Chapter 2.

Influence of temperature on the relationship between oxygen and fluorescence based estimates of photosynthetic parameters in a marine benthic diatom (*Cylindrotheca closterium*)

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**Abstract**

In this paper we investigate the temperature sensitivity of the photosynthetic process of the benthic diatom *Cylindrotheca closterium* grown in light-limited turbidostat cultures at two different growth rates. Photosynthesis was measured as the rate of oxygen evolution and as the PSII electron transport rate (ETR). The photosynthetic efficiency ($\alpha$), as measured by both methods, was rather insensitive to temperature, and decreased significantly only at the extreme temperatures used (5 and 35 °C). The maximum PSII quantum efficiency ($F_/F_m$) showed a small but significant trend of reduction with increasing temperature. However, the maximum rate of photosynthesis ($P_b max$ and $ETR max$) was extremely temperature sensitive. The effect of temperature on the relationship between $P_b$ and ETR was limited to the most extreme temperatures investigated; deviations from linearity were most extreme at 5 °C and different conversion factors were observed at 5 and 35 °C. A short-term change in temperature (10 - 30 °C), as might be experienced during emersion on a European tidal flat, will not significantly affect the relationship between $P_b$ and ETR. However, care should be taken when using a single conversion factor between $P_b$ and ETR at the extremes of the temperature range. We have also shown that algal absorption measurements are important for correct calculation of ETR. The facts that different species seem to have different conversion
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Factors and that changing environmental conditions will affect the absorption capacity and growth rate of the microphytobenthos (MPB) community suggest that it is wise to perform further calibrations of the relationship in the field before use in primary production modelling. Variable fluorescence measurements are quick and non-invasive and, with knowledge of the absorption properties of the MPB community, allow the quantification of photosynthetic parameters across large areas. Hence they are potentially useful for improving our estimates of ecosystem scale primary production.

Introduction

Benthic microalgae or microphytobenthos (MPB) form highly productive natural ecosystems in intertidal areas (Cahoon, 1999). They are an important food source for both benthic and pelagic communities (MacIntyre et al. 1996, Underwood & Kromkamp 1999) and have also been linked to sediment stabilisation (Underwood et al. 1995).

Three main factors appear to regulate the rate of photosynthesis in microphytobenthos; light availability (Barranguet et al. 1998, Perkins et al. 2001), mud surface temperature (Blanchard et al. 1996, Guarini et al. 1997) and CO₂ availability (Underwood & Kromkamp 1999 and references therein). Nutrients are not generally thought to be limiting in intertidal habitats (Underwood & Kromkamp, 1999). Both light and temperature change on seasonal, daily and hourly time scales. The two factors also co-vary, making their individual effects hard to separate in field situations (Guarini et al. 1997). In order to model and predict rates of primary production on estuarine flats successfully, it is necessary to understand the relationship between temperature and photosynthesis at both short (hourly, daily) time scales and long term (seasonal) time scales.

The temperature of the surface (upper 200 μm) in muddy (dominated by < 0.63 μm particles) sediments in temperate regions can easily change by 10 °C within an emersion period, with rates of temperature change as high as 4 °C h⁻¹ (Harrison, 1985). The magnitude of these changes is related to the timing of exposure to the atmosphere and the prevailing meteorological conditions, which jointly determine the nature of heat exchange between the sediment and the atmospheric boundary layer (Harrison & Phizacklea, 1987).

Previous authors have investigated the effect of temperature on the rates of primary production of MPB (Cadee & Hegeman 1974, Colijn & Van Buurt 1975, Admiraal 1977, Admiraal & Peletier 1980, Grant 1986). Blanchard et al. (1996) proposed a mathematical expression to relate maximum photosynthetic capacity (Pₘₐₓ [¹⁴C]) to temperature for MPB from a mudflat in the Marnes-
Oléron Bay, France and Blanchard & Guarini (1998) discuss in detail the effect of temperature on MPB productivity at the estuarine basin scale.

Various methods have been used to measure primary production by microphytobenthos, and all have their advantages and disadvantages (see Underwood & Kromkamp 1999 and references therein). Variable fluorescence could provide a fast and non-destructive way of measuring the in-situ photosynthetic electron transport rate (ETR) and the biomass of MPB at the time and spatial scales required for estuarine studies.

