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Comparison of different variable chlorophyll *a* fluorescence techniques to determine photosynthetic parameters of natural phytoplankton.

Author
Rüdiger Röttgers

Address
Institute for Coastal Research, GKSS Research Centre Geesthacht,
Max-Planck Strasse 1, D-21502 Geesthacht, Germany

Email
rroettgers@gkss.de

Short Title
Comparison of different variable chlorophyll fluorescence techniques

Abstract
Different methods for the determination of the maximum and the effective quantum efficiency of photochemistry using variable chlorophyll *a* fluorescence (Pump and Probe [P&P], Pulse Amplitude Modulation [PAM], and Fast Repetition Rate [FRRF]) were compared using natural samples. The methods differ in the applied light pulse used to saturate PSII photochemistry, regarded as single (STF) and multiple (MTF) turnover flashes. Measurements were conducted *in situ* and on natural phytoplankton samples during two cruises, one to the North Sea and one to the South Atlantic. In both cases *in situ* STF measurements using FRRF were identical to measurements on discrete samples using P&P, confirming that different instrumentation and methods agreed well for the determination of STF parameters. On the other hand, estimates of the quantum efficiencies calculated from MTF (PAM) measurements were higher than quantum efficiencies calculated from STF (P&P) measurements. However, the relative differences in the quantum efficiencies determined with MTF and STF measurements were the same in both cruises and agreed well with formerly reported differences determined on algal cultures. A simple factor can be used to convert the maximum quantum efficiency determined either by MTF or STF measurement, at least for diatom dominated phytoplankton. Similarly a non-linear function can be used for the conversion of the effective quantum efficiency. Finally, photosynthesis (P) vs. irradiance (E_0) relationships calculated from the effective quantum efficiency differed between MTF and STF measurement, but the basic parameters of the P vs. E_0 curve, i.e. the maximum electron transport rate, ETR_m, and the light efficiency factor, α, showed a linear relationship and can be converted by a linear function. These relationships solve some problems of converting results from STF measurements into those of MTF measurements or vice versa.

1. Introduction

For about 20 years active fluorescence techniques have been used to measure variable chlorophyll (Chl) *a* fluorescence, and the photophysiological parameters assessed thereby have been used to describe and quantify photosynthetic electron transport through photosystem II (Falkowski *et al.* 1986, Schreiber *et al.* 1986, Kolber & Falkowski 1993). These techniques have been compared to other oxygen- or carbon-based photosynthesis measurements, and a close correlation of the quantum yield of photochemical electron transport and that of photosynthesis has been shown (e.g. Kolber & Falkowski 1993, Flameling & Kromkamp 1998, Gilbert *et al.* 2000, Hartig *et al.* 1998), since Genty *et al.* (1989) observed that a simple fluorescence parameter ($\Delta F/F'_m$, $\Delta \Phi_F/\Phi_{Fm}$ in the original paper) correlates well with the quantum yield of CO_2 assimilation. Since then photosynthesis has often been quantitatively and qualitatively assessed by variable chlorophyll *a* fluorescence measurements for all kinds of autotrophs, like higher plants, symbiotic algae in corals and phytoplanktonic algae. Measurements of phytoplanktonic algae under natural conditions are most difficult because of low cell densities that necessitate highly sensitive instruments.

Different principles for measuring variable chlorophyll *a* fluorescence have been developed, which differ in the manner by which photochemistry is saturated to determine the maximum fluorescence yield, F_m. These are e.g. the "pulse amplitude modulation" (PAM) fluorometry (Schreiber *et al.* 1986), the "pump and probe" (P&P) fluorometry (Mauzerall 1972, Falkowski *et al.* 1986), the "fast repetition rate" (FRRF) fluorometry (Kolber *et al.* 1998), the "fluorescence induction and relaxation" (FIRe) technique (Gorbunov and Falkowski 2004) and the background irradiance gradient single turnover (BIG-STf) fluorometer (Johnson 2004). Commercial instruments for laboratory use are available for all techniques including the PAM (Walz, Effeltrich, Germany, for PAM and P&P), the Fl-200 (Photon System Instruments, Brno, Czech Republik, for PAM, P&P and FRRF), the FastTracka (Chelsea, West Molesey, UK, for FRRF), the FMS (Hansatech, King´s Lynn, UK, for PAM), the AFM (Opti-Sciences, Tynsboro, USA, for PAM) and the FIRe (Satlantic, Halifax, Canada, all protocols). A few instruments are sensitive enough to be used with dilute, natural phytoplankton (Xe-PAM, FastTracka, FIRe, Fl-200) and one (FastTracka) can be used *in situ* in the water column. The advantage of these techniques is that the fluorescence signal induced by a non-actinic (non photosynthetic) light source can be separated from the fluorescence induced by any actinic light, which allows the independent determination of variable fluorescence (F_v) under background irradiance. The PAM technique originally determines the fluorescence yield using a weak red LED whose excitation amplitude is modulated to separate the induced fluorescence signal from fluorescence induced by actinic light. The minimum fluorescence yield, F_0 or F' (see abbreviation list for details), is determined continuously, then photochemistry is saturated by a relatively long (500 to 1000 ms), strong light pulse of 5000 to 10000 µmol photons m^{-2} s^{-1}. During this pulse the maximum fluorescence, F_m or F'_m, is determined. In the presented study a Xe-PAM instrument is used, which uses weak and short (<2 µs), and thereby non-actinic, flashes to determine fluorescence with a flash frequency between 2 and 64 Hz. It can also be used for the P&P technique, where the maximum fluorescence is determined with a non-actinic "probe" flash following a strong "pump" flash (> 20000 µmol photons m^{-2} s^{-1}) coming from a second, stronger xenon flash lamp, which saturates photochemistry in <10 µs. F_m is typically determined when the "pump" to "probe" flash interval is between 30 and 100 µs (Mauzerall 1972, Falkowski & Raven 1997). The

short "pump" flash causes a single photochemical turnover by a reduction of the primary acceptor, Q_A, and is regarded as a single turnover flash (STF). It induces the photochemical phase of the chl a fluorescence only (Samson *et al.* 1999). The measured maximum fluorescence yield is called $F_{m(ST)}$. A longer flash duration of lower intensity induces multiple photochemical turnovers (MT), does fully reduce Q_A but probably reduces plastoquinone in addition. The maximum fluorescence increases because plastoquinone is known as a quencher of chlorophyll fluorescence. This so-called multiple turnover flash (MTF) is typically used by the PAM technique.

The kinetics of the rise of the fluorescence signal during a saturating flash ("Kautsky curve") is dependent on specific photophysiological parameters, like the functional absorption cross-section, σ_{PSII}, and the PSII reaction centre connectivity, p. The third variable Chl a fluorescence technique, FRRF, was developed to enable the absolute determination of these additional parameters together with the basic parameters F_0 and F_m and the fluorescence relaxation kinetics (Kolber *et al.* 1998). This technique is in principal highly flexible and allows the determination of STF and MTF characteristics. It uses a set of short LED flashlets (e.g. 100 - 2000), the duration and frequency of which can be independently adjusted, effectively resulting in lower or higher excitation energies. With a short (<150 µs) pulse of flashlets with higher excitation energy, STF events and, with a longer pulse (60 – 500 ms), MTF events are determined. At lower excitation energy fluorescence relaxation processes can be followed. However, the commercially available FastTracka instrument (Chelsea, UK) was designed for *in situ* profiling measurements using a high sampling rate. Because of that the FRRF protocol used is set to measure STF events only.

As stated above, the F_m values determined by STF and MTF differ considerably, and $F_{m(MT)}$ has been reported to be up to 50 % higher than $F_{m(ST)}$ (Kolber *et al.* 1998, Samson *et al.* 1999, Koblížek *et al.* 2001, Kromkamp & Forster 2003). The different methods used with each kind of instrumentation cause differences in obtained photochemical quantum efficiencies. This has led to confusion in the determined parameters and to wrong interpretation, as stressed by Kromkamp and Forster (2003), who suggested a terminology to separate parameters determined with a STF from that determined with a MTF in addition to the terminology of van Kooten and Snel (1990). In general the maximum quantum efficiency by a STF, $\Delta\Phi_m$, can be as high as 0.65, whereas that of a MTF, F_v/F_m, can be up to 0.83 (Samson *et al.* 1999), which is about 28 % higher. Samson *et al.* (1999) pointed out that "In practice, the use of MTF provides closer estimates of the actual quantum yields of O_2 evolution ... and CO_2 assimilation...". Hence, photosynthetic quantum efficiencies are underestimated by STF measurements. It would be helpful and important to convert MTF-related photochemical quantum efficiencies to STF-related efficiencies and vice versa.

