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# Application of fast repetition rate fluorometry to phytoplankton photosynthetic parameters in freshwaters

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**Abstract**

Fast repetition rate fluorometry (FRRF) was successfully applied to various studies in modern oceanography. In this study, for the first time, the seasonality of phytoplankton photosynthetic parameters in a deep alpine lake was observed using FRRF in combination with the traditional $^{14}C$ incubation technique. Special attention was given to the differences in photosynthetic behaviour during mixed and stratified conditions, characterised especially during summer by a deep chlorophyll maximum (DCM) dominated by the filamentous cyanobacterial species *Planktothrix rubescens*. Maximum light-utilisation efficiency ($\alpha^*_{14C}$) was in the range of 0.01–0.03 mgC (mg Chl-*a*)$^{-1}$ h$^{-1}$ (μmol phot. m$^{-2}$ s$^{-1}$)$^{-1}$, while maximum quantum yields for carbon fixation ($\Phi_{C,max}$) varied from 0.01–0.07 molC (mol phot.)$^{-1}$. Higher values occurred during thermal stratification indicating acclimation of the phytoplankton assemblage. These findings were supported by FRRF-based estimates, although cyanobacterial blooms could not be characterised by FRRF-excitation due to methodological deficiencies. In general, however, instantaneous photosynthetic rates measured by FRRF-excitation correlate well at sub-saturating light-intensities with conventional $^{14}C$-uptake rates, although they operate on different time-scales.

## Introduction

Photosynthetic parameters of phytoplankton in freshwaters are commonly derived from well established methods. *In situ* incubation techniques usually use either changes in oxygen concentration or the incorporation of radiocarbon into particulate matter, the latter being a method which is especially suitable under oligotrophic conditions. However robust these methods are, they suffer from shortcomings like isolating natural phytoplankton assemblages in bottles (Eppley 1980) or the differentiation between net and gross photosynthesis (Bender et al. 1987). Their main deficiency is that instantaneous photosynthetic rates of phytoplankton assemblages cannot be acquired.

In modern oceanography active fluorescence methods, in particular fast repetition rate fluorometry (FRRF, Kolber et al. 1998), were applied to various studies. FRRF offers the possibility to obtain non-destructive, real time and *in situ* estimates of photosynthetic parameters (Falkowski 1995). The technique was used to asses the response of marine phytoplankton to iron enrichment (Greene et al. 1994; Kolber et al. 1994; Behrenfeld et al. 1996; Behrenfeld and Kolber 1999), to study the spatial and temporal distribution (Strutton et al. 1997) as well as photosynthetic characteristics of phytoplankton (Olson et al. 1996; Suggett et al. 2001; McMinn and Hegseth 2004). Aside from measurements of phytoplankton assemblages, FRRF was applied to estimate

cyanobacterial photosynthetic rates (Raateoja et al. 2004a, b) and changes of chlorophyll fluorescence yields of reef corals (Gorbunov et al. 2000; Lesser and Gorbunov 2001; Table 1)

In this study we tested the applicability of FRRF to oligotrophic freshwater systems. This technique is relatively novel to freshwaters, and measurements covering the seasonality of an annual cycle in a temperate deep holomictic lake have not been performed so far. Special attention was given to the differences in photosynthetic behaviour during mixed conditions, where phytoplankton is homogeneously distributed in the water column, and stratified conditions during summer, when a deep chlorophyll maximum (DCM), mainly composed of the cyanobacterium *Planktothrix rubescens*, occurs at a depth of 10–12 m.

We hypothesise that the physiological performance of phytoplankton, expressed by instantaneous rates of phytoplankton photosynthesis varies (1) depending on mixing conditions which govern nutrient and light availability, and (2) seasonally with species composition. Additionally, the more traditional $^{14}C$ incubation technique (Steeman-Nielsen 1952) was used to validate fluorescence-derived primary productivity rates, since this technique has been routinely used for more than 20 years on Mondsee. We expect

