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Photosynthetic activity of natural microphytobenthos populations measured by fluorescence (PAM) and ^{14}C-tracer methods: a comparison

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ABSTRACT: PAM (pulse-amplitude-modulated) fluorescence measurements of motile microphytobenthic algae were carried out in June 1996 at Sylt, Germany. Comparisons between ^{14}C-based and fluorescence-based production rates were made. A very high correlation between ^{14}C- and fluorescence-based production rates was found for maximal production rates (P_{max} values). ^{14}C-based maximal production rates varied during the study period between 0.65 and 1.7 mg C mg chl a^{-1} h^{-1}, comparable to variations of P_{max} measured with the fluorescence-based method. For other photosynthetic parameters [α (maximum light utilization coefficient), E_k (light saturation index), E_{max} (light intensity at which P_{max} is reached)], differences between the 2 methods were much larger. Highest carbon quantum yields (ϕ_{ass}) (mol C mol quanta^{-1} absorbed) were obtained at low irradiances. Considering the whole range of investigated carbon quantum yields, we found that initially these values decreased at low to moderate irradiances without a concomitant decline of the actual photochemical efficiency (F_m' – F)/F_m' (F and F_m': minimal and maximal fluorescence signals in the light). Therefore, a high linearity between the actual photochemical efficiency and the carbon quantum yield could only be observed up to values of 0.018 mol C mol quanta^{-1}. This is different to higher plants, for which linearity can be observed up to carbon quantum yields of 0.042 mol C mol quanta^{-1}. It was shown that, for the calculation of the overall production rates based on the fluorescence method, it is necessary to carefully measure the mean specific absorption coefficient (a^*) of the algae. Unless this is achieved, PAM measurements cannot be used to calculate absolute production rates.

KEY WORDS: Photosynthetic activity · Fluorescence · PAM · Primary production · Microphytobenthos · German Wadden Sea

INTRODUCTION

The PAM (pulse-amplitude-modulated) instrument is a highly selective modulation fluorometer offering the potential to measure fluorescence yields in full sunlight. As a result of intensive research, methods are now available by which the fluorescence information can be quantitatively analysed and evaluated and the photochemical efficiency of PSII (Photosystem II) and relative electron flow rates can be obtained (for review see Bolhar-Nordenkampf et al. 1989, Demmig-Adams 1990, Foyer et al. 1990, Walker 1992, Edwards & Baker 1993, Schreiber & Bilger 1993, Schreiber et al. 1994). Most of these studies have been carried out on higher plant leaves or on isolated chloroplasts; only a few researchers have applied the PAM technique to study unicellular algae and phytoplankton (Kroon et al. 1993, Hofstraat et al. 1994). As has been pointed out by Ting & Owens (1992) and Büchel & Wilhelm (1993), there have been considerable limitations in the performance of available instrumentation for quenching analysis using dilute samples of unicellular algae with different antenna organization. Some of them were overcome by Schreiber et al. (1993) and Schreiber (1994), because they succeeded to measure fluorescence even in suspensions of very low chlorophyll concentrations (0.1 to 50 µg l^{-1}). The PAM system has only been used for a few years in phytoplankton research. There are

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only a limited number of scientific studies concerned with the estimation of the photochemical efficiency of natural phytoplankton or phytoplankton cultures conducted using the PAM technique (Ting & Owens 1992, Kroon et al. 1993, Hofstraat et al. 1994). So far this method has not been applied to microphytobenthos communities. A reliable estimation of microphytobenthos primary production is far from simple due to the patchy distribution of these communities. With most of the classical methods, e.g. the ^{14}C-tracer technique (Steemann Nielsen 1952) and different O_2 techniques, one can only make a few measurements per day and, moreover, most of them cannot easily be applied *in situ*. However, it has to be mentioned that measurements of oxygen fluxes by microelectrodes allow rapid estimations of temporal distribution patterns of primary production with high resolution, but the problem of spatial resolution remains. The use of bells jars is an alternative method to measure primary production at a relevant spatial scale, but the results obtained represent overall bulk measurements, from which one cannot derive physiological indicators [such as E_k (light saturation index), α (maximum light utilization coefficient) and P_{max} (maximal production rate) values]. In order to overcome these practical and methodological problems and to measure primary productivity *in situ* with a high frequency and/or on a relevant spatial scale, a strong need to introduce new methods exists.

In this study we evaluate the application of the PAM fluorescence technique with microphytobenthic algae as test organisms. Further, we tested the hypothesis that primary production rates obtained with the classical ^{14}C method are comparable to photochemical efficiency measurements obtained with the modulated fluorescence technique (PAM). Our ultimate long-term goal is to see whether this method can help to solve the problems associated with temporal and spatial resolution.

MATERIALS AND METHODS

Sampling. Sampling took place in June 1996 on the tidal flats of Keitum (KE) (54° 54' N, 8° 23' E), located on the coast of the island of Sylt in the German Wadden Sea. At several locations, thin layers of the muddy sediment surface were scraped with a small spatula and put in a jar. Each sample was thoroughly mixed and transferred to 20 × 30 cm containers and covered with 3 layers of lens tissue (Whatman 105). The samples were pre-incubated at a constant irradiance of 70 µE m^{-2} s^{-1} in a culture cabinet during both day and night. Additionally, during June 6 two parallel samples were pre-incubated outside in the shade at 210 ± 10 µE m^{-2} s^{-1}. The following morning, the lens tissue together with the part of the microphytobenthos which was able to migrate (Eaton & Moss 1966) into the thin tissue were harvested from the sediment. The algae in the upper 2 layers of the tissue were resuspended in a definite volume of prefiltered water (Whatman GF/F) taken from small tide pools in the sampling area. The algal suspension was cleaned from tissue fibres by decanting over a small sponge in a funnel (Colijn & van Buurt 1975). All measurements were conducted using this concentrated cell suspension. In addition, 3 experiments were performed at Königshafen (KO) (55° 2' N, 8° 25' E), which is located on the north coast of Sylt in a more marine setting. The samples from this site were taken and treated in the same way as those from Keitum.

