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Suitability of the Fluorescence Monitoring System (FMS, Hansatech) for measurement of photosynthetic characteristics in algae

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Abstract

A pulse-modulated fluorescence measuring system, originally developed for higher plant studies, the Fluorescence Monitoring System (FMS; Hansatech), was tested to study photosynthesis in algae. The main technical features of the FMS are described, and applications of the saturating pulse fluorescence method are presented on micro- and macroalgae. Some limitations of the apparatus for measuring fluorescence characteristics of microalgae are discussed. Chlorophyll *a* fluorescence quenching analyses were performed on macroalgae from different taxonomic groups (*Ulva* sp., *Palmaria palmata*, *Fucus serratus*), and the results of the fluorescence measurements are presented and discussed, in relation to the macroalgae characteristics. The FMS and the saturating pulse method may be successfully used with green and red algae, in contrast to brown algae, with which F'_m values higher than F_m were observed at low actinic light ($< 100 \mu\text{mol m}^{-2} \text{s}^{-1}$). This unconventional result is detected by error trapping routines and prevents employing the entire FMS potentiality. This point is discussed taking into account FMS specifications and limits.

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Abbreviations: F_0 , F_m , minimal and maximal fluorescence yields of dark-adapted samples; F'_0 , F_s , F'_m , minimal, steady-state, and maximal fluorescence yields of light-adapted samples, respectively; F_v , F'_v , variable fluorescence yields of dark- and light-adapted samples; Φ_{PSII} , quantum efficiency of PSII; Φ_{PSIIR} , FMS function that calculates Φ_{PSII} and measures F'_0 ; qP, qNP, NPQ, photochemical, nonphotochemical, and alternative nonphotochemical quenching; ETR, electron transport rate

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1. Introduction

In vivo chlorophyll fluorescence is a sensitive and nonintrusive method that provides rapid information on photosynthetic processes. In the laboratory as well as in the field, this method gives insights into the biochemical reactions and the partitioning of energy between photosynthetic components. In vivo chlorophyll fluorescence has, thus become a valuable tool for photosynthesis research. The yield of chlorophyll fluorescence is variable in intact cells, depending on their history and environment. At room temperature, most of the fluorescence emission originates from PSII antenna pigments. Changes in fluorescence yield essentially result from variations in the rates of photochemical energy conversion and nonphotochemical energy dissipation. The determination of these two competitive fluorescence lowering components, the principle upon which relies the fluorescence quenching analysis, has become facilitated with the saturation pulse method (Bradbury and Baker, 1981; Schreiber et al., 1986). This technique relies on the suppression of one of the two components of fluorescence quenching by a strong light pulse of several hundred milliseconds, ensuring complete Q_A (primary quinone acceptor of PSII) reduction (photochemical quenching = 0).

Among the different instruments intended to measure in vivo chlorophyll fluorescence, modulation fluorometers allow determination of the fluorescence yield even in full sunlight. These instruments comprise light-emitting diodes (LED) that emit a pulse beam sufficiently high to trigger off fluorescence but weak enough not to induce photochemistry. Fluorescence emission is collected by a sophisticated detection circuit that discriminates between the modulated fluorescence (induced by the pulsed beam) and the nonmodulated fluorescence (induced by other light sources, i.e. saturation pulses, actinic or ambient light).

Apart from laboratory or homebuilt instruments, several ready-to-use systems designed for measuring modulated fluorescence are commercially available. Pioneering the effort and currently the leader of the market, Walz (Effeltrich, Germany) offers a wide range of fluorometers and accessories, that are, by far, the most frequently used for fluorescence measurements in algae. Since 1997, Hansatech (Norfolk, UK) has offered the Fluorescence Monitoring System 1 (FMS1), a less expensive instrument primarily intended to use with higher plants, in either the laboratory or field. To our knowledge, the FMS1 has never been used in phycological studies. In the present work, we briefly describe the FMS1, give details on its specific advantages and drawbacks, and present fluorescence data collected with macroalgae and microalgae.

2. Materials and methods

2.1. Instrument description

The FMS1 consists of a portable control unit containing a battery, electronics, hardware, optics and light sources, a PAR/temperature probe and a series of clips supplied for specific use with higher plants, and a fibre optic that both delivers light to, and collects fluorescence signal from the sample. Data acquisition and recording software offers two different operating modes (“PC”, personal computer, or “local”). In the “PC mode”, the FMS1 is

controlled from a PC. Data points corresponding to the fluorescence signal value (bits) are stored every 110 ms. After conversion from analogic to digital format, the fluorescence signal is plotted against time and presented graphically in real time on the computer screen. For operation outside the laboratory (“local mode”), or when disconnected from the computer and the external power supply, the FMS1 can be used as a stand-alone modulated fluorometer powered by the internal rechargeable battery. Some experiments were run with the field version of the FMS1, the FMS2, that is not fundamentally different.