*Table 1.* Notation

| Parameter | Definition | Unit |
|---|---|---|
| FRRF | | |
| $F_0$, $F_m$ | Minimal and maximal *in vivo* fluorescence yields measured in dark adapted state | Arbitrary |
| $F'_0$, $F'$, $F'_m$ | Minimal, steady state and maximal *in vivo* fluorescence under ambient light | |
| $F_v$, $F'_v$ | Variable fluorescence ($F_v = F_m - F_0$) in dark adapted and light adapted state | Dimensionless |
| ($F_v/F_m$, $F'_v/F'_m$) | Maximum quantum yield of photochemistry ($\Delta\Phi_m/\Delta\Phi'_m$) in dark adapted and light adapted state | Dimensionless |
| $\sigma_{PSII}$ | Functional absorption cross-section of PS II | $m^2$ quanta$^{-1}$, Å quanta$^{-1}$ |
| $q_p$ | Photochemical quenching; the fraction of open reaction centres under ambient photon flux | Dimensionless (0–1) |
| $f$ | Fraction of potentially open reaction-centres in dark = $[(F_v/F_m)/0.65]$ | Dimensionless (0–1) |
| $\eta_{PSII}$ | Ratio of PS II reaction centres to Chl-*a* (1/500) | mol electrons (mol Chl-*a*)$^{-1}$ |
| $\alpha^*_{FRRF}$ | Maximum light-utilisation coefficient based on FRRF-measurements | mgC (mg Chl-*a*)$^{-1}$ h$^{-1}$ (µmol phot. m$^{-2}$ s$^{-1}$)$^{-1}$ |
| $E$ | Irradiance, ambient light at a distinct depth | µEinst m$^{-2}$ s$^{-1}$, µmol phot. m$^{-2}$ s$^{-1}$ |
| $M_C/M_{Chla}$ | Molecular masses of carbon and Chl-*a* | |
| $^{14}C$ | | |
| $a^*$ | Chl-*a* specific absorption cross section | m$^2$ (mg Chl-*a*)$^{-1}$ |
| $P^*_{14C}$ | Phytoplankton productivity based on $^{14}C$-estimates normalised to Chl-*a* | mgC (mg Chl-*a*)$^{-1}$ h$^{-1}$ |
| $P^*_{max}$ | Maximum photosynthetic rate | mgC (mg Chl-*a*)$^{-1}$ h$^{-1}$ |
| $E_k$ | Light saturation parameter = ($P^*_{max}/\alpha^*$) | µEinst m$^{-2}$ s$^{-1}$, µmol phot. m$^{-2}$ s$^{-1}$ |
| $\alpha^*_{14C}$ | Maximum light-utilisation coefficient based on $^{14}C$-estimates | mgC (mg Chl-*a*)$^{-1}$ h$^{-1}$ (µmol phot. m$^{-2}$ s$^{-1}$)$^{-1}$ |
| $\Phi_{C,max}$ | Maximum quantum yield of carbon fixation = ($\alpha^*/a^*$) | molC (mol phot.)$^{-1}$ |
| Physicochemistry | | |
| SRP | Soluble reactive phosphorus (orthophosphate) | µg l$^{-1}$ |
| DIC | Dissolved inorganic carbon | µg l$^{-1}$ |
| $Z_{eu}$ | Euphotic zone (1% light) | m |
| $Z_{mix}$ | Mixing depth | m |
| $Z_{mix}/Z_{eu}$ | Mixing – light correlative | Dimensionless |

that the parameters derived from instantaneous rates deviate more during stratification from parameters calculated from conventional 'long-term' $^{14}C$ incubations due to the presence of cyanobacteria, than during mixed conditions, as also reported in marine systems (Suggett et al. 2001; Raateoja et al. 2004a, b).

## Materials and methods

Vertical profiles of active fluorescence were acquired with a commercial Fast Repetition Rate (FRR) – Fluorometer (Fast$^{tracka}$, Chelsea Instruments) from April 2003 to December 2004 in a deep alpine lake, Mondsee, Austria for the top 20 m (Figure 1). Baseline, scatter and reference calibration were carried out following the user manual, the instrument response function was calibrated for gain 0, 1, 4 and 16. Blanks were determined for the light and the dark chamber using GF/C filtered lake water. The acquisition protocol was set to provide 100 saturation flashes per sequence with a saturation flash duration of approximately 1.5 μs, spaced 15 μs apart, followed by 20 relaxation flashes (~1.1 μs duration), 80 μs apart. The sleeptime between acquisition pairs was set to 50 ms. On each sampling tour, three vertical profiles were taken lasting in total 1.5–2 h. Data were quality controlled by deleting values of the instrument gain > 16, depth < 0 and photosynthetic active radiation (PAR) > 200. The controlled data were smoothed using a median filter and aggregated over fixed 0.5 m intervals. The initial fluorescence ($F_0$), the maximum fluorescence ($F_m$) and the functional absorption cross section of PS II ($\sigma_{PSII}$) were derived using the biophysical model of Kolber et al. (1998). The quantum efficiency of PS II was calculated from variable fluorescence ($F_v = F_m - F_0$) normalised to $F_m$, indicating the proportion of functional PS II reaction centres (Geider et al. 1993; Kolber and Falkowski 1993). Fluorescence-based productivity was calculated following the model of Kolber and Falkowski (1993) which qualitatively predicts photosynthetic rates from changes in the quantum yield of fluorescence:

$$P_{FRRF} = E\sigma_{PSII}q_p f\eta_{PSII}(3600/4)M_c/M_{Chla}A \quad (1)$$

where $A$ is a factor containing the conversion-factors from quanta to mol photons for irradiance, and $m^2$ to Å for $\sigma_{PSII}$. The term (3600/4) stands for

