

Determination of microphytobenthos PSII quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence

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ABSTRACT: A pulse amplitude modulated fluorometer (PAM) was used to investigate photosynthetic activity of microphytobenthos on an intertidal mudflat. Spectral irradiance measurements indicate that 75% of the signal detectable by the PAM originates in the upper 150 μm of the sediment. From the photosynthetic electron transport rate (*ETR*) measurements, it was concluded that the PAM could be used to observe changes in photosynthetic parameters during the day or the season. Photoacclimation to lower irradiance was indicated by changes in the maximum *ETR* and the saturating photon irradiance parameter I_k . When cores were exposed to a high photon irradiance for several hours, vertical migration could be followed using reflectance spectra. The data also showed that the benthic algae did not seem to experience photoinhibition or CO_2 limitation. To explain this, it is hypothesised that there is a continuous vertical migration in the top layer of the sediment, where algae can avoid photoinhibition due to prolonged periods of high irradiance and lack of CO_2 by migrating downwards while others migrate upwards.

KEY WORDS: Microphytobenthos · Chlorophyll fluorescence · Photosynthesis · Vertical migration · C-limitation

INTRODUCTION

Intertidal areas are a common feature of estuaries. The microphytobenthos colonising the intertidal flats form an important component of the estuarine ecosystem. Benthic algae are an important food-source for surface dwellers and deposit feeders (Sullivan & Moncreiff 1990, Heip et al. 1995) and are responsible for sediment stabilisation by excretion of carbohydrates (Holland et al. 1974, Paterson 1989, Yallop et al. 1994).

Microphytobenthos can reach high densities on intertidal flats and, consequently, microphytobenthos primary production can be an important fraction of total primary production in the system. Colijn (1983) estimated that microphytobenthos contributed 20% to total primary production in the Ems-Dollard estuary (The Netherlands). Sullivan & Moncreiff (1988) estimated

benthic microalgal production to be one third of total primary production in a Mississippi (USA) salt marsh.

Measuring microphytobenthos primary productivity is not easy because the activity seems highly variable, both on the hourly and monthly time scales. Microphytobenthos production seems to be positively correlated to elevation of the tidal flat, which is probably due to the influence of the length of the photoperiod, and to the clay content, which is related to hydrodynamic activity (de Jong & de Jonge 1995). Variation in photosynthetic parameters during low tide has also been observed (Blanchard & Cariou-LeGall 1994, Kromkamp et al. 1995a, MacIntyre & Cullen 1995) and has been attributed to vertical migration of benthic diatoms, which might be influenced by tidal stage and sun angle (Pinckney & Zingmark 1991), and CO_2 -limitation caused by high pH-values (Rasmussen et al. 1983, de Jong et al. 1988).

Methods to estimate primary production by microphytobenthos use chambers (see Heip et al. 1995 and references therein), the ^{14}C -technique, or oxygen micro-electrodes (e.g. Revsbech & Jørgensen 1983).

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All of these methods have their own advantages and disadvantages, which will be briefly discussed later. We chose to investigate whether it was possible to measure photosynthetic activity of microphytobenthos on intertidal flats using a pulse amplitude modulated fluorometer (PAM). This technique allows us in principle not only to measure photosynthetic activity rapidly in a non-intrusive way, but also to look at processes like quantum efficiency, photoprotection and photoinhibition, which will give extra information on the physiological state of the benthic microalgae.

Variable chlorophyll (chl) fluorescence originates mainly from light emission in chl *a*-containing photosystem II complexes (PSII). Excitation energy obtained by absorption of the light harvesting pigment protein complexes of PSII can not only be used for photochemistry, but can also be lost by thermal dissipation (heat) or fluorescence. These processes compete and influence the energy conversion efficiency or quantum yield (see Krause & Weis 1991, Kolber & Falkowski 1993, Dau 1994 and Horton & Ruban 1994 for reviews). Linear electron transport will only commence following charge separation. When the cells are fully dark adapted, and not photoinhibited, the primary electron

acceptor of PSII, Q_A , is in the oxidized state (all reaction centres are 'open'), non-photosynthetic energy dissipating mechanisms will be minimal, and the quantum yield will be maximal. Closure of PSII reaction centres by capture of irradiance will increase the fluorescence yield and induce energy-dissipating processes [non-photochemical (qN) quenching]. This will decrease the quantum efficiency. When all energy-dissipating mechanisms are saturated, photoinhibition will occur, leading to net degradation of the D₁-reaction centre II protein (Ohad et al. 1994). In this case, qN quenching will slowly relax when the cells are transferred to the dark. However, (normally short lived) qN quenching can also be induced at non-saturating irradiances. Hence the relaxation of qN quenching in darkness or dim irradiance conditions can be used to determine whether photosynthesis is down regulated ('dynamic inhibition') or whether chronic photoinhibition occurred (Osmond 1994).

In this paper we investigated whether the PAM technique could be used to follow changes in photosynthetic activity of microphytobenthos. Our results show that this is indeed possible.

