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Physiological *versus* behavioral photoprotection in intertidal epipelic and epipsammic benthic diatom communities

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ABSTRACT

Physiological and behavioral photoprotection are the two major mechanisms by which natural microphytobenthic assemblages protect themselves against high light. These mechanisms were investigated with high vertical resolution in intertidal epipelic (mud) and epipsammic (sand) benthic diatom communities. Photophobic cell migration was found in epipelic communities when exposed to high light, detected using pigment analysis of 200 μm sediment depth layers and Low Temperature Scanning Electron Microscopy. In the mud, significant differences between migratory and non-migratory (Latrunculin A-treated) biofilms were observed in the photosynthetic activity measured using rapid light curves: after exposure to high light, non-migratory biofilms showed lower light use efficiency (lower α) and lower maximum photosynthetic capacity (lower $rETR_{max}$). Increased de-epoxidation state (DPS) was observed in both epipelic and epipsammic diatom assemblages after exposure to high light: in the surface 400 μm for mud and throughout the sediment profile up to 1 mm for sand. The two diatom communities showed different photoregulatory strategies: the epipelic community of muddy sediments photoregulated using both physiological and behavioral photoprotection, while the epipsammic community of sandy sediments used exclusively physiological mechanisms.

1. Introduction

Microphytobenthos (MPB) inhabiting intertidal mud and sand flats of estuaries and shallow coastal zones has been identified as one of the most important primary producers in these ecosystems (MacIntyre et al., 1996; Underwood and Kromkamp, 1999). MPB is largely dominated by diatoms, although other groups of phototrophs occur frequently, such as cyanobacteria and euglenids.

Diatoms which live in muddy sediments – referred to as epipelic – are known to exhibit partially endogenous vertical migratory rhythms synchronized with diurnal and tidal cycles (Admiraal, 1984; Consalvey et al., 2004; Round and Palmer, 1966; Serôdio et al., 1997). These microalgae accumulate at the surface of the sediment during diurnal low tides, forming dense, highly productive biofilms, visible to the naked eye, and migrate downwards before tidal inundation or darkness. Diatoms attached to the particles of sandy sediments – referred to as epipsammic – do not seem to show such migratory patterns (Admiraal, 1984; Jesus et al., 2009).

It has been hypothesized that vertical migration is also a response to external stimuli such as irradiance, as diatoms position themselves at the sediment depth of optimum light condition, while avoiding photoinhibitory light levels. This is often referred to as behavioral photoprotection (Admiraal, 1984; Kromkamp et al., 1998; Perkins et al., 2001; Serôdio et al., 2006). Additionally, diatoms use the xanthophyll cycle, the reversible de-epoxidation of pigment diadinoxanthin (DD) into the energy dissipating form diatoxanthin (DT) as a physiological photoprotection mechanism. This non photochemical quenching (NPQ) mechanism diverts excessive light energy from photosystem II reaction centers limiting damage to the photosynthetic apparatus (see review by Goss and Jakob, 2010).

The development of an exceptional high capacity for rapid and large NPQ induction under light stress has been shown for planktonic diatoms (Lavaud et al., 2002; Ruban et al., 2004) and cell suspensions of benthic diatoms (Serôdio et al., 2005). In undisturbed benthic diatom communities, the characterization of photophysiological responses to high light as been hampered by the interference of vertical migration and depth-integration on the analysis of variable chlorophyll fluorescence (Jesus et al., 2006a; Perkins et al., 2002; Serôdio, 2004; Serôdio et al., 1997). Recently, Cartaxana and Serôdio (2008) used a diatom motility inhibitor (Latrunculin A, Lat A) to demonstrate the importance of vertical migration in regulating light

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exposure. Using the same method, Perkins et al. (2010) have compared behavioral and physiological down regulation in an epipelic diatom community and concluded that vertical cell movement was the primary response to increasing light dose. Although many studies have dealt with the photophysiology of intertidal epipelic diatoms (e.g. Cartaxana and Serôdio, 2008; Perkins et al., 2001, 2010; Serôdio et al., 1997) there is still a big gap in current knowledge concerning epipsammic communities (Jesus et al., 2009).

It was the objective of this study to compare physiological and behavioral photoprotection of intertidal epipelic and epipsammic diatom communities of the Tagus estuary mud and sand flats. This study compared sediment profiles using High Performance Liquid Chromatography (HPLC) pigment analysis performed on 200 μm sediment depth layers, as well as Low Temperature Scanning Electron Microscopy (LTSEM) to analyze behavioral *versus* physiological processes with high vertical resolution in these two contrasting biofilm types. Furthermore, the application of Lat A allowed the comparison of undisturbed non-migratory (depending exclusively on physiological processes) and migratory (potentially depending on both physiological and behavioral processes) biofilms.

