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Chlorophyll fluorescence measurements for assessment of primary production in aquatic ecosystems - the basics

Abstract

For many investigations on aquatic ecosystems it is desirable to assess the primary production with high resolution in time and space. Chlorophyll (Chl) fluorescence measurements have the potential to become the method of choice for such investigations. The biophysical and physiological background, the measuring techniques and the limitations of Chl fluorescence measurements as a tool for investigations on primary production in aquatic ecosystems are reviewed. The following aspects are discussed: contribution of Photosystem I fluorescence, variability of the Photosystem II fluorescence, photochemical quenching of Chl fluorescence (q_P), non-photochemical quenching (energy quenching, q_E; photoinhibitory quenching, q_I; state transitions, q_T), measuring technique, modulated fluorescence, special light programs (saturation pulse techniques using s or µs-pulses; 1 Hz-method); multi-color excitation and species-differentiation; basics of the use of fluorescence techniques for assesment of primary production.

Abbreviations

Chl, chlorophyll; ETC, electron transport chain; F_0, fluorescence yield after dark-adaptation, oxidized Q_A in all PS II; F_M, fluorescence yield after dark-adaptation and sudden application of saturating light, reduced Q_A in all PS II; F_0', fluorescence yield of light-adapted organisms for oxidized Q_A in all PS II ; F_M', fluorescence yield of light-adapted organisms for reduced Q_A in all PS II; $F_V = F_M - F_0$; $F_V' = F_M' - F_0'$; LED, light-emitting diode; LHC, light-harvesting chlorophyll-protein complex with molecular weights of 20-30 kDa; PAM, special fluorometer based on Pulse Amplitude Modulation; PS, photosystem; Q_A, primary quinone acceptor of PS II; REE, rapid exciton equilibration.

1 Introduction

Today, the chlorophyll fluorescence of algae and cyanobacteria can be accurately measured even on ultra-dilute suspensions of algae (Chl content below 100 ng/L). Precise *in-situ* measurements (e.g., sample collection and fluorescence measurement on a research vessel) or even *in-vivo* investigations (e.g., handy instruments controlled by divers or fully automated diving probes) are feasible using commercially available instruments. By means of special illumination programs reasonable estimates are obtainable on: (i) the amount of primary producers (i.e. algae and cyanobacteria), (ii) the actual rate of primary production, (iii) the 'fitness' of the primary producers (light stress effects, influence of toxic substances, etc.). However, it is rarely possible to obtain all this information simultaneously. Furthermore, heterogeneous samples (mixture of dissimilar species) may endanger the straightforward interpretation of fluorescence data and may require the use of instruments using a multi-color excitation technique. Thus, before starting a research program involving fluorescence measurements its is advisable to consider the strengths and limitations of the technique, to clarify the objectives and, then, to select the most appropriate instrumentation and experimental approach. The easiness of obtaining large heaps of fluorescence data and the knowledge on potential relations to various aspects of photosynthesis is seductive; not infrequently conclusions have been drawn on basis of misunderstood fluorescence data. Background knowledge on the involved biophysical basics and on the measuring technique is required to avoid experimental artefacts and to prevent misleading interpretations which might discredit this fascinating and powerful technique.

2 The variable chlorophyll fluorescence

In intact organisms the yield of Chl fluorescence is variable. In case the photosynthetic electron transport chain (ETC) is in a fully oxidized state (e.g. in dark-adapted organisms), the fluorescence yield is minimal (F_0-fluorescence); in case the ETC is in a fully reduced state (e.g. due to illumination with saturating light), the fluorescence yield is maximal (F_M-fluorescence). It is mainly a variability in the fluorescence yield; the shape of the emission spectrum changes only slightly (Fig.1).