METHODS

Sampling sites. Microphytobenthos cores were taken at several stations on an intertidal flat (Molenplaat) in the central part of the Westerschelde estuary (SW Netherlands, Fig. 1). Most of the cores (6 cm diameter) were taken at Stn B, which was visited at least monthly. The cores, each in a tube sealed with a rubber stopper at the bottom, were taken to the ship (RV 'Luctor') in a coolbox, where the PAM measurements were carried out. The first measurements were taken within half an hour after sampling. In order to allow repetition of the measurements during the day, the cores were exposed to the daily surface irradiance by placing them on the deck of the ship; the temperature was kept approximately constant by placing the tubes containing the cores in water, but leaving the surfaces of the cores dry. Nevertheless, the core temperature in summer could be raised by 2°C, which, however, also happened in the field. The cores did not dry out during the treatments, as a little water was always visible on the surface. Hence, the cores experienced conditions similar to those in the field. Four additional cores (2.5 cm diameter) were taken for pigment analyses. They were sliced immediately after being brought on board. The first 1 mm of the sediment was extruded using a piston manipulated with a screw, one whole turn equivalent to 1 mm. The four 1 mm slices were pooled and frozen at -80°C and lyophilized as soon as possible. They were stored in the dark at -80°C until

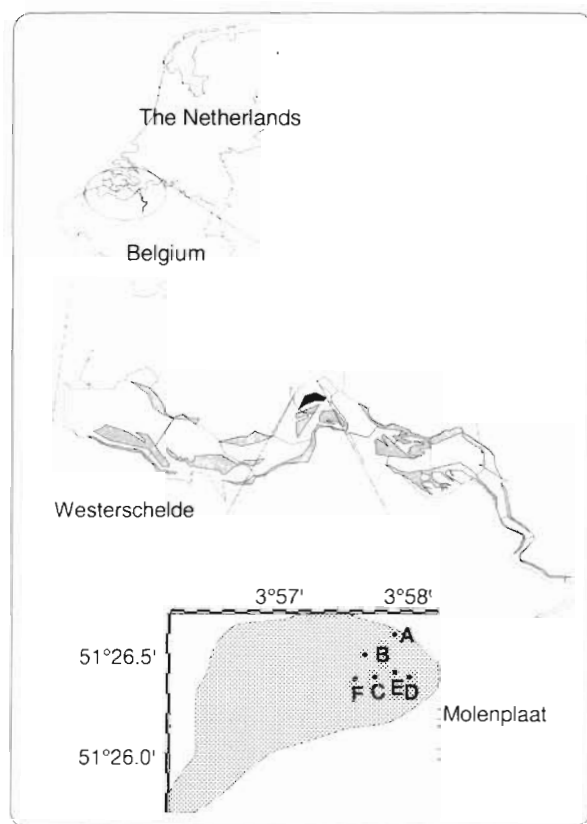


Fig. 1 Location of the Molenplaat in the Westerschelde estuary (The Netherlands) and the sampling stations on the intertidal flat

Table 1. Sediment characteristics of the sampling stations at the Molenplaat (Westerschelde estuary, The Netherlands) in March 1995. Grain sizes are given in parentheses

Stn	% silt ($<50\ \mu\text{m}$)	% fine sand ($50\text{ to }113\ \mu\text{m}$)	% medium sand ($262\text{ to }564\ \mu\text{m}$)
A	10.32	35.58	44.21
B	16.24	34.05	39.89
C	16.24	37.53	35.17
D	3.93	40.62	51.10
E	4.53	48.18	43.03
F	16.65	39.52	32.66

analyses. Water samples were taken near the intertidal flat. The average salinity of the water was approximately 20 psu. Some sediment characteristics of the Molenplaat are given in Table 1.

Fluorescence measurements. Variable fluorescence was measured with a PAM 101-103 fluorometer (Walz, Effeltrich, Germany), which controlled the Schott KL1500/E light source used for administering the saturating irradiance pulses. The photon flux density (PFD) of the irradiance pulse was more than $10\,000\ \mu\text{mol m}^{-2}\text{ s}^{-1}$, i.e. high enough to close all reaction centers. The pulses were administered at 20 s intervals. We made the pulse length, which varied between 500 and 700 ms, as short as possible in order to avoid an intrusive effect of the pulses. However, we carefully checked before each measurement whether maximum fluorescence (F_m) was correctly measured by changing the pulse length period and intensity. For actinic irradiance, a Schott KL1500 light source was used in combination with the PAM fiberoptics. Ten exposures (2 min each) were made with PFDs ranging from 0 to $1500\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ in order to construct photosynthesis (i.e. electron transport rate or *ETR*) irradiance curves. The resulting 6 datapoints per PFD were averaged, and these were fitted as a function of PFD according to Platt & Jassby (1976) using a least-squares method. The 95% confidence interval was generally less than 4% of the mean. The photon irradiance was adjusted by changing the voltage of the Schott KL1500 lamp. The tip of the fiberoptic was mechanically positioned perpendicular to the core surface, and the distance to the core surface was approximately 2 mm.

Quenching coefficients were calculated after van Kooten & Snel (1990).

The maximum energy conversion efficiency or quantum efficiency of PSII charge separation (ϕ_p^0) is calculated as:

$$\phi_p^0 = (F_m - F_o)/F_m = F_v/F_m \quad (1)$$

where F_o is the minimum fluorescence, F_m is the maximum fluorescence and F_v is the maximum variable

fluorescence yield of a minimally 15 min dark-adapted sample.