2.2. Programming of protocols

On either the “PC mode” or the “local mode”, the FMS1 can execute complex experimental protocols programmed using the software provided by the manufacturer (Hansatech Scripting Language). Each protocol consists of up to 30 script elements, corresponding to ca. 75 instructions and measurements, such as instrument setting, switching on and off the light sources, pausing between script instructions, and recording of fluorescence parameters (F_0 , F_m , F_s , F'_0 , F'_m), PAR and temperature. Other fluorescence parameters (F_v , F'_v , Φ_{PSII} , Φ_{PSIIR}) and values of quenching coefficients (qP, qNP, NPQ) and the electron transport rate (ETR) can then be computed by the software, using equations of [Schreiber et al. \(1986\)](#) or [Genty et al. \(1989\)](#):

$$F_v = F_m - F_0$$

$$F'_v = (F'_m - F'_0)^*$$

$$\Phi_{\text{PSII}} \text{ or } \Phi_{\text{PSIIR}}^* = \frac{F'_m - F_s}{F'_m}$$

$$qP = \frac{F'_m - F_s}{F'_m - F_0} \text{ or } \frac{F'_m - F_s}{(F'_m - F'_0)^*}$$

$$qNP = \frac{F_m - F'_m}{F_m - F_0} \text{ or } \frac{F_m - F'_m}{(F_m - F'_0)^*}$$

$$NPQ = \frac{F_m - F'_m}{F'_m}$$

$$ETR = PAR \times 0.5 \times \Phi_{\text{PSII}} \times 0.84$$

where * refers to the measurement of F'_0 , the fluorescence emission that should be obtained after exposure to far-red light, opening all reaction centres. It is worth noting that the ETR can be calculated by the FMS software only if a PAR value has been logged from the PAR/temperature leaf-clip. According to the manufacturer, FMS software capacities are usually adequate to do a multi-point light–response curve such as a quenching analysis; this point will be discussed further.

2.3. Technical specifications of light sources

The FMS1 used for these experiments was equipped with a 470 nm blue LED, the FMS2 with a 594 nm amber one. No differences in fluorescence parameters were evidenced during measurements on the macroalgae with one or the other apparatus. The modulation beam is characterized by very short duration pulses (1.8 μ s), and its intensity can be selected from four different levels, each setting having a different frequency of pulses. During actinic or pulse illumination, the modulating level is automatically switched to level 4 (highest intensity/frequency, i.e. 256 1.8 μ s pulses/110 ms). Actinic and pulse illuminations are provided by a halogen lamp (Osram 64255; 8 V, 20 W), and change in light intensity results from change in lamp voltage. Far-red light (unchanging intensity) is provided by an LED with a peak emission at 735 nm, and filtered with a long-pass filter (cut-on wavelength at ca. 705 nm).

2.4. Other light source and light measurements

Experiments were run to assess the effects of change in actinic light quality on fluorescence measurements. Low actinic light levels ($<25 \mu\text{mol m}^{-2} \text{s}^{-1}$) were provided either by the FMS (red-enriched spectrum) or by an external cold light source, Intralux[®] 4000-1 (Volpi, Zürich, Switzerland). The Volpi apparatus consists of a halogen lamp (Philips 13186 EPX/ERV; 14.5 V, 90 W) and power supply, providing variable white light output with a constant color temperature (3150 K) by way of a crescent-shaped diaphragm.

Cosine corrected sensors (Hansatech PAR/temperature leaf-clip or Li-Cor 189 quantum meter) were used for light measurements. The sensor of the Li-Cor 189 was mounted in place of the sample, either in a leaf-clip or close to the end of the optic fibre, depending on the algal species and the fluorescence emission level.

2.5. Biological material

Macroalgae (*Ulva* sp., *Palmaria palmata*, *Fucus serratus*) were sampled in July 1999 from tidal ponds near Roscoff (Marine Biological Station), located on the north coast of the Brittany peninsula (France). Quenching analyses were performed on different samples from several organisms. Additional experiments dealing with actinic light quality were conducted with *Fucus spiralis* and *Fucus vesiculosus* collected near Brest in September 2000. Macroalgae were maintained in air-bubbled sea water at room temperature under dim light conditions ($<50 \mu\text{mol m}^{-2} \text{s}^{-1}$) at the most for three days before running experiments.