^{14}C incubation. For ^{14}C-based primary production rates (PPR), small aliquots (2.5 ml) of the concentrated microphytobenthos suspensions were incubated simultaneously in a photosynthetron (Tilzer et al. 1993) at 18 ± 1°C at 11 different irradiances (23, 32, 53, 77, 126, 147, 235, 373, 588, 861 and 1134 µE m^{-2} s^{-1}) for 1 h. A quartz-halogen lamp served as the light source. The irradiance gradient was generated by metal nettings. Radioactive $NaH^{14}CO_3$ (0.5 µCi) was added to glass vials containing the algal suspension (2.5 ml in each vial). After the end of the incubation, the microphytobenthos cells were filtered onto a membrane filter (0.45 µm) and washed, and the radioactivity of the cells was measured with a liquid scintillation counter (Tri-Carb 1900 TR, Packard Instruments). Counting efficiency was determined by the external standard method. C-assimilation rates were fitted according to Megard et al. (1984). For standard nomenclature of photosynthetic parameters see Sakshaug et al. (1997).

Carbon quantum yields. Carbon quantum yields (ϕ_{ass}; mol C mol quanta^{-1}) based on the ^{14}C uptake were calculated according to the following equation:

$$\phi_{ass} = C_{fix} / (irra_{incid} \cdot a^{\bullet})$$

where C_{fix}: carbon fixed (mol C g chl a^{-1} h^{-1}); $irra_{incid}$: incident irradiance (mol quanta m^{-2} h^{-1}); and $a^{\bullet}$: mean specific absorption coefficient (m^2 g chl a^{-1}).

Fluorescence measurements. Chlorophyll fluorescence was measured with a PAM-101 fluorometer using the accessory module PAM-103 for saturation pulse control (Walz, Effeltrich, Germany). In the following, the chlorophyll fluorescence nomenclature and abbreviations of van Kooten & Snel (1990) are used. A 650 nm LED for pulsed measuring light (1.6 kHz) was used to determine minimal fluorescence in the dark (F_0) and fluorescence emission was measured at wavelengths above 710 nm (Schott RG9 long-pass filter). The basic system was extended by a new emitter detector-cuvette assembly (ED-101 Ultrasensitive

Assembly, Walz) which allows sensitive measurements of algae suspensions down to chl *a* concentrations as low as 20 µg l^{-1}. Experimental setup was the same as described in Schreiber (1994), the only exception being the actinic light source. In order to gain high levels of actinic light, a halogen lamp (Schott KL 1500) was used. Irradiance levels were stepwise increased electronically. To generate saturating light pulses, we used a second halogen lamp of the same type. To induce maximal fluorescence yield in the dark (F_m) or in the light (F_m'), saturation pulses with a length of 500 to 600 ms and an intensity of 1500 µE m^{-2} s^{-1} were applied.

An example of the measuring procedure with a suspension of microphytobenthos (sampled on 7 June) is shown in Fig. 1. The suspensions of microphytobenthos were kept in the dark in the photosynthetron at 18°C for at least 60 min. After dark incubation a small aliquot (1 ml) was transferred into the measuring cuvette and a saturating light pulse (SP) of length 600 ms and intensity 1500 µE m^{-2} s^{-1} was applied. The minimal (F_0) and maximal (F_m) fluorescence yields were determined on dark-adapted cells. From these signals the ratio between the variable ($F_m - F_0$) and the maximal (F_m) fluorescence was calculated according to:

$$(F_m - F_0)/F_m = F_v/F_m \quad \text{where } F_v = F_m - F_0 \quad (1)$$

Eq. (1) describes the potential photochemical efficiency of the open reaction centers of PSII.

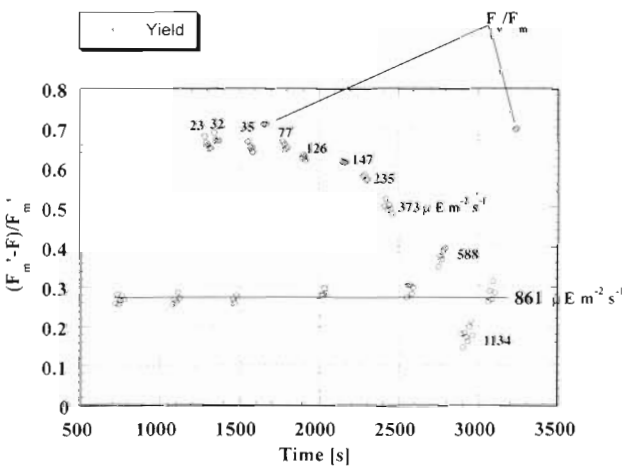

Fig. 1. Example of the PAM measuring procedure used on a microphytobenthos suspension sampled at Keitum, Sylt, Germany, on 7 June 1996 (KE7a). During 1 h, we measured the actual photochemical efficiency of PSII ($F_m' - F)/F_m'$ under the different irradiances applied in the photosynthetron (shown as numbers in plot) and, additionally, the potential photochemical efficiency of PSII (F_v/F_m) after at least 30 min in the dark. During the whole incubation period, we measured ($F_m' - F)/F_m'$ at 410 µE m^{-2} s^{-1} as frequently as possible to determine if this parameter remains constant over the whole incubation period