Rates of photosynthesis estimated from PAM (pulse amplitude modulated) fluorometry and oxygen evolution or C-fixation have been compared in a number of phytoplankton species (Flameling & Kromkamp 1998, Masojídek et al. 2001 & references therein), MPB (Hartig et al. 1998, Barranguet & Kromkamp 2000, Perkins et al. 2001, 2002) macroalgae (Beer et al. 2000, Franklin & Badger 2001) and seagrasses (Beer & Björk 2000). Above the saturating irradiance for photosynthesis (E_{s}), the relationship can be curvilinear, with an excess of electron transport compared to oxygen evolution. Non-linearity between oxygen evolution and ETR can also be observed at low irradiances, but this is probably due to light-enhanced rates of dark respiration (Flameling & Kromkamp 1998). There appears to be some interspecies variability in both the shape of the relationship and the value of the coefficient for the linear regression of P^{B}(O_{2}) on ETR (called the EE factor in Barranguet & Kromkamp 2000 or κ by Masojídek et al. 2001). If the PAM technique is to be successfully used as a means of estimating photosynthetic carbon fixation in intertidal ecotypes, then the conversion factor(s) should be well defined across a range of environmental conditions. This is particularly important in the dynamic estuarine environment where rapid changes in irradiance and temperature can occur.

The aims of this study were to describe and formulate the relationship between temperature and photosynthetic parameters of benthic microalgae grown in culture, using both PAM fluorescence and oxygen evolution. We investigated the relationship between P^{B} (µmol O_{2} (mg Chl a)^{-1} h^{-1}), and electron transport rate (ETR, µmol e^{-} mg Chl a^{-1} s^{-1}) over a range of temperatures, in cultures of the marine benthic diatom *Cylindrotheca closterium* growing at two different rates.

**Methods**

*Cylindrotheca closterium*, isolated from the Ems-Dollard Estuary, the Netherlands, was grown in continuous culture [turbidostat mode] in nutrient replete modified F/2 medium (Guillard & Ryther 1962, modified according to De Brouwer et al. 2002) at an average incident irradiance of 300 µmol
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m² s⁻¹ in the ‘high’ growth rate culture and 200 μmol m⁻² s⁻¹ in the ‘low’ growth rate culture. The cultures were continuously bubbled with air and illuminated for 16 h per day. Average incident irradiance was estimated by measuring the irradiance at the back of the vessel when filled with only medium and when the [Chl *a*] of the culture was constant as described by Van Liere & Walsby (1982, see below for equation). The temperature in the culture was 20±1 °C, salinity was 30 psu and pH 8.7. The turbidostat was not axenic, but bacterial numbers were always less than 5 % of the algal numbers. It was assumed that the culture was in steady state after 5 doublings once a constant cell number was reached. The first culture had a growth rate of 0.25 d⁻¹ and the second 0.42 d⁻¹ and for convenience these were designated as ‘low’ and ‘high’ growth rate culture. The cultures were sampled at the end of the dark period on successive days in order to measure photosynthesis-irradiance curves (P-E curves) using a Clark-type polarographic oxygen electrode (MI-730, Microelectrodes Inc, USA) and a pulse amplitude modulated (PAM) fluorometer (see below). Measurements of oxygen evolution and fluorescence parameters were made simultaneously on the same sample in a temperature controlled plastic cuvette over a range of temperatures (5–40 °C). A 100 ml sample of the *C. closterium* culture was taken from the turbidostat each morning before the light period. The sample was placed into a darkened, temperature controlled water bath set at one of the 8 experimental temperatures (5, 10, 15, 20, 25, 30, 35, and 40 °C). After 45 minutes adjustment time (in the dark), a 4 ml sub-sample was transferred to the cuvette (path length of 1 cm), which was placed in a temperature controlled aluminium jacket (±0.02 °C). The oxygen electrode and lid were inserted in the cuvette to create a sealed volume. The algae were kept suspended with the aid of a small magnetic stirrer. Respiration was measured for 10 min before measurements in the light were started. A P-E curve was then measured using 11 irradiance steps (0 to 1350 µmol m⁻² s⁻¹) each of 3 minutes duration. The light source was a standard slide projector fitted with neutral density filters (Balzers, Liechtenstein) and a heat filter. Light was measured at the back of the chamber with a PAR sensor. The average irradiance in the chamber was calculated by:

\[
E = \frac{(E_o - E_d)}{\ln(E_o) - \ln(E_d)}
\]

where *E*₀ and *E*ₐ are the irradiance at back of the chamber when filled with water and with algae, respectively (Van Liere & Walsby, 1982, Dubinsky et al. 1987). To ensure that the optics within the cuvette were well defined, a low biomass (1-2 mg Chl *a* l⁻¹) of *C. closterium* was used in the incubations. The low biomass allowed accurate irradiance measurements and, therefore, ETR determination, but meant that the oxygen electrode was operating at its detection limits.
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Fluorescence was measured using a PAM101-103 fluorometer (H. Walz, Effeltrich, Germany). The algal solution was excited by a weak red measuring light (1 µmol m⁻² s⁻¹, maximum emission at 650 nm) and fluorescence was detected at wavelengths above 695 nm. The maximum energy conversion efficiency or quantum efficiency of PSII charge separation (Fᵥ/Fₘ) was calculated as:

