Photosynthetic activity, photoprotection and photoinhibition in intertidal microphytobenthos as studied *in situ* using variable chlorophyll fluorescence

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**A B S T R A C T**

The photosynthetic activity of microphytobenthos biofilms was studied *in situ* on an intertidal mudflat of the Ria de Aveiro, Portugal. Time series of physical variables characterizing the microenvironment at the sediment photic zone (incident solar irradiance, temperature, salinity), photophysiological parameters and productive biomass of undisturbed microalgal assemblages were measured during daytime low-tide periods along one spring–neap tidal cycle, with the objective of (1) characterizing the short-term variability in photosynthetic activity *in situ*, (2) relating it with the changing environmental conditions and (3) with the operation of physiologically (xanthophyll cycle) and behaviorally (vertical migration) based photoprotective processes, and (4) assessing the occurrence of photoinhibition. Pulse Amplitude Modulated (PAM) fluorometry was applied to measure photosynthetic activity (the effective and maximum quantum yield of photosystem II, ΔF/Fm*′* and Fv/Fm*′*; the photosynthesis index E FY; rapid light-response curves (RLC)), the photoprotective operation of the xanthophyll cycle and photoinhibition (non-photochemical quenching, NPQ; quantum efficiency of open RCs, Fv′/Fm′; and vertical migration (productive biomass, Fv′)). The photosynthetic activity was found to be strongly affected by the cumulative light dose received during the morning low-tide periods. The fluorescence indices ΔF/Fm*′*, E FY, Fv′/Fm′* and RLC parameters were more depressed under high irradiances when clear sky was present during the morning low tide than when foggy conditions reduced the light dose received during a comparable period. Productive biomass exhibited maximum values in the first hours of the morning, followed by a steep decrease when irradiance reached moderate levels, due to the downward migration of the microalgae. This photophobic migratory response appeared to display a photoprotective role, allowing ΔF/Fm*′* to remain near optimum values until irradiance reached values as high as 750 μmol m⁻² s⁻¹. The response to high light also included the formation of NPQ, expected to represent mainly the operation of the xanthophyll cycle, which attained high values, above 5.9 for 1500 μmol m⁻² s⁻¹. Despite the photoprotection provided by energy-dissipation processes and photophobic behaviour, the light response of most photophysiological parameters showed a clear counter-clockwise hysteresis pattern, indicating the occurrence of photoinhibition. Hysteresis was due to the incomplete recovery of photosynthetic activity during the afternoon low tide, and its magnitude was dependent on the morning light doses.

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1. Introduction

Microphytobenthos, the communities of benthic microalgae that inhabit the intertidal flats of estuaries, form dense and highly productive diatom-dominated biofilms at the surface of the fine-grain sediments. The large amounts of microalgal biomass that accumulates in the photic layers of the sediment during diurnal low tides and the capacity for high photosynthetic activity under a wide range of environmental conditions enable intertidal microphytobenthos to reach community-level primary productivity rates comparable to those attained by phytoplankton (Underwood and Kromkamp, 1999). Being affected by the superimposition of diurnal and tidal cycles, the estuarine intertidal environment is characterized by short-term variability in various key factors controlling the photosynthetic activity of microphytobenthos. During daytime low tides, benthic microalgae are frequently exposed to extreme conditions, which frequently may include supersaturating irradiances, desiccation, and high salinity (Brotas et al., 2003). They are systematically subjected to extended periods of direct exposure to sunlight, alternating with long periods of darkness during immersion in turbid water or night, and, during eb and flow, experience sudden transitions in light, salinity and temperature. Moreover, the high photosynthetic rates attained by dense biofilms may cause the local depletion of...
nutrients (Kromkamp et al., 1998; Miles and Sundbäck, 2000) or of inorganic carbon (G lud et al., 1992; Cook and Ray, 2006).

The prolonged exposure to supersaturating irradiance is a major source of stress to the photosynthetic apparatus and a potential cause of limitation of microphytobenthos productivity. Excessive light energy absorbed by the light-harvesting complexes (LHC) and not used for photochemistry or dissipated as heat, may cause photoinhibition, the permanent or slowly reversible damages to the photosynthetic apparatus, mainly through the inactivation of photosystem (PS) II protein D1 (Kyle et al., 1984; Falkowski et al., 1994). Diatoms respond to excessive light by operating the xanthophyll cycle, through which the pigment diadinoxanthin (DD) is rapidly and reversibly converted in the energy-dissipating form diatoxanthin (DT) (Arsalane et al., 1994; Olaizola and Yamamoto, 1994; Casper-Lindley and Björkman, 1998). Under high light, the accumulation of DT in the LHC antennae reduces the excitation energy delivered to PSII RCs and minimizes potential damages to the photosynthetic apparatus. In the case of microphytobenthos dominated by epipelagic diatoms, a second form of photoprotection has been proposed to take place, based on the photophobic migratory response of motile diatoms to high irradiance. This ‘behavioural photoacclimation’ consists in the high light-induced vertical migration of the cells, from the surface to less illuminated zones of the sediment (Kromkamp et al., 1998; Perkins et al., 2001; Sérodio et al., 2001; Underwood, 2002).

Under natural conditions, the effective impact of excessive light may be enhanced by the combined effects of a number of other environmental factors affecting biofilm condition during low tide, such as air temperature and humidity, and wind speed. Environmental conditions such as these are very difficult to replicate indoors, and laboratory studies are thus likely to fail to realistically describe the responses of microphytobenthos in its natural environment. Considering this limitation, a growing number of studies on the photosynthetic performance and productivity of intertidal microphytobenthos have been carried out in situ (Miles and Sundbäck, 2000; Perkins et al., 2001; Underwood, 2002; Brotas et al., 2003; Migné et al., 2004; Honeywill et al., 2006; Spilmont et al., 2006, Migné et al., 2007). However, only a few of these have simultaneously characterized the photophysiological and migratory responses of microphytobenthos to changing environmental conditions (Bro tas et al., 2003; Perkins et al., 2001) and, to our knowledge, none has addressed quantitatively the functioning of photoprotective processes and the occurrence of photoinhibition.

The objectives of this study were (1) to characterize the short-term variability in photosynthetic activity in situ during periods of low tide; (2) to relate these changes to the natural variability of the main environmental factors and (3) to relate to the operation of photoprotective mechanisms, distinguishing the relative role of physiological and migratory processes, and (4) to evaluate the effectiveness of these processes in preventing photoinhibition.