During the ^{14}C incubation in the photosynthetron a small aliquot (1 ml) was transferred into the measuring cuvette and exposed to the corresponding irradiance applied in the photosynthetron. Before measuring fluorescence yields, aliquots were adapted for at least 10 min in the photosynthetron. Due to this preincubation time the photochemical efficiency reached a steady state within 1 min in the measuring cuvette. After this, a series of 5 to 20 saturating light pulses (500 to 600 ms of 1500 µE m^{-2} s^{-1}) with a delay of 10 s was applied. The minimal (F) and maximal (F_m') fluorescence signals under actinic irradiance were then determined. From the mean values of the fluorescence signals the actual photochemical efficiency of PSII (ϕ_P) was calculated according to

$$\phi_P = (F_m' - F)/F_m' \quad (2)$$

$(F_m' - F)/F_m'$ was calculated from the mean value of the 5 to 20 light pulses. 20 light pulses were necessary at high irradiances, because in this case larger variability of F_m' and F was obtained. By multiplying $(F_m' - F)/F_m'$ with the irradiance (E) the relative electron flow was obtained according to Hofstraat et al. (1994):

$$\phi_e = (F_m' - F)/F_m' \cdot E \quad (3)$$

By plotting the relative electron flow against incident irradiance, *P-E* curves were constructed. Calculation of the absolute electron flow was also possible, because the mean specific absorption coefficent (a^*) of the algae was measured (see below). a^* describes the overall absorption of light by the algae. Thus, an index of primary productivity was obtained:

$$PPR_{fluorescence} = (F_m' - F)/F_m' \cdot E \cdot a^* \quad (4)$$

Signals were recorded with the PAM aquisition system PAM-DA100 and card PAM100.

Irradiance. Photosynthetically active radiation (PAR; 400 to 700 nm) was measured as incident flux density in µmol quanta m^{-2} s^{-1} using a small calibrated 4π microquantum sensor with a 0.5 cm diameter (Zemoko, The Netherlands) inside the cuvette in combination with a LI-193SA data-logger (Li-Cor, USA).

Measurements of the mean specific absorption coefficient (a^*). Absorption spectra of cell suspensions were measured with an Uvicon dual-beam spectrophotometer. Absorption of cell suspensions was measured in 1 cm quartz glass cuvettes that were placed directly in front of an intregrating sphere. Filtered seawater was used as the blank. To determine the mean spectral absorption coefficient, the spectral values were integrated and averaged over the 400 to 700 nm wavelength range. To obtain the mean specific absorption coefficient (a^*), it was normalised to the concentration of chl *a*.

Chl *a* measurements. Chl *a*, a proxy for algal biomass, was measured by using a Thermo Separation Products

HPLC system according to the method of Mantoura & Llewellyn (1983), modified in the following way. A subsample of 25 to 50 ml of the algal suspension was filtered over a Whatman GF/C filter and frozen at −20°C. For analysis, the filter was extracted in 5 ml of acetone and placed in a cell mill for 3 min. The sample was then centrifuged for 10 min at 4000 rpm (72.5 × *g*) to remove filter debris. Finally, the extract was filtered onto a 0.20 µm teflon filter. 0.75 ml of the filtered extract was incubated with 0.25 ml ammonium acetate for 3 min, and then the chl *a* measurement was made. Chl *a* measurements were calibrated with standards from the Water Quality Institute (Hørsholm, Denmark).

RESULTS

^{14}C incubations

P-E curves were derived from the ^{14}C-incorporation measurements for all investigated samples (Fig. 2). P^B_{max} (biomass-specific P_{max}) values varied between 0.66 to 1.72 mg C mg chl a^{-1} h^{-1} between June 6 and June 9, 1996. In KE9b, P^B_{max} was nearly twice as high as in KE6a. For the investigation period no short-term temporal effects on the *P-E* parameters could be observed. Photoinhibition occurred on all days at irradiances higher than 861 µE m^{-2} s^{-1} (Fig. 2).

In Table 1, all relevant P-E parameters derived with the ^{14}C method are shown. E_k values varied between 135 and 287 µE m^{-2} s^{-1} and α values between 0.0023 and 0.012 (mg C mg chl a^{-1} h^{-1})(µE m^{-2} s^{-1})$^{-1}$. Maximal carbon quantum yields (Φ_{assmax}) varied between 0.018 and 0.077 mol C mol quanta^{-1} (Table 1).

For each incubation, the carbon assimilation values obtained at all irradiances were consistent (the r values of the fitted data were always >0.98). In addition, the experiments repeated at Keitum on June 8 and 9 showed very similar results. Therefore, we assume that the ^{14}C-derived values were a realistic estimation of primary production and could be taken as a basis for comparison with the fluorescence data.

PAM measurements

Before analysing the results obtained by the fluorescence technique, the time dependency of the actual photochemical efficiency $(F_m' - F)/F_m'$ has to be taken into account. Due to our experimental setup only 1 measurement of $(F_m' - F)/F_m'$ at each irradiance was possible during the whole ^{14}C-incubation period (1 h). In order to see whether the values for $(F_m' - F)/F_m'$ changed during this incubation period, we made time-resolved measurements. It was shown that, for all tested irradiances, $(F_m' - F)/F_m'$ was almost constant over time (Fig. 3). Therefore, we based all our calculations on the 1 fluorescence yield measurement taken at each irradiance during the ^{14}C incubation.