According to Genty et al. (1989), the effective quantum efficiency of charge separation in actinic irradiance is

$$\phi_p = (F_m' - F_s)/F_m' = \Delta F/F_m' \quad (2)$$

where F_m' is the maximum fluorescence in actinic light, F_s is steady-state fluorescence in actinic light, and ΔF is variable fluorescence in actinic light. ϕ_p can be used to calculate the linear rate of photosynthetic electron transport (*ETR*) as (Genty 1989, Kolber & Falkowski 1993, Hofstra et al. 1994):

$$ETR = \phi_p \times \text{PFD} \times \sigma_{\text{PSII}} \quad (3)$$

where σ_{PSII} is the functional cross section of PSII. The product of PFD and σ_{PSII} equals the amount of irradiance absorbed by a PSII unit. *ETR* is called J_e by Hofstra et al. (1994). As we could not measure σ_{PSII} , relative *ETR* was calculated as $\phi_p \times \text{PFD}$.

Irradiance measurements. During the PAM measurements the photon irradiance at the surface of the sediment was measured as the voltage applied to the lamp of the Schott KL1500 light source. This voltage was calibrated against a MACAM SD101 cosine corrected PAR sensor. Because we changed the irradiance by changing the voltage of the lamp, the spectral characteristics of the irradiance changed. However, from spectroradiometric measurements, carried out using a MACAM SR9910 PC spectroradiometer fitted with a cosine sensor, we concluded that this spectral change was not very important, as the amount of light energy between 400 and 550 nm relative to total PAR increased from 17 to 26% when going from low to saturating irradiances.

Spectral reflectance of the sediment was measured by feeding the reflected light collected by the fiberoptics of the PAM into a MACAM SR9910 PC spectroradiometer.

Attenuation coefficients of the sediment were determined by measuring the light penetration through a moisturised 1 mm thick layer of sediment. A 1 mm section of the core was sliced using the piston as described above. This was resuspended and left to settle in a bottle with a diameter identical to that of the core. As a reference measurement the bottle was filled with an identical volume of water. The Schott KL1500 halogen lamp was used as light source, and the photon irradiance was measured at 1 nm intervals using the MACAM spectroradiometer fitted with a quartz fiber and cosine sensor. The irradiance attenuation coefficient $K_{d,\lambda}$ (mm^{-1}) was calculated as:

$$\text{PFD}_{z,\lambda} = \text{PFD}_{0,\lambda} \times e^{-K_{d,z,\lambda}} \quad (4)$$

where z is the thickness of the sediment slice (1 mm).

Pigment analyses. From the 0 to 1 mm layer, 4 replicates per station were extracted in 95% methanol and buffered with ammonium acetate. Pigments were analyzed by reversed phase HPLC after Wright et al. (1991). The samples were injected through a Waters 171 Plus autosampler into an Alltech column (Econosphere C18). The signal was detected at 436 and 658 nm with a Waters 440 absorbance detector. From the 0 to 1 mm layer 4 replicates per station were extracted in 95% methanol and buffered with ammonium acetate before injection. See Barranguet et al. (1997) for more information.

RESULTS

Photoc depth and 'fluorescence' depth

Only 1 to 5% of the irradiance absorbed by a photosynthetic organism will be emitted as fluorescence. Therefore, in order to establish the 'measuring depth' of the PAM, we determined the irradiance attenuation in 1 mm sections of different cores using the MACAM spectroradiometer, assuming that irradiance decreased exponentially with depth (Eq. 4). As can be seen in Fig. 2, the attenuation of irradiance is very strong, but also rather variable among the different cores. The cores with a lower silt content (see Table 1) showed a lower irradiance attenuation. The increased absorption due to algae can be clearly seen as an absorption peak

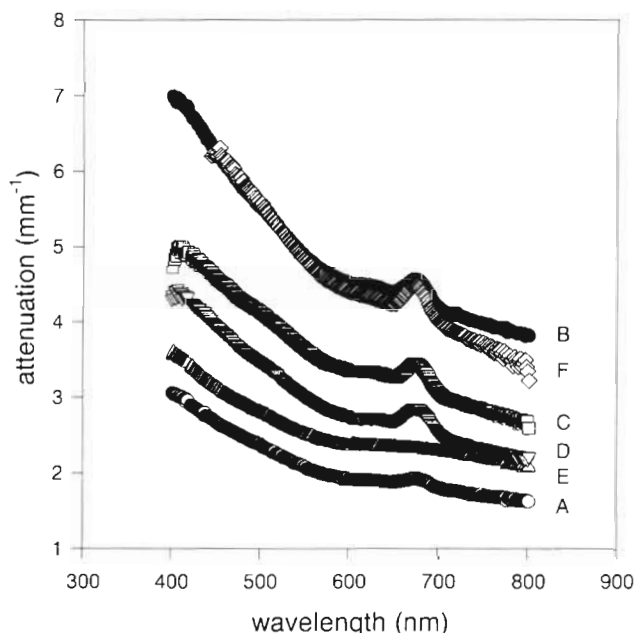


Fig. 2. Irradiance attenuation coefficients of sediments collected at Stns A to F