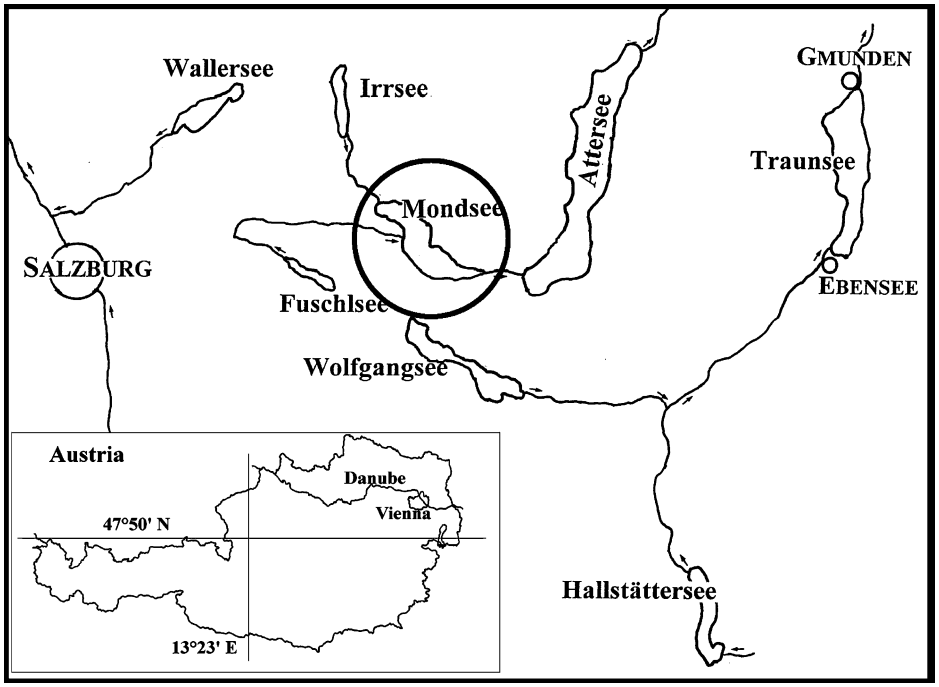


*Figure 1.* Location of Mondsee in the Salzkammergut lake-district. The insert indicates the position of the Salzkammergut in Austria, given by latitude and longitude.

the calculation from seconds to hours (3600), and 4 electrons, which are required to fix 1 molecule of carbon (1/4).

The maximum light-utilisation coefficient $\alpha$ (Equation (2)) was directly derived by re-arranging Equation (1), as also described by Moore et al. (2003), Babin et al. (1996), and Behrenfeld et al. (1998).

$$\alpha_{\mathrm{FRRF}} = \sigma_{\mathrm{PSII}} q_{\mathrm{p}} f \eta_{\mathrm{PSII}} (3600/4) M_{\mathrm{c}} / M_{\mathrm{chla}} A \quad (2)$$

*In situ* phytoplankton photosynthetic rates were estimated from carbon uptake applying the $^{14}$C technique after Steemann-Nielsen (1952). Water was collected from 11 different depths along a 15 m vertical profile (0, 1, 2, 3, 4.5, 6, 7.5, 9, 10.5, 12 and 15 m). For each depth one light and one dark glass-bottle was spiked with 4 μC (150 kBq) radioactive bicarbonate solution and horizontally suspended at *in situ* depth for 3 h. In the laboratory, 4 ml of 2N hydrochloric acid was added to 50 ml of the samples and bubbled directly in the incubation-bottles for 45 min (modified Acid bubbling method, ABM, Schindler et al. 1972). Ten millilitres sub-samples of each light and dark bottle were transferred to scintillation vials and mixed with 10 ml of scintillation cocktail (Packard Ultima Gold). The $^{14}$C content was measured as cpm in a Packard 1600 TR liquid scintillation counter, quench corrected and converted to dpm by internal efficiency. Based on these data, the chlorophyll-specific maximum carbon uptake rate ($P_{\mathrm{max}}$) and the maximum light-utilisation coefficient $\alpha^*_{14C}$ were calculated using the tangent hyperbolic function (Jassby and Platt 1976).

$$P = P_{\mathrm{max}} * \tanh(\alpha^* E / P_{\mathrm{max}}) \quad (3)$$

This non-linear regression model usually represents the best fit for light dependent pimary productivity observations.

Irradiance was measured as total photosynthetic available radiation (PAR) with a LiCor 4π optical sensor and a precise 3 nm interval spectroradiometer (RAMSES-SCC-VIS, TriOS).

Phytoplankton absorption spectra were measured applying the filter-technique after Kishino et al. (1986) and normalised to chlorophyll-*a* (Chl-*a*) to generate the chlorophyll-specific absorption ($a^*$ (λ)). To obtain the chlorophyll-specific absorption coefficient ($a^*$), the spectra were averaged between 350 and 700 nm.

Water temperature, pH, dissolved oxygen, specific conductivity and turbidity were measured with an automatic Yellow Springs 6502 profiler.

Orthophosphate (SRP, soluble reactive phosphorus) was analysed according to the conventional chemical technique after Greenberg et al. (1992). Alkalinity was titrated automatically using a 716 DMS Titrino. Total dissolved inorganic carbon (DIC) concentration was calculated from alkalinity and pH according to Golterman et al. (1978).

Chlorophyll-*a* concentration was determined following DIN 38 412 (1984). Two litres of water from each depth were filtered through 47 mm diameter Whatman GF/C filters (pore size 1.2 μm) and deep-frozen for 24 h. After extraction with hot ethanol, Chl-*a* was determined from spectrometric measurements at 665 and 750 nm and corrected for phaeophytin using acidification according to Lorenzen (1967).

Phytoplankton abundance and biovolume were determined from counting and sizing Lugol fixed samples from each depth after Utermöhl's sedimentation technique (Utermöhl 1958).