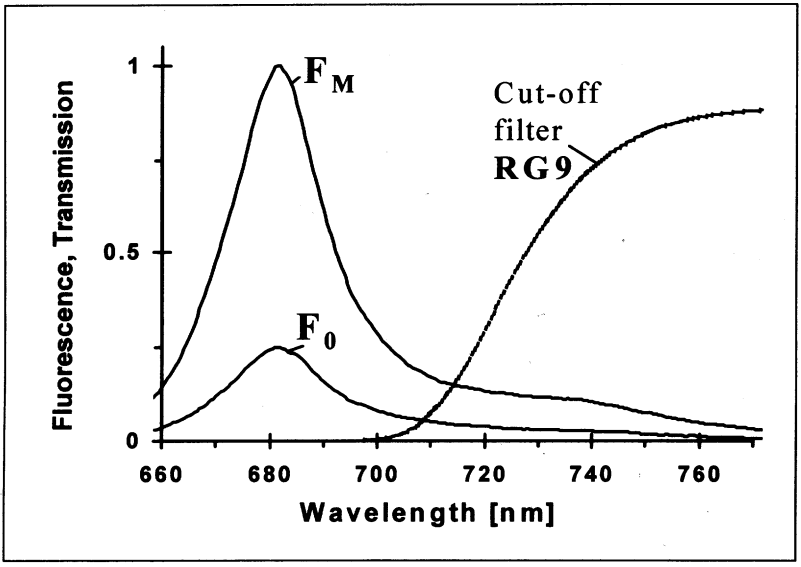

Fig. 1 Fluorescence emission spectra of a cell suspension of the green alga *Scenedesmus obliquus* in der F_M-state and in the F_0-state (collected at 17 °C by Dr. I. Heinze, Marburg). Additionally, the transmission characteristics of a long-pass filter (Schott glas RG9) are shown. The RG9 is often used as a cut-off filter for fluorescence detection in fluorometers which employ a red measuring-light LED. The F_0-to-F_M increase is due to the variability of the PS II fluorescence. In the F_0-state, roughly 5% of the emission at 683 nm stems from PS I; for wavelengths greater than 700 nm the PS I contribution may be as high as 30% (in green algae) or even higher (in cyanobacteria).

The variable fluorescence of plants and photosynthetic bacteria has been subject of research for decades. Instead of attempting an exhaustive review on this subject, a highly simplified extract of the results essential to the use of fluorescence measurements for assessment of primary production is presented; for a mechanistically more accurate discussion, see DAU 1994a. Fluorescence emission by anoxygenic photosynthetic bacteria is not considered. To avoid an overwhelming reference list, only a small selection of the relevant original works and review articles is cited. Numerous references on earlier works can be found in various chapters of GOVINDJEE et al. (1986); for more recent references see review articles of KRAUSE & WEIS (1991), DAU (1994a, 1994b), SCHREIBER et al. (1994) and GOVINDJEE (1995).

PS I fluorescence

In oxygenic photosynthesis fluorescence photons are emitted by the antenna pigments of PS II and PS I. The yield of the PS II fluorescence varies between about 2% (F_0-level) and 10% (F_M-level) as discussed further below in more detail. For reasons which are not fully understood, the PS I fluorescence yield is significantly lower (at least at temperatures above 0°C, see legend of Fig. 1) and not variable. Therefore, often the contribution of PS I-chlorophylls to the fluorescence emission of intact organisms is assumed to be negligible. However, in particular if fluorescence emission is detected at wavelengths greater than 700 nm, neglect of PS I contributions may not be a reasonably good approximation.

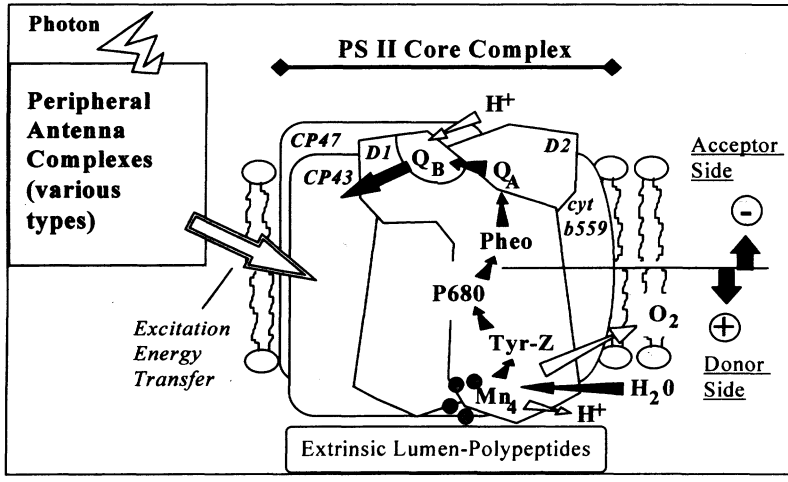

Fig. 2 The Photosystem II.
The type of the peripheral antenna is species-dependent. The core complex consists of a core antenna (CP 43 and CP 47) containing 35-80 Chl *a* (the precise number of Chl *a* per CP 43 and CP 47 in intact organisms is unknown) and the D1/D2/Cyt_{b559} reaction-center, where the light-induced electron transfer reactions proceed.

PS II light-harvesting and photochemistry.

The PS II consists of 'peripheral' antenna complexes and the PS II core complex (Fig. 2). The biochemical and spectroscopic characteristics of the peripheral antenna are species-dependent. For example, in most cyanobacteria the membrane-attached phycobilisomes serve as

peripheral PS II antenna, whereas in green algae membrane intrinsic chlorphyll *a/b*-protein complexes, the so-called LHC's, fulfil the same function. The species dependent differences in the peripheral PS II antenna system affect the color of the organisms which traditionally serves as a taxonomic criterium for algae. Thus, there are various types of peripheral PS II antenna which are each characteristic for a distinct group of organisms (*Chlorophyceae, Cyanophyceae, etc.*). In contrast to the peripheral PS II antenna, all relevant characteristics of the PS II core complex are evolutionary fully conserved (see Fig. 2). For this reason, various molecular mechanisms related to the variability of the PS II fluorescence are a common trait of all organisms of oxygenic photosynthesis. This holds in particular for the most prominent source of fluorescence variability, the so-called photochemical quenching of the PS II fluorescence.

