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Fast repetition rate fluorometry is not applicable to studies of filamentous cyanobacteria from the Baltic Sea

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Abstract

Fast repetition rate (FRR) fluorometry has been used successfully to investigate the variable fluorescence characteristics of cyanobacteria in oceanic Case 1 waters. In these waters, the effective absorption bands of the light-harvesting pigments for photosystem II (PSII) of the dominant cyanobacterial taxa overlap with the peak of the FRR excitation at 470 nm. The effective PSII absorption of the ecologically-significant filamentous cyanophytes in the Baltic Sea—*Nodularia spumigena* (Mertens ex Bornet and Flahault 1886) and *Aphanizomenon* sp. ((L.) Ralfs ex Bornet and Flahault 1886)—is, however, restricted to beyond 550 nm. We tested the applicability of a currently commercially available FRR fluorometer to studies of these two cyanobacterial taxa. We propose that the FRR technique should not be used in studies on these taxa, or on any cyanophyte containing phycoerythrocyanin instead of phycoerythrin, the former having inefficient PSII light harvesting in the wave band of the FRR excitation. This issue should be taken into account whenever field studies utilizing the FRR system are planned in those Case 2 water bodies, in which cyanobacteria lacking phycoerythin are among the dominant phytoplankton groups.

Since the introduction of its commercial version in the mid-1990s, the fast repetition rate (FRR) fluorometer (Kolber and Falkowski 1992) has been rapidly adopted in the field oceanographic research. Being capable of nearly instantaneous high-frequency in situ determinations of a suite of algal photophysiological parameters, this technique has helped us to comprehend, for example, the extent of iron limitations on phytoplankton growth in the world's oceans (Boyd et al. 2000).

Up to the present time, FRR-based estimates of the photosynthetic competence (F_v/F_m) of cyanobacteria (Cyanophyceae) at the species level are scarce, as the bulk of the published FRR datasets originate from field studies conducted in the world's oceans. So far, the FRR technique has mainly probed the photophysiology of natural phytoplankton populations containing the ubiquitous oceanic picocyanobacterial genera *Synechococcus* spp. and *Prochlorococcus* spp. Physiologically reasonable values of F_v/F_m, falling within the range of 0.20 to 0.55, have been observed in the context of Fe-enrichment experiments (Behrenfeld et al. 1996), and diurnal studies (Behrenfeld and Kolber 1999) on communities codominated by these two genera, as well as in water-column measurements on a *Prochlorococcus*-dominated community (Babin et al. 1996). Berman-Frank et al. (2001) used FRR metrics on a filamentous genus *Trichodesmium* spp., and noted F_v/F_m to vary from 0.25 to 0.55, depending on the severity of Fe-limitation in the strain IMS101.

The Baltic Sea is categorized as a Case 2 water body (Morel and Prieur 1977; Babin et al. 2003) due to its high load of suspended matter, but especially due to its high concentration, for a marine system, of chromophoric dissolved organic matter (CDOM) (Kirk 1994). The pelagic Baltic Sea represents a coastal type 3 in the Jerlov classification system (Jerlov 1976), with its peak transmittance from 550 to 570 nm, depending on the time of the year (Seppälä and Raateoja unpubl.). Here, the ecologically significant filamentous cyanobacterial taxa are the diazotrophic *Nodularia spumigena* and *Aphanizomenon* sp. Based on their spectral fluorescence and absorption characteristics, it is suggested that these species do not contain phycoerythrin (PE, λ_{Amax} from 495 to 575 nm, depending on the chromophores), but rather phycoerythrocyanin (PEC, λ_{Amax} from 570 to 595 nm), instead (Seppälä et al. 2004). As cyanobacteria cannot contain both PE and PEC (Bryant 1982), the photosystem II (PSII) absorption band of these species lies beyond the FRR excitation, 95% of which is within the range of 458 to 514 nm. Hence, we hypothesized that the FRR technique could not efficiently derive variable fluorescence characteristics from these taxa.

In this paper, we concentrate on two important filamentous cyanobacterial species in the Baltic Sea ecosystem, and we demonstrate that their photosynthetic activity as determined by the FRR technique is very low; much lower than the levels reported earlier for the ubiquitous oceanic cyanobacteria. Furthermore, the basic FRR measuring protocol is tested against two techniques based on 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)-induced variable fluorescence, and we show that the low photosynthetic activities observed are artifacts caused by the FRR methodology. The results obtained here are not universally applicable to other cyanobacterial taxa or oceanic regions.