$$Fᵥ/Fₘ = (Fₘ - Fₒ)/Fₘ$$

(2)

where Fₒ is the minimal fluorescence and Fₘ is the maximum fluorescence (during a saturating light pulse, 0.6 s, 6000 µmol m⁻² s⁻¹) of a sample dark-adapted for at least 15 minutes. The effective quantum efficiency of charge separation (i.e. the effective quantum efficiency of PSII) in actinic irradiance is:

$$ΔF/Fₘ = (Fₘ - F)/Fₘ$$

(3)

where F is the steady-state fluorescence and Fₘ' the maximum fluorescence after a saturating pulse when measured in the light (Genty et al. 1989). Using ΔF/Fₘ', the linear rate of electron transport (ETR) can be calculated for a single PSII unit (Genty et al. 1989, Hofstraat et al. 1994):

$$ETR = a*_{PSII} \times E \times ΔF / Fₘ'$$

(4)

where a*ₚₛᵮᵢ is the optical cross section of PSII. The product of E and a*ₚₛᵮᵢ is the amount of irradiance absorbed by a PSII unit. Because a*ₚₛᵮᵢ is difficult to measure, the spectrally averaged (400-700 nm) chlorophyll specific absorption cross section was determined using the opal glass method according to Shibata et al. (1954) using a double beam scanning spectrophotometer (UVIKON 940). Values of a* were 0.0159 ± 0.0005 and 0.0108 ± 0.0004 m² mg Chl⁻¹ (mean ± SE, n = 11) in the low and high growth rate cultures, respectively. For simplicity, it was assumed that absorbed irradiance was divided equally between PSI and PSII (Gilbert et al. 2000). Therefore, ETR was calculated as ΔF/Fₘ' × E/2 x a* (µmol e⁻ (mg Chl a)⁻¹ s⁻¹).

At least 3 replicate P-E curves (3 curves each day) were carried out at each of the 8 experimental temperatures for both growth treatments, except at 40 °C, at which no photosynthesis could be measured, and at 15 °C for the ‘high’ growth rate culture, where equipment failure prevented measurements. On each day, an experimental temperature was chosen at random (i.e. temperatures were examined in a random order over the experimental period). The 3 replicate P-E curves were carried out over a 4-5 hour period for each temperature treatment, meaning in effect that the sample used for the last P-E curve had up to 4 hours to acclimatise to the experimental temperature.
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Replicates from each day were examined but no pattern of acclimatisation in photosynthetic parameters could be found. The chlorophyll \( a \) (Chl \( a \)) concentration (mg l\(^{-1}\)) of the culture was measured spectrophotometrically according to Jeffrey & Humphries (1975) after extraction of the pigments in 90 % acetone. Net oxygen production was normalised to the daily [Chl \( a \)] and gross production was estimated by adding the initial rate of respiration. P-E curves, which did not show a significant degree of photoinhibition, were fitted to the model proposed by Webb et al. (1974) and, from the fit, the maximum photosynthetic capacity (\( P_{\text{B max}} \) and ETR\( _{\text{max}} \)) and the photosynthetic efficiency (\( \alpha^\text{B} \) and \( \alpha^\text{ETR} \)) were derived. The relationship between temperature and maximal photosynthetic rate was fitted using the non-linear model described by Blanchard et al. (1996):

\[
P_{\text{max}}(T) = P_{\text{MAX}} \left( \frac{(T_{\text{max}} - T)}{(T_{\text{max}} - T_{\text{opt}})} \right)^{\beta} \times \exp \left[ -\beta \left( \frac{(T_{\text{max}} - T)}{(T_{\text{max}} - T_{\text{opt}})} - 1 \right) \right]
\]

where \( P_{\text{max}}(T) \) is the maximum photosynthetic capacity (\( \mu \text{mol O}_2 \text{ mg Chl } a^{-1} \text{ h}^{-1} \) or \( \mu \text{mol } e^- \text{ mg Chl } a^{-1} \text{ s}^{-1} \)) at temperature \( T \) (°C), \( P_{\text{MAX}} \) is the maximum value of \( P_{\text{max}} \) reached at the optimum temperature (\( T_{\text{opt}} \)), \( T_{\text{max}} \) (°C) is the lethal temperature at which no photosynthesis is detectable, and \( \beta \) is a dimensionless shape parameter. To simplify the curve fitting and error estimation process, the shape parameter \( \beta \) was set to 1.3 (an average of the values reported by Blanchard, 1996) for all of the temperature versus \( P_{\text{max}} \) curves. Changing \( \beta \) between 0.5 and 2 resulted in only small changes in the other fitted parameters (< 5%). All curve fitting was carried out using ordinary least-squares criterion in Statistica 6 (StatSoft, Inc., Tulsa, USA, 2001).