The microalgae *Skeletonema costatum*, *Haslea ostrearia*, and *Phaeodactylum tricornerum* were grown in a modified Provasoli medium (Mouget et al., 1999) in a temperature-controlled room at $16 \pm 1^\circ\text{C}$. Illumination was provided by fluorescent tubes (Osram Cool White L 18 W/20) in a 14 h/10 h light/dark photoperiod, and cells were maintained in exponential growth phase by regular dilution with fresh medium.

Unless otherwise specified, the algae were dark adapted for at least 30 min before measuring F_0 and F_m . The correct setting of pulse saturation (intensity and duration) was determined beforehand for each alga.

3. Results and discussion

3.1. FMSI characteristics

According to the manufacturer's specifications, the intensity of the modulated beam ($<0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$) is supposed to be negligible as compared with the minimum actinic light level provided by the apparatus ($6.30 \mu\text{mol m}^{-2} \text{s}^{-1}$ at setting 1 or Act 1, as measured with the sensor of the Li-Cor 189 mounted in the FMS dark-adaptation leaf-clip). Nevertheless, fluorescence measurements showed that the modulated beam can be actinic (the dark-adapted fluorescence signal gradually increased with time), depending on algae and light history. This point justifies regular checking to ensure that the modulated light does not cause variable fluorescence from samples.

Concerning the light quality provided by the FMS, change in halogen lamp output (actinic light or pulse intensity) results from variation in lamp voltage, which also changes the color temperature of the light spectrum. The higher the voltage, the higher the light output, with a blue enrichment of the spectrum. The importance of these variations in light quality was assessed by comparing fluorescence parameters measured under low actinic light (ca. $20 \mu\text{mol m}^{-2} \text{s}^{-1}$) provided either by the FMS (red-enriched spectrum) or by a Volpi Intralux® 4000-1 (white light). Steady-state fluorescence emission (F_s) was higher, when actinic light was dispensed by the Intralux 4000-1 (Fig. 1). Expressed as the percent of the difference with F_s measured under the FMS actinic light, results of additional experiments conducted with *F. spiralis* and *F. vesiculosus* showed $6.1 \pm 2.2\%$ (mean \pm S.E., $n = 5$) higher F_s under the white actinic light (Intralux), in comparison to the red-enriched light (FMS). Although discrepancies between light scattering conditions (different angles between the two actinic sources and the fibre optic) were not taken into account, the changes in F_s are likely the consequence of the red-enriched actinic light, since far-red light ($<700 \text{ nm}$)

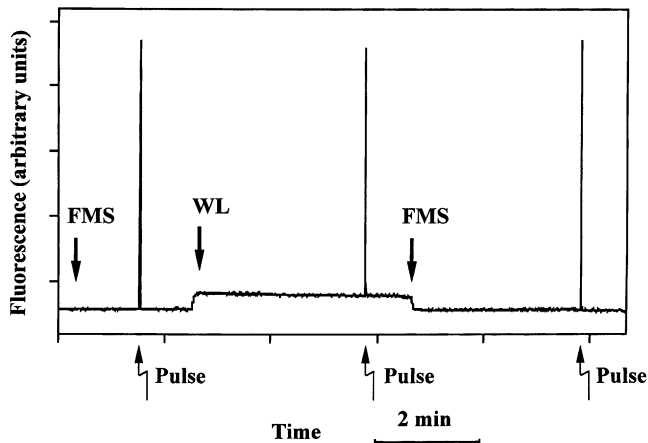


Fig. 1. Changes in fluorescence signal measured in *F. vesiculosus* exposed to low actinic light (ca. $20 \mu\text{mol m}^{-2} \text{s}^{-1}$) of different quality. Arrows indicate which actinic light is on: WL, cold white light provided by the Intralux 4000-1; FMS, red-enriched light provided by the FMS.

preferentially excites PSI and oxidises the plastoquinone pool and the PSII acceptors. However, changes in other fluorescence parameters are not high enough (difference in $F'_m < 2\%$; in $qP < 2\%$; in $\Phi_{PSII} < 3\%$) to question the technical choice of the actinic light control in the FMS.

The far-red LED operates at maximum output (ca. 15 mW m^{-2} at the end of the fibre) every time the “Far-red” button is clicked. This way the far-red light intensity cannot be changed other than by modifying the distance of the fibre optic to the sample, or by using neutral filters. Although the far-red source is claimed to be non actinic, it can induce photochemistry (not shown), and this point should be carefully checked before experiment.