Only a few comparisons between STF and MTF determination have been conducted under different actinic irradiance levels using tomato plants (Samson *et al.* 1999) and microalgal cultures (Koblížek *et al.* 2001, Suggett *et al.* 2003). The different comparisons did not agree regarding the correlation between effective quantum efficiencies for ST and MT events, $\Delta F/F_m'$ and $\Delta\Phi$ (see discussion). It is not known whether the relationship between quantum efficiencies determined by STF and MTF techniques is constant or dependent on other parameters, like temperature, algal physiology, algal type, etc. Species-specific differences were shown by Koblížek *et al.* (2001) but were not apparent in the study of Suggett *et al.* (2003). A constant factor

would allow a simple calculation of MTF parameters from STF-derived ones and vice versa.

The intention of the present work is to compare different instruments and variable Chl *a* fluorescence techniques in determining the basic parameters F_v/F_m and $\Delta\Phi_m$, $\Delta F/F_m'$ and $\Delta\Phi$, and the photosynthesis vs. irradiance parameters calculated from those, using natural phytoplankton communities. This was done under actual measuring and sampling conditions; i.e., we explicitly compared *in situ* measurements by FRRF (STF) to lab-based measurements on discrete samples using P&P (STF) and PAM (MTF) techniques, not just the instruments themselves. Hence the comparison includes error and changes induced by the sampling procedure and sample handling. Data from two cruises in different waters (South Atlantic and North Sea) are presented, during which *in situ* and on board laboratory measurements of variable Chl *a* fluorescence were performed with natural phytoplankton samples of different Chl *a* concentrations. Between the two geographical regions water temperature and Chl *a* concentrations varied strongly, and absolute differences in the determined fluorescence parameters were observed, but the specific relationship for each parameter when determined by STF and MTF did not differ significantly.

2. Materials & Methods

Sampling
The data were collected during two cruises, one with the RV "Heincke" in April and May 2003 in the German Bight and the central North Sea, and one with the RV "Polarstern" (ANT XXI/3, EIFEX) in the South Atlantic, lasting from January to March 2004. Water samples were taken from three to ten different depths using 12 l water sampling bottles. The bottles were fixed on a water sampling rosette (Sea-Bird Electronics, Bellevue, USA) together with a CTD system (Sea-Bird) and a FastTracka instrument (Chelsea, FRRF). Sub-samples from each depth were taken and filled into 500 ml black PE containers shortly after the sampling rosette was on deck. The PE containers were stored at the former ambient temperature of the water sample until all measurements had been performed (< 150 min).

Variable chlorophyll a *fluorescence measurements*
In situ measurement of the maximum and the effective STF quantum efficiency ($\Delta\Phi_m$ and Φ_m) were performed with a submersible FRRF fluorometer (FastTracka, Chelsea, UK). The instrument was fixed on the frame of the sampling rosette with the two cuvettes (light and dark cuvette) facing upwards to avoid shading of the examined water near the window of the light cuvette. Power was provided to the instrument by either a 30 m underwater cable or by the CTD (Sea-Bird 911). If used, the dark chamber of the instrument was connected to the underwater pump of the CTD system with a short, black silicon tubing. Two depth profiles were measured with a vertical velocity of less than 0.5 m s^{-1}, one during the down-cast and one during the up-cast of the instrument. If necessary, the background fluorescence of the specific water mass was determined by performing scatter corrections (see Fastracka processing software manual) with freshly 0.22 μm filtered seawater at ambient temperature with the same PMT gain as used during the cast. The specific PMT gain needed for different *in situ* conditions, i.e., different Chl *a* concentrations, was determined during the first cast of each cruise. Later

data of an online flow-through fluorometer were used to choose the suitable PMT gain setting. The measuring protocol was optimised in such a way that the fluorescence saturation was reached only after the 30 to 40 flashlet, but was still reaching the maximum fluorescence value (F_m), and that the relaxation was clearly seen, i.e., the fluorescence values decreased to below 60 % of F_m. The sampling frequency was maximised by using an average of only 3 single measurements and setting the sample interval to a short time (<100 ms). The instrument produces then 1 data set (an average of the three measurements) per second but alternates the channels (dark and light chamber). Hence, if both channels are used, the measuring frequency for each channel is one data set every 2 seconds; if one channel is used (light chamber), it is one data set each second. Averaging more measurements reduces the measuring frequency, which can be counteracted by a slower vertical velocity during the cast. At the end of each cruise the instrument response function (IRF) and the gain factors were determined as described in the FastTracka processing software manual (Chelsea, UK). The data were analysed with the processing software provided by Chelsea using the specifically determined background and scatter correction values, the determined instrument response function (IRF), and the gain factors and calibration values that were provided with the instrument. The following comparisons of FRRF data from the South Atlantic cruise are based on *in situ* measurements performed in the light chamber during casts conducted when the irradiance at the surface was below 1 μmol photons m^{-2} s^{-1} (late afternoon to early morning), and because of that photochemical and non-photochemical quenching was relaxed. Only the light chamber was active to increase the sampling rate (see above); the dark chamber was inactivated. During the North Sea cruise no night time measurements were performed, and data from the dark chamber were used. The light chamber data showed photochemical quenching under ambient irradiance, which prevents a comparison with data later determined on board but in the dark, when photochemical quenching is relaxed. Occasionally a small remaining photochemical quench near the surface was observed in the dark chamber, because for the conditions used in the present study the residence time in the dark chamber was not sufficiently long for a complete relaxation of the photochemical quenching. Measured efficiency values near the surface at high irradiance were up to 15 % lower than those at greater depths that were still in the actively mixed zone. This small remaining photochemical quenching was not corrected, and the values observed in the dark chamber were taken as $\Delta\Phi_m$. Each value used for the later comparison represents the average of five to ten single measurements (n=5-10) performed when the corresponding discrete sample was taken; hence, the instrument was at the same, constant depth.

Additional laboratory measurements on board were performed with two Xe-PAM instruments (WALZ, Effeltrich, Germany). One instrument was set up as a PAM fluorometer, the other as a P&P fluorometer. The PAM instrument was equipped with a red high power LED lamp (HPL-C) to provide the multiple turnover (MT) saturating light pulse, the P&P instrument with an additional xenon flash lamp (Xe-ST) to provide a short single turnover (ST) saturating "pump" flash. Two separated cuvette holders, holding a 1-cm quartz-glass cuvette each, were used. The specific detectors and saturation lamps were connected to the respective cuvette by light guides. Measuring and actinic light was provided to each cuvette by four-armed fibre-optics, which connected each cuvette with the specific measuring flash lamps and a slide projector. The slide projector was used to provide different actinic irradiance to the cuvettes, being able to perform photosynthesis vs. irradiance measurements (*P vs. E$_0$*) using short-term

incubations. Neutral density glass filters of different optical density that fit in the slide projector were used to provide 10 different irradiance levels. The filters were arranged in an order that the incubation started with darkness and that the irradiance increased stepwise until a total irradiance of about 1000 µmol photons $m^{-2} s^{-1}$ was reached. The light of the slide projector passed through a set of glass filters, which alter the original light spectrum of the 150 W halogen lamp and excluded wavelengths that would be "seen" by the detectors. The provided light spectrum was similar to an *in situ* light spectrum at greater depth. The relative spectral distribution was measured using a Ramses ACC spectroradiometer (TRIOS, Oldenburg, Germany). The total irradiance in each cuvette at each light step was measured regularly using a small, spherical PAR sensor (US-SQS, WALZ, Effeltrich, Germany). The samples in the cuvettes were stirred to prevent sinking of the algal cells using a small magnetic stirrer (WALZ, Effeltrich, Germany). With the sensitive setting of the instruments, necessary to measure natural samples of low algae concentration, the stirring induces artefacts in the measured fluorescence signal by strong movements of the algae in the cuvette. Therefore, the stirring was stopped 20 seconds before each F_m measurement. Before each incubation the instrument offsets were checked and the background fluorescence of the sample water compensated. This was done by filling the cleaned cuvettes with 0.22 µm filtered sample water and setting the two signals to zero by use of the instrument's "AutoCompensation" feature. During the saturation pulse of the PAM-type instrument the measuring flash frequency was automatically changed from 2 to 64 Hz; this increased the background fluorescence signal. Without actinic irradiance (darkness) the influence of this background signal on $F_{m(MT)}$ induced an error of only a few percent; at higher actinic irradiance the total $F_{m(MT)}$ signal decreases and the influence of the constant background signal increases dramatically and can reach 50 % of $F_{m(MT)}$. This background signal was compensated for by measuring the maximal signal with the 0.22 µm filtered sample water during 5 saturation flashes and than subtracting the averaged signal from the $F_{m(MT)}$ values measured later. The fluorescence signal induced by the strong ST flash was measurable but insignificant.