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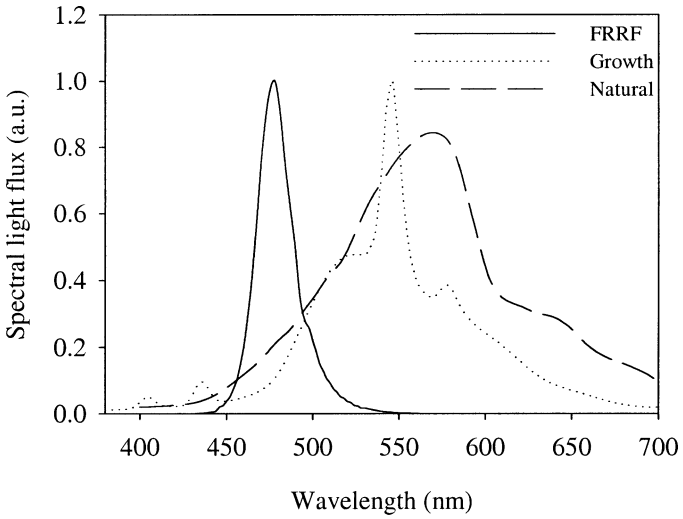

Fig.1. The excitation spectrum of the light source of the FAST*tracka* (FRRF: an array of Nichia NSPB300A LEDs with a BG39 filter), the light environment in which the four phytoplankton taxa studied were grown (Growth), and the natural Baltic Sea light environment (Natural). Spectra are relative to quanta.

Description of experiment

Unialgal batch cultures of Baltic Sea strains of *Nodularia spumigena* (strain HEM, Cyanophyceae) and *Aphanizomenon* sp. (strain KAC15, Cyanophyceae) were grown in a Z8 medium (salinity 7.0) at 18°C in 5-liter Erlenmeyer flasks. Cultures were acclimated to the 16 : 8 light : dark cycle at a low greenish irradiance (20 μmol quanta m^{-2} s^{-1}, Fig. 1) for several generations. For comparison, two eukaryotic phytoplankton species, *Prymnesium parvum* (strain KAC39, Prymnesiophyceae) and *Nannochloris* sp. (strain Tv14, Chlorophyceae), were cultured under similar conditions to those of the cyanophytes, except that a modified Erd-Schreiber growth medium (salinity 6.0) was used. Phytoplankton samples for fluorescence measurements were harvested while they were in the exponential phase of their growth curve.

The phytoplankton absorption coefficient ($a_{ph}(\lambda)$, m^{-1}, 380 to 800 nm) was measured using a filter-pad technique with a dual-beam spectrophotometer (Shimadzu 2101-UVPC) equipped with an integrating sphere (Tassan and Ferrari 1995). The fluorescence excitation spectra $F_{680}(\lambda)$ (excitation 380 to 670 nm, emission 680 nm) and $F_{730}(\lambda)$ (excitation 380 to 700 nm, emission 730 nm) of a DCMU-treated suspension (final concentration 20 μmol L^{-1}) were measured using a spectrofluorometer (Shimadzu RFPC-5001). All spectra were corrected for CDOM fluorescence and Raman scattering. The quanta-corrected $F_{680}(\lambda)$ and $F_{730}(\lambda)$ indicate the shape of the PSII absorption (Neori et al. 1988). The absorption coefficient for the photosynthetic pigments of PSII ($a_{ps}(\lambda)$, m^{-1}) was estimated by scaling quanta-corrected $F_{730}(\lambda)$ with $a_{ph}(\lambda)$ so that no overshoot took place in the wavelength interval of 500 to 690 nm (Johnsen and Sakshaug 1996).

The calibration of the baseline, scatter, and reference functions of the FRR fluorometer FAST*tracka* (Chelsea Technologies Group) was carried out according to the manufacturer's instructions, except that the instrument response function (IRF) was calibrated at each of the gain settings instead of at a gain of 16 only. Furthermore, an additional baseline measured from the 0.7 μm-filtered (Whatman GF/F) sample was used to subtract the effect of CDOM from the fluorescence transients. The FAST*tracka* excited light at 470 nm with a 30-nm half-bandwidth (Fig. 1), and the emission setup comprised a 685 nm band-pass interference filter, and a 665 nm long-pass filter. The samples were measured using a custom-built 100-ml quartz cuvette on the optical head of the ambient irradiance side of the FAST*tracka*. For the basic FRR determinations, an individual FAST*tracka* acquisition consisted of an average of five successive flash sequences. The flash sequence consisted of 100 saturation flashlets, each 1.52 μs in length (11 instrument units) at 2.80-μs intervals, and 20 relaxation flashlets of the same length at 50-μs intervals. The energy of a flashlet on the light-adapted side at which the quartz cuvette was supported was 7.9×10^{15} quanta m^{-2}, as calibrated by the manufacturer. The time gap between the acquisitions was 30 ms.