For all investigated samples taken at Keitum and Königshafen, the potential photochemical efficiency (F_v/F_m) varied between 0.4 and 0.725 (Table 2). The actual photochemical efficiency $(F_m' - F)/F_m'$ at 20 µE m^{-2} s^{-1} varied between a minimal value of 0.41 in KE6c and a maximal value of around 0.725 in KE9b. At irradiances higher than 50 to 80 µE m^{-2} s^{-1}, $(F_m' - F)/F_m'$ decreased linearly with increasing irradiances in all experiments (Fig. 4).

From the product of the incident light (E) and $(F_m' - F)/F_m'$ the relative electron flow (ϕ_e) of PSII could

Table 1. Compilation of results obtained with the ^{14}C-tracer method. P^B_{max}: biomass-specific maximal production rate; α: maximum light utilization coefficient; E_k: light saturation index; E_{max}: light intensity at which P_{max} is reached; Φ_{assmax}: maximal carbon quantum yield. For explanation of sample abbreviations see Fig. 2 legend

Sample	Pre-incubation irradiance of the sample (µE m^{-2} s^{-1})	P^B_{max} (mg C mg chl a^{-1} h^{-1})	α (mg C mg chl a^{-1} h^{-1}) (µE m^{-2} s^{-1})$^{-1}$	E_k (µE m^{-2} s^{-1})	E_{max} (µE m^{-2} s^{-1})	Φ_{assmax} (mol C mol quanta^{-1})
KE6a	70	0.96	0.00531	181	442	0.059
KE6b	210 ± 10	1.38	0.00862	160	511	0.052
KE6c	70	0.66	0.00230	287	655	0.018
KE6d	210 ± 10	1.62	0.00940	172	345	0.077
KO6a	70	1.20	0.00754	160	438	–
KE7a	70	1.59	0.01181	135	417	0.074
KO7a	70	1.33	0.00612	217	473	0.033
KO7b	70	1.46	0.00813	180	482	–
KE8a	70	0.67	0.00480	139	455	0.031
KE8b	70	0.66	0.00414	160	426	0.043
KE9a	70	1.61	0.00746	216	432	0.054
KE9b	70	1.72	0.00686	251	501	0.053

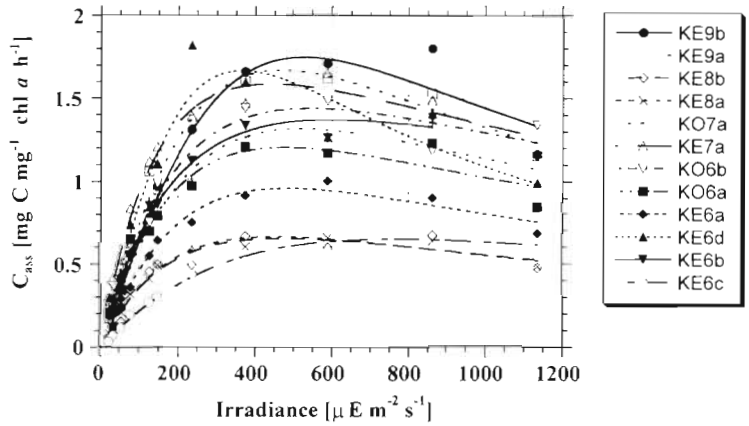

Fig. 2. *P-E* relationships [photosynthesis measured as assimilated carbon (C_{ass}) vs irradiance] for all investigated experiments obtained with the classical ^{14}C method. Sample abbreviations indicate the sampling location (KE: Keitum; KO: Königshafen, Sylt, Germany) with the sampling day [number: day in June 1996; letters: replicates (June 6: 2 pre-incubation irradiances; see Table 1)]

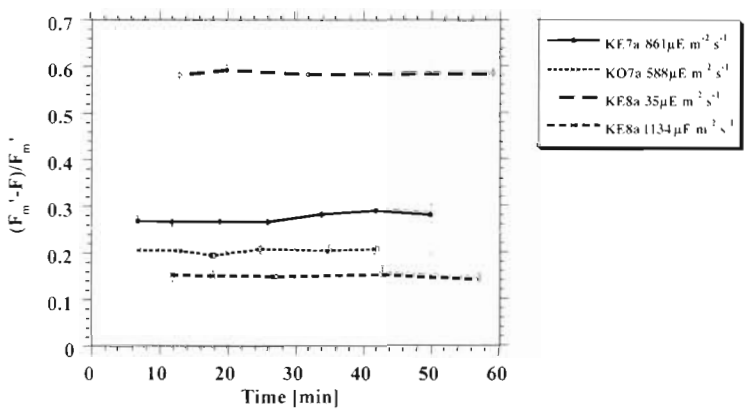

Fig. 3. Time dependency of $(F_m' - F)/F_m'$ (actual photochemical efficiency of PSII) over the whole incubation period. $(F_m' - F)/F_m'$ was measured several times at certain irradiances for different samples. Sample abbreviations as in Fig. 2 legend

be obtained (Fig. 5). Calculated maximal ϕ_e varied 2-fold between 110 and 240.

From the absorption spectra we calculated the mean specific absorption coefficient (a^*). a^* varied between 0.005 to 0.01 m^2 mg chl a^{-1} (Table 2).

