead, net photoinhibition simply proceeds more slowly in the absence than presence of lincomycin (Fig. 1A), or PSII function even recovers after an initial drop. In these cases Eq. (18) cannot be used, but fitting the kinetics to Eq. (3) is still practical. Application of Eq. (3) leads to higher k_{PI} and smaller k_{REC} for *O. tauri* than for *T. pseudonana* (Table 1).

In some phytoplankton studies, achieved rates of PSII repair have been estimated from the difference between rates obtained from exponential fits of PSII activity in samples with repair (−lincomycin)

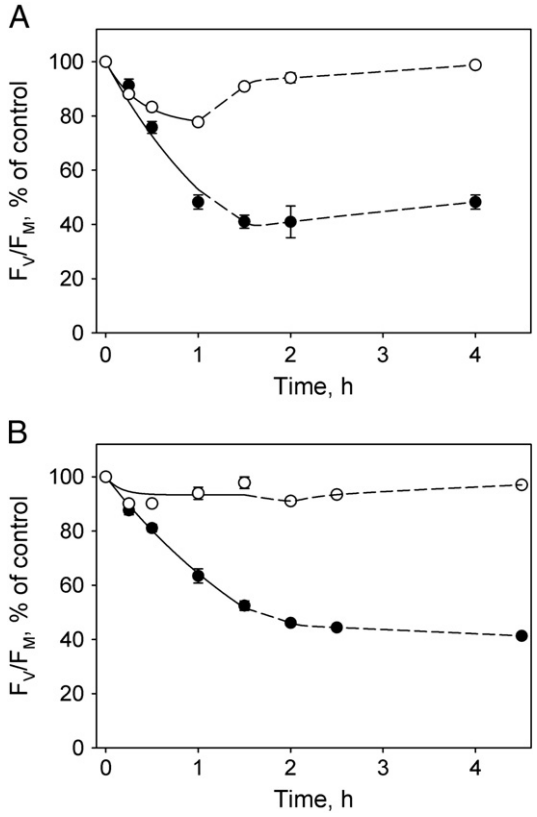

Fig. 1. F_V/F_M measurements from *Ostreococcus tauri* (A) and *Thalassiosira pseudonana* (B) cultures, grown at 30 μmol photons m^{-2} s^{-1} and then shifted the cultures to the PPFD of 300 (A) or to 450 (B) μmol m^{-2} s^{-1} (indicated with solid line) then back to 30 μmol photons m^{-2} s^{-1} for recovery (dashed line). Both the high-light treatment and the recovery treatment were done in the absence (open symbols) or presence (solid symbols) of lincomycin. F_V/F_M was measured after 10 min of dark incubation. Each data point represents an average of 4–5 independent experiments and the error bars, drawn if larger than the symbol, show SE. The solid lines show the best fit to Eq. (3), with k_{PI} and k_{REC} values listed in Table 1; $k_{REC}=0$ in the presence of lincomycin. The control values of F_V/F_M were 0.63 ± 0.015 for *O. tauri* and 0.69 ± 0.002 for *T. pseudonana*.

Table 1
Parameterization of photoinhibition and recovery with recommended methods using photoinhibition data from *Ostreococcus tauri* and *Thalassiosira pseudonana* and from the higher plant *Cucurbita pepo*. In calculation of k_{REC} and A_∞, data measured in the presence and absence of lincomycin were used; all other parameters were calculated from data from data measured in the presence of lincomycin. n.d. = not determined.

Species	Data	PPFD, μmol m^{-2} s^{-1}	k_{PI}, s^{-1}	k_{REC}, s^{-1}	A_∞, % of A_0	σi, $Å^2$ quanta^{-1}	Φ_{PI}
O. tauri	Figs. 1A, 3A	300	1.8×10^{-4}	5.8×10^{-4}	77	1.0×10^{-4}	n.d.
T. pseudonana	Figs. 1B, 3B	450	1.2×10^{-4}	1.8×10^{-3}	93	4.5×10^{-5}	n.d.
C. pepo	Tyystjärvi and Aro 1996	6.5–1500	0.0014–0.33	n.d.	n.d.	1.0×10^{-5}	7×10^{-8}

and without repair (+lincomycin) (e.g. [69,87,88,103]). If the control treatment with repair (−lincomycin) maintains steady PSII activity, while the treatment without repair (+lincomycin) declines through photoinactivation, the achieved rate of PSII repair is equal in magnitude to the initial photoinactivation rate. This estimator of the achieved PSII repair rate for a condition is not a simple rate constant and is not generally convertible to the Kok type k_{REC} formulation (Fig. 2).

PSII repair rates have also been inferred from rates of recovery of PSII function when light intensity decreases or UV exposure ends [40,56,75,104]. Fig. 1 (dotted lines) shows an example. In nature, this occurs during sunset or when phytoplankton mix from the upper to lower water column. The method is applicable to in situ measurements but k_{REC} reflects regulated biochemical processes that can depend on light intensity or other conditions associated with high light, and therefore this method may not correctly predict the k_{REC} value actually achieved during exposure to high light.