The post-processing software v5LITE (S. Laney), based on the equations of Kolber et al. (1998), was adopted to retrieve variable fluorescence parameters from the raw fluorescence data: (1) the initial and maximal in vivo fluorescence yields for dark-adapted (F_0 and F_m) and light-treated (F and F_m') samples, and (2) the corresponding photochemical energy conversion efficiencies (F_v/F_m and $\Delta F/F_m'$). By definition, F_v and ΔF refer to the variable fluorescence in the dark-adapted ($F_m - F_0$) and light-treated ($F_m' - F$) samples, respectively.

For the basic FRR determinations, the phytoplankton assemblages were dark-adapted for 30 min before the determination, during which no additional background light was provided. A total of five replicate acquisitions were measured. The resulting photochemical energy conversion efficiency is denoted as F_v/F_m(FRR).

The FRR transients were also monitored at 4- and 0.5-s intervals after the addition of DCMU (final concentration 20 μmol L^{-1}) to the dark-adapted, but also to the light-treated samples that were kept under light conditions matching the growth environment both before and during the determinations. DCMU was added ''on the run'' during the FRR measuring protocol, and the first few measurements served as a reference. The resulting photochemical energy conversion efficiencies were based on F_0 or F probed before the DCMU addition, and F_m or F_m' after the addition, respectively, and are denoted as F_v/F_m(D) and $\Delta F/F_m'$(D).

To optimize the instrument's output and to assure that the measurements were made within the instrument's linear range, we adjusted the sensitivity of the photomultiplier tube (PMT) separately for each of the cultures. The sensitivity of the gains was regulated by adjusting the internal PMT values of the gains. As a result, fluorescence emission triggered by an individual flashlet in the basic FRR determinations varied within a range from 3200 to 7300 instrument units regardless of the taxa studied. In our experience, the emission range from ~1,500 (for F_0) to ~9,000 (for F_m) instrument units provides reliable results.

We determined the wavelength dependence of the photochemical energy conversion efficiency by measuring the ini-