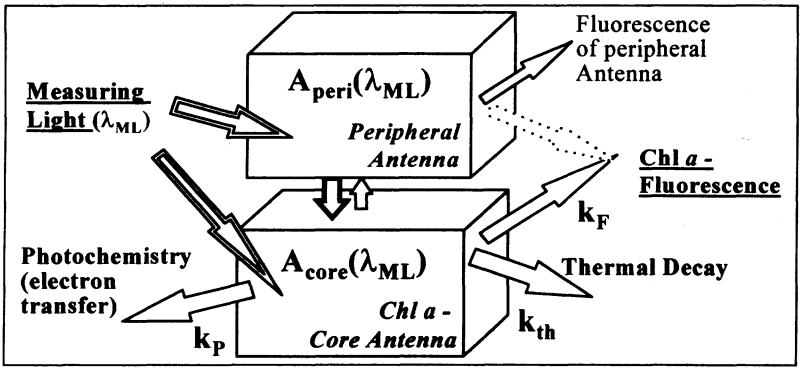

Fig. 3 Creation and decay of excited states in the PS II. An excited state is created by absorption of a photon; $A_{peri}(l_{ML})$ and $A_{core}(l_{ML})$ are the absorption coefficients (precisely spoken: the absorption cross-section at l_{ML}) of the peripheral and the core antenna, respectively. Due to rapid hopping between antenna pigments a so-called 'exciton equilibrium' is established. Then the excited state decays *via* four major pathways: (1) initiation of charge separation (electron transfer), (2) thermal dissipation (non-radiative decay), (3) emission of a fluorescence photon (radiative decay), (4) formation of triplet states (intersystem crossing). Triplet-formation is of particular relevance with respect to initiation of photodamage, but due to its extremely low yield this decay process is, in the present context, negligible. Due to REE occurence the rate constants for excited state decay (k_P, k_{th}, k_F) are independent of the excitation wavelength (i.e. l_{ML}, the wavelength of the measuring light). In case the primary quinone acceptor is singly reduced (F_M-state), photochemistry is inhibited ($k_P = 0$) and the fluorescence yield is maximal. For non-reduced Q_A (F_0-state), the fluorescence yield is minimal.

PS II fluorescence

The photon absorption event in the peripheral antenna or the PS II core complex is typically followed by 'rapid exciton equilibration' (REE, see DAU, 1994a; DAU & SAUER, 1996). This means that the excited state (or the exciton) is moving energetically down-hill to Chl *a* and then its is rapidly hopping back and forth between these chlorophylls until eventually (and clearly later) the excited state decays (see Fig. 3). Due to the random hopping the exciton 'forgets' where it has been created by an absorption event. Consequently, the chlorophyll fluorescence yield and emission spectrum are essentially independent of the excitation wavelength. Due to REE occurence it is usually sufficient to measure the PS II fluorescence at a single emission wavelength (or wavelength range); based on REE relatively uncomplicated models can be used for interpretation of fluorescence data (DAU, 1994a).

For the model depicted in Fig. 3, the fluorescence signals detectable in the F_0- and F_M-state are reasonably well described by (N_{PSII}, number of PS II in the sample volume; c_{instr}, a fluorometer-dependent constant; I_{ML}, intensity of the measuring light):

$$F_{0/M} = N_{PSII}\ c_{instr}\ I_{ML}\ \{A_{peri}(I_{ML}) + A_{core}(I_{ML})\}\ F^F_{0/M} \quad (1)$$

with $$F^F_0 = k_F / \{k_{th} + k_F + k_P\} \quad (2)$$

and $$F^F_M = k_F / \{k_{th} + k_F\}\ . \quad (3)$$

For the quantum yield of the photochemical reaction (here Q_A reduction) we obtain:

$$F^P_0 = k_P / \{k_{th} + k_F + k_P\} = F_V' / F_M' \quad (4)$$

with $$F_V' = F_M' - F_0' \quad (5)$$

Following the nomenclature of VAN KOOTEN & SNELLE (1990), F_M' and F_0' are used for organisms which are not dark-adapted, whereas F_M and F_0 are used exclusively in the case of complete dark-adaptation. The light-values, F_M'- and F_0', may differ from the dark-values, F_M- and F_0, due the non-photochemical quenching discussed below. Often the experimental protocol does not allow for a clear-cut distinction between light- and 'true' dark-values. Equations 1-5 hold for light- and dark-values.