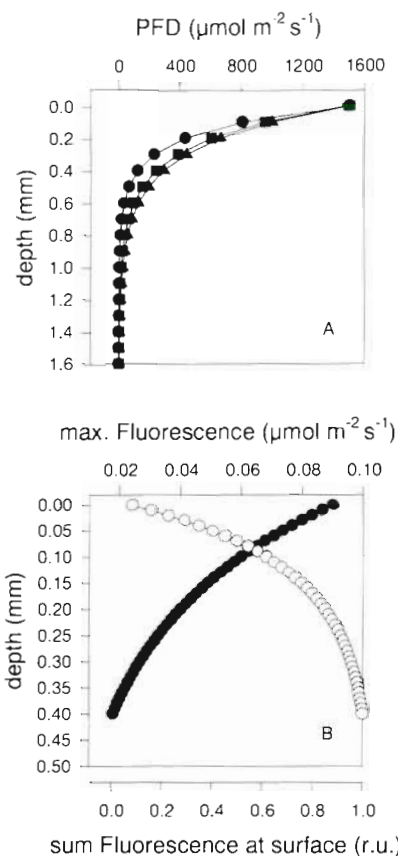


Fig. 3. Optical characteristics of the sediment. (A) Penetration of irradiance at 3 different wavelengths: 450 (●), 675 (■) and 720 (▲) nm. (B) Maximum fluorescence at depth (●), assuming that 5% of the light absorbed by chlorophyll is emitted as fluorescence. From this the total fluorescence at the surface was calculated (○) using the attenuation coefficient of red light ($K_{d,720nm}$)

near 675 nm. The spectral attenuation coefficient at 3 different wavelengths was used to calculate the PFD at several depths at Stn B (Fig. 3A). It must be taken into account, however, that our estimates may be too low due to the intense scatter of light in sandy sediments (Kühl et al. 1994). In the range of the grain sizes of the Molenplaat, scattering can eventually double the total scalar irradiance just below the surface. As we did not possess fiber optic microprobes, we could not verify this. Like Kühl et al. (1994), we observed that near-infrared light penetrates deeper into the sediments. As the light attenuation between 700 and 800 nm hardly varied, we chose to use the attenuation coefficient of light at 720 nm to calculate the amount of fluorescent light measured at the sediment surface. We assumed a diatom chlorophyll-specific absorption cross section of $0.008 \text{ m}^2 (\text{mg chl})^{-1}$ (Kromkamp & Limbeek 1993) and a homogeneous distribution of 15 mg chl m^{-2} in the top 1 mm. From this we calculated the amount of PAR

(400 to 700 nm) absorbed at depth, assuming a surface photon irradiance of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$. At most, 5% of the irradiance absorbed is emitted as fluorescence. Using this percentage, we calculated the maximum fluorescence emission at depth, and from this, using Eq. (4) and $K_{d,720\text{nm}}$, we calculated how much of the emitted fluorescence reached the sediment core surface (Fig. 3B). From this calculation, which gives only an indication of the depth detectable by the PAM, we conclude that most of the fluorescence (75%) emanates from a depth less than $150 \mu\text{m}$.

Photosynthetic performance at different sites

Fluorometric measurements were performed on all 6 stations in March, April and May only. Chlorophyll contents varied considerably, both over time and between stations, although the central stations, B and F, always contained the highest benthic algal biomass (Table 2). Despite the sharp differences in microphytobenthic biomass in March and May, the optimal quantum efficiency F_v/F_m of dark adapted samples was quite similar, and varied between 0.53 and 0.66, with the exception of Stn D (Table 2). This indicates that the physiological state of the benthic algae was similar, despite the differences in biomass. In April the maximum quantum efficiency was below 0.3, with the exception of Stn F. As fluorescence signals were low in April (and for Stn D in March), we had to use a high setting for measuring light and gain, and the data were very noisy and probably not reliable.

Table 2. Chl *a* contents (mg m^{-2}) and maximum quantum efficiency (F_v/F_m) of benthic algae at the 6 different sampling stations. nd: not determined

Stn	March		April		May	
	Chl <i>a</i>	F_v/F_m	Chl <i>a</i>	F_v/F_m	Chl <i>a</i>	F_v/F_m
A	2.6	0.58	1.3	0.23	2.7	0.54
B	23.0	0.63	14.1	0.22	13.3	0.53
C	20.0	0.66	2.8	0.21	12.0	0.54
D	0.5	0.31	1.6	0.13	2.5	0.60
E	4.3	nd	4.4	0.26	9.7	0.61
F	25.6	0.54	15.9	0.53	8.0	0.56

From June onwards only Stns B and A were visited. F_v/F_m values were in general comparable between both stations, so we conclude that, despite the patchiness in biomass, the (photosynthetic) physiological state was not different.

Temporal changes in photosynthetic performance

Fig. 4 shows an example of changes in minimum and maximum fluorescence yields (F_o and F_m) and optimal quantum efficiency (F_v/F_m) during the day of a core taken at Stn B on June 2, 1995. As can be seen, there was a gradual increase in both the minimum and maximum fluorescence yield, whereas F_v/F_m remained constant. The proportional increase in both F_o and F_m indicates an increase in biomass near the surface,

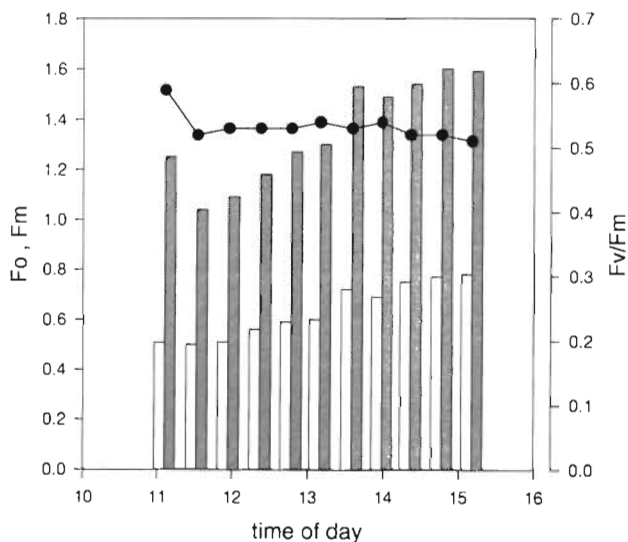


Fig. 4. Change in minimum (F_o , open bars) and maximum (F_m , hatched bars) fluorescence yield and optimal quantum efficiency (F_v/F_m , ●) with time (h) measured on June 2, 1995