All measurements were performed at the same temperature as during *in situ* sampling. Therefore, the measurements took place in a temperature-controlled room, and the cuvettes were fitted with small thermostats (US-T, WALZ). The actual temperature inside each cuvette was regularly controlled with an electronic thermometer and a small PT-100 flexible sensor. The electronic thermometer was calibrated against a mercury thermometer, with a resolution of 0.1 °C.

A dark "acclimated" sample was poured into each cuvette in dim light, and after additional two minutes in the dark the maximum quantum efficiencies (F_v/F_m and $\Delta\Phi_m$) determined, three times for the MTF and six times for the STF measurements. Afterwards the first light interval started. Each of the ten light intervals lasted 110 seconds. During the last 20 seconds of each interval three MTF measurements were recorded in the cuvette with the PAM-type instrument and six STF measurements with the P&P-type instrument in the other cuvette to measure the effective quantum efficiencies, $\Delta F/F'_m$ and $\Delta\Phi$, respectively. The 90 seconds were enough to reach steady state conditions and hence to measure a constant background fluorescence signal F. The whole P *vs.* E_0 measurement, consisting of changing the neutral density filters, starting and stopping the stirring, and triggering each individual Xe-PAM measurement, was computer-controlled and automated using a self-programmable control unit (Fl-100) and the FluorWin-software (both: PSI, Brno, Czech Republic). The three MTF-PAM and

the six STF-P&P measurements of each interval were averaged. During the South Atlantic cruise additional F_v/F_m and $\Delta\Phi_m$ measurements were performed after all P vs. E incubations were finished, and the samples were in the dark for more than 120 min.

Determination of photosynthesis (P) vs. irradiance (E₀) parameters
Effective quantum efficiencies can be used to estimate photosynthetic rates. A simple approach is the calculation of the relative electron transport rate (*rETR*) as

$$rETR = \Phi E_0, \tag{1}$$

where Φ is either $\Delta F/F_m'$ or $\Delta\Phi$, and E_0 the specific scalar irradiance. The resulting *rETR* vs. E_0 curves were analysed by least-squares regression of the data to a simplified model of Platt & Gallegos (1980) not using the photoinhibition part of the formulation. This was used to determine the maximum *rETR*, $rETR_m$. The slope of the light-limited part of the *rETR* vs. E_0 curve, α, was determined by a simple linear regression of the first 3 data points, which gave more consistent results than the former non-linear regression of all data points, because usually more data points were in the saturation part of the curve and the simple regression analysis did not account for the general error, which is smaller at lower irradiance levels.

Statistical analysis
The data of two independent parameters (e.g. F_v/F_m vs. $\Delta\Phi_m$, $\Delta F/F_m'$ vs. $\Delta\Phi$, $rETR_{m,ST}$ vs. $rETR_{m,MT}$) of either the North Sea or the South Atlantic cruise were examined by linear or non-linear regression analysis. Differences between the regression results for the North Sea and the South Atlantic data were statistically analysed using the Bootstrap method (Efron & Tibshirani, 1993).

3. Results

General
The cruise in the North Sea during early spring 2003 covered most of the German Bight, some areas along the west-, east- and north-Frisian coast and parts of the central North Sea, an area from 53.5° N to 55.4° N and 3.6° E to 8.5° E. The area includes coastal waters with minimum salinity of only 29.5 and oceanic waters with a salinity of up to 34.5. Water temperature ranged from 6.3 to 11.2 °C. Solar surface irradiance was relatively high due to sunny days, and maximum noon values reached more than 1500 µmol photon m^{-2} s^{-1} during half of the cruise days. Samples were taken from near the surface down to 20 m; this includes some samples taken from below a shallow mixed layer in offshore regions. The phytoplankton consisted of either a very diverse community of different species of diatoms (Bacillariophyceae) and dinoflagellates or a single-species dominated plankton bloom of *Phaeocystis globosa* (Prymnesiophycaee) or *Chaetoceros socialis* (Bacillariophyceae). The mean Chl *a* concentration varied between 0.5 mg m^{-3} in the central North Sea to about 4 mg m^{-3} in coastal waters. In local blooms Chl *a* was as high as 24 mg m^{-3}. The maximum MTF quantum efficiencies (F_v/F_m) determined on board varied between 0.24 and 0.73. This variability was induced by high or low non-photochemical quenching and by the different physiological status of the bloom-forming algae. A large *Phaeocystis* bloom along the west Frisian coast showed variations in $\Delta F/F_m'$ over short distances (< 5 km), which were correlated with

visually observable changes in colony size and shape. Small, round colonies showed higher values than large and irregularly formed colonies, the latter most likely indicating degrading parts of the bloom. Similarly, blooms of *Chaetoceros socialis* had lower $\Delta F/F'_m$ values when high Chl *a* values were observed and these blooms were likely to be already degrading.

During the cruise in the South Atlantic in late austral summer 2004, an iron fertilisation experiment was conducted (European Iron Fertilisation Experiments, EIFEX). Samples were taken from different water masses in the Antarctic convergence zone over a period of 8 weeks, inside and outside a fertilised water mass, covering the geographic area from 48.5° S to 51.5° S and 1° E to 19° E. Salinity was always high and around 34.0, water temperature was low and between 1.0 and 6.0 °C. Solar surface irradiance was low, reaching values above 1500 µmol photon m^{-2} s^{-1} during noon on only one fifth of all cruise days. Vertical mixing was strong and the mixed layer reached 100 m depth on most stations; hence, samples were taken from inside the mixed layer with only a few exceptions. The regular phytoplankton was diverse and consisted of pico- and nanoplankton with some large diatom cells. After iron addition the plankton community changed and was dominated by large diatom cells of different species. Chl *a* concentrations varied between 0.2 mg m^{-3} in untreated waters to 3.0 mg m^{-3} in the iron fertilised water patch. F_v/F_m varied from 0.21 to 0.65. The variability was induced by the iron addition, which led to an increase in the mean F_v/F_m over a period of 4 weeks in the fertilised water patch, whereas the surrounding waters had low values throughout (0.31 - 0.38).

Comparison of the maximum quantum efficiency $\Delta\Phi_m$ determined by FRRF and P&P
A depth profile of the maximum quantum efficiency measured *in situ* (FRRF) in the South Atlantic is shown in Figure 1, together with profiles of the maximum quantum efficiency measured on discrete samples on board (P&P and PAM). The two different measurements using STF (FRRF and P&P) techniques agreed well at greater depths, despite the fact that in situ measurements were compared to measurements on discrete sample after "dark adaptation" on board, whereas near-surface values showed lower *in situ* values due to some photochemical quenching as the profile was measured in late afternoon. Higher values were observed for the MTF-PAM method.
All relevant data (see methods for details) of the FRRF and P&P measurement were plotted against each other for the North Sea (Fig. 2 upper panel) and the South Atlantic (Fig. 2 lower panel). Simple linear regression forced through the origin yielded slopes not significantly different from a 1:1 relationship (South Atlantic: m = 1.011±0.006, r² = 0.91, n = 90; North Sea: m = 1.028±0.015, r² = 0.88, n = 74).