The parameter F_V/F_M is rarely greater than 80%. There is, however, considerable evidence that the 'real' quantum yield for Q_A reduction can exceed 90%. In the model of Fig. 3 the reversibility of the primary charge separation is neglected. One consequence of this approximative treatment is that by using Eq. 4 the value for the Q_A-reduction quantum yield is underestimated (DAU 1994a).

Photochemical quenching (q_P)

Because the PS II fluorescence yield is significantly diminished in comparison to (isolated) chlorophyll in organic solvents, the variability of the PS II fluorescence yield is usually discussed as a fluorescence quenching of variable strength. 'Photochemical quenching' denotes a decrease in the fluorescence yield which results from excited state decay by initiation of a photochemical reaction. In PS II the initiated photochemical reaction is the transfer of an electron from the primary chlorophyll donor, P680, *via* the primary pheophytin acceptor, Pheo, to the quinone acceptor, Q_A, which becomes singly reduced by this process. If Q_A is already reduced, this process cannot take place and the photochemical quenching is assumed to be absent. Thus, for reduced Q_A the fluorescence yield is maximal (F_M) whereas for oxidized Q_A it is minimal (F_0). In intact organisms illuminated with light of sub-saturating intensity typically Q_A is reduced in a fraction of all PS II. Consequently, the measurable fluorescence yield, F_S, is determined by the percentage of PS II with reduced Q_A, and the extent of photochemical quenching is described by a so-called quenching coefficient:

$$q_P = \{F_M'-F_S'\} / \{F_M'-F_0'\} \quad (6)$$

Per definitionem, the photochemical quenching coefficient (q_P) is zero in case all PS II are in the F_M-state (all Q_A reduced). For a more accurate discussion, however, it needs to be considered that the excited state decay by initiation of pheophytin reduction can proceed even in the presence of reduced Q_A; for details see DAU 1994a.

Based on the model sketched in Fig. 3, we obtain for the rate of PS II electron flux (which is, more or less closely, related to the rate of CO_2-fixation):

$$R_{ET} = q_P \, F_P^{0\prime} \, I_{abs} = F_P I_{abs} \quad (7)$$

with $$F_P^{0\prime} = \{F_M'-F_0'\} / F_M' \quad (8)$$

and $$F_P = \{F_M'-F_S'\} / F_M' \quad (9)$$

where I_{abs} denotes the rate of photon absorption by all the PS II in the sample volume (thus, I_{abs} is proportional to the light intensity).

Non-photochemical quenching (q_N)

Various quenching mechanisms which are not directly related to changes in the Q_A redox state affect the PS II fluorescence. The three most prominent ones are: energy quenching (q_E), photoinhibitory quenching (q_I) and state transition quenching (q_T) (see KRAUSE & WEIS 1991; DAU 1994b). Minor effects include quenching by oxidized plastoquinone (of the PQ-pool), fluorescence increase due to an effect of the thylakoid membrane voltage on charge separation reactions, quenching by oxidized P680 and quenching of excited Chl-singlet states by carotenoid triplets (see DAU 1994a, GOVINDJEE 1995). Resolution of various quenching effects by fluorescence measurements on intact organisms is often possible by utilization of differences in the rates for formation or decay.

Thylakoid energization (q_E)

The q_E-formation parallels the acidification of the thylakoid lumen (therefore also denoted as 'pH-dependent quenching'). Upon illumination q_E is built up within a few seconds (3-30 s); q_E relaxes in the dark in about 30-120 s (the 'rapidly-reversible non-photochemical quenching'). The F_M value is strongly quenched (by 10-60%); the F_0-quenching is clearly smaller (2-25%). Even though this phenomenon has been intensively studied for higher plants (but rarely for algae and cyanobacteria), its biophysical basis and physiological role is only inadequately understood (see RUBAN & HORTON 1995). There is, however, considerable evidence that the q_E-quenching is caused by an increased rate of thermal deactivation of excited chlorophylls (increase of k_{th} in Eqs. 1-5 as demonstrated for green algae by HEINZE & DAU 1996); a role in protection against photoinhibitory damage is often assumed.

Photoinhibition (q_I)

Prolonged exposure (e.g., 30 min) to high-intensity light causes PS II-inactivation (photoinhibition, photodamage, ARO et al. 1993, BAKER & BOWYER 1994) and an accompanying effect on the PS II-fluorescence which is irreversible in the minute range. This q_I-effect is typically characterized by a clear increase of F_0' and decrease of F_M' and, consequently, in a pronounced, not rapidly-reversible decrease of F_V'/F_M'. Several stress factors (e.g. aging of algae cultures) result in a fluorescence phenotype which resembles photoinhibitory quenching.