It is worth noting that when the actinic light is on (“Actinic” button pressed), changing the halogen lamp output is impossible by simply increasing or lowering the value in the numeric box. As a consequence, if the actinic light is already on, e.g. during data recording, the lamp must be switched off before changing the illumination intensity (e.g. see Fig. 3). Nevertheless this drawback is avoided with the use of a script.

The FMS software allows for the programming of complex experimental scripts or protocols, which consist of a series of script elements, corresponding to instrument setting, data measurements and calculation of fluorescence parameters. The maximum number of script elements generating a protocol is supposed to be usually adequate to do a multi-point light–response curve. On the other hand, the effective number of steps of a protocol depends upon the kind of script elements selected, especially the number of instructions per script element. As an example, the Φ_{PSII} function results in four measures (PAR, temperature, F_s , and F'_m) and five calculated parameters (Φ_{PSII} , qP , qNP , NPQ , and ETR), given that F_0 and F_m have been previously stored. As a consequence, the number of script elements actually available can become inadequate, especially if comments (e.g. actinic light settings recording) are inserted by way of the TEXT element of the Hansatech Scripting Language.

3.2. Experiments on algae

Before running experiments, the correct setting of pulse saturation (intensity, much variable according to the species and set-up, and duration, most of the time, 700 ms) was determined for each alga. The PAR/temperature probe intended for use with higher plants is of no use with algae, since it contains electronics and cannot be immersed. On the other hand, although the cable is not designed for underwater use, it is possible to insert just the end of the fibre optic in water without damaging it. This way fluorescence can be measured directly on macroalgal blades or microalgal solutions, which avoids resorting to temperature-controlled cuvettes that could dramatically lower the fluorescence signal, even though these kinds of cuvette are specifically intended for oxygen or fluorescence measurement. As an example, when compared to fluorescence measured directly (glass cuvette, GC) on algal solutions of low Chlorophyll *a* concentrations (the lowest, $0.5 \mu\text{g Chlorophyll } a \text{ ml}^{-1}$, being imposed by the FMS sensitivity), optical windows (water jacket) of the Hansatech DW3 cuvette (1–20 ml) lower the fluorescent signal of *S. costatum* by a factor of two (Fig. 2a). This problem is not particular to the DW3, since the Hansatech DW2 cuvette (0.2–2.5 ml), specifically intended for use with microalgae, decreases the fluorescent signal by a factor up to five. Similar results were obtained with *H. ostrearia* and *P. tricornutum* (not shown). Moreover, Fig. 2b shows that F_m/F_0 ratios (hence, F_v/F_m values) not only depend on the Chlorophyll

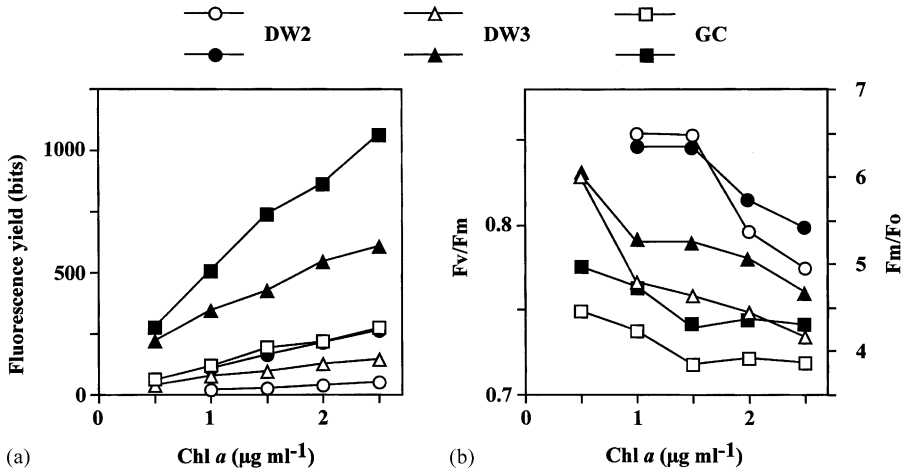


Fig. 2. Changes in parameters of fluorescence measured in Hansatech cell DW2 (circles) or DW3 (triangles), or in a GC (squares), as a function of Chlorophyll *a* concentration in *S. costatum*. Cells were dark adapted for 30 min prior to measurements. (a) Closed symbols represent F_m , and open symbols represent F_0 . (b) Closed symbols represent F_v/F_m , and open symbols represent F_m/F_0 .

a concentration used for the measurements, due to light scattering and reabsorption phenomena, as already shown by Ting and Owens (1992) or Büchel and Wilhelm (1993), but also on the type of cuvette containing the algae, at least the way fluorescence signal is recorded from. Better results are obtained with the probe positioned at the open top of the GC, rather than through the side of either the DW3 or the DW2 cuvette. Measuring fluorescence directly on the microalgal solution gives the highest fluorescence yields as well as an almost constant F_v/F_m value with low Chlorophyll *a* concentrations (Fig. 2b). Since light conditions prevailing during F_0 and F_m determination differently influence these parameters, choosing the best algal concentration and experimental device remains an important step for the validation of fluorescence measurements.