Comparison of F_v/F_m vs. $\Delta\Phi_m$ determined by PAM and P&P, respectively
During the South Atlantic cruise F_v/F_m and $\Delta\Phi_m$ were determined two times with the same sample but at different times after a period of darkness (30 min vs. 120 min). $\Delta\Phi_m$ did not change significantly but F_v/F_m increased slightly and was on average about 5 % higher after a longer dark incubation (data not shown). This might be due to the longer "dark acclimation" itself or due to slight temperature changes during this incubation. This 5 % difference in F_v/F_m was corrected for in the later comparison. The comparison of all lab-based F_v/F_m and $\Delta\Phi_m$ data (Fig. 3) showed a good linear correlation with similar slopes for the South Atlantic and North Sea data, respectively (South Atlantic: m = 1.19±0.01 , r² = 0.81, n = 377; North Sea: m = 1.23±0.01, r² = 0.88, n = 77; mean m =

1.21). The two data sets (South Atlantic, North Sea) were not significantly different from each other; hence, F_v/F_m values would generally be about 21 % higher than those of $\Delta\Phi_m$, so that

$$F_v/F_m = 1.21 \; \Delta\Phi_m. \tag{3}$$

Comparison of $\Delta F/F_m'$ vs. $\Delta\Phi$ determined by PAM and P&P, respectively
In Figure 4 the effective quantum yields, $\Delta F/F_m'$ and $\Delta\Phi$, from different P vs. E_0 incubations were plotted against each other, independently of the specific irradiance level of each light interval and considering that the difference in irradiance between the two cuvettes (see method) can be ignored (the difference was less than 10 %). The relationship between $\Delta F/F_m'$ and $\Delta\Phi$ values showed a non-linear pattern with a high linear slope at low values and a lower one at higher values, converging with the slope of the former comparison of the maximum quantum efficiencies. For comparison the data of each cruise were empirically fitted by the function

$$\Delta F/F_m' = a \; (1\text{-exp}[-b\Delta\Phi/a]). \tag{4}$$

The resulting parameters for the two cruises were not significantly different from each other (North Sea: $a = 0.74\pm0.03$ and $b = 2.32\pm0.06$, n = 2101; South Atlantic: $a = 0.79\pm0.02$ and $b = 2.40\pm0.06$, n = 780, $\pm$SE). Hence, the analysis of all data from both cruises resulted in a = 0.75$\pm$0.02 and b = 2.34$\pm$0.04 (n = 2881, $\pm$SE), so that

$$\Delta F/F_m' = 0.75 \; (1 - \text{exp}[-2.34 \; \Delta\Phi / 0.75]) \tag{5}$$

Comparison of calculated rETR and rETR vs. E_0 parameters
For the South Atlantic cruise, *rETR* values were calculated from results of both lab fluorescence techniques (P&P, PAM). All *rETR* values determined by P&P are plotted against the corresponding values determined by PAM in Figure 5. A linear regression analysis resulted in a slope of 1.95$\pm$0.02 ($\pm$SE, r^2 = 0.82, n = 2005). The strong increase in scatter is due to the fact that the relative error of the quantum efficiency increased with higher irradiance, since F' approaches $F_m' (F_v \to 0)$, and that this error is then multiplied by a high irradiance value.

The influence of the different techniques in determining P vs. E_0 parameters was tested using the calculated *rETR* values. Figure 6 shows a typical set of the two *rETR* vs. E_0 curves and the respective maximum relative electron transport rate, $rETR_m$, the light efficiency factor, α, and the irradiance at the onset of light saturation, E_k. A good linear correlation between $rETR_{m,MT}$ and $rETR_{m,ST}$ was observed (Fig. 7). The factors (m) for $rETR_m$ (as $rETR_{m,MT} = m * rETR_{m,ST}$) were 2.14$\pm$0.04 and 2.08$\pm$0.02, ($\pm$SE, r^2 = 0.77 and 0.68, n = 84 and 190) for the North Sea and South Atlantic data, respectively. The two factors were not significantly different from each other, and the data sets were therefore combined. This resulted in a factor of 2.11$\pm$0.02 ($\pm$SE, r^2 = 0.78, n=274), and

$$rETR_{m,MT} = 2.11 \; rETR_{m,ST}. \tag{6}$$

The relationship between α_{MT} and α_{ST} could be described by a linear function as well, except that the line did not go through the origin, following $\alpha_{MT} = m\ \alpha_{ST} + b$ (Fig. 8). The factors (m) were 0.88±0.04 and 0.99±0.03, and the offsets (b) were 0.20±0.01 and 0.15±0.01 (r^2 = 0.83 and 0.87, n = 83 and 194) for the North Sea and South Atlantic data, respectively. The difference between North Sea and South Atlantic data was not significant. The linear regression of the combined data yielded a factor m of 0.96±0.02 and an offset b of 0.16±0.01 (±SE, r^2 = 0.85, n=277, see Fig. 8), hence

$$\alpha_{MT} = 0.96\ \alpha_{ST} + 0.16. \tag{7}$$

As can be seen from Fig. 7 and Fig. 8 the absolute values of α and $rETR_m$ were higher in the North Sea, but the relationships between ST and MT measurements were the same. A slight physiological difference in these relationships was observed in the rETR data (Fig. 5), where small clusters were visible at $rETR_{st}$ values of ca. 4, 7 and 14, which deviate from the straight line found by simple regression analysis. These clusters can be assigned to the rETR values of iron-depleted water at low irradiance values (ca. 10, 25, and 40 µmol photons m^{-2} s^{-1}, respectively). At higher irradiances these clusters disappeared, so their effects were not visible in the $rETR_m$ data, because $rETR_m$ was typically reached at irradiances above 100 µmol photons m^{-2} s^{-1}. For α this effect is visible. The lower α values (<0.3) of the South Atlantic data, which are below the regression line, are mainly from iron-depleted water, whereas the higher values (>0.3) are from the iron-fertilized water but are very similar to the higher North Sea values. However, the error for low α values induced by these differences will be in the range of 10 %.

4. Discussion

Theoretically the results determined by a STF should be independent of the type of variable Chl a fluorescence technique used. However, differences can occur due to differences in the instrumentation, e.g. the excitation light spectrum. The two types of instruments used here did differ in the type of light source used to excite fluorescence. The FastTracka instrument uses a set of blue LEDs with a narrow bandwidth (~20 nm) peaking at 470 - 480 nm. The Xe-PAM uses a white xenon flash lamp and a halogen lamp. Both spectra are altered by a blue short-pass filter. The resulting light spectra are broad and ranged from 400 to 600 nm with a maximum at about 530 nm (for spectral details see Suggett *et al*. 2003). A frequent criticism (e.g. Kromkamp & Forster 2003) is that blue light would be highly selective for certain algal groups and would, e.g., underestimate cyanobacteria, which possess a relatively low absorption cross section in the blue spectral region. However, as can be seen from the FRRF vs. P&P comparison (Fig. 2), the much broader light spectrum of the Xe-Pam instrument does not give a different response compared to the narrow spectrum of the FastTracka instrument under the examined conditions, which included strong dynamic changes in the phytoplankton community induced by different geographical positions and iron fertilisation. However the observed algae were of a few taxonomic groups only, mostly diatoms and dinoflagellates, and occasionally small pico- and nannoflagellates. Comparisons with results from cyanobacteria and prochlorophytes are not done, since problems were reported for the FastTracka instrument when used with filamentous cyanobacteria (Raateoja *et al.* 2004).

The advantages of the FastTracka instrument are that it is employable *in situ*, i.e., it is able to measure under ambient conditions, and that it is more sensitive. However the Xe-PAM instrument can be tuned to a sufficient sensitivity and can be used to determine the maximum quantum efficiency, which on the other hand is difficult when the FastTracka is used during day time, since the short dark period in the dark chamber is not sufficient for a complete relaxation of the energy quenching and, hence, to determine F_0'. However, equations are given by Gorbunov et al. (2001) which can be used to calculate $\Delta\Phi_m$ without knowledge of F_0'. More advantages and disadvantages of the two different techniques are discussed in Kromkamp & Forster (2003).

As shown here the STF results of both instruments (and with both variable fluorescence techniques) are identical for night-time measurements (Fig. 2), and a prolonged dark incubation of discrete samples did not significantly alter these results. It is concluded that the different STF techniques (P&P, FRRF) used in different instruments and with different sample procedures (in situ vs. discrete sampling) are giving the same results and can be interchangeably used without further treatment of the raw data. However FRRF has the advantage that parameters like the functional cross section, the connectivity parameter, and relaxation kinetics can be measured additionally.

ST measurements can be repeated often and at short intervals, even with the same sample, and the precision can be increased by averaging large numbers of single measurements. The relatively long flash duration of a MTF leads to additional quenching, and the interval between two single measurements with the same sample has to be much longer to relax this quenching and to reach steady state conditions again. At low light or in the dark this quenching lasts up to a few minutes, preventing any repetitive measurements. On the other hand the higher value of $F_{m(MT)}$ provides a larger dynamic range between the minimum and maximum fluorescence yield, and the long flash duration enables a better determination of the maximum fluorescence yield. Both lead to a better signal to noise ratio, i.e., a smaller number of averaged measurements is needed. At higher actinic light levels the additional quenching by the MTF does relax fast, and repetitive measurements can be conducted with intervals of less than 5 seconds.