State-transitions (q_T)

In higher plants and green algae the q_T-formation is related to LHC-phosphorylation and the resulting decrease in the PS II-antenna size (change of $A_{peri}(I_{ML})$ in Eqs. 1-5), a phenomenon denoted as State 1-State 2 transition (BONAVENTURA & MYERS, 1969; ALLEN 1992); there seem to be analogous state transitions in cyanobacteria (and perhaps red algae) involving the phycobilisome system (see, e.g., CANAANI 1986). State tranistions are involved in adaptation not only to changes in spectral quality, but also in intensity of light (see DAU 1994b). State transitions proceed within 5-20 min; their effect on F_M' and F_0' is usually relatively small (5-25%).

3 Measuring technique

Already the basic experimental set-up shown in Fig. 4A allows Chl fluorescence measurements, but it is neither suited for *in-situ* (and *in-vivo*) measurements on dilute suspensions, nor for an experimental approach involving special light programs.

The type of instrument sketched in Fig. 4B is superior due to the following features: (1) By using a modulated measuring light and a correlation amplifier solely the magnitude of the fluorescence stemming directly from the modulated measuring light is detected; ambient light artefacts are supressed and a signal is obtained which is a (relative) measure of the fluorescence yield. (2) The perpendicular arrangement of light sources and fluorescence detector minimizes stray-light artefacts. (3) Color filters are never fully non-fluorescent; the use of interference cut-off filters minimizes artefacts due to filter fluorescence. Instruments for measurement of modulated fluorescence are commercially available (various companies).

The employment of a modulated measuring light does not completely solve all technical problems related to measurements on ultra-dilute suspensions. In particular, there seems to exist no perfect combination of optical filters which fully prevents artefactual contributions of scattered measuring light to the fluorescence signal. Furthermore, fluorescent humic substances in the sample suspension inevitably give rise to an undesired contribution to the fluorescence signal. These problems can be overcome, at least partially, by using special light programs (see 4 and 6).

Considerable inaccuracies which result from quantitatively significant absorption or scattering of the measuring light and the emitted

fluorescence by turbid suspensions is another potential problem. This problem can, at least partially, be solved by a correction on basis of the simultaneously-measured attenuation of the measuring light (MOLDAENKE & al. 1995). Presently, only a few commercially available instruments are equipped with an attenuation correction.

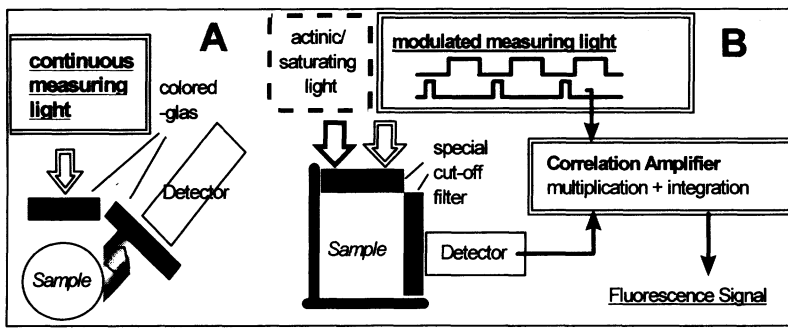

Fig. 4 Set-up for fluorescence measurements using a continuous (**A**) or a modulated (**B**) measuring light. The modulated light may be either symetrically modulated (equal length of on- and off-period, approach in use for decades) or asymetrically modulated (e.g., short light-on period, PAM-approach, SCHREIBER 1986). Today the source of the modulated light typically is either a LED, or Laserdiode, or a Xenon-Flash lamp. The Correlation Amplifier (correlator, Lock-in amplifier, phase-controlled rectifier or demodulator) provides an output signal proportional to the amplitude of the modulated fluorescence which originates from the modulated measuring light. The correlation facilitates, first, the discrimination between modulated fluorescence and the non-modulated fluorescence resulting from additional non-modulated light-sources (ambient light, actinic light, saturation light) and, second, a significant noise reduction. An increased integration time-constant of the correlation amplifier results in reduced noise, but also in a lengthened response time. In some commercially available instruments the correlation is done by a micro-processor unit.

4 Special light programs

Due to the variability of the PS II fluorescence special light programs can be utilized for (1) suppression of light-scattering contributions and of fluorescence of 'dead' substances (to obtain an estimate of the amount of chlorophyll in the sample which is not affected by artefactual contributions), (2) obtaining estimates on the actual rate of

photosynthesis, (3) gathering information on PS II quantum yield, 'fitness', stress effects and influences of toxic substances (biotest).