Hansatech leaf-clips can help grip macroalgal blades, but actual usefulness depends on the species and the fluorescence emission level. These clips are equipped with a fibre optic adapter, so as to mount the end of the fibre at 60° to the plane of the sample. With *F. serratus* or *Ulva* sp., leaf-clips and the fibre optic adapter insure a fluorescence signal strong enough for the sensitivity of the FMS1. In contrast to the brown or the green macroalga, *P. palmata* held in the leaf-clip emits a fluorescence signal too low, even if the end of the fibre optic is close to the algal blade (minimum distance, ca. 1.5 cm). To increase the fluorescence signal detected, blades have to be roughly fixed against the end of the optic fibre.

For experiments with macroalgae, after previous determination of F_0 and F_m , saturating flashes were applied to measure F'_m and calculate Φ_{PSII} on algae adapted to different actinic light levels from ca. 10 to 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Fluorescence parameters were then calculated by the FMS software from the recorded measures: F_v/F_m , the maximal PSII quantum efficiency of dark-adapted sample; Φ_{PSII} , the effective PSII quantum efficiency of illuminated sample, and the different quenching coefficients, qP and qNP or NPQ. Fig. 3 shows the

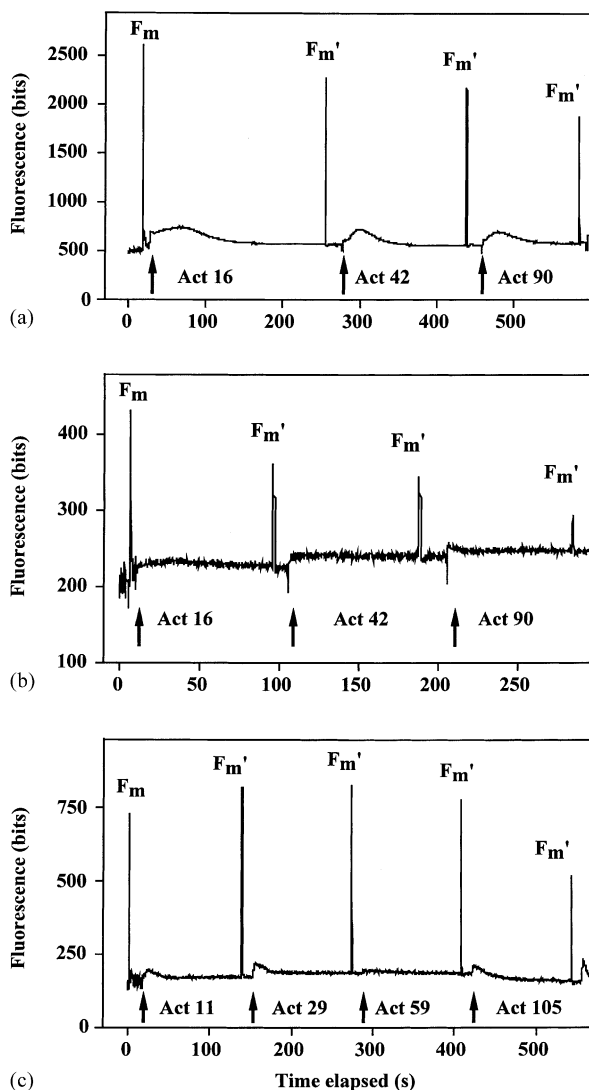


Fig. 3. Typical results of saturating pulse fluorescence measurements for (a) *Ulva* sp., (b) *P. palmata*, and (c) *F. serratus* in dark (F_m)- or light (F_m')-adapted state. Arrows indicate changes in actinic light level; numbers correspond to the intensity measured at the algal blade level ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Undershoots in the fluorescence signal result from the manual change in actinic light level.