The higher values of $F_{m(MT)}$ resulted in higher maximum and effective quantum efficiencies, F_v/F_m and $\Delta F/F_m'$. The constancy of this difference has not so far been thoroughly examined under natural conditions. Samson *et al.* (1999) estimated a factor of 1.28 (see above). Koblížek et al. (2001) observed variable, species-specific factors between 1.0 and 1.22 for cultures of different microalgal species. Similar to the former study, Suggett *et al.* (2003) compared MTF (Xe-PAM) and STF (FastTracka) measurements with algal species from five different algal groups but reported, in contrast, a constant factor of ca. 1.23. Suggett *et al.* (2003) also reported results for the effective quantum efficiencies $\Delta F/F_m'$ and $\Delta\Phi$ and found a factor similar to that for the maximum quantum efficiencies at low irradiance levels (1.17). At higher irradiance the value increased to 1.5 (Suggett *et al.* 2003). The factors found in the present study were 1.23 for samples from the North Sea and 1.19 for those of the South Atlantic. The two factors were not significantly different from each other, despite the different geographical region and the different abiotic conditions (water temperature and depth, total irradiance, etc.). The mean value of 1.21 is similar to the results of Suggett et al. (2003) and the maximum values observed by Koblížek et al. (2001). The results presented here support a practically constant factor, which can be used to convert the

different maximum quantum efficiencies determined with different techniques and instruments under most natural conditions, and also with cultured algae.

For the effective quantum efficiency the conversion factor increased to about 2.0 at the highest irradiance level when the quantum efficiency was below 0.1 (Fig. 4 and 5). I.e., without actinic light the reduction of further electron acceptors by the MTF, following Q_A down to the PQ pool leads to a constant relative enhancement of the F_m, whereas the enhancement of F'_m is dependent on the actinic light level and varies between 1.2 and 2.0 but can be approximated by a non-linear function dependent on the absolute values only (Fig. 4). As an approximation the effective quantum efficiencies can empirically be converted into each other by an averaged non-linear function (Eq. 5). The influence of the actinic irradiance on this conversion can be counteracted by comparing the calculated rETR values for each data set of STF and MTF measurements. This is shown in the comparisons for $rETR_m$ and α. The obtained α and $rETR_m$ values can be converted by Eq. 6 and 7, respectively.

5. Conclusion

The two different instruments used here (Xe-PAM, FastTracka) were both sensitive enough to determine variable chlorophyll a fluorescence parameters even at the lowest chlorophyll a concentration found (<0.3 mg m^{-3}), and the new FIRe system is equally sensitive (Satlantic). The Xe-PAM is not sensitive enough to work at Chl a concentration below 0.2 mg m^{-3} in tropical waters (pers. observation). All instruments have their advantages and disadvantages. The FastTracka can be used for *in situ* measurements, but because of its size is less practical for laboratory use, which is needed when determinations of photophysiological parameters under controlled conditions are required. The basic methodological differences between ST and MT protocols led to differences in the determined parameter, which seem to be independent of the abiotic conditions and species composition and less dependent on phytoplankton physiology. This makes it possible to convert parameters based on ST measurements to those based on MT measurements and *vice versa*. This allows not only comparison of measurements done with different instrumentation but also done with the different variable chlorophyll a fluorescence techniques. This will make results obtained by different variable fluorescence techniques during the last 20 years and in the future more comparable. However, measurements in subtropical/tropical, picoplankton-dominated regions are yet to be investigated.

Appendix
Abbreviations

α	Light efficiency factor, initial slope of the P *vs.* E_0 curve
α_{MT}	α measured using MTF
α_{ST}	α measured using STF
$\Delta\Phi$	Effective PSII quantum efficiency measured using a STF

$\Delta\Phi_m$	Maximum PSII efficiency measured using a STF
$\Delta F/F'_m$	Effective PSII quantum efficiency measured using a MTF
σ_{PSII}	Functional absorption cross section
E_0	Scalar irradiance
E_k	Irradiance at the onset of light saturation of the P vs. E_0 curve
F	Steady-state fluorescence yield in the light
F_0	Minimal fluorescence yield after dark acclimation
F'_0	Minimal fluorescence yield in light acclimated state
F_m	Maximum fluorescence yield after dark acclimation
F'_m	Maximum fluorescence yield in light acclimated state
$F_{m,MT}$	F_m measured using a MTF
$F'_{m,MT}$	F'_m measured using a MTF
$F_{m,ST}$	F_m measured using a STF
$F'_{m,ST}$	F'_m measured using a STF
FRRF	Fast repetition rate fluorometry
F_v	Variable fluorescence yield after dark acclimation measured using a MTF ($=F_{m(MT)} - F_0$)
F_v/F_m	Maximum PSII efficiency measured using a MTF
MT, MTF	Multiple turnover, multiple turnover flash
P	Photosynthesis, photosynthetic rate
PAM	Pulse amplitude modulated fluorometry
P&P	Pump and probe fluorometry
$rETR_{MT}$	Relative electron transport rate measured using a MTF
$rETR_{m,MT}$	Maximum $rETR_{MT}$
$rETR_{ST}$	Relative electron transport rate measured using a STF
$rETR_{m,ST}$	Maximum $rETR_{ST}$
ST, STF	Single turnover, single turnover flash