More or less complex light-programs which involve determination of the fluorescence yield in the presence of reduced Q_A^- (F_M or F_M') by application of high-intensity light-pulses (see Figure 5) are frequently used. The duration of the light-pulse employed for reduction of Q_A mostly is either greater than 300 ms or less than 20 µs. In the former case, the frequently used 'saturation pulse method' , Q_A and the total ETC are reduced (BRADBURY & BAKER 1981; QUICK & HORTON 1984; SCHREIBER et al. 1986; SCHREIBER et al. 1995). In the latter case, Q_A is reduced by a µs 'pump pulse' and the fluorescence yield is determined roughly 100 µs later by a µs 'probe pulse' (MAUZERALL 1972; FALKOWSKI et al. 1986; KOLBER & FALKOWSKI 1993).

An alternative light-program involves determination of the variability in the fluorescence yield induced by an actinic light modulated with 1 Hz (SCHROETER et al. 1991; MOLDAENKE et al. 1995, see Fig.6), a method derived from analysis of the variable PS II fluorescence in the frequency domain (DAU & HANSEN 1989). The 1-Hz fluorometer is particularly useful for continuous environmental monitoring.

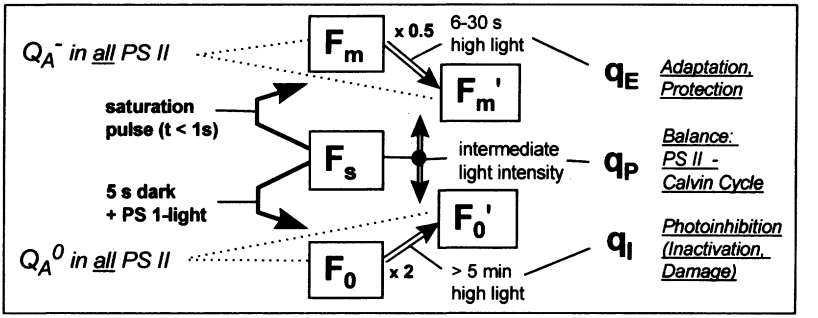

Fig. 5 Fluorescence yield and its response to special light programs (e.g. by the saturation pulse method). The corresponding experimental set-up involves a fluorometer for measurement of modulated fluorescence as sketched in Fig. 4B (e.g., the PAM fluorometer, Walz, Effeltrich). It should be noted that photoinhibition is, typically, related to a F_0-increase (as indicated in the above scheme) and a significant F_M-decrease (not indicated in the above scheme). Determination of F_0' is often troublesome because full reoxidation of reduced Q_A is not readily achieved. In particular, in many cyanobacteria the plastoquinone pool redox-state is affected by the respirational activity of the organism; therefore, accurate determination of F_0 and F_0' by special light programs is not possible.

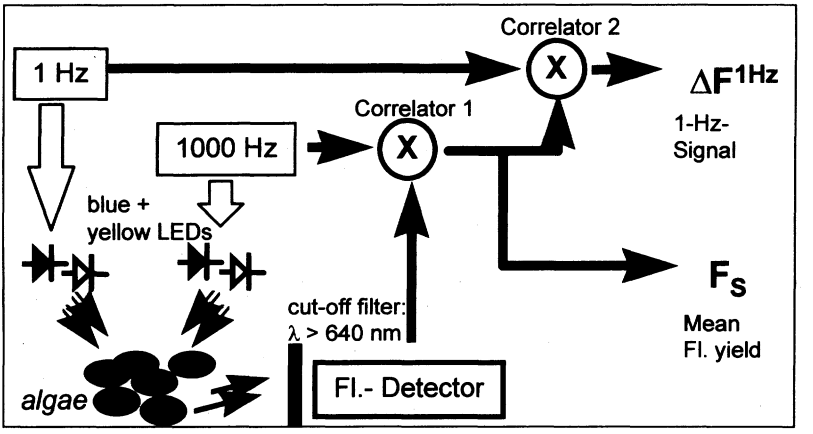

Fig. 6 Scheme of the 1-Hz-fluorometer. The 1-Hz-signal represents the magnitude of the variations in the fluorescence yield which are induced by the actinic light LED modulated with 1 Hz (MOLDAENKE et al. 1995). The F_S-determination involves measurement of modulated fluorescence as depicted in Fig. 4B. Due to the double-correlation technique exceptional sensitivity can be achieved. In contrast to the saturation-pulse techniqe, the light-program (i.e. the actinic light modulated with 1 Hz) is running all the time and the so-called 1-Hz-signal is continuously provided. The value of the ratio DF^{1Hz} / F_S is related, but not identical to the the ratio F_V'/F_M' as obtained by the saturation pulse method.