fluorescence trace from different macroalgae after dark adaptation and following actinic illumination (low light levels only, i.e. $<100 \mu\text{mol m}^{-2} \text{s}^{-1}$). Both the green (Fig. 3a) and the red (Fig. 3b) macroalgae showed a standard fluorescence trace, particularly concerning F_m' values that were lower than F_m . In contrast, the brown macroalgae exhibited unconventional F_m' , higher than F_m at actinic light $<100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3c). Quenching analyses

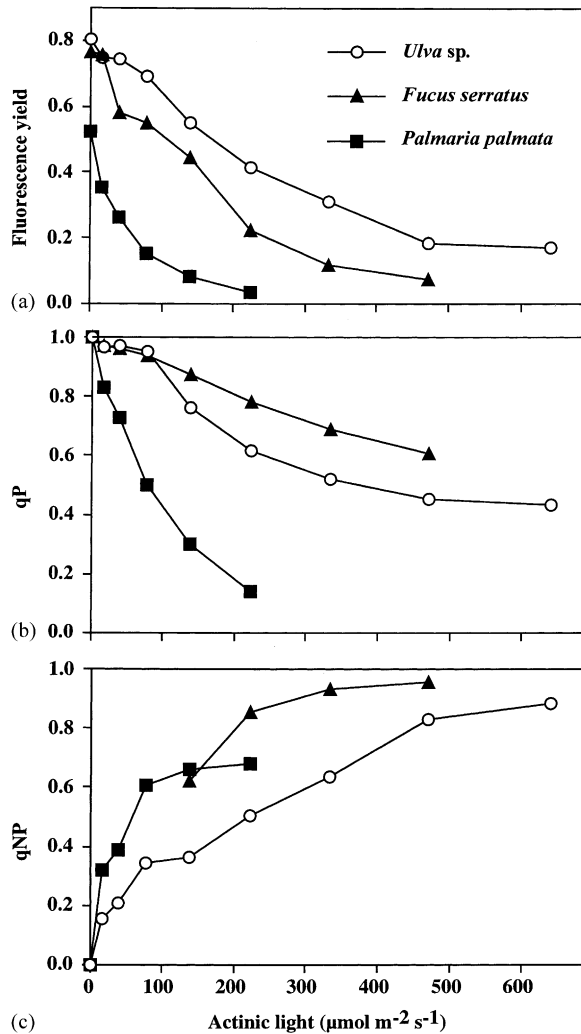


Fig. 4. Changes in fluorescence parameters and quenching coefficient, as a function of actinic light intensity, for: *Ulva sp.*, *P. palmata*, and *F. serratus*: (a) F_v/F_m (after dark adaptation) and PSII (actinic light on); (b) photochemical quenching qP; (c) nonphotochemical quenching qNP. For qNP calculation, during exposure to actinic light higher than ca. $200 \mu\text{mol m}^{-2} \text{s}^{-1}$, F_s was often lower than F_0 in *F. serratus*. In these conditions, quenching analyses were made possible by the measure of F'_0 , fluorescence emission obtained after exposition to far-red light (PSIIR function), i.e. corresponding to the fluorescence signal in a light-adapted state with all PSII reaction centres open. In an alternative way, F'_0 can be estimated according to Oxborough and Baker (1997).

were performed with the three macroalgae, and values of F_v/F_m and Φ_{PSII} are presented in Fig. 4a. The effective PSII quantum efficiency decreased with increasing light, the decrease being more pronounced for *P. palmata* and *F. serratus* than for *Ulva sp.*, illustrating their respective capacity to acclimate to high irradiances in relation with their usual bathymetric

levels. Fluorescence quenching coefficients are presented in Fig. 4b (qP) and Fig. 4c (qNP), keeping in mind that, when F'_m was higher than F_m in *F. serratus*, qNP was not calculated by the FMS software from recorded values. Excessive absorbed light is more effectively dissipated at high irradiance in *Ulva* sp. and *F. serratus* than in *P. palmata*, which exhibits the lowest qP and the highest qNP as long as actinic light increases. Both *Ulva* sp. and *F. serratus* can use light energy efficiently to drive photosynthesis at high irradiances, but the green macroalga dissipates less energy as nonradiative processes. Φ_{PSII} , higher in *Ulva* sp. than *F. serratus*, is not the consequence of a lower decline in photochemical quenching, qP (Fig. 4b), but of a slower increase in nonphotochemical quenching, qNP (Fig. 4c).