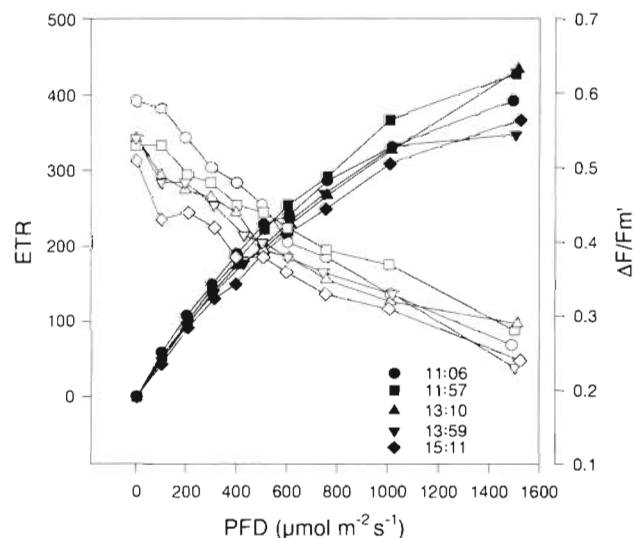


Fig. 5. Change in effective quantum efficiency ($\Delta F/F_m'$, open symbols) and relative photosynthetic electron transport (ETR, closed symbols, relative units) with photon flux density (PFD) at different times during low tide. Core taken on June 2, 1995.

Key shows time of day measurements were taken

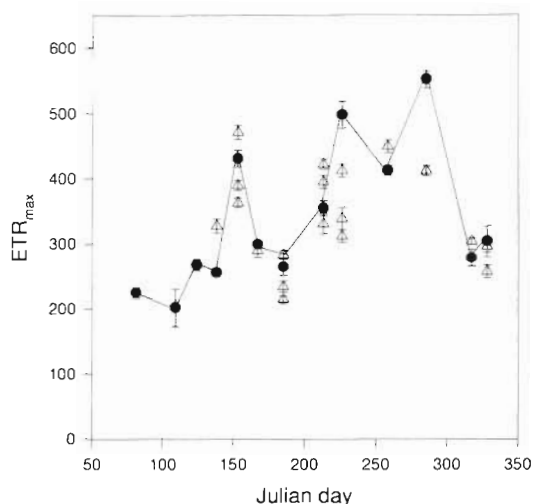


Fig. 6. Seasonal change in maximum rate of ETR at Stn B. Error bars give the 95% confidence interval calculated from the Platt & Jassby (1976) fit. The line connects the first measurements taken each day (●), normally 1 h before low tide. As can be seen, on a number of days ETR_{max} changed during low tide (Δ). No consistent pattern could be observed in these daily changes

probably due to upward vertical migration. This will be discussed below.

Fig. 5 shows a typical example of the change in effective quantum efficiency ($\Delta F/F_m'$) when exposed to increasing PFDs at different times of the day of a core taken on June 2 at Stn B. The quantum efficiency of charge separation decreased from 0.6 to 0.25, as more and more PSII traps closed with increasing PFD. The changes in $\Delta F/F_m'$ in time were small. From the change in $\Delta F/F_m'$ the relative ETR was calculated as a measure of photosynthetic activity (Fig. 5). Because, in this example, the pattern in $\Delta F/F_m'$ with PFD hardly varied at different times of the day, daily changes in ETR were very small. The pattern illustrated in Fig. 5, i.e. a rather constant photosynthetic activity throughout low tide, was observed on a number of days, whereas on other days, changes in $\Delta F/F_m'$ with increasing PFD were larger, causing a decrease in photosynthetic activity during the day. The relative electron transport rates at different PFDs were fitted according to Platt & Jassby (1976), and the maximum ETR , the 'photosynthetic affinity' α (i.e. the initial slope of an ETR vs PFD-plot) and the saturating PFDs ($I_k = ETR_{max}/\alpha$) were calculated. The change in maximum ETR , a parameter comparable to the photosynthetic capacity P_{max}^B , is plotted in Fig. 6. The line connects the first samples (closed symbols), which were taken approximately 1 h before low tide. The triangles show the other measurements made on that day. In winter, ETR_{max} was low, most probably due to low temperatures; it rose to higher values in summer. The change in ETR_{max} observed on sev-

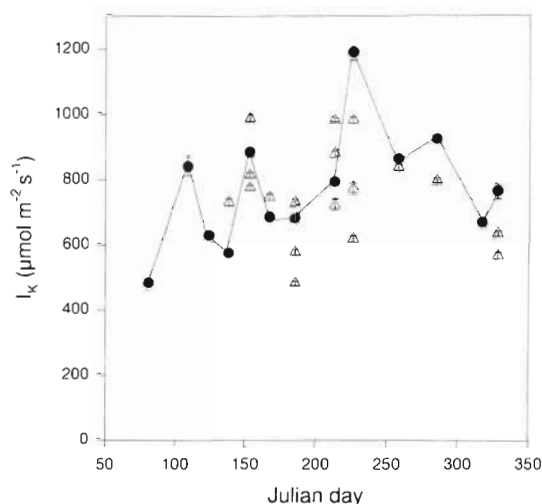


Fig. 7. Seasonal change in the light saturation parameter I_k . It shows the same pattern as ETR_{max} (in Fig. 6). Symbols as in Fig. 6

eral days during low tide is not due to measuring errors, as the noise level is quite low, as indicated by the error bars (see also Fig. 9), but is caused by a change in photosynthetic activity during low tide. Note that on some days there was no significant change (Day 167) or a small change in maximum ETR (Days 317 and 328), whereas on most days there was a considerable change in ETR_{max} during low tide ($p < 0.05$). We could not find any consistent pattern in the changing activity, indicating the absence of nutrient limitation.