The photosynthetic activity, photoprotection and photoinhibition of microphytobenthos were studied in situ by using Pulse Amplitude Modulated (PAM) fluorometry (Schreiber et al., 1986). This technique allows to simultaneously and in real time measure the activity of PSII, assess the operation of the xanthophyll cycle and the occurrence of photoinhibition, and quantify vertical migration-induced changes in the surface biomass of undisturbed biofilms. The photosynthetic activity of biofilms was assessed by measuring the effective and maximum quantum yield of PSII ($\Delta F/F_m$ and $F_0/F_m$ respectively; see Table 1 for notation), a fluorescence-based index for gross depth-integrated photosynthetic rates (EFY), and by constructing rapid light-response curves (RLC). Xanthophyll cycle operation and occurrence of photoinhibition were assessed by the non-photochemical quenching (NPQ) coefficient and the quantum efficiency of open RCs ($F_o/F_m$). Behavioural photoprotection was investigated by monitoring $F_o$, a proxy for the photosynthetic biomass present in the photic zone.

### Table 1

Notation

<table>
<thead>
<tr>
<th>Notation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$F_0$</td>
<td>Initial slope and photoinhibition parameter of a RLC ($\mu$mol·m$^{-2}$·s$^{-1}$)</td>
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<tr>
<td>$F_m$</td>
<td>Maximum daily $F_0$ value ($\mu$mol·m$^{-2}$·s$^{-1}$)</td>
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<td>$\Delta rETR$</td>
<td>High light-induced decrease of $rETR$ ($\mu$mol·m$^{-2}$·s$^{-1}$)</td>
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<td>$E$</td>
<td>Spectrally averaged irradiance of PAR (400–700 nm) ($\mu$mol m$^{-2}$·s$^{-1}$)</td>
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<td>EFY</td>
<td>Photosynthesis index (dimensionless)</td>
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<td>$F_m$</td>
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<td>$F_o/F_m$</td>
<td>Quantum efficiency of open PSII RCs (dimensionless)</td>
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<tr>
<td>NPQ</td>
<td>Non-photochemical quenching coefficient (dimensionless)</td>
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<tr>
<td>PSII</td>
<td>Photosystem II</td>
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<tr>
<td>RC</td>
<td>Reaction center</td>
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<tr>
<td>$rETR$</td>
<td>Relative electron transport rate (dimensionless)</td>
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<td>$rETR_{RLC}$</td>
<td>Maximum relative electron transport rate in a RLC (dimensionless)</td>
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<td>S</td>
<td>Salinity</td>
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<tr>
<td>$T$</td>
<td>Temperature (°C)</td>
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### 2. Materials and methods

#### 2.1. Study site and periods

The study was carried out on an intertidal mudflat located near Vista Alegre, on the Ria de Aveiro, a mesotidal estuary located in the west coast of Portugal (Fig. 1). The study site consisted of fine muddy sediment (97% particles <63 µm), colonized throughout the year by dense microphytobenthos assemblages, dominated by diatoms of the genera Navicula, Nitzschia, Gyrosigma and Pleurosigma. Measurements were made during periods of low tide, from sunrise to sunset, on a total of 5 days, from 14 to 27 July 2004, distributed along a complete spring–neap tidal cycle. The presented results refer to 3 days, chosen as representative of the fortnightly variability in the timing of light exposure and tidal submersion: 2 days when diurnal low-tide exposure occurred twice during the photoperiod (14 and 27 July) and 1 day during which a single light exposure period took place (23 July). Days 14 and 27 July were chosen to illustrate the effects of contrastingly different sunlight exposure conditions: clear sky during the whole of day 14, and a dense fog during most of the morning low tide on day 27. All times are reported in UTC+1.

#### 2.2. In situ measurements: physical variables

During each period of low tide, incident irradiance ($E$, spectrally averaged solar irradiance, PAR, 400–700 nm), sediment temperature ($T$) and salinity ($S$), were measured at regular intervals at the site of the measurements. Irradiance was measured using a PAR sensor (LI-193SA and LI-250 light meter, Li-Cor, Lincoln, Nebraska, USA). Sediment surface temperature was measured non-invasively using an infrared thermometer (CA. 872, Chauvin Arnoux, France). Salinity was measured on sediment surface interstitial water, collected from lens tissues placed on the sediment surface, using a salinity refractometer ($S$/Mill, Atago, Japan).
2.3. In situ measurements: variable fluorescence

Variable chlorophyll fluorescence was measured using a portable PAM fluorometer (Portable Junior-PAM, Gademann Instruments GmbH, Germany). This fluorometer applied a modulated blue light (LED-lamp peaking at 470 nm, half-bandwidth of 31 nm) as source for measuring, actinic and saturating light, emitted at a frequency of 25 Hz when measuring the minimum fluorescence level \( F_o \) or 1.2 kHz when measuring other fluorescence parameters. Fluorescence was measured in situ using a 1.5 mm-diameter plastic fibreoptics (Edmund Optics, UK), maintained at a constant distance of 2 mm from the sediment surface, at a approx. 45° angle, and facing South to minimize shading effects. To facilitate the adjustment of the relative position of the fibreoptics and the sediment surface, measurements were made on undisturbed sediment samples (3.6 cm diameter), collected using plastic corers. Samples were kept moist during the whole period of measurements, by carefully adding site water as necessary to replace evaporated interstitial water, without disturbing or submerging the sediment surface. This was found necessary because preliminary tests showed that, mainly on sunny and windy days, core samples not kept hydrated suffered very rapid desiccation, not being representative of the conditions on the tidal flat. A total of 6 fibreoptics were used to measure fluorescence in the same number of replicated samples. Measurements on different samples were made sequentially, by main-
taining one end of each fibre fixed in its measuring position pointing to the sample, and by connecting the free end to the fluorometer.