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Figure legends
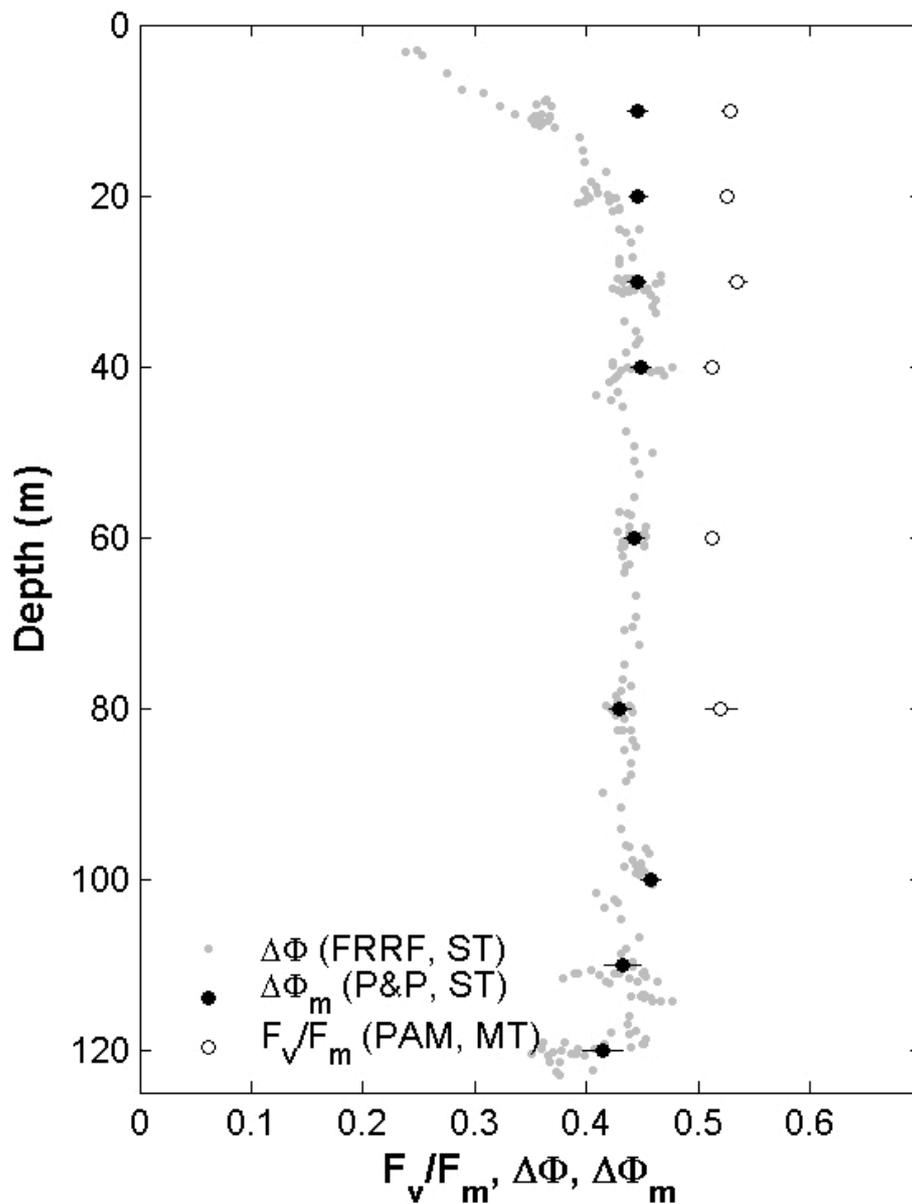
Fig. 1. An *in situ* depth profile of the effective quantum efficiency, *ΔΦ*, measured by FRRF (FastTracka) in the South Atlantic together with profiles of the maximum quantum efficiency measured by P&P, *ΔΦ_m*, and PAM fluorometry, *F_v/F_m*, on discrete samples taken during the cast. The data were obtained during late afternoon. Some photochemical quenching near the surface is visible, whereas no non-photochemical quenching was observed in the discrete samples. Vertical mixing was strong and ranged down to 100 m. Values for *ΔΦ_m* and *F_v/F_m* are given with ± S.D. (n=6 and n=3, respectively).
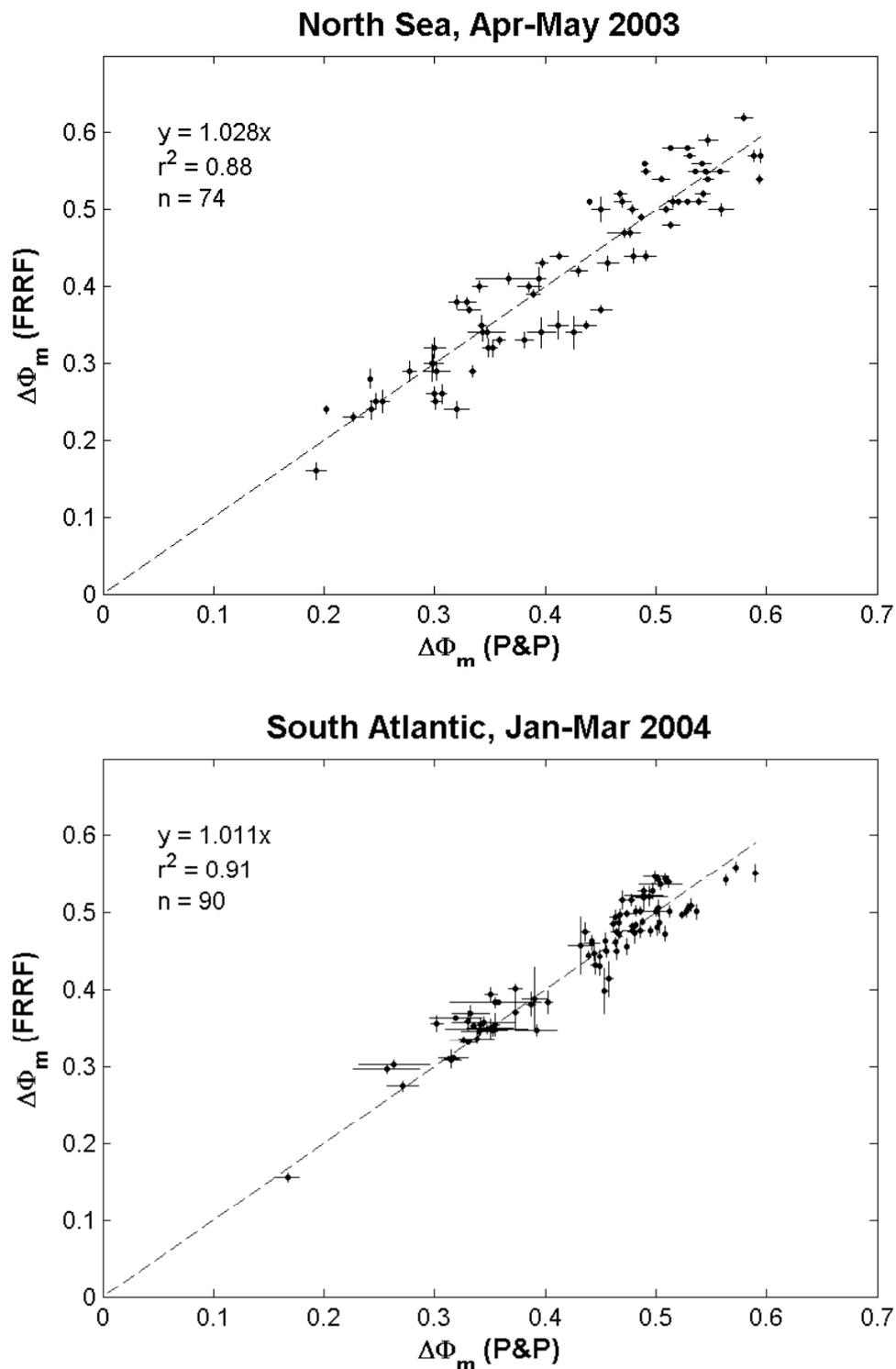
Fig. 2. Comparison between maximum quantum efficiency, $\Delta\Phi_m$, measured *in situ* using FRR fluorometry (FastTracka) and $\Delta\Phi_m$ measured later in the laboratory using P&P fluorometry. The data were obtained in the North Sea in April and May 2003 (upper panel), and in the South Atlantic from January to March 2004 (lower panel). Standard deviations for both parameters are indicated (thin lines). Shown in numbers are the results of a linear regression analysis forced through the origin. Indicated is the 1:1 relationship (dashed lines).