Comparison of fluorescence- and ^{14}C-based production rates

By using Eq. (4) the fluorescence-based production rates were calculated (Fig. 6). In contrast to ϕ_e, the fluorescence-based production rates were quite similar to the ^{14}C-based production rates. When all experiments were taken into account, the correlation between ^{14}C-based production rates and fluorescence-based production rates was highly significant (r = 0.89) (Fig. 7). The correlation coefficient increases (0.95) when results at irradiances above 770 µE m^{-2} s^{-1} were excluded, but with this the fluorescence-based production rates underestimated the ^{14}C-based production rates (Fig. 8). This becomes clear in a more detailed analysis of single experiments, e.g. in experiments KE6d and KE9a (Figs. 9 & 10). Fluorescence-based production rates underestimated the ^{14}C-based production rates at low incident irradiances (<400–500 µE m^{-2} s^{-1}). At intermediate incident irradiances (400 to 600 µE m^{-2} s^{-1}) fluorescence-based production rates nearly equalled ^{14}C-based production rates, whereas at higher irradiances (>700 µE m^{-2} s^{-1}) fluorescence-based production rates overestimated ^{14}C-based production rates.

Concerning the *P-E* parameters, highest correlations between the 2 methods were obtained when comparing P_{max} values (r = 0.82) and α values (r = 0.83) (Fig. 11), whereas for the other parameters (E_k and E_{max}) there was no linear correlation between the 2 methods (Table 3). α was on average 0.67 times lower with the fluorescence-based method as compared to the ^{14}C method (Table 3). For E_k values, no correlation between the 2 methods could be found (r = 0.087). The same held for E_{max} values.

Highest carbon quantum yields (ϕ_{ass}; mol C mol quanta^{-1}) were obtained under low irradiances. Considering the whole range of measured ϕ_{ass}, we observed that initially these values decreased from low to moder-

Table 2. Potential photochemical efficiency of PSII (F_v/F_m), mean specific absorption coefficient (a^*) and chl *a* values. Sample abbreviations as in Fig. 2 legend

Sample	F_v/F_m	a^* (m^2 mg chl a^{-1})	Chl *a* (mg l^{-1})
KE6a	0.607	0.006793	0.175
KE6b	0.690	0.008327	1.608
KE6c	0.400	0.006993	0.138
KE6d	0.680	0.008327	1.608
KO6a	0.665	0.006509	0.175
KE7a	0.725	0.007954	0.420
KO7a	0.565	0.009948	0.096
KO7b	0.575	0.009948	0.096
KE8a	0.625	0.004982	0.192
KE8b	0.617	0.004982	0.192
KE9a	0.713	0.006809	1.132
KE9b	0.710	0.006809	1.132

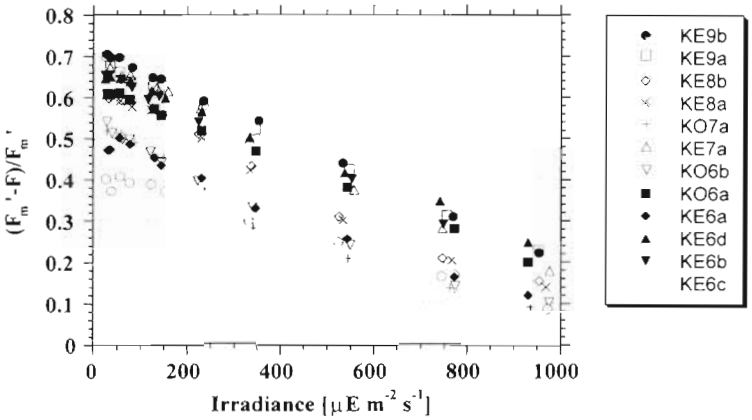

Fig. 4. $(F_m' - F)/F_m'$ at different incident irradiances. Sample abbreviations as in Fig. 2 legend

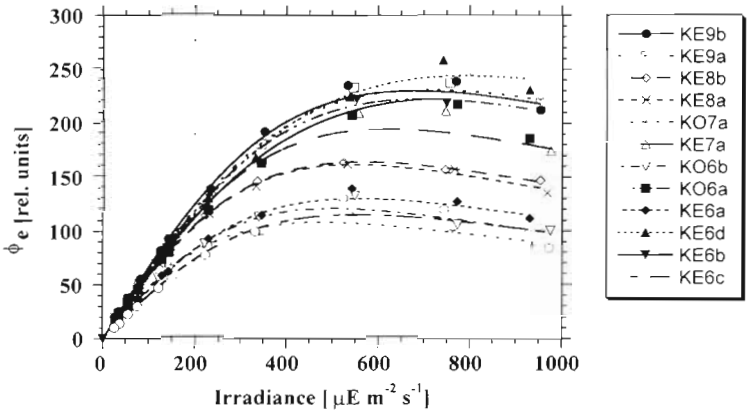

Fig. 5. Relative electron flow (ϕ_e) values calculated using Eq. (3) at different incident irradiances. Sample abbreviations as in Fig. 2 legend