## Results

Sampling was carried out over a 20-month-period providing data acquired under variable physical stratification, changing light conditions and differing phytoplankton productivities.

Mixed and stratified seasons were defined from temperature profiles and corresponding density differences. Winter mixed conditions lasted from end of October 2003 to mid-April 2004 and from November until the end of the sampling programme in December 2004 and were associated with surface water temperatures between 2.7 and 9.8 °C. Stratification occurred from May to mid-October 2003 and from end of April to end of October 2004 reaching peak temperatures of 25.8 °C in the surface water layer (Figure 2a). Under isothermal conditions during winter, the water column in Mondsee was completely mixed to the bottom, while during summer the mixing depth varied from 6 to 8 m. Photosynthesis was restricted to the euphotic zone with an average depth of 11.7 m (7.9–18.4 m) as illustrated in Figure 2b.

Soluble reactive phosphorus concentrations during spring overturn were in the range of

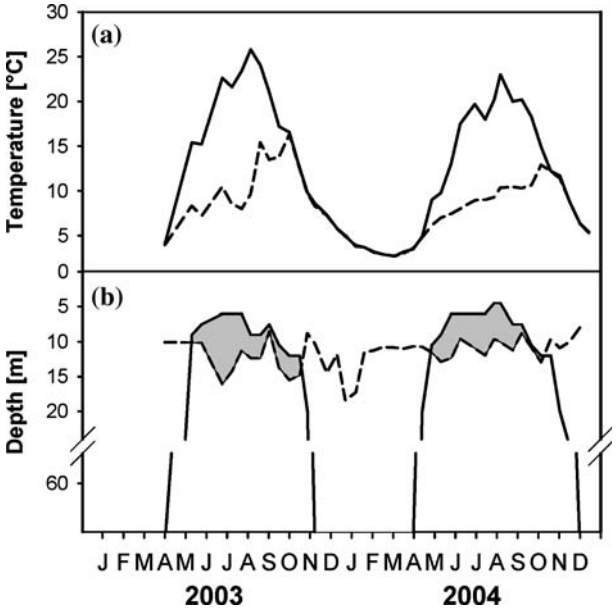


*Figure 2.* Physical variability during the sampling period 2003/2004. (a) surface water temperature (solid line) and metalimnetic temperature (dashed line) in °C; (b) mixing depth ($Z_{mix}$, solid line) and eupthotic zone ($Z_{eu}$, dashed line) in m. The shaded area indicates the stratified period.

1.3–1.7 μg $l^{-1}$ and not detectable during the stratified period. DIC ranged from 2.6 to 3.6 mM (pH 7.6–8.4) under mixed conditions and varied between 2.2 and 3.3 mM (pH 7.1–8.6) in the epilimnion during stratification. In the metalimnion, DIC increased up to 3.9 mM at a pH of about 8.6.

The depth distribution of Chl-*a* was closely linked to thermal stratification. During the circulation period Chl-*a* was homogeneously distributed, while a DCM was observed in the thermocline during stratification, reaching a maximum of 16.2 mg $m^{-3}$. Microscopic evaluation of phytoplankton abundance indicated that the phytoplankton assemblage in the DCM was dominated by the filamentous cyanobacterium *P. rubescens*. During the rest of the year the phytoplankton assemblage was mainly composed of diatoms.

The quality of FRRF measurements near the water-surface was strongly influenced by light intensity. Maximum fluorescence in the light and the dark chambers ($F_{mL}$ and $F_{mD}$) showed lower values in the high-light regions near the surface, caused by corruption of the detector. As a consequence, FRRF-measurements below 200 μEinst $m^{-2}$ $s^{-1}$ were exclusively used to calculate photosynthetic parameters. The inter-calibration of the light and dark chambers was tested by regression of all available data below 200 μEinst $m^{-2}$ $s^{-1}$ from the casts. The data were highly correlated (slope = 0.97, $r^2$ = 0.98, $n$ = 910, $P < 0.001$), which was proved by filtered freshwater blanks.

The maximum quantum yield of photochemistry ($F_v/F_m$) varied from 0.49 to 0.60 (average 0.57, SD = 0.02) under mixed conditions and was more or less uniform with depth. Under stratified conditions, $F_v/F_m$ was generally lower (average 0.50, SD = 0.04) and showed a characteristic vertical pattern: quenching near the surface, highest efficiencies at the bottom of the mixed zone and decreasing values further down the column corresponding to the light gradient.

$\sigma_{PSII}$ varied from 195.4 to 366.2 (average 252.4 Å $m^{-2}$) between 6 and 15 m depth during mixed conditions and from 173.2 to 311.1 (average 221.9 Å $m^{-2}$) during stratification. $\sigma_{PSII}$ depends on the vertical mixing rate (Moore et al. 2003), which is shown in Figure 3 depicting examples of typical profiles from August to December 2004. While the typical winter profile showed a uniform distribution, $\sigma_{PSII}$ followed the vertical gradient of stratification in summer.