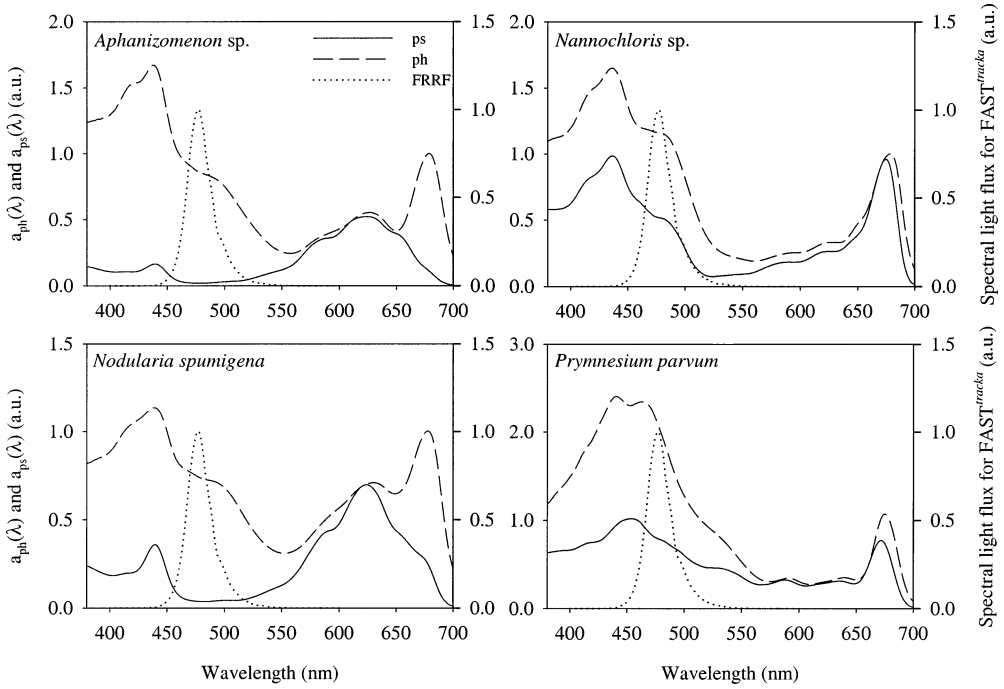

Fig. 2. Spectral absorption characteristics of the four phytoplankton taxa studied: the absorption coefficients of phytoplankton ($a_{ph}(\lambda)$, here ph), and of the photosynthetic pigments of PSII ($a_{ps}(\lambda)$, here ps). $a_{ph}(\lambda)$ was normalized at the Chl *a* red peak at 679 nm. The dotted line in each panel is the excitation spectrum (relative to quanta) of the light source of the FAST^{tracka}.

tial (steady state) fluorescence yield before, and the maximal yield after the addition of DCMU (final concentration 20 μmol L^{-1}, and illuminated 1 to 3 min with white light to saturate PSII) at 680 nm using a spectrofluorometer (Shimadzu RFPC-5001) with excitation and emission slits of 3 nm, except that 5 nm was used for *Aphanizomenon.* A total of 12 excitation wavelengths from 420 to 590 nm were specifically selected for each species, based on their absorption properties. Due to the continuous spectrofluorometric excitation, this approach cannot directly assess F_0. Thus, instead of the ''true'' photochemical energy conversion efficiency for each wavelength ($F_v/F_m(\lambda)$), we obtained an underestimate of it ($F_v/F_m(\lambda)$*). To obtain an estimate of $F_v/F_m(\lambda)$* as close to its maximum attainable level as possible, it was modeled as a function of various levels of excitation deliveries (EDs). Various combinations (4 to 7) of neutral screens (Rosco) were placed in the excitation light path of the spectrofluorometer. For wavelength λ and screen combination i, the ED (μmol quanta m^{-3} s^{-1}) was defined as

$$ED(i, \lambda) = a_{ps}(\lambda) \times E(\lambda) \times T_i(\lambda)/100$$

where $E(\lambda)$ is the spectral excitation of the spectrofluorometer (μmol quanta m^{-2} s^{-1}), and $T_i(\lambda)$ is the measured transmittance (%) of the screen combination i. As a primary empirical model, a second-order polynomial was fitted to ED and $F_v/F_m(\lambda)$*, the intercept giving the target value of $F_v/F_m(\lambda)$ when ED is 0 μmol quanta m^{-3} s^{-1}. If the regression coefficients were not significant ($p > 0.05$), a linear model was used instead. If no ED-dependent trend was observed in $F_v/F_m(\lambda)$*, an average value of the observed $F_v/F_m(\lambda)$* was adopted. In the calculations, the fluorescence originating from the 0.7-μm-filtered sample (Whatman GF/F) was subtracted from the results for every wavelength and screen combination to compensate for the effect of CDOM.

The field sampling was carried out in the southwest Finnish archipelago in the Baltic Sea. The sample was taken from a developing cyanobacterial bloom at a depth of 3 m (at an optical depth of ~1) in order to ensure that the algal community was not severely photo-inhibited. The sample, concentrated using a plankton net of 50-μm-mesh size, consisted almost exclusively of the cyanobacterial taxa *Nodularia spumigena.* The sample was kept in darkness until the FRR measurements were made. This took place within 1 h after the sampling. The natural light spectrum was measured at the same location, at a depth of 5 m, but clear of bloom patches, using a spectroradiometer RAMSES (Trios GmbH) (Fig. 1).

Results

The chlorophyll *a* (Chl *a*) absorption in *Aphanizomenon* and *Nodularia* was overlapped by the absorption of phycobilins at 570 to 650 nm (Fig. 2). For all taxa, the Chl *a* blue peak at 440 nm was overlapped by the absorption of various carotenoids, and additionally by the absorption of chlorophyll *b* (Chl *b*) in the case of *Nannochloris,* and by that of chlorophyll *c* (Chl *c*) in the case of *Prymnesium.* Clearly, for *Nannochloris* and *Prymnesium,* the shapes of $a_{ph}(\lambda)$ and $a_{ps}(\lambda)$ were quite similar, the difference being mainly indicative of photo-protective carotenoids. These two spectra differed more in *Aphanizomenon* and *Nodularia,* as PSII emission is almost solely influenced by the phycobilins, and most of the Chl *a* is located in low-fluorescing photosystem I

Table 1. The photochemical energy conversion efficiency (F_v/F_m) of the four phytoplankton taxa studied, as estimated by the basic FRR transients (F_v/F_m(FRR)), by the natural sample (F_v/F_m(FRR) in situ)), by the DCMU-enhanced FRR kinetics (F_v/F_m(D) and $\Delta F/F'_m$(D)), and by the spectrofluorometric method using the excitation/emission combination 470 nm/680 nm (F_v/F_m (470)). The 95% confidence intervals in parentheses are given. nd = no data.