2.4.4. A plot of F_V/F_M versus cumulative incident photons, in the presence of lincomycin

For Fig. 3, Eq. (15) was used to fit the results, assigning values of F_V/F_M to $[A]_0$ and $[A]_t$ in the same way as in Eq. (17). Assuming that a photon-dose reciprocity holds across ecophysiologically reasonable irradiance levels, a σ_i determined under a particular light level and quality converts to k_{PI} if multiplied by the incident irradiance level of interest. The cumulative incident photons per area can be measured directly or estimated by multiplying measured photon flux density by elapsed time. A σ_i value can be determined even across temporally varying light levels by measuring cumulative photons per area or by intermittently monitoring light and interpolating to estimate cumulative photons.

To date we have done most of our photoinactivation trials on phytoplankton under moderately high blue light, for both conceptual reasons, since blue light often dominates in marine situations, and for technical reasons, since our system for measuring PSII functional antenna sizes (σ_{PSII}) uses blue light (Satlantic FIRe flash induction fluorometer, Halifax, Canada). We are then able to directly compare the probability of exciton delivery to PSII with the probability of PSII photoinactivation, by dividing σ_{PSII}/σ_i . For studies of the mechanisms of photoinhibition, it is useful to measure photoinactivation in a range of taxa under different wavelength ranges, since the absorbance spectra of the photoreceptor(s) of photoinactivation determine σ_i and Φ_{PI} at each wavelength [4,5,86].

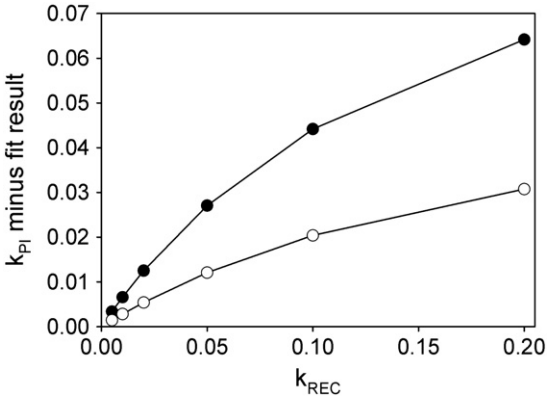

Fig. 2. Comparison of simulation results for two parameterizations of PSII repair.The X-axis plots k_{REC} values generated using Eq. (18). The Y-axis plots the difference between the rate constants obtained by applying an exponential fit to PSII function from samples measured in the presence and absence of lincomycin. Photoinactivation and repair of PSII were simulated using Eq. (3) with $k_{PI}=0.05$ (open circles) and with $k_{PI}=0.1$ (solid circles). The data were linearized using Eq. (7).

2.5. Practical guidelines for measurement of photoinhibition

The following instructions are written to gain insight into photoinhibition when the work focuses on analyses of ecological interactions or characterization of a mutant.

1. If you can easily isolate thylakoids before and after illumination, then measure the light-saturated rate of oxygen evolution, using a quinone electron acceptor. If rapid isolation of active thylakoids is not a good option, then measure F_V/F_M. Preferably, do both. Remember to dark adapt your samples before measuring F_V/F_M. The appropriate dark incubation duration depends upon the taxa and the physiological condition of the cells. Do pilot tests to detect dark relaxation (or even dark induction) of NPQ.
2. If your data can form a time series, as when starting from a control condition and illuminating to cause photoinhibition, then measure several data points, not just before illumination and after illumination. This can help uncover confounding effects like induction of NPQ.
3. Illuminate in the presence of lincomycin and fit your data to Eq. (5) to obtain k_{PI}. To parameterize a rate constant for recovery, do measurements without lincomycin, use Eq. (3) for fitting but fix k_{PI} to the value obtained with lincomycin.
4. If you want to predict behavior in different light intensities, then assume that k_{PI} is directly proportional to light intensity if other conditions do not change.
5. The quantum yield measures σ_i and Φ_{PI} are essentially equivalent but σ_i is simpler to measure.

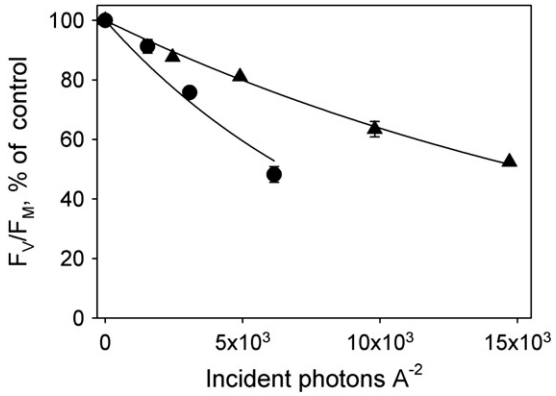

Fig. 3. The F_V/F_M values from Fig. 1 plotted versus cumulative incident photons, in the presence of lincomycin for *Ostreococcus tauri* (circles) and for *Thalassiosira pseudonana* (triangles). The lines show the best fit to Eq. (14) with σi values listed in Table 1.

3. Concluding remarks

PSII is the photochemical engine of the biosphere [105], and yet it is an unstable complex that must be continually regenerated. The rate constant, quantum yield and target size parameterizations for photoinactivation are interconvertible, each with experimental and operational advantages for particular purposes. Quantum yields are readily defined for planar optical situations such as leaves. Target sizes are more useful for three-dimensional suspensions of phytoplankton. PSII repair is a multi-step process, with sequential steps through multiple pools of repair cycle intermediates. Its kinetics can be parameterized with various mathematical methods, depending upon the nature of the available data and the purpose of the analysis.

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