3.3. FMS defect, troublesome brown algae, or misuse of the fluorescence theory?

Measuring F'_m higher than F_m conflicts with the fluorescence theory, but it is not restricted to *F. serratus*; *F. vesiculosus* reacted the same way (Fig. 5), as did *F. spiralis* (not shown). Furthermore, preliminary experiments conducted with microalgae possessing different light-harvesting pigments showed that at low actinic light levels ($< 100 \mu\text{mol m}^{-2} \text{s}^{-1}$),

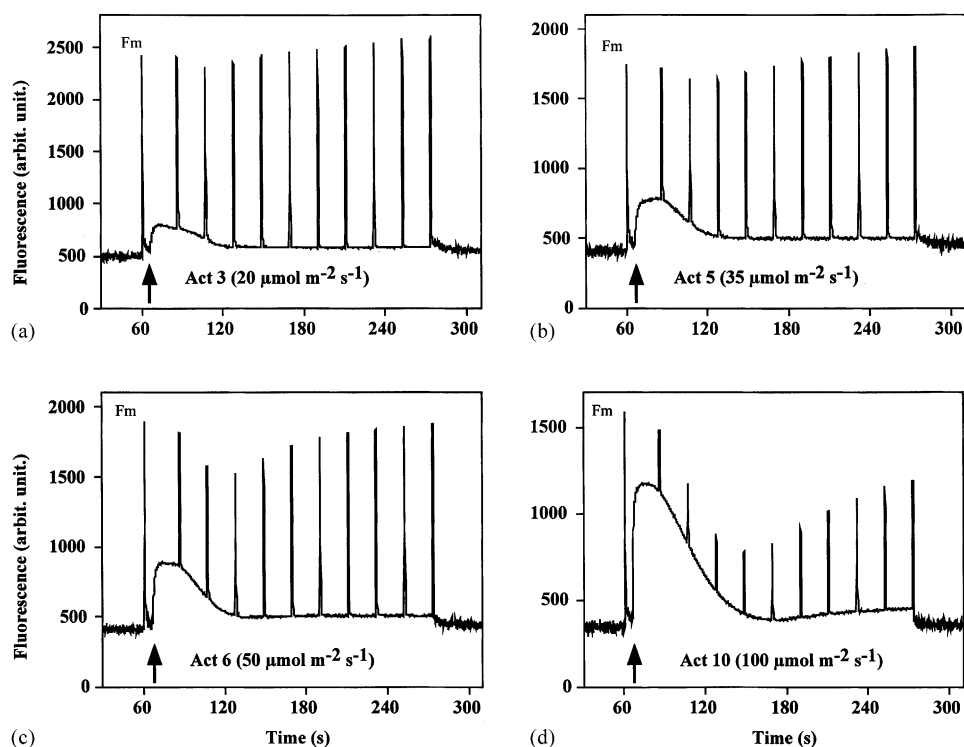


Fig. 5. Typical saturating pulse fluorescence measurements for *F. vesiculosus* dark adapted for 30 min, and then exposed to different levels of actinic light (arrows): (a) 20, (b) 35, (c) 50, or (d) $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (FMS Act 3, 5, 6, and 10 settings, respectively). The first saturating pulse corresponds to F_m , the others ones illustrate the evolution of F'_m with time.

fluorescence measurements in a red (*Porphyridium cruentum*) or a green microalga (*Dunaliella* sp.) consistently resulted in F'_m lower than F_m , while in some diatoms (*S. costatum*, *H. ostrearia*, or *P. tricornutum*), of which photosynthetic pigments are very similar to those of brown algae, $F'_m > F_m$ were quite often observed (unpublished data). Could these intriguing results be restricted to FMS measurements? First of all, most FMS fluorescence measurements were done with 700 ms saturating pulses, a flash duration that favors F_m overestimation as well as F'_m underestimation (Schreiber et al., 1995a,b). Moreover, similar results, not dependent on the fluorometer type, were obtained with microalgae by other authors. Falkowski et al. (1986) with the pump and probe method (on different chlorophyll *c*-containing algae), and both Ting and Owens (1993) and Geel et al. (1997) with a PAM 101 (on *P. tricornutum*), also observed F'_m higher than F_m at irradiances lower than $100 \mu\text{mol m}^{-2} \text{s}^{-1}$. As a common hypothesis, this particular comportment could stem from a nonphotochemical quenching of chlorophyll fluorescence in darkness resulting from chlororespiration (Ting and Owens, 1993). The development of a transmembrane proton gradient by chlororespiratory electron transport can make determination of true (i.e. maximum) F_m value almost impossible in dark-adapted cells (Jakob et al., 1999). However, addition of uncouplers (carbonylcyanide *m*-chlorophenylhydrazide, CCCP or nigericin) did not support this hypothesis in *P. tricornutum* (Geel et al., 1997). Schreiber et al. (1995a), working on the cyanobacterium *Synechocystis*, measured (with a modified PAM 101) low F_m on dark-adapted cells, and higher F'_m levels after illumination by moderate blue light. Interestingly, Torzillo et al. (1996), working on outdoors cultures of *Spirulina platensis*, also observed $F'_m > F_m$. Using a PAM 2000, under red light illumination ($150 \mu\text{mol m}^{-2} \text{s}^{-1}$), these authors measured “true” F_m in the presence of 10^{-5} M DCMU (dichlorophenyl-dimethylurea).