	Aphanizomenon	*Nodularia*	*Nannochloris*	*Prymnesium*
F_v/F_m(FRR)	0.090 (0.073–0.107)	0.065 (0.027–0.104)	0.495 (0.487–0.502)	0.441 (0.433–0.449)
F_v/F_m(FRR) in situ	nd	0.116 (0.064–0.168)	nd	nd
F_v/F_m(D)	0.278 (0.196-0.359)	0.294 (0.269–0.318)	0.659 (0.648–0.670)	0.724 (0.719–0.729)
$\Delta F/F'_m$(D)	0.553 (0.531–0.575)	0.638 (0.627–0.648)	0.642 (0.593–0.692)	0.645 (0.599–0.692)
F_v/F_m(470)	0.487 (0.464–0.509)	0.463 (0.425–0.502)	0.580 (0.569–0.591)	0.508 (0.468–0.548)

(PSI). For each species, the shapes of $F_{680}(\lambda)$ and $F_{730}(\lambda)$ were identical (data not shown).

Aphanizomenon and *Nodularia* presented very low levels of photosynthetic competence, determined by the basic FRR transients (F_v/F_m(FRR)), whereas in *Nannochloris* and *Prymnesium*, the values stayed at physiologically reasonable levels (Table 1, Fig. 3). We tried to accelerate the saturation rate of PSII in *Nodularia* by increasing the FRR excitation delivery. We gradually extended the duration of the flashlet up to 3.92 μs (~2.6-fold of the default), but this did not lead to any changes in F_0, F_m, or F_v/F_m(FRR). To explore the lowest boundary of F_v/F_m(FRR) in *Nodularia*, DCMU was added to the dark-adapted sample, but the sample was subsequently kept in strong daylight-mimicking light before the FRR measurements to ensure as full a blockage of the electron transport chain (ETC) as possible. The resulting values were ~0.10 (data not shown). Increases in the fluorescence yield in the course of the FRR saturation protocol, leading to F_v/F_m value >0, are observed even without any photosynthetic sample (Laney 2003). This characteristic of the instrument is rectified by the IRF calibration. In the study by Laney (2003), the IRF correction of v5LITE lowered F_v/F_m by 20%, whereas in our study, the corresponding decrease was 5% (data not shown). Even though a zero-level of F_v/F_m was not achieved due to our IRF correction, we consider F_v/F_m(FRR) ~0.10 as a boundary value representing the lowest value the iterative procedure could retrieve using our calibration setup. Consequently, the basic FRR measurements suggested that the photosynthetic activity of *Aphanizomenon* and *Nodularia*, as estimated by F_v/F_m(FRR), were near zero.

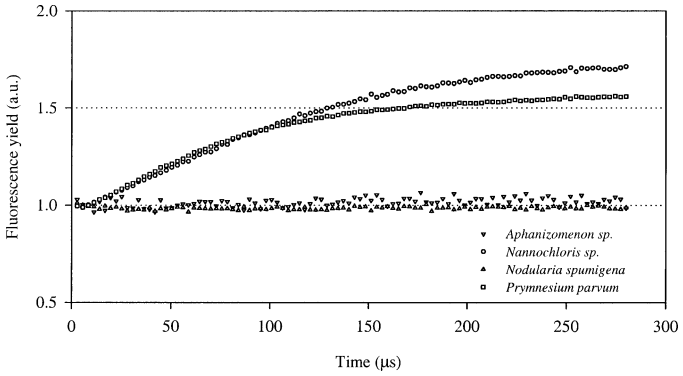

Fig. 3. The raw FRR fluorescence induction kinetic curves for the four phytoplankton taxa studied, showing that the FRR protocol used failed to saturate PSII of *Aphanizomenon* and *Nodularia* within a single turnover of PSII. The phytoplankton assemblages were dark-adapted for 30 min before the determination, during which no additional background light was provided. The fluorescence yield equals the ratio of the light flux on the emission side of the FAST*tracka* to that on the excitation side. The instrument gains were adjusted so that fluorescence emission did not increase above 7,300 instrument units in any taxa studied. Thus, the flat induction curves for *Aphanizomenon* and *Nodularia* did not result from an excessive fluorescence emission reaching the internal limiter (at ~11,000 units).