Linear regression (model 1) analysis was carried out on the relationship between \( P^\text{B} \) and ETR (with ETR as the predictor) across the full range of experimental temperatures and for both of the experimental growth rates. Data were log transformed (\( \ln(x) \)) to ensure statistical assumptions were fulfilled (normal distribution, homoscedasticity of variance).

Three-way mixed model ANOVA was used to evaluate significant effects of growth rate (fixed factor), temperature (fixed factor) and experimental replication (random factor, nested within growth rate and temperature) on the slope coefficients of the relationship between \( P^\text{B} \) and ETR (with \( P^\text{B} \) and ETR as the dependent and continuous predictor variables, respectively). Homogeneity of slopes was tested to see if temperature affected the slope of the regression estimates (tested separately within each growth treatment). \( F_r/F_m \) data were arcsine transformed [\( x' = \text{arcsin} (\sqrt{x}) \)] before analysis. One-way or two-way (model I) ANOVA was used to test for significant variation
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of the data. The post-hoc unequal means HSD test was used to determine the significant differences between group means within the one-way ANOVA setting. Bartlett’s test was used to check for heteroscedasticity of variances. All confidence intervals (CI) are at the 95 % level. All statistical analyses were performed in Statistica 6 (StatSoft, Inc., Tulsa, USA, 2001).

Results

Influence of temperature on the maximum rate of photosynthesis

Photosynthetic capacity ($P_{B,max}$). The relationship between $P_{B,max}$ and temperature for both low and high growth rate cultures of Cylindrotheca closterium was characterised by a steady rise in $P_{B,max}$ with temperature from a value of about 200 $\mu$mol O$_2$ (mg Chl a$^{-1}$) h$^{-1}$ at 5°C, to a maximum value of between 1100 and 900 $\mu$mol O$_2$ (mg Chl a$^{-1}$) h$^{-1}$ between 20 and 30 °C (Fig 1). At temperatures above 30 °C, $P_{B,max}$ began to decline rapidly, and photosynthesis was undetectable at 40 °C. Although the fitted curves give an optimum temperature of approximately 30 °C for both growth rates, the highest $P_{B,max}$ -value recorded for the higher growth rate was measured at the growth temperature of 20 °C. This may be a coincidence or indicate that acclimation to the growth temperature has occurred. There were no significant differences between parameter values estimated from the temperature-$P_{B,max}$ equation for the high and low growth rate cultures (Table 1).

Maximum ETR ($ETR_{max}$). The shapes of the relationships between ETR$_{max}$ and temperature for the low and high growth rate cultures were similar to those for $P_{B,max}$ (Fig. 2). The fitted temperature response curves for the 2 growth rates were also similar (Table 2). Both growth treatments began with an ETR$_{max}$ of approximately 0.38 $\mu$mol e$^{-}$ mg Chl a$^{-1}$ s$^{-1}$ at 5 °C, and ETR$_{max}$ steadily rose to an optimum of between 1.7 and 2 $\mu$mol e$^{-}$ mg Chl a$^{-1}$ s$^{-1}$ at 30 - 35 °C.

Table 1. Parameter values and standard errors (SE) estimated from Equation 5 for maximum photosynthetic capacity at low (0.25 d$^{-1}$) and high (0.42 d$^{-1}$) growth rates in Cylindrotheca closterium grown at 20 °C

<table>
<thead>
<tr>
<th>Growth rate d$^{-1}$</th>
<th>$P_{B,max}$ $\mu$molO$_2$ (mg Chl a$^{-1}$) h$^{-1}$</th>
<th>SE</th>
<th>$T_{max}$ °C</th>
<th>SE</th>
<th>$T_{opt}$ °C</th>
<th>SE</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>1070</td>
<td>116</td>
<td>41.5</td>
<td>3.2</td>
<td>30.0</td>
<td>1.3</td>
<td>0.61</td>
</tr>
<tr>
<td>0.42</td>
<td>961</td>
<td>92</td>
<td>38.0</td>
<td>1.0</td>
<td>29.2</td>
<td>0.8</td>
<td>0.69</td>
</tr>
</tbody>
</table>
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The estimates for $T_{opt}$ derived from oxygen evolution and ETR measurements were slightly different (1 °C) but, since the measurements were made at 5 °C temperature intervals, the significance of these differences cannot be evaluated. No photosynthesis was measurable at 40 °C with either method.