Every 15 min, a saturating pulse was applied and the effective quantum yield of PSII, \( \Delta F/F_{m'} \) \( = (F_m'-F_o)/F_{m'} \) (Genty et al., 1989), was measured on each sample. On 3 of these samples, one Rapid Light Curve (RLC; White and Critchley, 1999) was constructed every 30 min. Each sample was darkened for 10 s, using a black plastic cover, after which it was exposed for 10 s to each of 8 increasing actinic light levels (provided by the fluorometer), from 80 to 1600 \( \mu \)mol m\(^{-2}\) s\(^{-1}\). Under each light level, \( F_o \) and \( F_{m'} \) were determined and the relative electron transport rate was calculated by \( \text{rETR} = E \times \Delta F/F_{m'} \). The first value of the each RLC, measured after 10 s of darkness, was taken as an estimation of \( F_o/F_{m'} \) \( = (F_{m'}-F_o)/F_{m'} \), the quantum efficiency of open PSII RCs (Ralph et al., 1999). The sample remained in the dark for 2 min, after which \( F_o \) and the maximum quantum yield of PSII, \( F_m/F_{m'} \) \( = (F_m-F_o)/F_m \), was determined, before it was returned to ambient light. The fluorescence parameter \( F_o \) was used as a proxy for productive biomass, the photosynthetic biomass present at each moment in the photic zone of the sediment (Serôdio et al., 2001). The \( F_o \) value measured after only 2 min of darkness cannot be taken as representing the full dark-adapted state of the sample, but was shown to be a good estimator of microalgal biomass (Serôdio et al., 2006a, 2007). Using the values of \( E \) and \( \Delta F/F_{m'} \) determined before the start of the RLC, the fluorescence-based photosynthesis index, \( \text{EFY} \), was calculated by (Serôdio et al., 2007)

\[
\text{EFY} = E \times F_o \times \Delta F/F_{m'}
\]

RLCs were characterized by estimating the parameters \( a_{RLC} \) (initial slope), \( \text{rETR}_{m,RLC} \) (maximum rETR) and \( D_{RLC} \) (photoinhibition parameter), through fitting the model of Platt et al. (1980) to \( \text{rETR} \) vs \( E \) curves. The model was fitted iteratively by minimizing a least-squares function using MS Excel Solver. Curve fit was very good in all cases \( (r \geq 0.913 \text{ in a total of 133 RLCs}) \).

2.4. NPQ estimation

As methodological problems impede the determination of NPQ, generally used to quantify the operation of the xanthophyll cycle in microphytobenthic biofilms, an alternative method was applied in this study, based on the variation of the initial slope of RLC, \( \varphi_{RLC} \). Shown to relate linearly to NPQ (Serôdio et al., 2006b; Cruz & Serôdio, 2008). To confirm the applicability of the method, NPQ and \( \varphi_{RLC} \) were compared on microphytobenthos suspensions (to avoid migratory effects on fluorescence parameters) exposed to a wide range of light conditions. Surface sediment (ca. 1 cm deep) was collected in the days of field work and taken to the laboratory, where microalgae were collected using the ‘lens tissue technique’ as described in Serôdio et al. (2005), in the following day. Variable fluorescence was measured using a PAM fluorometer comprising a computer-operated PAM-Control Unit (Walz, Germany) and a WATER-EDF-Universal emitter-detector unit (Gademann Instruments GmbH, Germany). A modulated blue light (LED-lamp peaking at 450 nm, half-bandwidth of, 20 nm) was applied as source for measuring, actinic and saturating light, delivered through a 1.5 mm-diameter plastic fibreoptic connected to a fluorescence cuvette (KS-101, Walz, Germany). The fluorescence cuvette was connected to a recirculating water bath (Frigiterm-10, Selecta, Spain) and all measurements were made at 20 °C.

NPQ and RLCs were compared as described in Serôdio et al. (2006b). Microalgae were dark incubated for 30 min, after which a saturating pulse was applied and fluorescence levels \( F_o \) and \( F_{m'} \) were measured. Samples were then exposed to 12 increasing
irradiance, from 12 to 920 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Under each level, \( F_{d} \) and \( F_{m}^{i} \) were determined each 90 s, until a steady state in \( \Delta F/F_{m}^{i} \) was reached (minimum 7.5 min). For each light level \( E \), \( \Delta F/F_{m}^{i} \) and NPQ were calculated and \( \Delta F/F_{m}^{i} \) vs \( E \) and NPQ vs \( E \) curves were constructed. Due to the possible occurrence of \( F_{m}^{i} \) values higher than the \( F_{m} \) value measured after dark adaptation, NPQ was calculated by adapting the non-photochemical quenching coefficient (Schreiber et al., 1994):

\[
\text{NPQ} = (F_{m}^{i} - F_{m}^{i}/F_{m}) \quad (2)
\]

where \( F_{m}^{i} \) is the maximum \( F_{m}^{i} \) value measured during the light curve (Serôdio et al., 2005). One RLC was constructed after a steady state was reached (constant \( \Delta F/F_{m}^{i} \) values) under each light step of the NPQ vs \( E \) curve and immediately after NPQ had been determined. RLCs were constructed by exposing the sample for 10 s to each of 12 increasing actinic light levels (the same used in the NPQ vs \( E \) curves). NPQ was compared with the variation (decrease) of \( \alpha_{\text{RLC}} \) under high light, as calculated by (Serôdio et al., 2006b):

\[
\Delta \alpha_{\text{RLC}}(E) = \alpha_{m,\text{RLC}} - \alpha_{\text{RLC}}(E) \quad (3)
\]

where \( \alpha_{m,\text{RLC}} \) is the maximum \( \alpha_{\text{RLC}} \) value measured during the light curve. Time series of NPQ were generated by applying Eq. (3) to observations of \( \alpha_{\text{RLC}} \) obtained \textit{in situ} and estimating \( \alpha_{m,\text{RLC}} \) from the maximum value observed during each day.

### 2.5. Taxonomic composition

The species composition of the biofilms was determined by analysing replicated samples of the microalgal suspensions used in the laboratory measurements. Microalgal suspensions were fixed in 1% \textit{v/v} formaldehyde and viewed using bright-field microscopy for determination of the relative abundance of diatoms, euglenophytes and cyanobacteria. Identification of diatom species was carried out on subsamples oxidized using concentrated HCl.

### 3. Results

#### 3.1. Physical variables

During the study period, daylength was approx. 14 h 30 min (from sunrise at 6h30 to sunset at 22h00). The submersion during high tide reduced the effective photoperiod to ca. 10 h (two periods of 7 h and 3 h, corresponding to low tide), 7 h, and 9 h 30 min (4 h 30 min and 5 h) on days 14, 23 and 27 July, respectively. The days with two periods of low tide were characterized by clear sky throughout the day, with irradiance reaching maximum values over 1750 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Fig. 2a) and 1430 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (day 27, Fig. 2c) during the low-tide periods. During the morning of day 27, foggy conditions temporarily reduced \( E \) to values below 400 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Dissipation of fog beginning at 10h00 caused a rapid increase in incident irradiance, that raised by more than 3.5-fold in 1 h. On day 23, partial cover by high clouds reduced irradiance throughout the day, which nevertheless remained above 500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) during the whole low-tide period (Fig. 2b).