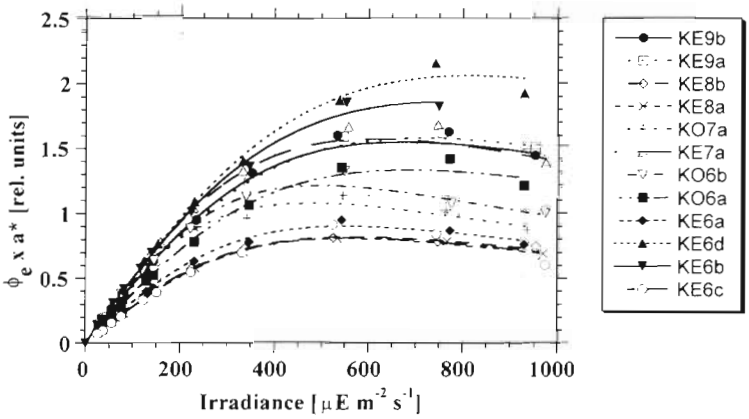

Fig. 6. Fluorescence-based production rates calculated using Eq. (4) at different incident irradiances. Sample abbreviations as in Fig. 2 legend

ate irradiances without a concomittant decline of $(F_m' - F)/F_m'$ (Fig. 12). A high linearity between $(F_m' - F)/F_m'$ and Φ_{ass} could only be observed up to values of 0.018 mol C mol quanta^{-1}.

DISCUSSION

This is the first study to use the PAM technique to obtain an index for primary production rates of microphytobenthos communities. A strong correlation between the fluorescence-based method and the radioactive tracer technique was observed, reconfirming earlier results (Hartig & Colijn 1996). At low irradiances (<400–500 μE m^{-2} s^{-1}) and at high irradiances (>700 μE m^{-2} s^{-1}) we observed deviations between the 2 methods. At low irradiances this deviation might be caused by the spectral differences between the light sources of the photosynthetron and the PAM. Whereas the irradiance levels in the photosynthetron were attenuated by metal nettings, which do not affect the spectral irradiance, the irradiance level of the actinic light source of the PAM system was stepwise increased by regulation of the voltage. This causes changes in the spectral irradiance, which result in a shift of the peak wavelength from red to a 'white' spectrum. Boyd et al. (1997) who made a rigorous comparison of fluorescence-derived (pump and probe) and ^{14}C-based estimates consider spectral deviations to be the explanation for the observed differences between the 2 methods. After correction for spectral differences through spectral normalisation, the majority of the ca 3-fold differences observed by Boyd et al. (1997) between the absolute values derived by the 2 techniques could be explained. Compared to these differences, our differences between the 2 methods were small. For the discrepancies between the fluorescence-based method and the radioactive tracer technique at high irradiances, a decent explanation is lacking. One possible explanation might be the existence of alternative electron sinks, e.g. the Mehler reaction (Geel 1997) or nitrogen reduction, which is known to increase with increasing irradiances (Weger & Turpin 1989).

Few other studies have attempted to compare conventional ^{14}C- and fluorescence-based measurements of primary production. A strong correlation between the 2 methods was described by Falkowski et al. (1991) in a study of 21 stations from the Northwest Atlantic and the subtropical Pacific, concluding that the pump and probe technique may be used to estimate photosynthesis *in situ*. Unlike the pump and

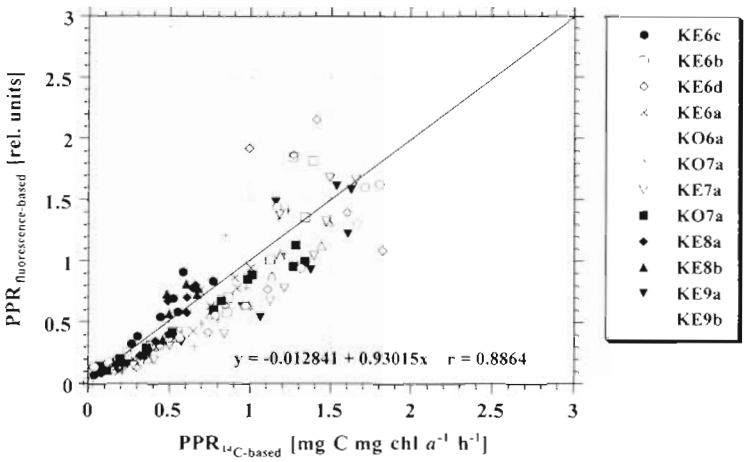

Fig. 7. Correlation between ^{14}C-based and fluorescence-based [$(F_m' - F)/F_m' \cdot E \cdot a^*$] production rates (PPR) for all performed experiments. Sample abbreviations as in Fig. 2 legend

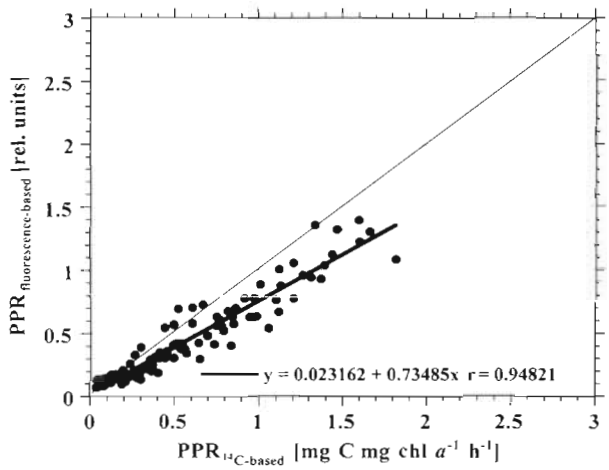

Fig. 8. Correlation between ^{14}C-based and fluorescence-based PPR for all performed experiments. Data at irradiances over 770 µE m^{-2} s^{-1} are excluded