![Figure 1. Influence of temperature on the maximum photosynthetic capacity ($P_B^{\text{max}}$, μmol O$_2$ mg Chl a$^{-1}$ h$^{-1}$) of Cylindrotheca closterium grown at 20 °C. Filled circles, culture with a growth rate of 0.25 d$^{-1}$; open circles, culture with a growth rate of 0.42 d$^{-1}$. Error bars show 95 % confidence intervals.](image)

Table 2. Parameter values and standard errors (SE) from Equation 5 for maximum electron transport rate at low (0.25 d$^{-1}$) and high (0.42 d$^{-1}$) growth rates in Cylindrotheca closterium grown at 20 °C.

<table>
<thead>
<tr>
<th>Growth rate d$^{-1}$</th>
<th>$\text{ETR}_{\text{MAX}}$ μmol e$^{-}$ mg Chl a$^{-1}$ s$^{-1}$</th>
<th>SE</th>
<th>$T_{\text{max}}$ C</th>
<th>SE</th>
<th>$T_{\text{opt}}$ C</th>
<th>SE</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>1.64</td>
<td>0.143</td>
<td>38.0</td>
<td>1.0</td>
<td>31.0</td>
<td>0.4</td>
<td>0.82</td>
</tr>
<tr>
<td>0.42</td>
<td>1.81</td>
<td>0.108</td>
<td>40.9</td>
<td>1.1</td>
<td>31.5</td>
<td>0.8</td>
<td>0.86</td>
</tr>
</tbody>
</table>
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Figure 2. Influence of temperature on the maximum electron transport rate (ETR max, μmol e⁻ (mg Chl a)⁻¹ s⁻¹) of Cylindrotheca closterium grown at 20 °C. Other details as in Fig. 1.

Influence of temperature on photosynthetic efficiency

Photosynthetic efficiency per unit chlorophyll a (αBP). There was no clear trend in αBP across the range of experimental temperatures (Fig. 3). In the low growth rate culture, αBP was between 3.75 and 5.9 μmol O₂ (mg Chl a)⁻¹ h⁻¹ (μmol m⁻² s⁻¹)⁻¹ in the 10 - 35 °C range. The lower value at 5 °C was not statistically significant. In the high growth rate culture, αBP was about 2.5 μmol O₂ (mg Chl a)⁻¹ h⁻¹ (μmol m⁻² s⁻¹)⁻¹ across the 5 - 35 °C range. The mean value of αBP across the range of experimental temperatures was lower for the high growth rate than for the low growth rate culture (two-way ANOVA, F(1, 37) = 21.14).

αETR. There was little influence of temperature on αETR in either culture (Fig. 4). The high growth rate culture had a mean αETR of 3 nmol e⁻ (mg Chl a)⁻¹ (μmol photons)⁻¹ m² across the full range of experimental temperatures. The low growth rate culture had a mean αETR of 5.7 nmol e⁻ (mg Chl a)⁻¹ (μmol photons)⁻¹ m² from 10 - 30 °C, but αETR was reduced at both 5 and 35 °C when compared to the adjacent temperature class (post-hoc unequal means HSD test, p<0.05). Again, the mean αETR
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across the full range of experimental temperatures was lower for the high than for the low growth rate culture (two-way ANOVA, $F_{(1,37)} = 382$).

The growth rate did not significantly affect the maximum PSII activity ($F_v/F_m$; one-way ANOVA; Fig. 5). $F_v/F_m$ showed a slight but significant downward trend with increasing temperature (6% decrease per 10 °C; model I regression, $F_{(1,52)} = 14.52$). At temperatures above 30 °C, the decrease in $F_v/F_m$ values was more marked and, at 40 °C, $F_v/F_m$ was almost zero. The effective quantum efficiency of charge separation ($\Delta F/F_m$‘), taken from the PE curves at irradiances close to the growth irradiance, followed a similar pattern to that for $ETR_{max}$ with increasing temperature (Fig. 5), as might be expected because the growth irradiance is close to the light saturation parameter $E_k$.