The periods of low tide were also characterized by large variations in the temperature and salinity of the surface of the sediment (Fig. 2a–c). Large increases in temperature (up to 75%, from 16 to 28 °C; Fig. 1a) were observed in the morning low-tide periods, closely following the increase in incident solar radiation, a dependency particularly evident when the morning periods of days 14 (clear sky) and 27 July (fog) are compared (Figs. 2a, c).

Large variations were also observed regarding salinity, particularly during the afternoon period, when it continued to increase whilst irradiance and temperature were already decreasing (Fig. 2b, c). Increases of more than 100% during the same low-tide period were observed, with maximum values reaching above 65, and values of 50 being common.

#### 3.2. Photosynthetic activity

A clear inverse relationship was found between \( \Delta F/F_{m}^{i} \) and \( E \) on all days (Fig. 2d–f). On day 14 July, \( \Delta F/F_{m}^{i} \) decreased with the morning increase in \( E \), this decrease becoming more marked when \( E \) reached values above 600 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Fig. 2d). For \( E > 900 \mu \text{mol m}^{-2} \text{s}^{-1} \), \( \Delta F/F_{m}^{i} \) remained very low, below 0.15. During the afternoon low tide, \( \Delta F/F_{m}^{i} \) recovered monotonically following the decrease in \( E \). A similar response pattern was observed on day 27, but with the difference that, during the earlier part of the morning \( \Delta F/F_{m}^{i} \) started by increasing until it reached a maximum before decreasing (Fig. 2f). Maximum \( \Delta F/F_{m}^{i} \) values attained on this day were higher than those of day 14 (\( p = 0.047 \), t-test), and remained relatively high during most part of the morning low tide, starting to decrease significantly only when \( E > 500 \mu \text{mol m}^{-2} \text{s}^{-1} \) (Fig. 2d, f). On 23 July, when low tide occurred during the middle of the day, \( \Delta F/F_{m}^{i} \) decreased steadily during most of the exposure period, even when \( E \) remained constant, around solar noon (ca. 1500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Fig. 2e). Recovery of \( \Delta F/F_{m}^{i} \) started only when irradiance decreased below 1000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \).

\( F_{r}/F_{m} \) displayed the same overall pattern of variation of \( \Delta F/F_{m}^{i} \), mostly characterized by inverse variation with \( E \) (Fig. 2g–i). \( F_{r}/F_{m} \) was generally higher than \( \Delta F/F_{m}^{i} \), the difference between the two decreasing with \( E \) (Fig. 2d–i). Exceptions to this tendency occurred on the morning of 27 July, during which both indices showed almost identical values (and higher than on day 14) (Fig. 2f, i), and, at the end of the photoperiods of both days, when \( \Delta F/F_{m}^{i} \) increased at a higher rate than \( F_{r}/F_{m} \) and reached values markedly higher than \( F_{r}/F_{m} \) (respectively, 14.8 and 32.9% higher; t-test, \( p = 0.005 \) and 0.010). As a consequence, while the recovery of \( \Delta F/F_{m}^{i} \) in comparison with early morning value was almost complete (for days 14 and 27 July, respectively, –23.5% and –13.0%; t-test, \( p = 0.134 \) and 0.051), \( F_{r}/F_{m} \) appeared to recover only partially, attaining values considerably lower than those observed at the beginning of the day (respectively, –56.6% and –37.8%; t-test, \( p < 0.001 \) and \( p = 0.004 \)) (Fig. 2d, f, g, i). The irradiance level for which \( \Delta F/F_{m}^{i} \) equalled \( F_{r}/F_{m} \) was similar on both days, of ca. 150–200 \( \mu \text{mol m}^{-2} \text{s}^{-1} \).

The pattern of variation of \( E_{FY} \) was also strongly dependent on light history, as evident by comparing the morning low tides of days 14 and 27 July. In the first day, \( E_{FY} \) displayed a bi-phasic pattern, with a marked increase due to the large increase of \( E \) and slow decrease of \( \Delta F/F_{m}^{i} \). Maximum values were reached around 8h30, which was then followed by a gradual decrease, corresponding to the decrease of \( \Delta F/F_{m}^{i} \) and \( F_{r} \) (Fig. 3j, see below), until it remained relatively constant for the rest of the low tide period (Fig. 2j). On the afternoon low tide, a second peak was observed, due to the recovery of \( \Delta F/F_{m}^{i} \) and the rise in \( F_{r} \) immediately after ebb, and to the later decrease in \( E \). In contrast, on day 27 July, \( E_{FY} \) increased during most of the morning low tide, reaching the highest value shortly before flood, and showing some decline only after the fog has cleared out (Fig. 2i). The variation pattern observed in the afternoon low-tide period was generally similar to the one observed on day 14, but presenting higher values. On 23 July, \( E_{FY} \) showed a steep decline during the first hours of the low-tide period, after which it remained relatively stable (Fig. 2k).
The hourly variation of \( \frac{F_v}{F_m} \) and \( \Delta \frac{F}{F_m} \) displayed a similar pattern, generally characterized by an accentuated decrease with the morning rise in \( E \) and a partial recovery during the afternoon period (days 14 and 27; Fig. 3a, d and d, f), or by maintaining low values during the mid-day high light exposure, that gradually increased under the afternoon decline in irradiance (day 23; Fig. 3b, e). As with \( \frac{D}{D_m} \), the light history of the morning period conditioned significantly the hourly variation of these indices, with clear differences found between days 14 and 27 July. In the morning of day 14, when \( E \) rose rapidly, both indices decreased steeply to very low values, particularly \( \frac{F_v}{F_m} \), which remained very close to 0 during the rest of this period (Fig. 3a). In contrast, during 27 July, a significant decrease started only shortly before flood, with much higher values measured for comparable \( E \) levels (Fig. 3c, f). On day 27, an increase in the two indices was observed during the first hours of the morning period (Fig. 3c, f). This was also observed for \( \Delta \frac{F}{F_m} \) on day 14, although in a much less extent (Fig. 3d).