South Atlantic, Jan-Mar 2004

y = 1.19x
r^2 = 0.81
n = 375

North Sea, Apr-May 2003

y = 1.23x
r^2 = 0.86
n = 68
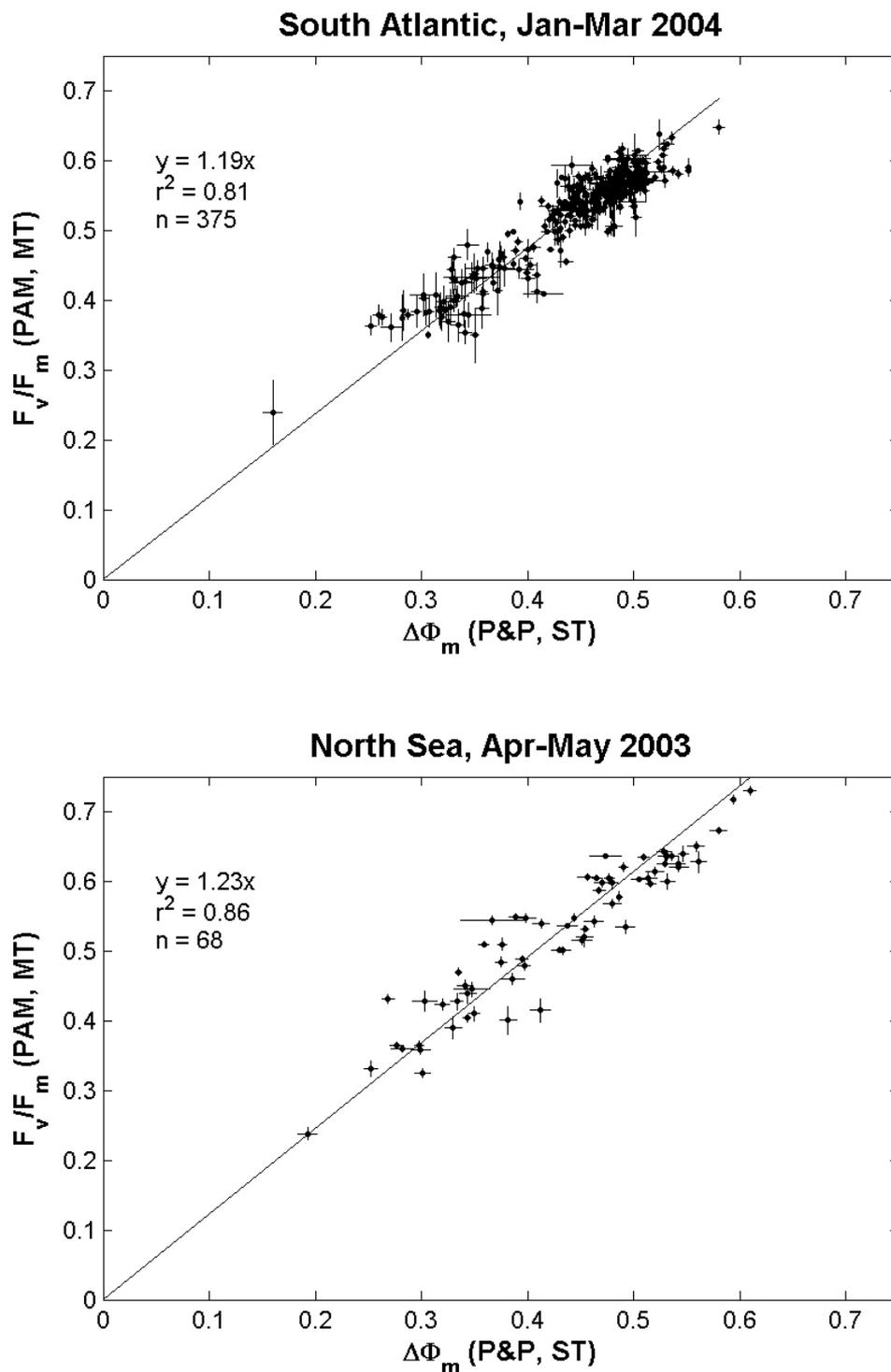
Fig. 3. Comparison between the maximum quantum efficiency measured by P&P fluorescence, $\Delta\Phi_m$, and that measured by PAM fluorescence, F_v/F_m. The data were obtained in the North Sea in April and May 2003 (upper panel), and in the South Atlantic from January to March 2004 (lower panel). Standard deviations for both parameters are indicated (thin lines). Shown are the results of linear regressions forced through the origin (solid lines), and the corresponding values of the regression analysis.
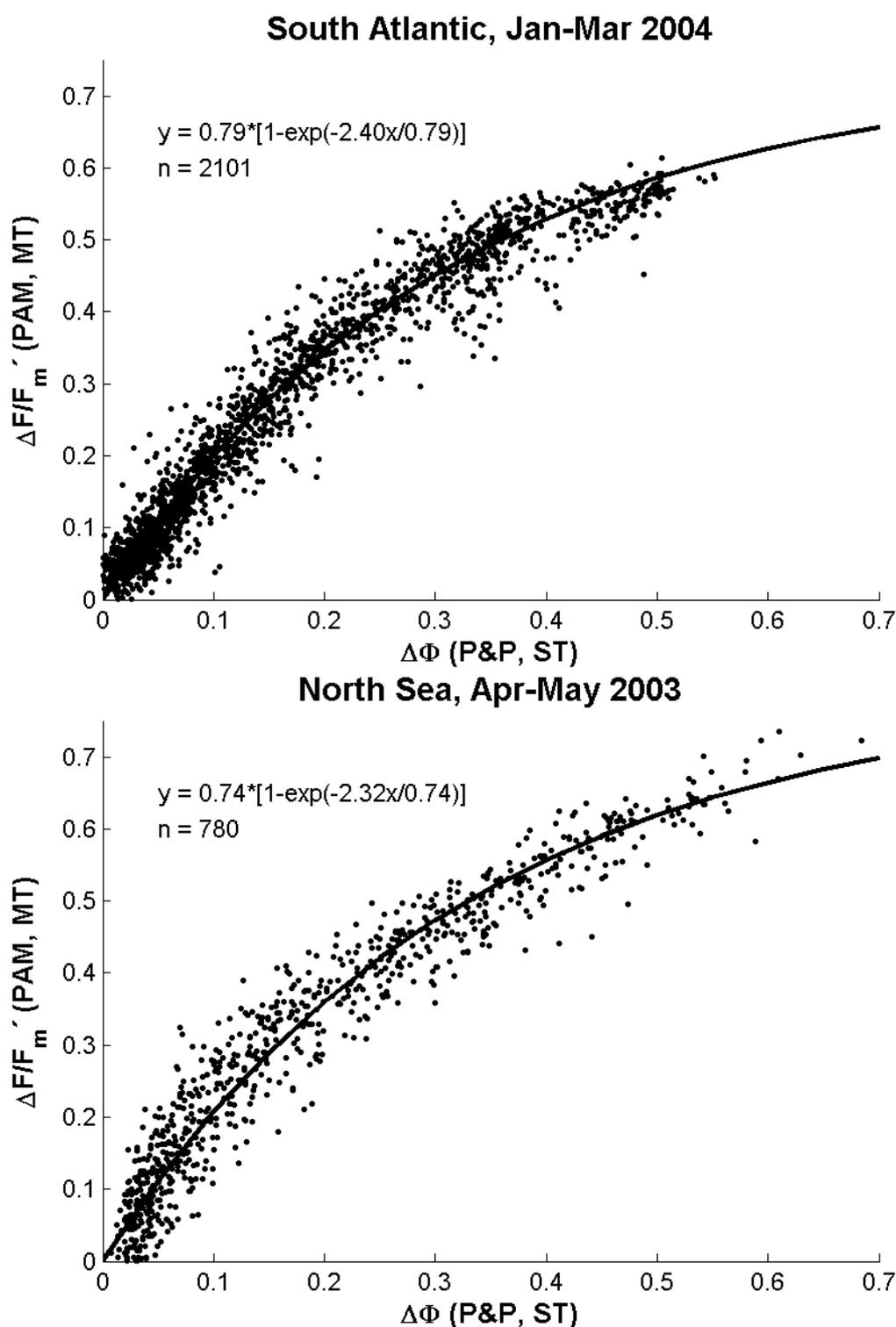
Fig. 4. Comparison between the effective quantum efficiency measured by P&P fluorescence, $\Delta\Phi$, and that measured by PAM fluorescence, $\Delta F/F_m'$. The data were obtained during laboratory photosynthesis vs. irradiance incubations in the North Sea in April and May 2003 (upper panel), and the South Atlantic from January to March 2004 (lower panel). Shown are the results of non-linear regressions (solid lines), and the corresponding values of the regression analysis.
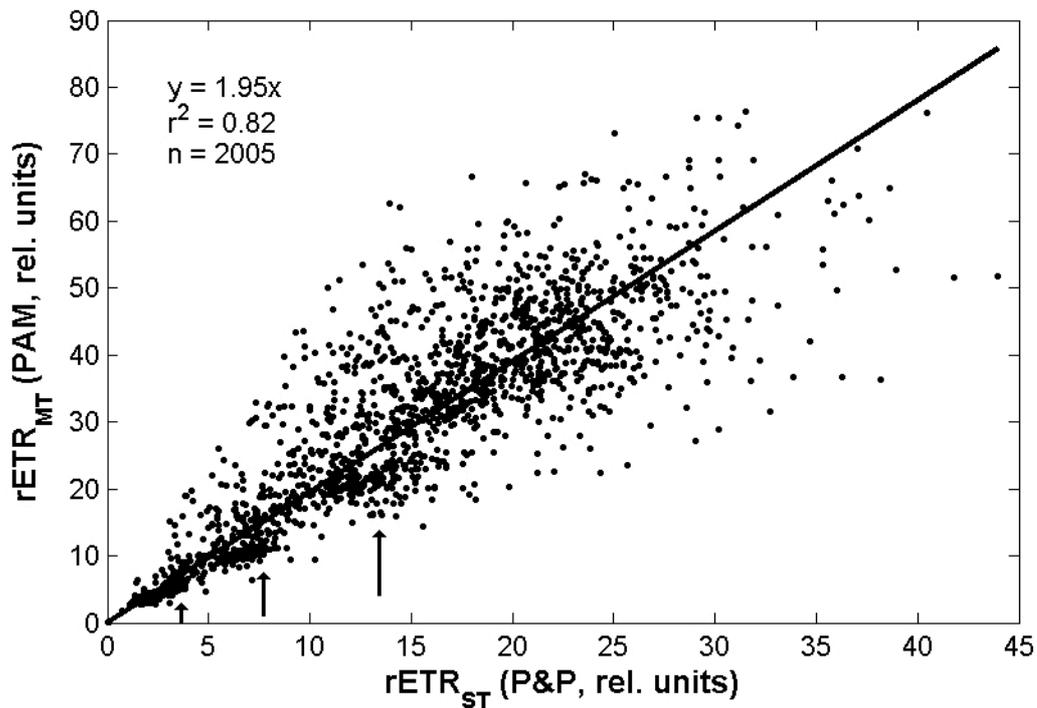
Fig. 5. Comparison between the relative electron transport rates (rETR) determined by P&P fluorescence (rETR$_{ST}$) and by PAM fluorescence (rETR$_{MT}$). Data were obtained during photosynthesis vs. irradiance incubations in the South Atlantic. A simple calculation of rETR was used (see method) and the data are plotted independently of the incubation irradiance. Shown are the results of linear regressions forced through the origin (solid line), and the corresponding values of the regression analysis. The arrows mark the positions of values from samples of iron depleted water (see text).