The measurement and interpretation of variable fluorescence parameters in cyanobacteria is problematic, and the cyanobacterial light-harvesting machinery has characteristics that may cause lower F_v/F_m levels than those observed in eukaryotic phytoplankton (Büchel and Wilhelm 1993; Campbell et al. 1998). To discover whether the observed difference in F_v/F_m(FRR) between *Aphanizomenon/Nodularia* and *Nannochloris/Prymnesium* was real or whether it was just caused by the methodology, a series of tests was carried out that led to the following conclusions.

First, the low F_v/F_m(FRR) values for *Aphanizomenon* and *Nodularia* were not due to the special characteristics of the laboratory strains, because F_v/F_m(FRR) in situ, measured from a natural *Nodularia* sample, showed equally low values (Table 1).

Second, the DCMU-induced values of photosynthetic competence (F_v/F_m(D)) were clearly higher than the corresponding F_v/F_m(FRR) values, indicating that the observed low F_v/F_m(FRR) values for *Aphanizomenon* and *Nodularia* were artifacts (Table 1). Even higher values ($\Delta F/F'_m$(D)) for *Aphanizomenon* and *Nodularia* were obtained when background light was provided.

Third, the spectral photochemical energy conversion efficiency ($F_v/F_m(\lambda)$) corroborated the hypothesis that the observed F_v/F_m(FRR) did not reflect the true photosynthetic activity of either *Aphanizomenon* or *Nodularia*. $F_v/F_m(\lambda)$ did not vary significantly between wavelengths (data not shown), but was always >0.38 in the waveband 420 to 590 nm regardless of species. F_v/F_m(470), which is most closely comparable to the excitation wavelength of the FAST*tracka*, was always >0.46 regardless of species, and the values for *Aphanizomenon* and *Nodularia* were almost as high as those for *Nannochloris* and *Prymnesium* (Table 1).

We determined $F_v/F_m(\lambda)$ following a protocol that can be perceived as a piece-wise fluorescence excitation spectrum composed of a sequence of excitation/emission combinations, in which the emission wavelength was fixed at 680

nm, corresponding to the emission of the FAST*tracka*. Our rationale for this approach was that previously scanned wavelengths would affect the light-adaptive state of the phytoplankton assemblage if regular fluorescence excitation spectra were to be used. The outcome of the protocol is very sensitive to the excitation light flux of the spectrofluorometer, and the modeling of $F_v/F_m(\lambda)$* as a function of ED is needed to obtain an estimate of $F_v/F_m(\lambda)$ as close as possible to the ''true'' value, which is, by definition, not lowered by fluorescence quenching mechanisms.

Discussion

Eukaryotic phytoplankton possessing pigment-protein complexes with chlorophylls and carotenoids as pigment parts (Jeffrey and Vesk 1997) usually exhibit efficient PSII absorption of FRR excitation. Cyanophytes show much more variability in their efficiency to absorb light in this wave band, due to their heterogenous pigmentation, with the presence or absence of PE as the shortest wavelength-absorbing rod element and, in the case of PE being present, the domination of either phycourobilin (PUB, λ_{Amax} at 495 to 500 nm), or phycoerythrobilin (PEB, λ_{Amax} 540 to 575 nm) (Sidler 1994). The difference in the light-harvesting characteristics of PSII between the *Aphanizomenon/Nodularia* and *Nannochloris/Prymnesium* is illustrated by F_{730} (Fig. 2). *Nannochloris* and *Prymnesium* ensure an efficient excitation delivery to their PSII in the blue/green wave band by harboring Chl *a* and Chl *c* (*Prymnesium*), and Chl *a* and Chl *b* (*Nannochloris*) in their PSII antenna (Anderson and Barrett 1986). They exhibit pronounced fluorescence triggered by the excitation between 450 and 500 nm—the wave band of the FRR excitation—but hardly any fluorescence is emitted by *Aphanizomenon* and *Nodularia* when excited with light in that wave band. As the capability of the PSII of *Aphanizomenon* and *Nodularia* to absorb the FRR excitation seems to be very low, only a small part of the FRR excitation delivery reaches their PSII.