The relationship between fluorescence-based and $^{14}C$-derived photosynthetic parameters was examined correlating estimates of primary productivity from both methods (Figure 4). Under

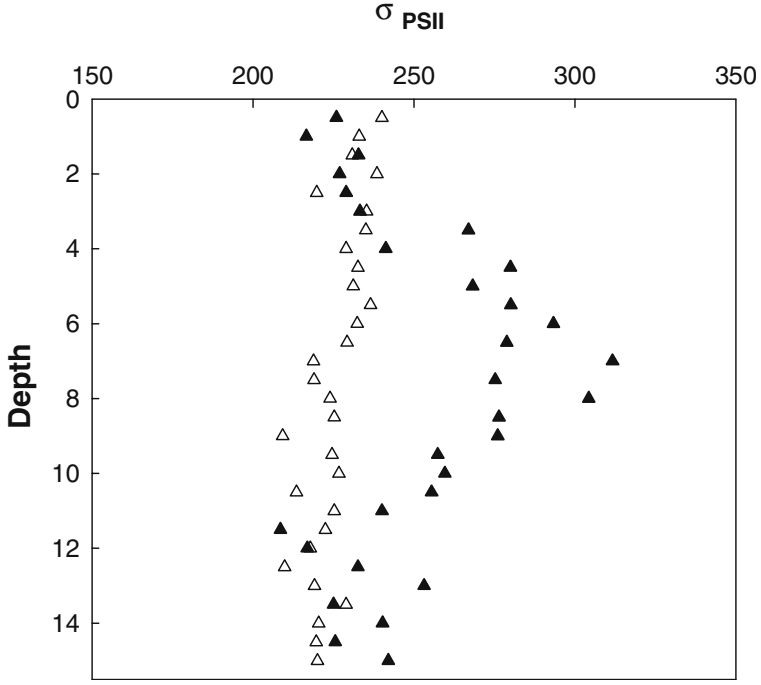


*Figure 3*. Effect of vertical mixing on the distribution of $\sigma_{PS\ II}$. Example profiles from August to December 2004 reflecting stratified (▲) and non-stratified (Δ) conditions.

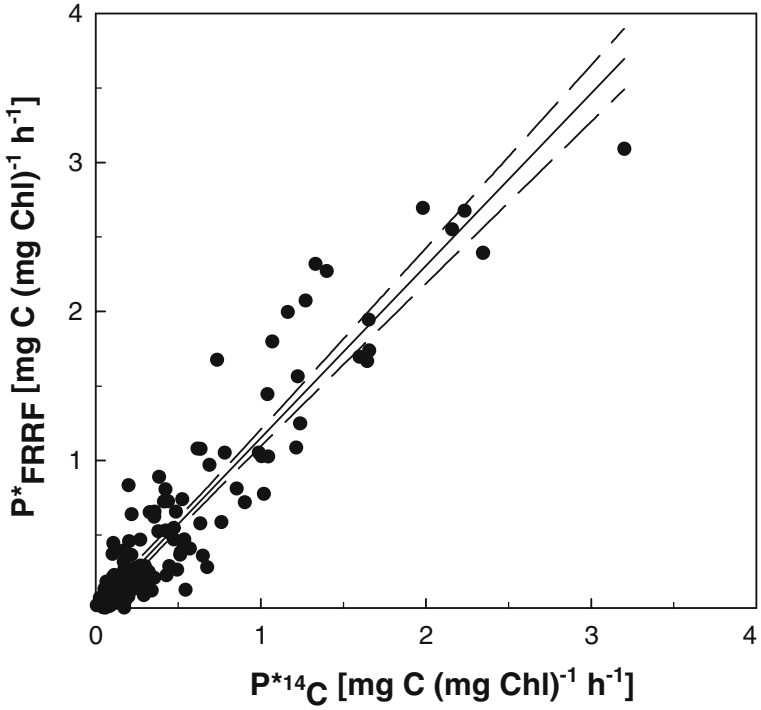


*Figure 4*. Correlation between fluorescence-based ($P^*_{FRRF}$) and $^{14}$C-based ($P^*_{14C}$) phytoplankton productivity under sub-saturating conditions ($r^2 = 0.88$, $n = 140$, $P < 0.001$, slope = 1.15). The dashed lines indicate the 95% confidence limits.

sub-saturating conditions $^{14}$C-derived productivity explained 88% of the variance of FRRF-based estimates (140 pairs of measurement, slope = 1.15, $r^2 = 0.88$, $P < 0.001$). Under high-light conditions as well as during the appearance of the DCM dominated by *P. rubescens*, fluorescence-based carbon fixation rates did not correlate with estimates from carbon uptake. The mismatch of fluorescence-based data and $^{14}$C-uptake during blooms of *P. rubescens* is illustrated in Figure 5, where all productivity-rates of both methods are plotted against irradiance at sub-saturating light conditions.

The maximum light-utilisation coefficient $\alpha^*_{FRRF}$ was calculated from FRRF-based estimates using Equation (2). $^{14}$C-based estimates of $\alpha^*$ were calculated using the tangent hyperbolic function. The two derived parameters were not correlated. Except in two cases, FRRF-derived values of $\alpha^*$ were higher than $^{14}$C-based values. Splitting the data in a mixed and stratified sub-set, fluorometry-based data ranged in both cases between 0.02 and 0.04, while values based on $^{14}$C measurements formed two distinct clusters (Figure 6a). Phytoplankton was characterised by higher light-utilisation efficiency (0.01–0.03 mgC (mg Chl-*a*)$^{-1}$ h$^{-1}$ (µmol phot. m$^{-2}$ s$^{-1}$)$^{-1}$) during the circulation period, while it was at maximum 0.1 during stratification.