5 Current development: species-differentiation by multi-color excitation

For a homogenous algae population (single species, identical developmental and adaptational state) the fluorescence parameters $F_M^{(\prime)}$, $F_0^{(\prime)}$, F_S and DF^{1Hz} are proportional to the Chl *a* concentration; measurements of Chl fluorescence (as shown in Fig. 4b) can, e.g., be used to obtain a relative measure of the Chl *a* content. Frequently, however, the population is heterogeneous. For a mixture of various green algae species, the resulting inaccuracies may be tolerable. However, the errors are likely to be unacceptable for, e.g., a mixture of green algae and cyanobacteria because excitation spectra, $A_{peri}(I_{ML})$ in Eq. 1, and fluorescence yields, $F^F_{0/M}$ in Eq. 1, of these organisms are pronouncedly different.

On basis of Eq. 1 (see also Fig. 3) we obtain for the fluorescence signal, F_x, of a heterogeneous organism population (i.e. a mixture of various species):

$$F_x(I_{ML}) = \sum_{i=1,2\ldots} C_{Chla}^{\,i}\, f_x^{\,i}(I_{ML}) \qquad (10)$$

$$\text{with} \quad f_x^{\,i}(I_{ML}) = c_{instr}\; I_{ML}(I_{ML})\; (N_{PSII}^{\,i} / C_{Chla}^{\,i})\; \{A_{peri}^{\,i}(I_{ML}) + A_{core}(I_{ML})\}\; F_x^{\,F,i} \qquad (11)$$

where the superscript 'i' indicates the reference to the i'th species and $C_{Chla}^{\,i}$ the concentration of Chl *a*-molecules (e.g., in µg/L) associated with the i'th species.

Using modern optoelectronics and special correlation schemes, it is technically feasible to detect, at a single emission wavelength, separately the fluorescence induced by measuring light sources (LEDs) of different wavelengths ($I_{ML} = I_1, I_2, ..I_j, ..I_N$). By collecting 'norm spectra' on samples with known Chl concentration which contain exclusively the i'th species, the $f_x^{\,i}(I_j)$ are obtainable. In case the number of excitation wavelengths is equal to or greater than the number of discernible species, determination of the $C_{Chla}^{\,i}$, the species-resolved chlorophyll content, is achievable by using appropriate fit algorithms.

Major limitations of this multi-color excitation approach are: (i) differentiation between species with similar PS II antenna systems is usually not feasible; (ii) the number of resolvable species or organism groups can never exceed the number of the number of excitation wavelengths; (iii) variations in the developmental or adaptational state of one species (or organism group) may cause significant variations in the norm-spectra and, consequently, misleading results (a potential problem particularly for cyanobacteria). Some 'resistance' against the third problem is achievable by intelligent fit algorithms, but only in case the number of excitation wavelengths exceeds the number of organism groups.

The multi-color excitation approach for 'algae differentiation' is perhaps the most promising recent development with respect to *in-situ* and *in-vivo* Chl-fluorescence measurements on algae suspensions. Potentially, this approach enables the fast and convenient, spatially-resolved and differentiated assessment of the amount primary producers and the rate of primary production in aquatic ecosystems by means of diving probes which employ on-line data transfer to a personal computer on board of a research vessel.

Recently, the first five-color diving-probe system became commercially available (l_{ML} = 450, 525, 570, 590 and 610 nm; detection at l > 670 nm; turbidity correction; measurement of water pressure for depth determination; C. Moldaenke, bbe Moldaenke, Schauenburgerstr. 116, D-24118 Kiel, Germany). A four-color table-top instrument (l_{ML} = 450, 590, 620 and 650 nm; detection at l > 710 nm; possibility to employ complex, computer-controlled light-programs; see KOLBOWSKI & SCHREIBER, 1995; J. Kolbowski, Büro für Umweltmeßtechnik, W.-v.-d.-Vogelweide-Str. 56, D-97422 Schweinfurt, Germany) has been presented at the workshop on determination of primary production in Zingst (October 97) and has been announced to become commercially available soon.

6 Assessment of primary productivity by Chl-fluorescence measurements

In the following it is assumed that the rate of oxygen evolution by PS II is a suitable measure for the gross rate of primary production. The O_2-evolution rate (e.g. in Mol evolved $O_2/(m^3\ h)$) is determined by:

(I) the amount and species composition of primary producers (the total number of PS II, $N_{PS\,II}$, in the sample volume per species),
(II) for each species the 'effective' quantum yield for O_2-evolution of the illuminated sample (F_{ox}), and
(III) the integrated product between photon-fluence rate, I(l), and the species-dependent absorption cross-section of PS II ($A(l) = A_{peri}(l) + A_{core}(l)$). Thus,

$$R_{ox} = \sum_i N_{PSII}^{\ i}\, F_{ox}^{\ i}\, L^i \qquad (12)$$

with

$$L^i = \int I(l)\, A^i(l)\, dl \quad . \qquad (13)$$

(I) Amount of primary producers.