The FRR excitation delivery to PSII is a function of excitation light flux and spectral matching between the excitation light and the absorption characteristics of a phytoplankton assemblage. Because of the poor spectral matching between the FRR excitation and cyanobacterial PSII absorption, the extremely high efficiency although short lived ($\sim$4700 μmol q m^{-2} s^{-1} during the 280-μe) excitation of FAST*tracka* failed to accomplish the key requisite for the FRR technique (i.e., to saturate PSII within a single turnover of PSII (Falkowski and Kolber 1995)). The incomplete saturation of the PSII resulted in the underestimations of F_m (Fig. 3), and hence, F_v/F_m(FRR). Thus, it seems obvious that the FAST*tracka* with the current excitation setup cannot provide reliable estimates of F_v/F_m for *Aphanizomenon* and *Nodularia*. Similar low F_v/F_m(FRR) levels for these taxa have been observed under various growth conditions (light levels, spectral environments, nutrient limitation), and in a range of biomasses (from 5 to 500 μg Chl *a* L^{-1}, n = 43, Ylöstalo et al., unpubl.).

The FRR technique relies on excitation delivery to PSII to overcome the reoxidation rates of the stable electron acceptors downstream of P680, which is the primary electron donor in PSII (Kolber and Falkowski 1992). DCMU, in turn, has a chemical function that blocks the ETC downstream of P680 permanently by inhibiting the reoxidation of Q_A^-, which is the primary stable electron acceptor in PSII. The addition of DCMU with subsequent illumination leads to the simultaneous increase in the PSII fluorescence yield with the gradual closing of the reaction centers of PSII, as light energy is no longer channeled to maintain the linear electron transport. Thus, the DCMU effect is dependent on light dose (e.g., the fluorescence rise toward F_m depends not only on the excitation delivery, but also the time window of the delivery). DCMU provides a more profound way to assess the variable fluorescence transients (especially F_m) of *Aphanizomenon* and *Nodularia* than the FRR technique, and we can consider the F_v/F_m(D) and $\Delta F/F_m'$(D) values as being closer to the ''true'' values of F_v/F_m.

The poor FRR excitation delivery to the cyanobacterial PSII was reflected to lower F_v/F_m(D) than $\Delta F/F_m'$(D) values in *Aphanizomenon* and *Nodularia.* The rationale is that after DCMU has stemmed the photosynthetic electron flow, a degree of excitation delivery to PSII is still needed to saturate the ETC downstream of P680, before the fluorescence yield increases to F_m. However, in the course of DCMU treatment, the FRR excitation delivery alone could not provide as complete a blockage of the cyanobacterial ETC as when it was supported by the background light. Consequently, the increase in the fluorescence yield was not maximal for the dark-adapted samples, and our way of calculating F_v/F_m(D) led to the observed values near 0.30 instead of $>$0.50 when supported by the background light ($\Delta F/F_m'$(D)). This phenomenon was not observed with *Nannochloris* and *Prymnesium,* because their PSII absorbed the FRR excitation efficiently enough to provide complete blockage of the ETC, and no supporting background light was needed.

In addition to the lowered F_m, the low FRR-based F_v/F_m in *Aphanizomenon* and *Nodularia* may be partly caused by a high F_0 in relation to F_m. In the variable fluorescence theory it is traditionally assumed that the parts of F_v/F_m (i.e., F_0 and F_v) should both originate from PSII (Krause and Weis 1991). In cyanobacteria, F_v does seem to originate from PSII, but phycobiliprotein fluorescence, the small fraction of cellular Chl *a* that is in PSII, and possibly also Chl *a* in PSI all form a part of F_0, which may lower F_v/F_m (Campbell et al. 1998). Strictly speaking, this traditional view of the origin of fluorescence does not entirely hold true. PSI is responsible for 1% to 2% of F_m at 685 nm (a wavelength matching the emission peak of the FAST*tracka*), as observed in a microalgal species (Holzwarth 1990), and in a higher plant (Franck et al. 2002), and as much as 10% of F_0 at 685 nm (Franck et al. 2002). A high F_0 in relation to F_m in phycobilisome-containing algae may also result from large phycobilisome antenna serving PSII, but only a small antenna serving PSI (Büchel and Wilhelm 1993). If the decrease in F_v/F_m(FRR) were caused to any marked extent by an increased F_0, both the DCMU induction and the spectrofluorometric method should give low values. This was, however, not observed. Consequently, we argue that it was the incomplete saturation of PSII that caused the observed low F_v/F_m(FRR).