![Figure 3. Influence of temperature on the photosynthetic efficiency per unit chlorophyll a ($\alpha$) of Cylindrotheca closterium grown at 20 °C. Other details as in Fig. 1.](image)

**Relationship between PB and ETR: influence of growth rate and temperature.**

A scatter plot of ln (PB) against ln (ETR) reveals that both growth rates appear to have large variances and to overlap one another (Fig. 6). Significant interaction was found between the effects of growth rate and temperature on the $P_B$-ETR relationship for both growth treatments measured at all temperatures (three-way mixed model ANOVA, $F_{(5, 28)} = 7.34$, $p < 0.001$), so that differences
between the slope coefficients ($b_i$) and slope intercept coefficients ($a_i$) from the two growth rates could not be statistically evaluated.

Figure 4. Influence of temperature on the photosynthetic efficiency ($\alpha^{ETR}$ $\mu$mol e$^{-}$ (mg Chl a)$^{-1}$ (\(\mu$mol photons)$^{-1}$ m$^2$) of Cylindrotheca closterium grown at 20 °C. Other details as in Fig. 1.

Figure 5. Influence of temperature on maximum PSII activity ($F_v/F_m$; circles) and effective quantum efficiency of charge separation ($\Delta F/F_m'$) at irradiances close to the growth irradiance.
(200 – 300 µmol m\(^{-2}\) s\(^{-1}\); triangles) of Cylindrotheca closterium grown at 20 °C. Other details as in Fig. 1.

To investigate the influence of temperature at each growth rate separately we used linear regression analysis of the P\(^B\)-ETR relationship at each temperature treatment (replicates pooled together). Temperature was found to affect the slope of the P\(^B\)-ETR relationship significantly in both the low growth rate culture (homogeneity of slopes, F\(_{6, 241}\) = 3.87) and the high growth rate culture (homogeneity of slopes, F\(_{5, 188}\) = 8.42). The lowest values for both the slope coefficient (b\(_i\)) and the intercept (a\(_i\)) were observed at 5 °C for both growth treatments (Figs 7, 8), but a reduced value for a\(_i\) was also recorded at 35 °C in the high growth rate treatment (Fig. 8). Little variability in a\(_i\) was observed between 10 and 30 °C and the average value for both growth rates was 6.4 (Fig. 8).

**Discussion**

Kromkamp et al. (1998) have demonstrated that variable fluorescence techniques can be used to measure photosynthetic activity of benthic microalgae. Barranguet & Kromkamp (2000) found no significant change in the relationship between P\(^B\) (\(^{14}\)C) and relative electron transport rate (rETR = \(\Delta F/Fm' \times E\)) as a function of the season (although changes during low tide were observed) and were able to use a single regression coefficient, which they termed EE (ETR efficiency for C-fixation) to convert ETR into units of C-fixation. This may signify that the relationship between P\(^B\) and ETR is quite robust and not influenced by temperature. However, in their case, the algae were acclimated to the different temperatures existing in the different seasons. Short-term changes in temperature could still affect this relationship. If this occurred, EE would be affected, and could result in inaccurate estimates of primary production. Our results demonstrate that, in general, short-term changes in temperature can affect the relationship between P\(^B\) and ETR, although this effect was mainly limited to the extremes of the temperature range examined (5 and 35 °C; Figs 7 and 8).

Temperature acclimation of light harvesting pigment complexes involves changes in the ratio and quantity of several photosynthetic pigments (see Davison 1991, for a review). However, our cultures were grown at a constant temperature (20 °C) and it is unlikely that the light harvesting capacity changed in our short-term temperature experiments. Indeed, no significant change in photosynthetic efficiency was observed except at 35 and 5 °C, indicating that, at low irradiance, only the most extreme temperatures affect photosynthesis. After conversion to units of carbon (using a PQ of 1.4 as suggested by Williams & Robertson 1991 for growth on nitrate), values of α\(^B\)
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ranged from 0.018 to 0.069 mg C (mg Chl a)$^{-1}$ (µmol m$^{-2}$ s$^{-1}$) h$^{-1}$. These values are comparable to $\alpha^B$ values found in sediment slurries from the Westerschelde Estuary, the Netherlands (Barranguet et al. 1998, Barranguet & Kromkamp, 2000) and are within the range of those found for other natural MPB populations (Blanchard & Montagna, 1992, MacIntyre & Cullen, 1998). The relative stability of $F_v/F_m$ over a wide temperature range, as found in this study, is often observed in terrestrial plant research (Briantais et al. 1996, Pospisil et al. 1998, Pospisil & Tyystjarvi, 1999). Field measurements of MPB carried out on tidal flats in the Westerschelde, Netherlands over a seasonal cycle also showed rather stable $F_v/F_m$ values throughout the year Kromkamp et al. 1998).

Figure 6. Scatter plot of natural logarithm of gross production normalised to Chl a against natural logarithm of electron transport rate of Cylindrotheca closterium grown at 20 °C. Fitted lines represent linear regressions of the high and low growth rate cultures. Inset is the regression fit and 95 % confidence intervals of the whole data set back transformed to its power function. Other details as in Fig. 1.