Convenient *in-vivo* or *in-situ* determination of $N_{PSII}^{\ i}$ (which corresponds to the organism concentration) by fluorescence measurements is, in principle, possible. For a homogeneous population the values of F_0 or F_M are proportional to N_{PSII} (Eq. 1). However, due to non-photochemical quenching (see 2) there is a potential error in the fluorescence-based N_{PSII}-determination of, roughly, a factor 2. In case pronounced q_E- and q_I-formation can be excluded, the fluorescence-

based N_{PSII}-determination should be relatively precise. For concentrated or turbid samples, however, an attenuation correction is mandatory (MOLDAENKE et al. 1995). For heterogeneous populations with varying species compositions, N_{PSII}-determination for each organism group is required (see 5).

(II) Quantum yield for photochemistry. It is often possible to obtain a reasonable estimate for F_{ox} by fluorescence measurements. For a homogeneous population to a first approximation, 4 times F_{ox} (4 PS II-turnover are required per O_2-molecule) equals to the fluorescence paramter F_P of Eq. 9 (first experimental proof: GENTY et al. 1989, 'corrected' theory: DAU 1994a; application to algae, e.g., HEINZE et al. 1996, GEEL et al. 1997). Also the (alternative) fluorescence parameter DF^{1Hz} / F_S is closely related to F_{ox} (VANSELOW et al. 1997).
For ultra-dillute organism suspensions, a contribution to the fluorescence signal due to scattered measuring light and fluorescence of humic substances is unavoidable (see 3). In this context, it could be profitable to employ directly the fluorescence parameters F_V' or DF^{1Hz} for obtaining an estimate on the product 'N_{PSII} F_{ox}'. Furthermore, using this approach the errors due to q_E- or q_I-formation are, presumably, relatively small. Presently, however, this approach is merely an untested suggestion.

(III) Number of incident and absorbed photons. Determination of the L^i in Eq. 12 is, unfortunately, troublesome. Using a homogenous 'norm-sample', the wavelength dependence of the PS II absorption cross-section, $A_{PSII}(l)$, can be accurately determined by measurement of the F_V-fluorescence excitation spectrum (using a wavelength-scanning laboratory fluorometer). These measurements are relatively difficult (various lurking artefacts), but 'affordable' because frequent repetition is not required. However, spectrally resolved under-water measurements of the photon-fluence rate (for determination of $I(l)$ in Eq. 13) are required which are costly in terms of financial resources and manpower because spacial and temporal resolution is needed.

Approximative approaches

Occassionally a highly approximative, semi-empirical approach might be applicable involving:

(i) determination of the Chl-content of the sample (or N_{PSII}),
(ii) measurement of the photosynthetically active radiation (PAR) with a simple instrument and

(iii) calculation of R_{ox} for *in-situ* or *in-vivo* measurements of fluorescence yield ratios (F_V'/F_M' or $\Delta F^{1Hz} / F_S$) on basis of calibration factors. For example, the rate of oxygen evolution of the green algae *Scenedesmus obliquus* has been found to be :

$$R_{ox} = I_{PAR} \cdot F_V'/F_M' \cdot (0.9 \pm 0.16)\ (\mu mol\ mg^{-1}\ h^{-1}) / (\mu E\ s^{-1}\ m^{-2}) \qquad (14)$$

with R_{ox} in µmol O_2 per mg Chl and h, I_{PAR} in µE per s and m^2 (for lab cultures grown at various conditions, but in the absence of significant photoinhibition; see HEINZE et al. 1996).

For a more intricate, but perhaps more general approach see GEEL et al., 1997. New approximative approaches are needed for handling multi-color excitation data obtained on species mixtures (see 5).

Stress and toxicity

Often it is the objective to search for an influence of 'stress factors' (high light intensities, unfavorable O_2-concentration, pollutants, toxic substances, etc.) on photosynthetic organisms. The molecular basis of the involved phenomena is complex and can not be derived from fluorescence measurements. However, fluorescence parameters may be used as convenient and sensitive 'indicators' for the presence of a stress effect. The parameter F_V/F_M is sensitive to light stress, but it is usually insensitive to substances which inhibit reactions of the ETC or the Calvin cycle (e.g. various herbicides); the parameter $\Delta F^{1Hz} / F_S$ responds to both types of 'stress'. Thus, the former fluorescence parameter is more specific in its response, whereas the latter is senses a clearly broader spectrum of stress or toxicity effects.

7 Conclusion

For many investigations on aquatic ecosystems, it is desirable to assess the primary production with high resolution in time and space. However, additional work needs to be spend for development of instrumentation and the (approximative) methods which are indispensable for relating fluorescence data to primary production. Presently, a strict standard for instrumentation and data evaluation is neither existent, nor desirable because it would impede future developments. The choice of method and instrumentation depends on character and objectives of the intended investigation.

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