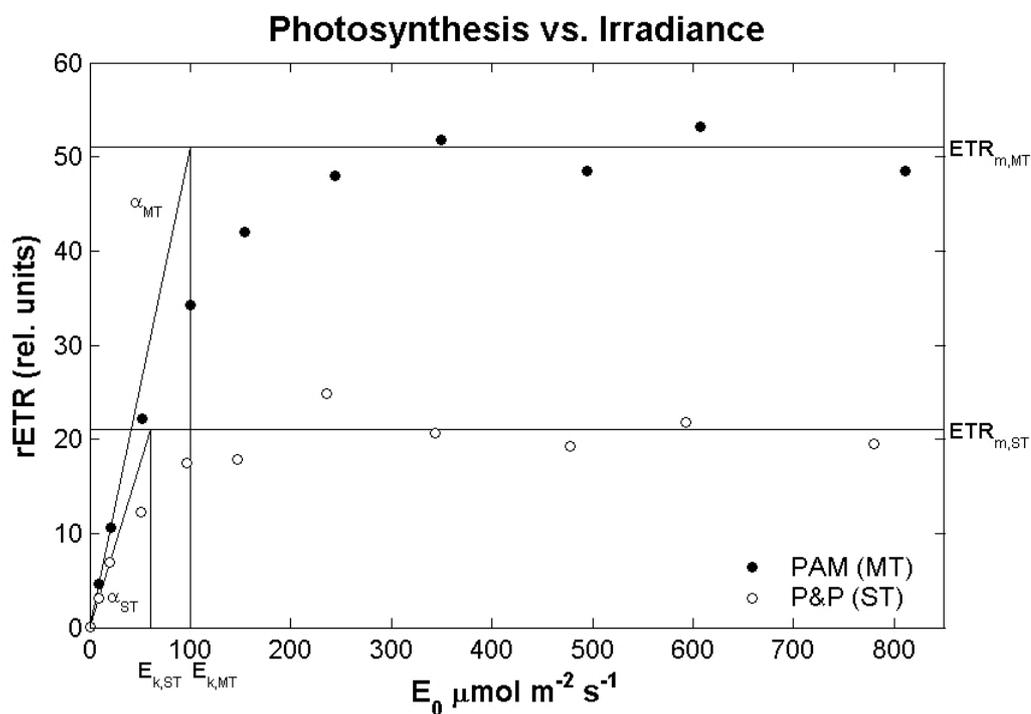
Fig. 6. Typical relative photosynthesis vs. irradiance curves determined by rETR measurements using either ST-P&P fluorescence (closed circles) or MT-PAM fluorescence (open circles). A simple calculation of rETR was used (see method). Indicated (solid lines) for both techniques are the maximum rETR, $rETR_{m,ST}$ and $rETR_{m,MT}$, the light efficiency (initial slope), α_{MT} and α_{ST}, and the onset of light saturation, $E_{k,ST}$ and $E_{k,MT}$.
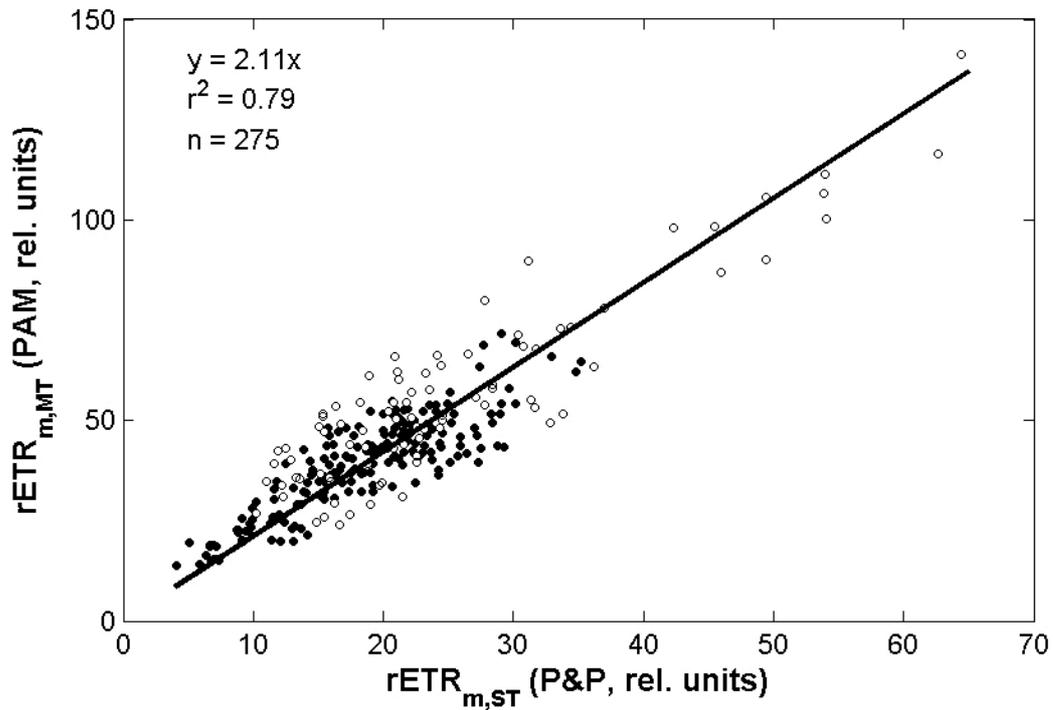
Fig. 7. Relationship between the maximal rETR obtained from ST-P&P fluorescence measurement $rETR_{m,ST}$ and that obtained from MT-PAM measurements, $rETR_{m,ST}$. The data were collected during April and May 2003 in the North Sea (open symbols), and from January to March 2004 in the South Atlantic (closed symbols). Shown are the results of a linear regression forced through the origin of all data points (solid line), and the respective values of the regression analysis.
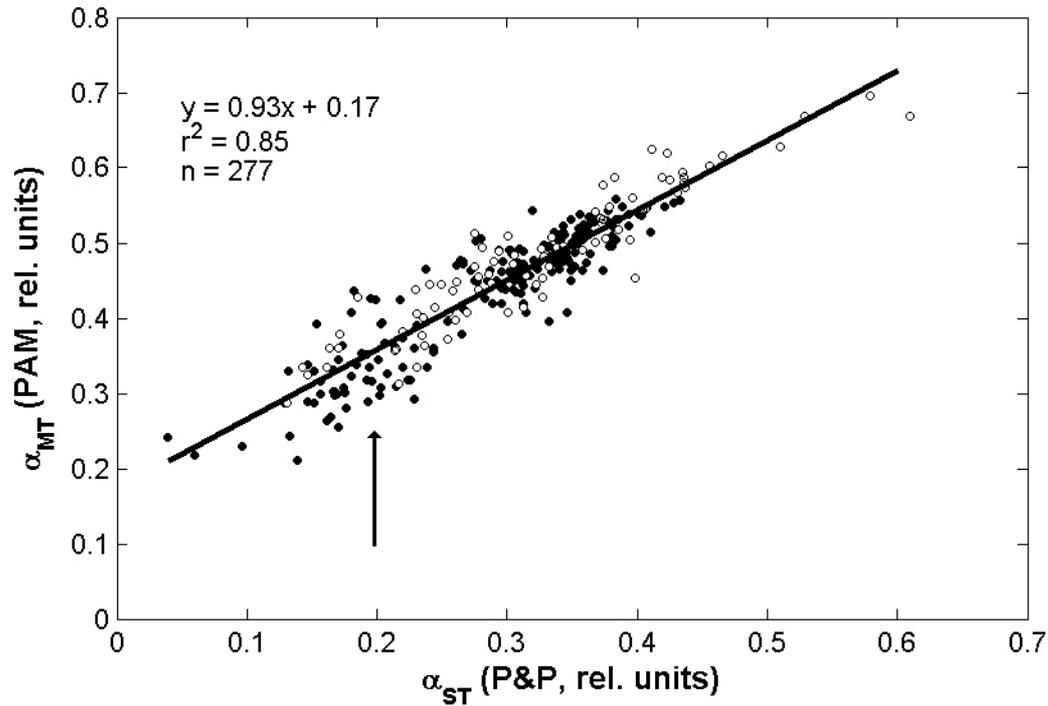
Fig. 8. Relationship between the light efficiency factor obtained from ST-P&P fluorescence measurement, α_{ST}, and that obtained from MT-PAM measurements, α_{MT}. The data were collected during April and March 2003 in the North Sea (open symbols), and from January to March 2004 in the South Atlantic (closed symbols). Shown are the results of a linear regression forced through the origin of all data points (solid line), and the respective values of the regression analysis. The arrow marks the position of values from samples of iron depleted water (see text).