Between Days 160 and 210 there was a sudden dip in the maximum ETR ; the possible reason for this will be discussed later. The photosynthetic affinity, α , remained more or less constant during the year. During the day (or tidal exposure), α decreased, but the changes were not always significant. Because of this, I_k , the saturating irradiance for ETR , showed more or less the same pattern as ETR_{max} : relatively low values in winter, higher values in spring and summer, and a strong decrease in values between Days 150 and 220 (Fig. 7), although the pattern was less clear than for ETR_{max} . In general the values were rather high as compared to I_k values previously measured for phytoplankton (determined from ^{14}C -uptake rates), which varied between 250 and 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Kromkamp & Peene 1995), indicating that the microphytobenthos community of the upper surface layer seems adapted to high light conditions.

The maximum quantum efficiency did not show a pattern similar to ETR_{max} or I_k (Fig. 8): values in winter and spring were similar and varied between 0.6 and 0.65. Daily or tidal patterns were less pronounced. Notice the decrease in F_v/F_m between Days 200 and

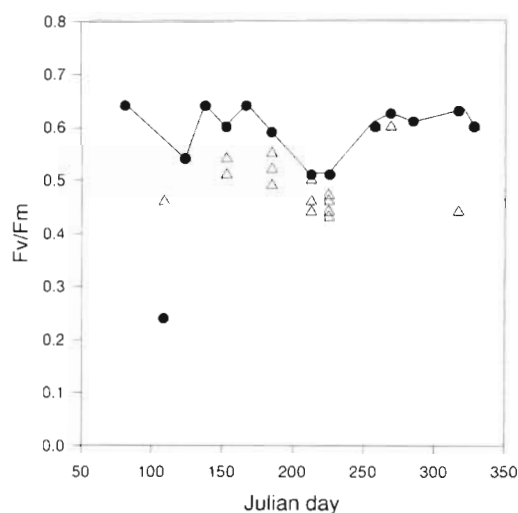


Fig. 8. Seasonal change in maximal PSII quantum efficiency. The line connects the first measurements of the day, usually taken 1 h before low tide. Notice that the decrease between Days 200 and 250 was observed later than the decrease in ETR_{max} or I_k . Symbols as in Fig. 6. The low F_v/F_m at around Day 105 was most likely due to a noisy signal

250: it did not correspond to the temporary decrease in ETR_{max} or I_k , which was observed earlier that year.

Vertical migration and absence of photoinhibition

On June 29, a core, taken at Stn B, was exposed for 4 h to a PFD of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$, during which the change in fluorescence was followed. As can be seen in Fig. 9, both F_s and F_m' increased with time. From this it could be calculated that the effective quantum efficiency of PSII ($\Delta F/F_m'$) did not decrease, as might be expected after a prolonged exposure to the high PFD. On the contrary, $\Delta F/F_m'$ increased slightly from 0.35 to 0.4 during the first 90 min of the incubation. This makes it likely that photoinhibition did not occur.

To see whether the increase in F_s and F_m' was due to vertical migration, the reflected light was measured by a spectroradiometer. The reflectance (R) spectra showed a decrease in reflection, especially in the blue and around 675 nm, due to an increased absorption by chlorophyll (not shown). To make this more clear, we divided the measured reflectance spectrum at time zero by that at time t in order to obtain a measure of the absorption spectrum. The first R spectrum was taken as a reference value. Because the amount of microphytobenthos already present, as well as the sediment composition, may vary between days, it is not possible from these measurements to quantitatively estimate the chlorophyll content present. Nevertheless, the spectra are very well suited to demonstrate changes in

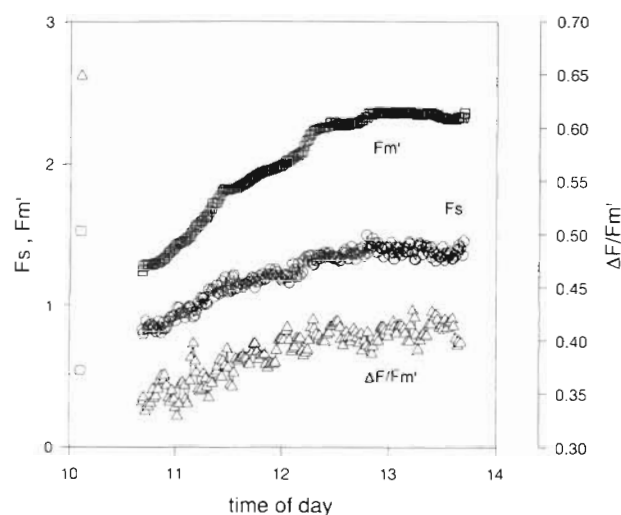


Fig. 9. Change in F_m' , F_s and $\Delta F_v/F_m'$ with time when a core taken on June 29, 1995, was exposed to a PFD of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$. Note that the noise is low, indicating that the change in ETR_{max} during the day (see Fig. 6) is not due to noise, but due to a change in activity