The response of maximum photosynthetic capacity to short term changes in temperature in Cylindrotheca closterium is typical of most unicellular algae (Davison, 1991). Physical processes such as diffusion and cellular pH are also influenced by temperature and may contribute to the observed temperature effects. At higher temperatures, denaturation of chlorophyll-proteins (Briantais et al. 1996) and inactivation of the oxygen evolving mechanism occur (Samson et al.
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This explains the complete abolition of oxygen evolution and variable fluorescence at 40 °C found in this study.

Figure 7. Influence of temperature on the slope coefficient ($b_i \pm$ confidence interval) of the linear regression of log-transformed gross production normalised to Chl a against log-transformed electron transport rate (see Fig. 6) in cultures of Cylindrotheca closterium grown at 20 °C. Other details as in Fig. 1.
Figure 8. Influence of temperature on the intercept coefficient \((a_i \pm \text{confidence interval})\) of the linear regression of log-transformed gross production normalised to Chl a against log-transformed electron transport rate (see Fig. 6) in cultures of Cylindrotheca closterium grown at 20 °C. Other details as in Fig. 1.

The response of photosynthesis at high temperatures may also be affected by photorespiration. The \(K_m\) of Rubisco/oxygenase for \(O_2\) increases more slowly with increasing temperature than the \(K_m\) of Rubisco/carboxylase for \(CO_2\), so that the potential for photorespiration increases with increasing temperature. This is amplified by temperature dependent changes in the relative solubility of \(CO_2\) and \(O_2\) (Raven & Geider, 1988). The occurrence of a \(CO_2\) concentrating mechanism will reduce the incidence of photorespiration. The appearance of a sharp optimum (peak) indicates that the algae are not limited by carbon availability, unlike many macroalgae, which show a broad temperature optimum (Davison, 1991). Field samples of MPB measured by us at local sites also seem to show a peak rather than a broad optimum response of photosynthesis to temperature.

The \(T_{opt}\) values found in this study (30 °C) are higher than those found by Blanchard et al. (1997) with field derived MPB (about 25 °C). A possible explanation is that the algae in this study were acclimated to a stable growth temperature (20 °C), unlike the natural populations of MPB studied by Blanchard et al. (1997), which grew in a highly fluctuating temperature regime. However, differences in the dominant species composition of the MPB community could also explain the differences in \(T_{opt}\). The lethal temperature (\(T_{max}\); about 40 °C) was the same as that found by Blanchard et al. (1997). As \(T_{max}\) seems to be controlled by the thermolabile properties of the PSII complex components, it is unlikely to vary much with acclimation and is probably comparable for most algae from similar climatic regions.

We also fitted temperature response curves to the maximum relative ETR (\(rETR = \Delta F/Fm' \times E\)), and observed a significant difference in the magnitude of \(rETR_{max}\) values between the low growth rate (\(rETR_{MAX} = 206\)) and the high growth rate (\(rETR_{MAX} = 335\)). The fact that this difference in \(rETR_{max}\) between cultures grown at different growth rates disappeared when absolute rates of ETR were calculated demonstrates the importance of knowledge of the absorption properties of the algae for calculation of fluorescence-based photosynthetic rates.

**Relationship between \(P^b\) and ETR**

When the regression equation \((\ln (P^b) = a_i + b_i \ln (ETR); \text{Fig. 6})\) is back transformed, it takes the form of a power function: \(P^b = e^{a_i}.ETR^{b_i}\). A slope coefficient \((b_i)\) of 1 for the log transformed
regression equation indicates a linear relationship between $P^B$ and ETR. A $b_i$ below 1 indicates a curvilinear relationship with ETR higher than $P^B$ at high irradiances and $P^B$ higher than ETR at lower irradiances. The relationship between $P^B$ and ETR was non-linear at most temperatures because $b_i$ was below 1 (Fig. 7), although the deviation from linearity was small (inset Fig. 6). Differences in $b_i$ between growth rates could not be evaluated because of significant interaction between the effects of temperature and growth rate. With respect to temperature, the relationship between $P^B$ and ETR became increasingly curvilinear, as indicated by lower $b_i$ values, at 5 °C (Fig. 7). The relationship between C-fixation or oxygen evolution and ETR is not always linear and this non-linearity is sometimes observed in microphytobenthos (Hartig et al. 1998, Barranguet & Kromkamp, 2000, Perkins et al. 2001) as well as in planktonic algae (Geel et al. 1997, Flameling & Kromkamp, 1998, Masojídek et al. 2001). This non-linearity is most pronounced at irradiances exceeding $E_k$, and can be due to alternative electron sinks like the Mehler-ascorbate-peroxidase reaction and photorespiration, or to changes in the optical cross section. Recent comparisons of oxygen evolution and ETR measured using mass spectrometry have suggested that cyclic electron flow around PSII or nonphotochemical energy quenching within PSII centres are more likely causes of non-linearity than oxygen consuming processes (e.g. Mehler-ascorbate-peroxidase reaction; Ruuska et al. 2000, Badger et al. 2000, Franklin & Badger, 2001). At low irradiance, the most likely cause is variable, light stimulated rates of respiration (see Flameling & Kromkamp, 1998 for a discussion on possible reasons for non-linearity between oxygen evolution and ETR).