Maximum quantum yields for carbon fixation ($\Phi_{C,max}$) were calculated for 12-month data, as the Chl-*a* specific absorption coefficient $a^*$ was only available since January 2004. There was an exponential relationship between $\Phi_{C,max}$ and $F_v/F_m$ (Figure 6b), indicating that changes in the photochemical quantum yield $F_v/F_m$ account for 60% of the variation of $\Phi_{C,max}$ ($R = 0.78$, $P < 0.001$). Highest values of $\Phi_{C,max}$ ranging from 0.028 to 0.063 mol C (mol phot.)$^{-1}$ were found in accordance with higher estimates of $F_v/F_m$ during mixed conditions and were associated with higher nutrient availability.

## Discussion

Mondsee is a deep oligotrophic alpine lake with a seasonal phytoplankton succession characterised

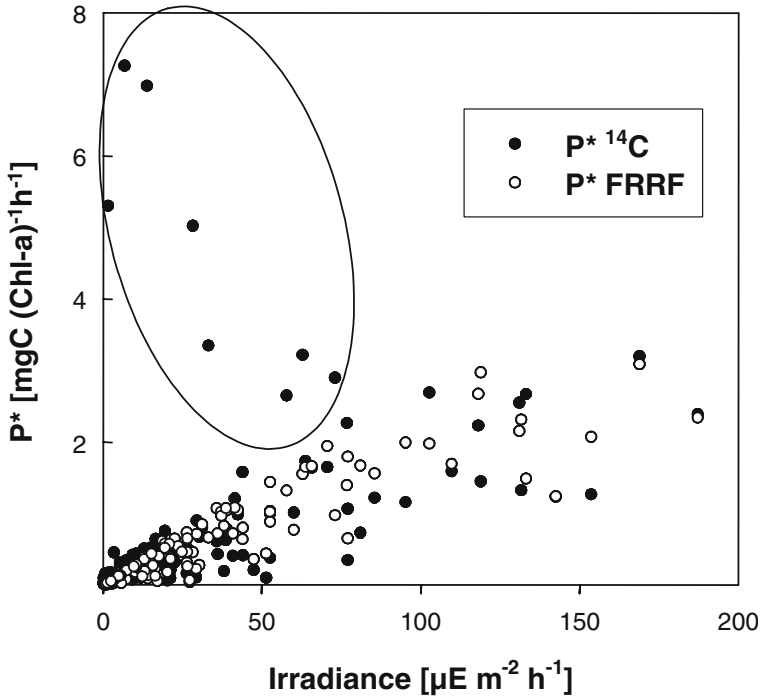


*Figure 5.* Relationship between all estimates of FRRF- and $^{14}$C-productivity in mgC (Chl-a)$^{-1}$ h$^{-1}$ and total available radiation in µEinst m$^{-2}$ s$^{-1}$. $^{14}$C-based measurements during *P. rubescens* blooms are marked. As discussed in the text, these values are underestimated by FRRF due to the excitation band-width of the cyanobacterial species, which is not exactly in the spectrum of the Fluorometer.

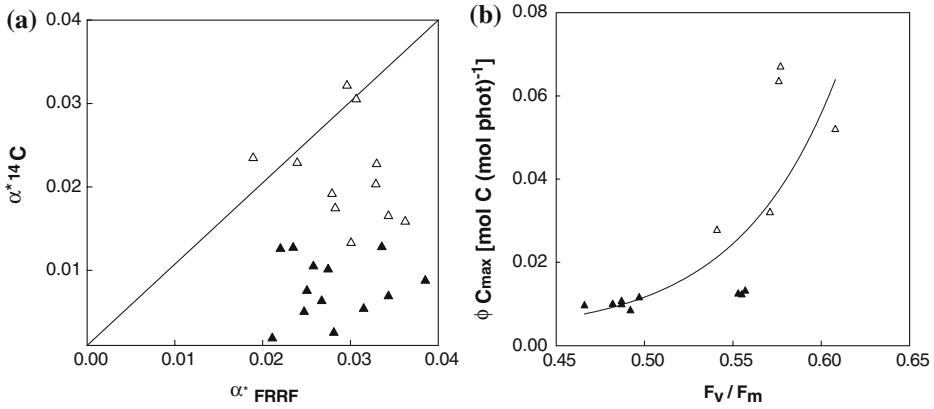


*Figure 6*. Comparison of physiological parameters derived from $^{14}$C-based and FRRF-based estimates. (a) $\alpha^*_{14C}$ as a function of $\alpha^*_{FRRF}$ both in mgC (Chla)$^{-1}$ h$^{-1}$ (µmol phot. m$^{-2}$ s$^{-1}$)$^{-1}$; (b) $\Phi_{C,max}$ as a function of $F_v/F_m$. Symbols of the plots correspond to stratified (▲) and non-stratified (() conditions.

by diatoms during winter and spring and a mass occurrence of the filamentous cyanobacterium *P. rubescens* during summer and early autumn (Dokulil and Skolaut 1986; Dokulil 1987; Teubner et al. 2003). During the stratification period, it dominated the phytoplankton assemblage in the thermocline reaching up to 95% of the fresh-weight biomass (Findenegg 1969; Dokulil 1993; Dokulil and Teubner 2003).