In the world's oceans, the FRR excitation matches the

natural light habitat rather well (Suggett et al. 2001), and it is apparent that the ubiquitous cyanobacterial taxa adapted to the bluish oceanic light regime—*Prochlorococcus, Synechococcus,* and *Trichodesmium*—respond strongly to FRR stimulation. PE, with PUB and PEB as chromophores, serves as the main light-harvesting pigment for *Synechococcus* and *Trichodesmium* (Wood et al. 1998; Subramaniam et al. 1999). *Prochlorococcus* in turn harbors pigment complexes containing Chl a_2 (λ_{Amax} 443 to 450 nm) and Chl b_2 (λ_{Amax} 476 to 480 nm) (Ting et al. 2002). These pigmentations are efficient in harvesting the FRR excitation peaking at 470 nm, especially as PUB predominates over PEB in bluish marine waters (Wood et al. 1998). In the Baltic Sea, which is categorized as a Case 2 water body, the spectral matching between the FRR excitation and the natural light habitat is, however, poorer (Fig. 1, Raateoja et al. 2004). The light-harvesting machinery of the dominant filamentous cyanobacterial taxa in the Baltic Sea—*Nodularia* and *Aphanizomenon*—respond to the Baltic Sea light environment by being able to harvest light in a longer wave band than the ubiquitous oceanic cyanobacterial taxa; the former harbor PEC instead of PE, thus missing most of the FRR excitation delivery.

Generally speaking, due to the flexible light harvesting characteristics of cyanobacteria, the feasibility of the FRR technique for use in cyanobacterial research depends ultimately on the particular aquatic system under study, and its inherent optical properties. As it seems obvious that PEC cannot harvest the FRR excitation with the efficiency required for sound quantitative results, the precondition for efficient FRR absorption seems to be the possession of PE. Furthermore, as 95% of the FRR quantum flux is within the range of 458 to 514 nm, even the dominance of either PUB or PEB may play a role in determining whether the FRR technique is able to provide quantitatively reliable estimates of the variable fluorescence parameters.

Cyanobacteria show a low ratio of in vivo fluorescence to Chl *a* when measured using conventional fluorometers employing blue excitation (Cunningham 1996). As FAST*tracka* can be regarded as a conventional fluorometer in this respect, the cyanobacterial fluorescence signal easily gets swamped by the eukaryotic one in water column measurements. We conducted a seasonal study at the entrance to the Gulf of Finland in the Baltic Sea in the year 2000, and observed that even though cyanobacteria comprised up to 60% of the total biomass of the nanophytoplankton and microphytoplankton community (wet weight) in late summer (Raateoja et al. 2004), no variability in the F_v/F_m values in situ could be related to the taxonomy (Raateoja and Seppälä unpubl.). The effect of the presence of cyanobacteria on F_v/F_m, if it existed, was overridden by fluorescence-quenching mechanisms driven by environmental forcing. The natural cyanobacterial blooms in the Baltic Sea, apart from the actual surface scums, usually consist of more than one species, with representatives from eukaryotic taxa as well. Thus, the FRR parameters can be retrieved reasonably from a mixed algal community of this kind, but the results describe the eukaryotic part of the community, not the blooming cyanobacteria, as the bulk of the fluorescence signal originates from the eukaryotic taxa. In this light, the low F_v/F_m(FRR) levels measured from our field sample were simply due to the sample consisting almost exclusively of *Nodularia.* Further study is needed on natural phytoplankton populations containing or dominated by cyanobacteria in different aquatic environments in order to assess to what extent the observed variable fluorescence characteristics actually originate from the prokaryotic part of the community.

The dominant cyanobacterial species in the Baltic Sea, *Nodularia spumigena* and *Aphanizomenon* sp., cannot efficiently harvest the FRR excitation, and thus the FRR technique is not recommended for quantitative research on these taxa. The inability of the FRR system to work with cyanobacterial species lacking PE is one of few defects of a system that can monitor a suite of algal photophysiological parameters from a number of systematic groups. Quite reasonably, FAST*tracka* was originally targeted to excite Chl *a*—the ubiquitous pigment within the oceans. The requirement of exciting the phycobiliproteins will be met in the future (J. Dunning, Chelsea Technologies Group, pers. comm.). With this development, the FRR system should then be applicable for probing the variable fluorescence characteristics of cyanobacteria lacking PE. As evidenced by various authors, this apparent deficiency of the FRR system does not seem to apply to the world's oceans described as Case 1 waters, in which the FRR excitation matches well the light environment to which the dominant cyanobacterial species are adapted.

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