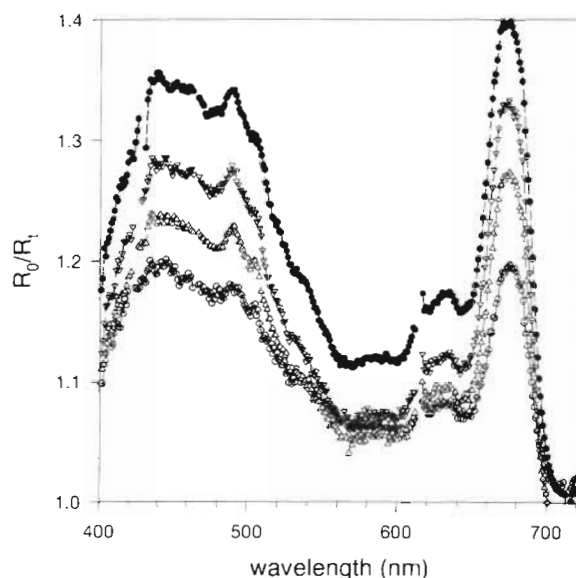


Fig. 10. Change in the reflection spectrum with time of a core taken on June 29, 1995. The R -spectrum, which resembles an algal absorption spectrum, was calculated by dividing the initial spectral scan (R_0) by a scan at time t (R_t). (○) 10:13 h; (△) 11:13 h; (▽), 12:13 h; (●) 14:13 h

the amount of algae at the sediment surface. As can be seen in Fig. 10, the spectrum very much resembled an algal absorption spectrum. There was no clear absorption due to phycoerythrin (562 nm) or phycocyanin (620 nm), indicating that cyanobacteria, if present in the microphytobenthos community at this station,

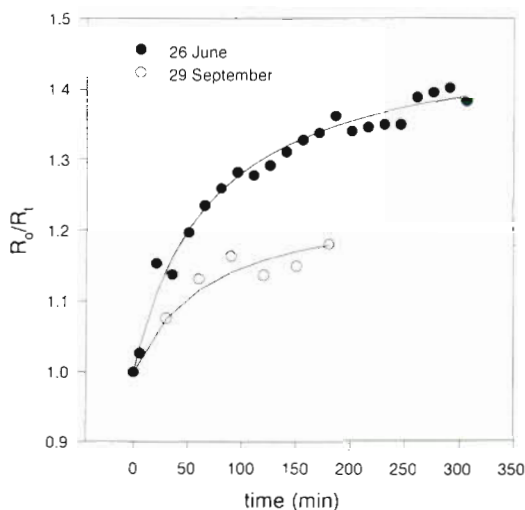


Fig. 11. Change in reflection at $\lambda = 675$ nm with time on 2 different days. Notice that 50% of the increase in reflection occurs within the first hour

played only a minor role at this time. It is also clear that the absorption at the sediment surface increased considerably, demonstrating a vertical migration of microphytobenthos to the sediment surface. A similar result was found when the experiment was repeated on September 29. When the change at 675 nm is plotted against time, it is clear that the initial migration activity is highest: 50% of the change in reflectance was observed in the first hour of the measurement (Fig. 11). This rapid migration will complicate areal primary production estimates, because the time scale of migration is within the time scale of the photosynthetic measurements normally employed.

DISCUSSION

The aim of this paper was to investigate whether active fluorescence, measured using a PAM fluorometer, can be used to follow changes in photosynthetic activity of microphytobenthos. It is clear from the results that the measured fluorescence only monitors the upper part of the microphytobenthic community, hence the technique as we employed it cannot be used directly to estimate areal primary productivity.

One has to be aware though that the PAM measures PSII quantum efficiency and that photosynthetic electron transport is calculated from this. Because the electrons are split from water, one might expect a close coupling between *ETR* and oxygen evolution (see also Kolber & Falkowski 1993). However, due to the presence of electron sinks which can consume oxygen, like the Mehler reaction, and photorespiration, the rela-

tionship between oxygen production and *ETR* is not necessarily linear, and especially at higher irradiance the relationship becomes non-linear (Geel et al. 1997, Flaming & Kromkamp 1998). A change in the functional cross section of PSII might also influence the absolute rate of *ETR* (Kolber & Falkowski 1993). Nevertheless, some interesting features were observed: the PAM could be used to construct *ETR*-PFD curves, analogous to the well known photosynthesis irradiance (P-I) curves. Because this was done *in situ*, the possible influence of microgradients in the upper surface layer were not disrupted, as is the case when P-I curves are made using slurries with the ^{14}C -technique to measure C-fixation. Hence, the change in photosynthetic parameters can be more easily linked to changes in the environment than when using C-fixation methods.

When this was done, it was noticed that during low tide, a change in photosynthetic activity sometimes occurred, as has been observed before using ^{14}C (Blanchard & Cariou-Le Gall 1994) or oxygen methods (e.g. Pinckney & Zingmark 1993, Kromkamp et al. 1995a). Whether this change in activity was due to a true rhythmic activity, as suggested by Pinckney & Zingmark (1993), or was due to some other factors, e.g. photoinhibition, CO_2 -limitation or backpressure, is not clear at present (but see below).