Vertical scales in freshwaters differ from those in marine systems by an order of magnitude (10 m versus 100 m), as indicated by the depth of the euphotic zone ($Z_{eu}$) and the mixing depth ($Z_{mix}$) (Figure 2b). Restricted by the small-scale water-column, we had to optimise the sampling procedure to obtain sufficient and high quality FRRF-data. Error due to rapid sampling, irradiance and acclimation of the instrument to water-temperature was overcome by lowering the Fluorometer slowly by a hand-operated winch. In the topmost layers, the fluorescence signal was interfered by ambient red light (Raateoja et al. 2004a, b). The light chamber was more affected by high incoming radiation than the dark chamber as also reported by Smyth et al. (2004).

Maximum yields of photochemistry ($F_v/F_m$) indicate that thermal stratification has a strong impact on the physiological state of phytoplankton assemblages. Lower values of $F_v/F_m$ during stratification, indicating a lower proportion of functional reaction centres *f* (Falkowski and Kolber 1993; Geider et al. 1993), were explained by nutrient limitation (Babin et al. 1996; Moore et al. 2003) and a higher proportion of non-photosynthetic pigments (Babin et al. 1996).

In fact, Mondsee is a phosphorus-limited system with maximum SRP of 1.7 µg l$^{-1}$ during spring overturn and undetectable amounts during summer. Highest $F_v/F_m$ (0.51) during summer occurred below the mixed zone at depths between 6.5 and 10.5 m, where the density gradient prevented phytoplankton from being advected and exposed to high light intensities. During stratification, PAR was available down to $Z_{eu}$(max) 11.8 m resulting in a ratio of $Z_{mix}/Z_{eu} < 1$. Summer phytoplankton in Mondsee is dominated by a deep maximum of *P. rubescens*, an extremely shade-adapted cyanobacterium which utilises the green light spectrum at depths between 10 and 12 m. In this metalimnetic *Planktothrix* poulation, $F_v/F_m$ was 0.39–0.44. These values most probably underestimate the efficiency of PS II. As Kromkamp et al. (2001) point out, thylakoids of cyanobacteria are also used for respiration and phycobilisomes produce fluorescence both leading to underestimations of $F_v/F_m$.

The functional absorption cross section ($\sigma_{PSII}$) was higher during stratification and also showed a distinct vertical pattern. During the mixed period, cells are moved faster through the vertical light gradient, than they can acclimate their photosynthetic apparatus, the distribution of $\sigma_{PSII}$ will be rather uniform within different depths (Moore et al. 2003). During stratification, phytoplankton cells can acclimate to the light regime, leading to characteristic vertical patterns. As indicated in

Figure 3, the highest values of $\sigma_{PSII}$ during stratification were observed at a depth around 7.5 m, declining to lower values down to 15 m depth. The mixing depth extended to an average of 7.9 m during stratification, and in combination with high incoming radiation near the surface, $\sigma_{PSII}$ was expected to be low in the epilimnion. The depth of the euphotic zone was on average 11.8 m in summer, providing an usable amount of light for photosynthesis in the metalimnion. Uniformly low values in the *Planktothrix* layer below 12 m depth may be caused by species composition.

Generally, observations on $\sigma_{PSII}$ can be interpreted by the light history of the cells (Kolber et al. 1988, 1990). Non-photochemical quenching can cause a reduction of $\sigma_{PSII}$ up to 50% during a day (Falkowski et al. 1986; Olaizola et al. 1994), whereas photoacclimation over a longer period can cause changes of $\sigma_{PSII}$ by a factor of 3 (Kolber et al. 1988). Moreover, there is variability in $\sigma_{PSII}$ due to species composition (Kolber et al. 1988). Lower values of $\sigma_{PSII}$ during the mixed period, when diatom populations dominated the phytoplankton assemblages, were consistent with observations from Moore et al. (2003) and taxon-specific data from Olson et al. (1996). The nutrient status of the cells also influences $\sigma_{PSII}$. In accordance with our findings, studies of Berges et al. (1996) have shown higher $\sigma_{PSII}$ following nutrient limitation.