The daily pattern of \( rETR_{m,RLC} \) was also strongly affected by light conditions occurring during the morning. On day 14 July, a large initial increase was followed by a steep decline for \( E > 600 \mu \text{mol m}^{-2} \text{s}^{-1} \), after which \( rETR_{m,RLC} \) decreased at a relatively low rate (Fig. 3g). On day 27, \( rETR_{m,RLC} \) increased gradually during most of the morning low tide, only decreasing when fog dissipated and incident irradiance rapidly increased (Fig. 3i). On all days, \( rETR_{m,RLC} \) decreased when \( E \) declined by the end of the day (Fig. 3g–i).

The results obtained for these 3 days were representative of the whole spring–neap tidal cycle. Observations made on the remaining days of the tidal cycle (summarized in Table 2) were in
general agreement with the range and patterns of variation presented in detail above. ΔFm'/Fm', Fv'/Fm, Fv'/Fm', and αRLC displayed the same overall inverse variation with E, with lowest values generally coinciding with highest light levels, and highest values being observed for lower irradiances. EFY and rETRm,RLC were also depressed under high E levels, the higher values corresponding to moderate irradiances (Table 2).

3.3. Productive biomass

The hourly variation of Fo, the proxy for microphytobenthos productive biomass, was characterized by high values during the early part of the morning low tides, which gradually decreased as E increased (Fig. 3j, l). This early morning peak corresponded to the accumulation of large numbers of microalgae in the uppermost layers of the sediment, and was better defined on day 27 July, when morning irradiance was lower (Fig. 3l). On both days 14 and 27 July, Fo started to decrease at roughly the same time (8h30–9h00), to values similar to those observed on the afternoon light period (Fig. 3j, l). On day 14 July, this decrease in Fo became noticeable when irradiance reached over ca. 750 μmol m⁻² s⁻¹, whilst on day 27 July it started under considerably lower irradiance levels, of 150–200 μmol m⁻² s⁻¹ (Fig. 3j, i). An inverse relationship between Fo and E was also observed, although more attenuated, in the afternoon low tide. An increase in Fo following the decrease in E also occurred on day 23, although this tendency was later inverted, anticipating the flood by high tide (Fig. 3k).

3.4. Light responses

To better assess the effects of light history on the hourly variability of the various fluorescence indices, light-response
curves were reconstructed from the time series of observations during the entire effective photoperiod (Fig. 4). In most cases, a clear counter-clockwise hysteresis pattern was observed, with the decrease observed during the morning not being matched by the subsequent recovery during the afternoon (e.g. Fig. 4a, c). A clear effect of the morning light dose was found for all indices, the difference between the morning and the afternoon light responses being generally higher on day 14 than on day 27 (Fig. 4).

In the case of ΔF′/F′m and F′/Fm (as well as zRLC, data on Fig. 3), a higher light dose in the morning caused a larger relative decrease of the values measured during the afternoon, particularly under lower irradiance levels (E<500–600 µmol m⁻² s⁻¹; Fig. 4a–d). During the morning low tides, the light responses of ΔF′/F′m and F′/Fm were characterized by an initial phase when values remained constant or increased slightly, followed by an almost linear decrease until E>1000 µmol m⁻² s⁻¹ (Fig. 4e–d). In the afternoon period, the two indices recovered following a linear trend virtually parallel to the observed during the afternoon period, the two indices recovered following a complete match was observed on day 27 July (Fig. 4e, f).

The comparison between zRLC and the NPQ level attained in situ, data on Fig. 3), a single equation could be used to describe all data (Fig. 5a).

On the basis of this linear relationship, NPQ could be estimated from RLCs and its short-term variability in situ could be characterized (Fig. 5b, c). On 14 July, the NPQ induction started early in the morning, when irradiance levels reached over ca. 500–600 µmol m⁻² s⁻¹ (Fig. 5b). NPQ increased following a saturation-like pattern, stabilizing at maximum values of 5.6–5.9 for E>1300 µmol m⁻² s⁻¹. On day 27, NPQ started by decreasing from a relatively high initial value of ca. 1.2 to nearly zero. During the rest of the morning, NPQ induction was more gradual, reaching only ca. 50% of the maximum values of day 14, although after a shorter exposure period (Fig. 5c).

The magnitude of the NPQ induction was inversely related to the difference between ΔF′/F′m measured in situ and on suspensions, under optimal, stress-free conditions. This difference was substantially larger on day 14 July, when ΔF′/Fm measured in situ were, apart from the initial part of the morning, much lower than the values measured in suspensions (a difference up to 0.40 for E = 1200 µmol m⁻² s⁻¹). On day 27, ΔF′/Fm measured in situ was much nearer optimal conditions, the difference between in situ and suspensions remaining almost constant for most of the E range (E>300 µmol m⁻² s⁻¹), and not exceeding 0.10.

3.6. Taxonomic composition

The species composition of the microalgal suspensions did not vary substantially along the study period, the assemblages being dominated by five species of motile diatoms, which accounted for 76–83% of total cell counts: Navicula cf. gregaria Donkin, Nitzschia draveilensis Coste and Ricard, Nitzschia frustulosa (Kützing) Grunow, Nitzschia perspicua Cholnoky, Parlibellus crucicula (W. Smith) Witkowski, Lange-Bertalot and Metzeltin. Euglenophytes and cyanobacteria accounted for less than 1% of cell counts on all days.