Seasonal changes in ETR_{max} and I_k were not related to changes in nutrient concentrations: in the overlying water, nitrate concentrations were above 100 μM all the time. Ammonia concentrations during Days 160 to 180 changed from 12 to 7 μM . Silicate (>20 μM) and phosphate (>3 μM) also did not reach limiting concentrations. Pore water concentrations in the upper cm were even higher (ammonia up to 600 μM , silicate up to 400 μM and phosphate up to 175 μM ; K. Perry pers. comm.). Nevertheless, in a dense mat local depletion cannot be ruled out completely, but we do not think this is likely. Between Days 160 and 210, lower values for ETR_{max} and I_k were found. In the period from Day 160 to 180, the average daily light dose was approximately 25 mol photons m^{-2} , whereas in the preceding and following fortnight the light dose was nearly twice as high (not shown). This indicates that the observed pattern in ETR_{max} and I_k might reflect photoacclimation.

According to Falkowski et al. (1992), nutrient limitation will decrease the optimal quantum efficiency. This has been demonstrated for nitrogen limitation (Kolber et al. 1988), iron limitation (Geider et al. 1993) and for phosphorus limitation (Geider et al. 1993, Flaming & Kromkamp unpubl.). As noted above, the change in optimal quantum efficiency of PSII (see Fig. 8) is unlikely to be due to a limitation in the macronutrients N, P or Si. The fact that no consistent pattern in the change in F_v/F_m during low tide could be observed also

corroborates the hypothesis that nutrient limitation did not occur. As the CO_2 concentrations in the water are high, we find it unlikely CO_2 will be limiting at the start of the low tide. Additional experiments in which CO_2 or bicarbonate were added confirmed this. The same was observed after enrichment of a core with ammonia (not shown). On the other hand, we cannot rule out that, as the time progresses during the day at low tide, a CO_2 limitation develops. On the contrary, this is very likely and has been argued before (de Jong et al. 1990, Glud et al. 1992).

The F_v/F_m values are below 0.8, the value typically found in higher plants. However, algae, especially chl *c* containing microalgae, show a lower optimal F_v/F_m (Ting & Owens 1992, Ibelings et al. 1994, Flameling & Kromkamp 1995, Kromkamp et al. 1995b; see Büchel & Wilhelm 1993 for a review on algal fluorescence). We therefore conclude from the F_v/F_m ratio that the PSII reaction centres were fully functional and that nutrient limitation by N, P or Si did not occur. This argument leaves unexplained the decrease in F_v/F_m between Days 200 and 250. For this we have no explanation, only that it seems to coincide with a change in species composition: normally the microphytobenthos community at Stn B is composed of benthic diatoms, but for the period that coincided with the lower F_v/F_m values, the intertidal flat was coloured green instead of brown, and the dominant benthic alga in this period was a *Euglena* species.

When a sediment core was exposed to a high PFD for several hours, upward vertical migration could be clearly demonstrated (see Figs. 10 & 11). The observed pattern in apparent quantum efficiency $\Delta F/F_m$ showed a small increase, and not, as expected by us, a decrease. Exposure for a prolonged period to a high photon irradiance will most likely initiate energy quenching, i.e. energy dissipation as heat. This (dynamic) down-regulation of photosynthesis is viewed as a protective mechanism against chronic photoinhibition (Horton & Ruban 1994, Osborne 1994). Down-regulation (i.e. energy quenching) as a result of sunlight has been observed in leaves (e.g. Bilger et al. 1995), macroalgae (Osmond et al. 1993, Hanelt et al. 1994) and unicellular algae (Kroon et al. 1994, Flameling & Kromkamp 1995, Ibelings et al. 1995, Kromkamp et al. 1995b) and is a process which can be induced (and relax) very rapidly. On intertidal flats, high PFDs will lead to high rates of photosynthesis. This will cause a pH-rise and probably a lack of CO_2 . CO_2 limitation has been inferred before (Rasmussen et al. 1983, de Jong et al. 1988, 1990, Glud et al. 1992). If Rubisco is limited by substrate, this will induce backpressure effects at PSII and cause accumulation of reduced Q_A ; this will decrease the efficiency of charge separation, and thus lower $\Delta F/F_m$. A decrease in $\Delta F/F_m$ was, however, not

observed during the experiments in high irradiance, indicating no shortage of CO_2 to the upper part of the benthic population. It is, however, unlikely that high rates of photosynthesis can be sustained by CO_2 and that down-regulation of photosynthetic activity will not occur. We therefore think it is more likely that during the overall vertical migration to the upper layer, there is a vertical 'micro'-migration within the first mm of the sediment where algae at the surface migrate to deeper layers in order to prevent photoinhibition and CO_2 limitation, and are replaced by others.

These results also show that it is impossible to estimate the areal primary productivity of microphytobenthos accurately using current techniques. When the light-dark oxygen micro-electrode technique is used (Revsbech & Jørgensen 1993), construction of gross photosynthesis-irradiance curves takes too much time: within the time scale of the measurement, vertical migration will have occurred. On the other hand, when the ^{14}C -technique is used and the benthic algae are resuspended in filtered seawater (e.g. Blanchard & Cariou-Le Gall 1994), the vertical distribution of the algae will be disrupted, as will all the chemical gradients, or in the case of chamber techniques, the specific activity of the ^{14}C in the sediment is not known. Maybe the most realistic method therefore is to measure the oxygen profile using micro-electrodes, and from sediment and diffusion characteristics, calculate the net primary production with the aid of a 1-dimensional diffusion-reaction model (Wiltshire 1992, Epping & Jørgensen 1996).

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