Estimates of primary productivity from FRRF-based measurements and $^{14}C$-incubations showed a high consistency throughout the sampling period with slightly higher estimates of $P_{FRRF}$. This is in agreement with the findings of Suggett et al. (2001), while large differences between photosynthetic parameters derived from FRRF – and $^{14}C$-estimates were observed by Boyd et al. (1997), who explained the inconsistency of both methods by spectral differences of the light regime. During the occurrence of *P. rubescens* in the metalimnetic layer, FRRF was not able to give similar results as the $^{14}C$ incubation technique. Findings of Raateoja et al. (2004a, b) indicate that the applicability of FRRF to phycoerythrocyanin-rich cyanobacterial taxa is restricted due to their spectral fluorescence and absorption characteristics. In contrast to phycoerythrocyanin-rich cyanobacteria with an excitation band width between 570 and 595 nm (Bryant 1982), phycoerythrin-rich cyanobacteria ($\lambda$ 495–575 nm) lie within FRRF excitation ($\lambda$ 458–514 nm). Although *P. rubescens* is counted to the group of phycoerythrin-rich cyanobacteria, FRRF was not applicable during mass appearance of the taxon. The amount of phycoerythrin or their derivates phycourobilin ($\lambda$ 495–500 nm) and phycoerythrobilin ($\lambda$ 540–575 nm) were not determined (Sidler 1994; Raateoja et al. 2004a, b).

Excluding *P. rubescens*-rich layers, it is none the less astonishing that estimates of primary productivity based on two totally different timescales show an $r^2$ of 0.88.

Phytoplankton assemblages dominated by diatoms have a higher light-utilisation efficiency, as indicated by $\alpha^*_{14C}$. The indifferently high values of $\alpha^*_{FRRF}$ did not correspond to $^{14}C$-based $\alpha^*$, which clearly separated winter- from summer-phytoplankton assemblages. Observations of Moore et al. (2003) described similar results, and Suggett et al. (2001) found $\alpha^*_{FRRF}$ to be 1.5–2.5 times higher than $\alpha^*_{14C}$. These results might be interpreted by the differences of the two techniques. The $^{14}C$ technique estimates net photosynthetic rates, while instantaneous FRRF-based estimates represent gross photosynthetic rates (Bender et al. 1987; Marra 2002). Respiration can cause up to 10% of the difference between FRRF-based and $^{14}C$-based light-utilisation efficiencies (Grande et al. 1989; Weger et al. 1989; Danieri et al. 1992). Further processes which affect $\alpha^*_{FRRF}$ are discussed by Flameling and Kromkamp (1998): the higher $\alpha^*_{FRRF}$ can be caused by a combination of cyclic electron flow around PS II (Kolber and Falkowski 1986; Prasil et al. 1996), photorespiration and the Mehler reaction, which has also been observed in cyanobacteria (Kana 1992, 1993).

Maximum yields for carbon fixation ($\Phi_{C,max}$) were associated with higher quantum yields of photochemistry ($F_v/F_m$) under mixed conditions. Typical for diatom dominated phytoplankton, the optical absorption cross section decreases with the increase of cellular pigmentation and pigment packaging during adaptation to low light conditions, hand in hand with a higher light-utilisation efficiency (Bracher and Wiencke 2000). Under light-limited conditions, the maximum quantum yield of charge separation has been reported to reach values of 0.07–0.09 (Greene et al. 1994; Babin et al. 1995; Arbones et al. 2000) which is in accordance with our findings during winter, when

light availability is generally lower. The decrease of $\Phi_{C,max}$ and $F_v/F_m$ was consistent with a lower proportion of functional reaction centres under phosphorus depletion during stratification, similar to findings by Kolber et al. (1990) in the sea under nitrogen limitation. The nutrient status affects the optical absorption cross section (a*) of phytoplankton because it is directly related to the synthesis of components of the light harvesting apparatus.

Although FRRF is a convenient tool to estimate phytoplankton productivity under sub-saturating conditions, there are some major deficiencies in the applicability to freshwater conditions. Limited by the depth of the lake, FRRF-data acquisition has to be carried out in a rather small-scale water-column. Highly interesting information from the epilimnion is hard to interpret using a single acquisition protocol for the whole water-column because of the high incoming radiation. This may be overcome by using two different protocols with less sensitive settings for the topmost layers. But this also means acquisition and, most importantly, interpretation of the incoming radiation prior to each FRRF cast. Changing the protocol during the casts will mean another prolongation for the time consuming procedure of data acquisition, always in mind that this is done by a hand-operated winch. Another point is, that specific phytoplankton assemblages like cyanobacteria cannot be characterised sufficiently due to their low *in vivo* fluorescence to Chl-*a* ratio (Cunningham 1996). Generally speaking, we do not recommend FRRF as a stand-alone tool for deriving phytoplankton photosynthetic parameters. Choosing the proper operational scale, it provides additional high quality information on phytoplankton physiology, which is not obtainable with the conventional $^{14}C$ method.

## Conclusions

- During the mixed period, phytoplankton was dominated by diatoms. The acclimation to lower radiation due to the short day-length and higher nutrient availability was expressed by higher quantum yields of photochemistry ($F_v/F_m$) and light-utilisation efficiency ($\alpha^*_{14C}$), whereas the functional absorption cross section ($\sigma_{PSII}$) decreased.
- Summer phytoplankton is efficiently acclimated to the light environment expressed by highest $F_v/F_m$ and $\sigma_{PSII}$ right below the mixed zone under sub-saturating conditions. In the metalimnion, *P. rubescens* shows a lower proportion of functional reaction centres which is most probably due to alternative electron sinks.
- Estimates of primary productivity from FRRF showed a general consistency to conventional $^{14}C$-incubations under sub-saturating conditions.

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