4. Discussion

4.1. Photosynthetic activity

The photosynthetic activity of microphytobenthos during daytime low tides was characterized by a large short-term...
variability associated to the rapidly changing environmental conditions, with $\Delta F/F_{m}'$ varying from physiological maxima to values close to zero during the course of single exposure periods. The differences found between the photosynthetic light responses of days 14 and 27 July, with the high light-induced decrease of $\Delta F/F_{m}'$ being much more pronounced on the first day, indicate that the pattern of variation of photosynthetic activity is strongly determined by the cumulative effects of prolonged exposure to high light. Also, the larger difference found on day 14 July between the $\Delta F/F_{m}'$ light response of biofilms in situ and of microalgal suspensions (considered as close to optimal), further shows that the photosynthetic activity at a given moment is not determined by current irradiance level alone, but also by the previous light history. Probable causes for this enhanced decrease of the photosynthetic activity include the photoprotective downregulation of photosynthesis or the effects of photoinhibition, resulting from the continued exposure to high light. Other possible causes are the limitation of photosynthesis due to depletion of nutrients or carbon within the dense biofilm, which can be expected as a result of the high photosynthetic rates during the early part of the morning (Ludden et al., 1985; Glud et al., 1992; Kromkamp et al. 1998; Miles and Sundbäck, 2000; Cook and Ray, 2006). Salt and osmotic stress may also contribute to depress photosynthesis during prolonged low-tide exposures (Rijstenbil, 2003), but their

**Fig. 4.** Light-response curves of effective ($\Delta F/F_{m}'$; a, b) and maximum quantum yield of PSII ($F_v/F_{m}$; c, d), of the proxy for photosynthetic rate, $E_{FY}$ (e, f), and of the productive biomass, $F_o$ (g, h), reconstructed from the time series of data measured during the morning (open circles) and afternoon (closed circles) low-tide periods of 2 days of a spring–neap tidal cycle. Vertical bars represent±1 standard error (a, b: $n=6$; c–h: $n=3$). Arrows indicates the temporal order of the data points.
influence in this study was likely minimal as sample desiccation was prevented during the measuring periods.

Because microphytobenthic biofilms are dominated by motile microalgae, their community-level, depth-integrated photosynthetic properties are also affected by migration-induced changes in the cell vertical distribution. As such, another possible cause of the accentuated decreases in biofilm photosynthetic activity is the downward migration of the more physiologically competent microalgae, leaving in the photic zone cells in worst physiological condition (e.g. non-migratory, therefore more prone to photodamage). On the other hand, migration into the sediment could have accentuated the overestimation effect of depth-integration of fluorescence emission on \( \Delta F/F_m \), when measured on intact biofilms (Forster and Kromkamp, 2004; Serôdio, 2004), particularly under high irradiances, and has masked an actually larger depressing effect of light. This depth integration effect must also be taken in consideration when comparing \( \Delta F/F_m \) measurements made on sediments and on suspensions, since it implies that the observed differences may actually represent a larger difference in the inherent physiological response of the microalgae.

The causes for the response of \( \Delta F/F_m \) to high light may also explain the pattern observed for \( r_{\text{ETR}_{\text{RLC}}} \), also characterized by a marked decrease under moderate to high light, both on days 14 and 27 July. These results are partially in agreement with previous observation made on intact biofilms, but contradict what is expected for microphytobenthos cells under optimal conditions, for which an increase in \( r_{\text{ETR}_{\text{RLC}}} \) with increasing irradiance was consistently observed (Serôdio et al., 2006b; Cruz and Serôdio, 2008). Although determined from light-response curves too short to allow reaching of steady state under each light step, \( r_{\text{ETR}_{\text{RLC}}} \) is related to the maximum photosynthetic capacity, reached when the rate of photosynthesis is limited by the activity of the electron transport chain or Calvin cycle enzymes (Behrenfeld et al., 2004; Ralph and Gademann, 2005). As such, increases of \( r_{\text{ETR}_{\text{RLC}}} \) with irradiance have been observed and explained by the high light-induced activation of the carbon metabolism, which represents a photoprotective response against potential photoinhibition cause by excessive light (Ralph et al., 1999).

The simultaneous decrease of \( \Delta F/F_m \) and \( F_m \), as observed on days 14 and 27 July, caused the photosynthesis index \( E_{\text{FY}} \) not to display maximum values under the highest light levels, as predicted by productivity models based on laboratory observations of the migratory control of productive biomass (Pinckney and Zingmark, 1991; Guarini et al., 2000; Serôdio and Catarino, 2000). This illustrates the potentially erroneous information retrieved from laboratory studies and the value of realistic, in situ observations.

4.2. NPQ and photoprotection

The operation of the xanthophyll cycle is often investigated by measuring the quenching of variable fluorescence, NPQ, which has been found to be linearly related to the relative DT content (Arsalan et al., 1994; Olazola and Yamamoto, 1994; Serôdio et al., 2005). However, due to methodological problems related to the confounding effects of vertical migration on fluorescence parameters, the calculation of the NPQ coefficient for intact microphytobenthos samples is problematic if not impossible (Serôdio et al., 2005; Jesus et al., 2006). In this study, we were able to monitor the evolution of NPQ on intact biofilms in situ by following the short-term changes in \( \Delta \alpha_{\text{RLC}} \). This approach is based on the co-variation of \( \Delta \alpha_{\text{RLC}} \) and NPQ, due to their common dependence on the efficiency of light capture and energy transfer from the LHC antennae to the PSII RCs (Serôdio et al., 2006b). The finding of a single linear relationship between \( \Delta \alpha_{\text{RLC}} \) and NPQ measured on the different days ensured that, even if the value of \( \alpha_{\text{RLC}} \) varied among days, changes in \( \Delta \alpha_{\text{RLC}} \) could be taken as proportional to changes in NPQ.

The maximum values estimated for NPQ, up to 5.9 for \( E > 1500 \mu\text{mol m}^{-2} \text{s}^{-1} \), are among the highest recorded for microalgae or plants, for which typical maximum NPQ values remain below 4.0 (Roháček, 2002; Ruban et al., 2004; Serôdio et al., 2005), but similar to the maximum values documented for diatom-dominated microphytobenthos (Serôdio et al., 2005, 2006b). The NPQ coefficient is determined by all non-photochemical processes capable of lowering the fluorescence emission, which include both rapidly reversible, photoprotective processes as the xanthophyll cycle (‘energy-dependent quenching’, \( q_E \)) and slowly reversible, photoinhibitory processes resulting from damages to
the photosynthetic apparatus (‘photoinhibitory quenching’, \( q_v \); Müller et al., 2001). However, the NPQ estimated on the basis of \( \Delta F_{\text{RLC}} \) may be expected to relate mainly to \( q_v \), since the formation of DT in the antennae bed is the cause for the high light-induced decrease in light capture efficiency detected by \( \Delta F_{\text{RLC}} \) (Sérodi et al., 2006b). Moreover, the observed correlation between \( \Delta F_{\text{RLC}} \) and \( F_v/F_m \) further supports that the main features of the patterns of variation of \( q_v \) are associated to the photoprotective component of NPQ (\( q_v \)), since \( F_v/F_m \) measures the quantum efficiency of energy capture by open RCs, largely determined by downregulation of surface irradiances, and not under minimum light levels, were corroborated by the early morning peaking of \( \Delta F_{\text{RLC}} \) (Roháček, 2002). As such, the measured high NPQ values are likely to represent an elevated capacity for photoprotection, developed as an adaptation against the damaging effects of prolonged exposure to direct sunlight that characterizes the estuarine intertidal environment. It may be added that the high NPQ observed in this study may represent an understimation of the values actually reached by the microalgae present at the sediment surface, due to the effects of depth integration of upwelling fluorescence emitted by cells in deeper layers, exposed to lower light (Forster and Kromkamp, 2004; Sérodi, 2004).