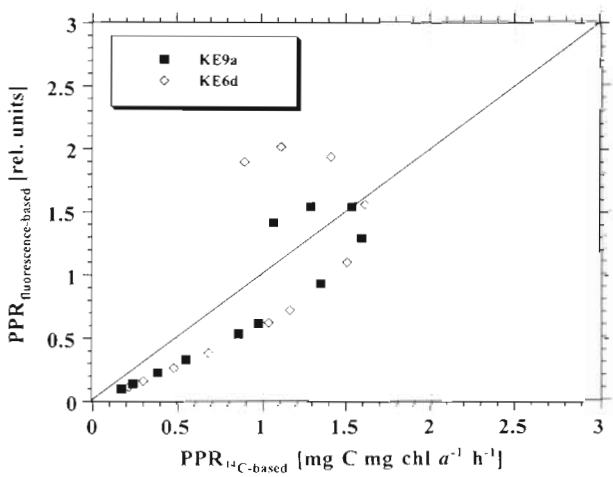

Fig. 9. Correlation between ^{14}C-based and fluorescence-based PPR for 2 different experiments at Keitum (KE9a and KE6d). Sample abbreviations as in Fig. 2 legend

probe technique the PAM method we used uses halogen lamps instead of xenon flash lamps. These instruments cannot be compared directly because of the different half width times of the flashes. A comparison of the 2 methods was carried out by Schreiber et al. (1995); the most distinctive difference is related to the method of F_m' determination. Therefore, the pump and probe technique works in a different manner to the PAM technique and comparisons between the results derived from the 2 methods are not easily made.

For higher plants a linear relationship between $(F_m' - F)/F_m'$ and the carbon quantum yield efficiency has been observed with the PAM technique (Harbinson et al. 1990, Seaton & Walker 1990). Edwards & Baker (1993) concluded that, under a wide range of conditions, $(F_m' - F)/F_m'$ can be used to predict accurately and rapidly CO_2-assimilation rates in maize. Genty et al. (1989) measured CO_2-assimilation rates and actual fluorescence photochemical yields for a variety of plants (C3 and C4) at different irradiances and CO_2 concentrations. They found an excellent linear correlation between the quantum yield of CO_2 fixation and $(F_m' - F)/F_m'$. These findings were confirmed and extended by Horton (1989), Keiller & Walker (1990), Seaton & Walker (1990), and Krall & Edwards (1991), who compared the quantum yield of O_2 evolution or CO_2 fixation and $(F_m' - F)/F_m'$ for leaves of numerous plant species and under a variety of physiological conditions.

Considering the whole range of investigated carbon quantum yield efficiencies, we found that for micro-

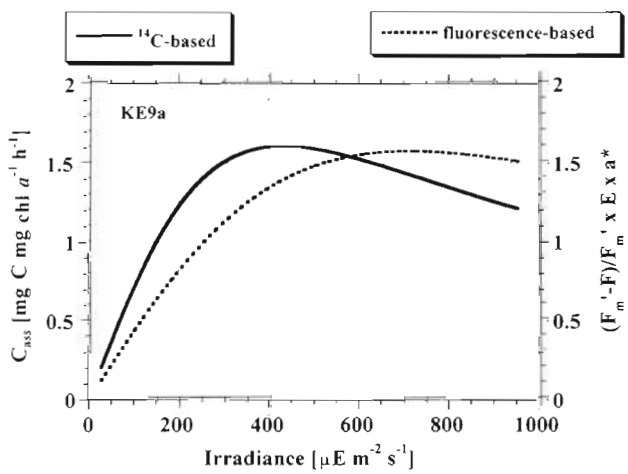

Fig. 10. *P-E* curves obtained with the radiocarbon and fluorescence estimations for one of the experiments at Keitum on June 9 (KE9a)

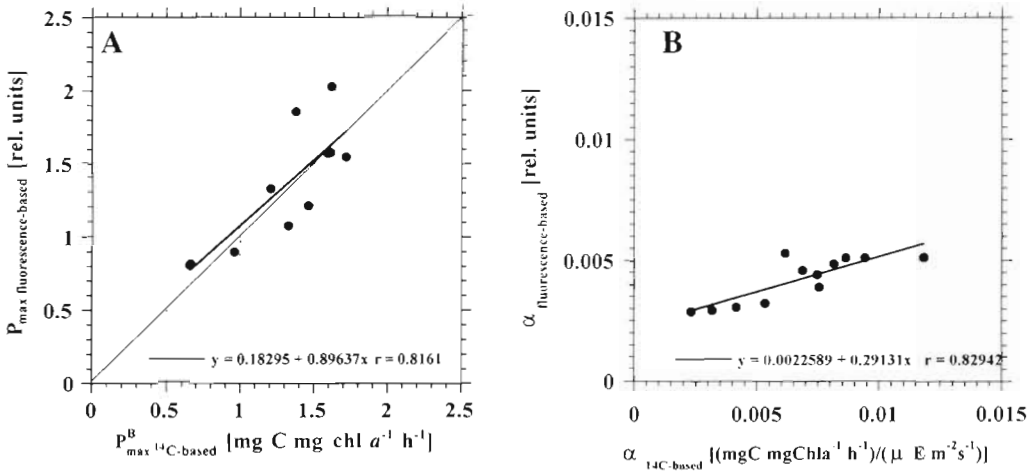

Fig. 11. Correlation between the P-E parameters (A) P_{max}, and (B) α obtained with the radiocarbon and fluorescence estimations

phytobenthos these values initially decreased from low to moderate irradiances without a concomitant decline of $(F_m' - F)/F_m'$. Therefore, a high linearity between the photochemical efficiency $(F_m' - F)/F_m'$ and the carbon quantum yield could only be observed up to values of 0.018 mol C mol quanta^{-1}. This is different to higher plants where this linearity could be observed up to carbon quantum yields of 0.042 mol C mol quanta^{-1} (Seaton & Walker 1990).