In natural environments, dark-adaptation state is indeed rare or difficult to observed, except before dawn. Moreover, neither plants nor algae experience sudden transitions from complete darkness to light similar to those of a saturation pulse, although natural light supply is usually dynamic. On the other hand, it is well known that in higher plants, a reproducible dark-adaptation state is established after 10–30 min, whereas in some green algae and chromophytes (Chrysophyta, Pyrrophyta, Phaeophyta), 90 min or longer can be required (Büchel and Wilhelm, 1993). Last but not least, quenching in the dark-adapted state may be present in other brown algae (e.g. *Laminaria saccharina*, Axelsson, personal communication), although it is not so frequently reported in the literature. Whatever the origin of this quenching in darkness, and apart from being a consequence of the algal light history still to be investigated, it is curious that this phenomenon seldom appears in the literature. The reason for this last point could be quite straightforward; the lowest actinic light level available with a widely used PAM fluorometer, e.g. the PAM 2000 (Walz), is ca. $80 \mu\text{mol m}^{-2} \text{s}^{-1}$. With higher actinic light intensities, F'_m becomes lower than F_m in *F. serratus* and *F. vesiculosus*, as it is in *Ulva* sp. and *P. palmata*, rendering the FMS entirely operational for quenching analyses.

Returning to Fig. 3c, clearly, $F'_m > F_m$ does not happen, when *F. serratus* is exposed to actinic light higher than ca. $100 \mu\text{mol m}^{-2} \text{s}^{-1}$. The same is true for other Fucaceae, e.g. *F. vesiculosus* (Fig. 5). For this set of experiments, F_m was measured after a 30-min dark adaptation, after which saturating flashes were applied every 30 s to samples illuminated with actinic light of different intensities, ranging from 20 (Act 3) to $100 \text{ (Act 10)} \mu\text{mol m}^{-2} \text{s}^{-1}$. F'_m

was first lower than F_m , afterwards it increased to become higher than F_m , reaching a plateau in more than 10 min. In the same time, qP decreased during the first 2 min, then it increased to reach also a plateau (not shown). Not only is observation of $F'_m > F_m$ a time-dependent phenomenon, but it also depends on the actinic light intensity. From Act 3 to 10, the higher the actinic light, the lower the F'_m values, so far as to be $< F_m$. Of course, the general pattern of fluorescence signal follows a classical slow-fluorescence kinetics: sharp increase, followed by a slow decrease, sometimes a long and smaller increase, and then an F_s . Below $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Act 10), $F'_m \text{ max}$ (i.e. the plateau) was higher than F_m ; from Act 10 and above, F'_m was always lower than F_m . In the last case, $F'_m < F_m$ is likely the consequence of a light-induced nonphotochemical quenching, while $F'_m > F_m$ seems to be a light-induced reorganization of the antennae, rather than the dissipation of a dark-induced quenching.

4. Conclusion

Our results clearly show that the FMS1 may be satisfactorily used to measure *in vivo* chlorophyll fluorescence on algae in laboratory. For experiments in the field, the FMS2, which is a very similar apparatus, should be preferred, since it is powered by removable rechargeable batteries. Both instruments are simple to use, with convenient software control, especially when connected to a PC. However, in comparison with a widely used similar Walz instrument (PAM 2000), both FMS offer fewer possibilities of settings and controls, like variation of far-red light intensity, fine assessment of flash characteristics, or the so-called damping, which is an electronic means of increasing the signal/noise ratio. On the other hand, the wide range of actinic light setting provided by the FMS could be an important advantage, since it allows the observation in some brown algae of an unusual fluorescence phenomenon ($F'_m > F_m$ at low actinic light) that seems to be less often detected with other fluorometers like the PAM 2000. Although the present work was not intended to deal with fluorescence theory, some of these results could raise the question whether some fluorescence parameters and prerequisites, first defined for higher plants or green algae, could or should be used so universally with organisms from other taxonomic groups.

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