The observation of minimum NPQ values for low to moderate irradiances, and not under minimum light levels, were corroborated by the early morning peaking of \( \Delta F_{\text{RLC}} \), \( F_v/F_m \) and \( F_v/F_m' \), particularly on day 27 July, and can be explained by the formation of NPQ in the dark, and by its gradual dissipation during the first hours of the morning. The induction of NPQ in the dark has been reported for diatoms grown in culture, and was shown to occur to a great extent for microphytobenthos assemblages (Sérodi et al., 2005, 2006b; and references therein). The most likely causes are the occurrence of chlororespiration or the reverse operation of ATP synthase, both processes capable of forming a transthylakoidal proton gradient and inducing the production of energy-dissipating pigment diatoxanthin, DT (Ting and Owens, 1993; Olazola and Yamamoto, 1994; Jakob et al., 1999; Dijkman and Kroon, 2002; Lavaud et al., 2002). The production of DT in the dark would also explain the incomplete recovery of \( \Delta F_{\text{RLC}} \) during the end of the afternoon period, which could thus result from the counteracting of the relaxation of NPQ following the decrease in irradiance.

4.3. Migration and photoprotection

The results showed evidence for the occurrence of photophobic downward migration of microalgae as a response to high irradiance. On both days when ebb took place before sunrise and the low tide coincided with the beginning of the morning, microalgae accumulated at the surface but subsequently started to migrate into the sediment. This pattern of behaviour was more pronounced on day 27 July, when microalgae migrated to the surface when irradiance remained low due to foggy conditions. The downward migration started when \( E \) reached 150–200 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), a range of values that coincides with the threshold level found to trigger a photophobic migration in laboratory experiments (Sérodi et al., 2006a). On the contrary, on day 14, descending migration appeared to start at a much higher light level, which could lead to conclude that this migratory response was not controlled by light. Although downward migration prior to high tide can occur independently of light conditions as a result of a tidal endogenous rhythm (Brotas et al., 2003), this is unlikely the case, as the decrease in \( F_o \) started much before (3–5 h) the actual tidal flood.

However, it must be considered that the above-mentioned results of laboratorial experiments were obtained by testing the induction of downward migration only after a dense biofilm had been formed at the surface, under low light. The light conditions in the morning of day 27 July resembled this protocol, since they allowed for the biofilm to form under low light before it was exposed to high light. In contrast, the early and rapid rise in irradiance on day 14 July makes the in situ results not directly comparable to those obtained in the laboratory. In this case, the biofilm was formed simultaneously with the increase in irradiance, which may have caused the cells to accumulate at some depth below the surface where light levels were lower. In agreement with this is the fact that \( \Delta F_{\text{RLC}} \) remained near optimum values until surface irradiance reached values as high as 750 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The relatively constant values of \( F_o \) during this period is thus likely to result from a balance between the endogenously controlled or low light-induced upward migration and the downward photophobic response to the increase in surface irradiance.

The descending migration under high light has been interpreted as a behavioural photoacclimation or photoprotective strategy, by which motile microalgae modulate their light exposure by moving through the steep vertical light gradient within the sediment (Kromkamp et al., 1998; Perkins et al., 2001; Sérodi et al., 2001; Underwood, 2002). Vertical photophobic migration and the xanthophyll cycle are thus likely to act as complementary photoacclimation processes, the former minimizing the extent of the activation of the latter, both reducing potential damages to the photosynthetic apparatus. Probably due to the mentioned methodological problems regarding the measurement of NPQ in biofilms, the combined operation of these two processes has not been studied. The results of this study suggest that vertical migration may display a preventive role, reducing or delaying the induction of the xanthophyll cycle. This idea is supported by the observations of day 14 July, when the early morning upward migration resulted in the accumulation of microalgae at subsurface layers, preventing the exposure to already high light levels. This enabled the photosynthetic efficiency (\( \Delta F_{\text{RLC}} \)) of the biofilm to remain near optimum values, and NPQ to remain low, until considerably high irradiance levels were reached.

On the other hand, the migratory response to high light seems to occur only after the induction of NPQ, as on both days the irradiance threshold for the start of downward migration was higher (although not much) than the one for the NPQ increase. In a situation of prolonged exposure to high light, downward migration might represent an advantageous strategy to prevent damages resulting from the photoprotective capacity of the xanthophyll cycle being exceeded. On day 27 July, the rapid increase in irradiance impinging on the microalgae accumulated at the surface caused NPQ to start earlier (under lower \( E \)) than on day 14. However, the rapid and massive downward migration of microalgae, together with the relatively short exposure to high light, may have reduced the need for photoprotection through the xanthophyll cycle, and NPQ attained lower values for comparable light levels.