By multiplying $(F_m' - F)/F_m'$ with the irradiance (E) we obtained the relative electron flow (ϕ_e) according to Hofstraat et al. (1994). It should be stressed that the ratio $(F_m' - F)/F_m'$ describes the actual quantum yield of photochemical conversion of excitons already migrating in the light-harvesting complex of PSII. To estimate the absolute rate of linear electron flow through PSII, it is necessary to take into account the process of incident irradiance absorption by the PSII light-harvesting complex, which, in turn is subject to environmental regulation (Cleveland & Perry 1987, Herzig & Falkowski 1989). Because we determined the mean specific absorption coefficient (a^*), which is a parameter for the overall absorption cross section of the algae (Dubinsky 1992), we were also able to calculate the absolute electron flow rates. If the ratios between the fluorescence-based and ^{14}C-based production rates with and without a^* are compared, the correlation was much better if a^* is incorporated. But it should be pointed out that a^* represents the overall absorption of the phytoplankton, which means the absorption by both PSII and PSI. At room temperature, about 95% of the fluorescence is emitted by PSII (Dau 1994, Falkowski 1994, Schreiber et al. 1995), which means that it might be much better to estimate the absorption cross section of PSII, which is called the effective absorption cross section (Dubinsky 1992). Kolber & Falkowski (1993) were able to estimate the effective absorption cross section, because they used

Table 3. Quotients between fluorescence-based and ^{14}C-based P-E parameters (P_{max}, α, E_{max}, E_k) from different P-E curves (fluorescence-based/^{14}C-based). Sample abbreviations as in Fig. 2 legend

Sample	P_{max}	α	E_{max}	E_k
KE6a	0.94	0.61	1.26	1.54
KE6b	1.35	0.59	1.49	2.27
KE6c	1.23	1.26	0.86	0.98
KE6d	1.25	0.55	2.29	2.29
KO6a	1.10	0.52	1.56	2.14
KE7a	0.99	0.43	1.47	2.28
KO7a	0.81	0.87	1.01	0.93
KO7b	0.83	0.60	1.03	1.39
KE8a	1.21	0.62	1.21	1.95
KE8b	1.23	0.74	1.33	1.66
KE9a	0.98	0.59	1.65	1.65
KE9b	0.90	0.67	1.34	1.34
Mean	1.07	0.67	1.38	1.70
SD	0.18	0.22	0.37	0.49

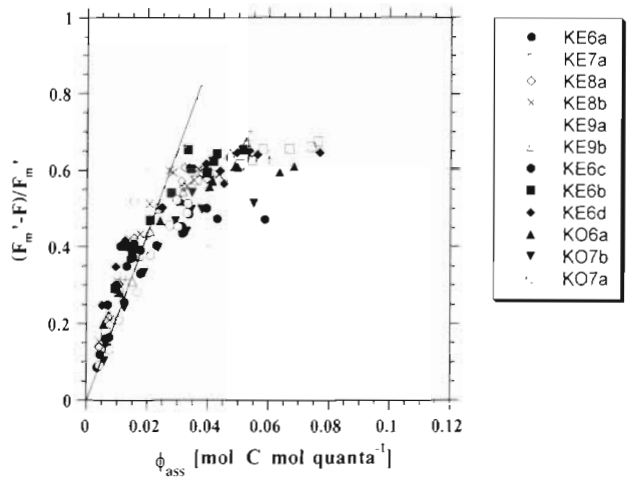

Fig. 12. Carbon quantum yields (ϕ_{ass}; mol C mol quanta^{-1} absorbed) vs $(F_m' - F)/F_m'$. Sample abbreviations as in Fig. 2 legend

the pump and probe method. An advantage of this method is that it can measure the effective absorption cross section directly. For the calculation of the overall production rates based on the fluorescence method, it is necessary to carefully investigate the effective absorption cross section of the algae. Unless this is achieved, PAM measurements cannot be used to calculate absolute production rates.

Considering the *P-E* parameters, highest correlations between the 2 methods were obtained when comparing P_{max} values, whereas for the other parameters (α, E_k and E_{max}) there were larger differences. This is contrary to the findings of Boyd et al. (1997), who found significant correlations for E_k.

Apart from the positive results, some problems still exist. We had to concentrate the samples using the lens tissue method. With this method we were able to study only the motile fraction of the microphytobenthos (see Wolfstein & Hartig 1998, this volume). Also, other processes (PSII heterogeneity, energy transfer from PSII to PSI, dissipative photochemistry at PSII, contribution of PSI to the overall fluorescence signal, and other electron sinks) may affect the final conversion of irradiance into fixed C (Slooten 1996). But, although we did not take into account the effects of these processes, we found a very good correlation between the ^{14}C-based method and the fluorescence-based method when using only the simple equation $(F_m' - F)/F_m' \cdot F \cdot a^*$

Considering that such fluorescence measurements can be carried out in seconds using submersible and more sensitive fluorometers in the field, we predict that this method will greatly impact future ecophysiological research. For instance, this method can be used for rapid estimations of spatial and temporal distribution patterns with high resolution, which is needed particularily for microphytobenthos and phytoplankton communities due to their patchy distribution.

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