As explained above, the exponential of the intercept $a_i$ in Fig. 8 is equivalent to slope coefficients called EE by Barranguet & Kromkamp (2000) or $\kappa$ by Masojídek et al. (2001). The intercept ($a_i$) was significantly affected by temperature, but only at the extremes of the temperature range examined (5 and 35 °C; Fig. 8). In the 10 - 30 °C range, most values of $a_i$ were similar (range 6.2 - 7.0).

As the effect of growth rate could not be statistically evaluated and the influence of temperature was relatively small, model 1 linear regression of the full data set was carried out in order to evaluate the magnitude of the error incurred in the prediction of $P^B$ from ETR across the range of temperatures and at two growth rates. The resulting slope coefficient $b_i$ was 0.80 (95% CI 0.76 - 0.85) and $a_i$ was 6.37 (95% CI 6.29 - 6.45; $r^2 = 0.75$; $F_{(1, 406)} = 1248$). The predictive equation provides reliable estimates of $P^B$ at higher values of ETR (95% CI about 10% of the predicted mean) but, at the lowest values of ETR, the 95% CI was about 50% of the predicted mean (Fig. 6, inset; see below).
To allow comparisons with literature values for the regression between $P^B$ ($\mu$mol O$_2$ (mg Chl a)$^{-1}$ h$^{-1}$) and ETR ($\mu$mol e$^-$ (mg Chl a)$^{-1}$ s$^{-1}$), the regression equation was back transformed to a power function ($P^B = e^{a_i}.ETR^{b_i}$; Fig. 6, inset). Converting both variables to the same time units gives an EE value of 0.16 (95 % CI 0.15 - 0.18) mol O$_2$ (mol e$^-$)$^{-1}$. This value is close to the theoretical minimum value of 0.25 mol O$_2$ (mol e$^-$)$^{-1}$ (e.g. 4 electrons required at PS II per O$_2$ molecule; Gilbert et al. 2000). After conversion to comparable units (assuming $a^* = 0.02$ and 50% of absorbed irradiance is transferred to PSII), EE values of between 0.019 and 0.39 molO$_2$ (mol e$^-$)$^{-1}$ have been reported in the literature for a range of microalgal species (Masojidek et al. 2001, and references therein). If we take the coefficients calculated in this study, and convert these to mg C, assuming a PQ of 1.4, we find a value of 0.114 mol C (mol e$^-$)$^{-1}$. This value is a little higher than the average value of 0.0995 mol C (mol e$^-$)$^{-1}$ (estimated using the same assumptions as above) found by Barranguet & Kromkamp (2000) in a field-based comparison of MPB photosynthesis.

When the EE factors are examined at the different temperatures, photosynthesis appears to be less efficient at 5 $^\circ$C, which could be due to the Calvin cycle being more strongly affected by temperature than PSII charge separation. As a consequence, algae are more susceptible to photoinhibition at low temperatures (Tyystjarvi et al. 1994).

**Conclusions**

A short-term change in temperature (10 - 30 $^\circ$C), such as might be experienced during emersion on a European tidal flat, will not significantly affect the relationship between $P^B$ and ETR. However, care should be taken when using a single conversion factor between $P^B$ and ETR at the extremes of the temperature range. Algal absorption measurements are important for correct calculation of ETR. The facts that different species seem to have different conversion factors, and that changing environmental conditions will affect the absorption capacity and growth rate of the microphytobenthos community, suggest that it is wise to perform further calibrations of the relationship in the field before use in primary production modelling. Estimates of primary production at the estuarine basin scale may suffer from patchiness in the photosynthetic response of the MPB community. Variable fluorescence measurements are quick and non-invasive and, with knowledge of the absorption properties of the MPB community, allow the quantification of photosynthetic parameters across large areas. Hence they are potentially useful for improving our estimates of ecosystem scale primary production.