4.4. Hysteresis and photoinhibition

Diatoms are known to have the capacity to develop very high levels of non-photochemical quenching under high light stress (‘super-quenching’; Ruban et al., 2004; Lavaud et al., 2004), which has been shown to provide effective photoprotection against photoinhibition in estuarine species, both planktonic (Lavaud et al., 2007) and benthic (Cruz and Sérodi, 2008). However, the observed hysteresis patterns in the light response of most fluorescence indices are a strong indication of the occurrence of photoinhibition. Counter-clockwise hysteresis caused by the ‘afternoon depression’ (Schanz and Dubinsky, 1988) of photosynthetic
activity has been attributed to slowly reversible quenching of fluorescence associated to the \textit{de novo} synthesis of the D1 protein (Falkowski et al., 1994; Gorbunov et al., 2001; Levy et al., 2004). Although hysteresis may be due to other causes, like circadian rhythms, nutrient depletion, or photosensitization (Levy et al., 2004), its strong dependence on the light exposure dose found in this study supports the idea that it was mainly due to damages to the photosynthetic apparatus resulting from prolonged light stress. The importance of the light dose received during the morning period is reinforced by the observation that its effects are not reversed even when the afternoon photoperiod was relatively short and with moderate irradiance levels, as in the case of day 14 July. On the basis of these results may be the prolonged exposure to irradiances exceeding the photoprotective capacity, which was seen to saturate at 1300 \mu mol m^{-2} s^{-1}. This is also supported by the observed light dose dependence of the relative variation of \( F_{v}/F_{m} \) and \( \Delta F/F_{m} \). The short dark incubation (2 min) used for measuring \( F_{v}/F_{m} \) impeded the correct measurement of the true maximum quantum yield of PSII, the index most commonly used to assess the extent of photoinhibition (Falkowski et al., 1994). However, the comparison of \( F_{v}/F_{m} \) with the \( \Delta F/F_{m} \) level measured immediately prior to the darkening can be informative for the distinction between \( q_{e} \) from \( q_{p} \) as the cause for the observed high light-induced decrease of photosynthesis efficiency. Under high light, larger differences between consecutive \( \Delta F/F_{m} \) and \( F_{v}/F_{m} \) values indicate a faster dissipation of the NPQ previously established, which can therefore be deduced to be mostly due to \( q_{e} \), whilst smaller differences between the two indices are indicative of \( q_{p} \). The large differences found between \( \Delta F/F_{m} \) and \( F_{v}/F_{m} \) on day 14 July and the similarity observed on day 27 reinforces that the difference between the 2 days is caused by light exposure during the morning periods. On the morning of the day 14, \( F_{v}/F_{m} \) values remained very low, only slightly above the corresponding \( \Delta F/F_{m} \) levels, indicating a slow reversal of the established NPQ, therefore likely to be constituted by a significant \( q_{e} \) component. In contrast, on day 27, \( F_{v}/F_{m} \) decreased only by 0.1 during most of the morning period (from ca. 0.6 to 0.5; with the exception of the last measurement), remaining well above \( \Delta F/F_{m} \), suggesting a much larger NPQ relaxation, thus likely due to \( q_{e} \). This rapid relaxation of a significant fraction of NPQ is in agreement with the data available for NPQ relaxation measured on microphytobenthos, which was shown to attain 45\% in 2 min after exposure to 1700 \mu mol m^{-2} s^{-1} \cite{serodio2005}. During the second low tide, the two indices became similar as irradiance decreased, until \( F_{v}/F_{m} \) became less than \( \Delta F/F_{m} \) by the end of the photoperiod. This pattern was observed on both days 14 and 27 July, and can be explained by the formation of NPQ in the dark. The finding of photoinhibition in microphytobenthos biofilms may be considered as unexpected, since decreases in community-level photosynthesis under high light are usually not observed (Kromkamp et al., 1998; Underwood and Kromkamp, 1999; Underwood, 2002). Hysteresis in the photosynthetic light response of microphytobenthos has been described before, but of a much reduced importance \cite{serodio2007}. Furthermore, hysteresis patterns were found despite the interruption of the high light exposure provided by high tide, which additionally allowed to ‘reset’ the high levels of salinity, temperature and other potentially stressful conditions such as supersaturated oxygen concentrations and low carbon and nutrient levels. These results may thus be seen as contradicting the often put forward hypothesis that photosynthetic vertical migration represents an effective mechanism against photoinhibition. In particular, the hypothesis of ‘micromigration’, by which stressed cells in the photic zone are continuously replaced by others in better physiological condition \cite{Kromkamp1998, Underwood2005}, would predict a full recovery during the afternoon low-tide periods and the absence (or large reduction) of hysteresis. In contrast, the obtained results suggest that, under natural conditions, either micromigration does not occur or it does not result in significant beneficial effects regarding community-level photoinhibition. However, these results may not be generalized to the whole year, as the photoprotection capacity of microphytobenthos communities from the same sampling site was found to vary (increase) significantly from summer to winter \cite{serodio2005}, which may allow a reduced sensitivity to high light and lower levels of photoinhibition.

The question remains open as to whether the hysteresis detected for the biofilm as a whole, is due to the change in the physiological status of the same microalgal population, or to the change in the microalgal population present at the sediment surface. The method used for characterizing the species composition of the biofilms did not allow for detecting short-term changes in the species present in the uppermost layers. Considering the downward migration of large numbers of microalgae during the morning low tide, it seems possible that a surface population change has occurred and that the observed biofilm-level photoinhibition resulted from the worst physiological condition of the microalgae remaining in the photic zone during late morning and afternoon. It may be hypothesized that the large productive biomass observed in the morning corresponds to a large population of motile microalgae that surfaces only during the first hours of the day, when irradiance is below damaging levels, the light absorbed during this period being sufficient to satisfy their carbon needs for the whole day. This would explain the considerable changes in the physiological condition of the biofilms from the morning to the afternoon photoperiods, and also the apparent overnight recovery of their physiological status, as denoted by the high values of the photophysiological parameters observed in early morning.

5. Conclusions

Benthic microalgae living on estuarine intertidal flats must cope with large and simultaneous changes in various environmental factors that affect their photosynthetic performance. In the case of diatom-dominated biofilms, the short-term responses to fast changing environmental conditions include not only physiological processes common to other photoautotrophs but also microalgal motility, through the regulation of the vertical position of the cells within the of the photic zone. The obtention of time series of chlorophyll fluorescence parameters \textit{in situ} permitted to realistically characterize both the photophysiological and migratory responses of microphytobenthos biofilms during diurnal low-tide periods, and to identify a number of aspects, some of them not previously described:

1. Photosynthetic activity is strongly dependent on recent light history, being severely depressed when the biofilm receives a high light dose during morning low tides.
2. Benthic microalgae undergo photophobic downward migration under high light, causing the maximum accumulation of cells at the sediment surface to occur during the early morning hours.
3. Microalgae respond to high light by activating energy-dissipating processes based on the xanthophyll cycle, reaching large NPQ levels which denote a high capacity for protection.
4. The light response of most photophysiological parameters may display hysteresis patterns, due to the incomplete recovery of photosynthetic activity during the afternoon. The magnitude of the hysteresis depends on the morning light history,
occurs despite the submergence in high tide during the period of the day of higher light stress.

5. The photoprotective mechanisms available to benthic microalgae, both physiological and behavioural, may not be sufficient to prevent the occurrence of photoinhibition.

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