2. Materials and methods

2.1. Experimental design and sampling

Sediment cores (8 cm diameter) were collected on the 7th and 9th of April 2008 from Alcochete mud and sand flats, located on the eastern shore of the Tagus Estuary (38 44′ N, 9 08′ W). Two sites were sampled: a muddy site (silt fraction, <63 μm: 97%) on the first day, and on the second sampling day a sandy site composed of a mixture of very fine to coarse sand, between 125 and 1000 μm (for more details see Jesus et al., 2006b), hereafter called mud and sand, respectively.

For each sediment type, one core was randomly selected and the minimum fluorescence (F_0) measured every 7 min using a Pulse Amplitude Modulation (PAM) fluorometer (Diving PAM, Walz, Germany), in total darkness to detect and follow endogenous vertical migration rhythms. Light attenuation throughout the sediments was measured using a fiber optic microsensor controlled with a micromanipulator (MM33, Märtzhäuser, Germany), using 7 replicates for mud and sand. The procedure was adapted from Lassen et al. (1992). Light attenuation coefficients (k) were estimated by fitting an exponential function to the vertical profiles of irradiance. Remaining sediment cores were left overnight in the laboratory with a shallow depth of *in situ* water (±2 cm), carefully added so as not to re-suspend the sediment.

The day following sampling, the overlying water of the cores was carefully removed according to the tidal cycle (i.e. when the sampling site became emerged) and the cores were exposed to low light (15 μmol photons m^{-2} s^{-1}) to promote cell migration to the sediment surface. Light was measured with the Diving PAM fiber quantum sensor. Minicores (2 cm diameter) were then sampled and the establishment of the biofilm at the sediment surface was assessed by the stabilization of the Normalized Vegetation Index (NDVI) measured by an USB2000 spectroradiometer (Ocean Optics, Dunedin, USA). Rapid-light curves (RLCs) were performed using a PAM fluorometer (Diving PAM, Walz, Germany) in three sediment minicores. These minicores were frozen in liquid nitrogen (LN) and placed face down on a foil (so that the surface was always identifiable), wrapped, and stored at −80 °C for High Performance Liquid Chromatography (HPLC) pigment analysis and Low Temperature Scanning Electron Microscopy (LTSEM).

The other minicores were divided between 2 treatments: Control (addition of filtered site water only), and Latrunculin A (Lat A, dissolved in filtered site water) to inhibit cell motility. After 30 min at low light (15 μmol photons m^{-2} s^{-1}), three minicores of each treatment were transferred to high light (HL, 1200 μmol photons m^{-2} s^{-1}) for 1 h. RLCs were then performed on the samples after HL exposure and the minicores immediately frozen in LN for HPLC and LTSEM. Finally, three minicores for each treatment were transferred from low light to the dark for 2 h, after which the minicores were frozen in LN for LTSEM. The experiment described was done for the two sediment types: mud and sand.

2.2. Chemical treatments

A concentrated Latrunculin A (Lat A) stock solution (1 mM) was prepared by dissolving purified Lat A (Sigma-Aldrich) in dimethylsulfoxide. A solution of 20 μM Lat A was then prepared by diluting the appropriate amount of the concentrated stock solution in filtered water collected at the sampling site. Small volumes of this solution (total of 200 μL) were applied to undisturbed sediment samples by carefully pipetting directly onto the sediment surface, until forming a continuous thin layer that completely covered the sample. The amount of Lat A used was previously determined to be sufficient to inhibit diatom migration in benthic biofilms (Cartaxana and Serôdio, 2008). 200 μL of *in situ* filtered water was added to all control minicores to mimic chemical treatments but without addition of Lat A.

2.3. Rapid light response curves

RLC were carried out with the Diving PAM internal halogen light source using 9 incremental light steps (0, 100, 200, 295, 404, 592, 807, 1200 and 1700 μmol photons m^{-2} s^{-1}) each with 30 s duration. RLC parameters $rETR_{max}$ (relative maximum electron transport rate), α (initial slope of the RLC), and E_k (light saturation coefficient) were estimated by adjusting the model by Platt et al. (1980) to the experimental data.

2.4. HPLC pigment analysis

The frozen sediment minicores were divided in two halves, one for HPLC pigment analysis and the other for LTSEM. The sediment blocks for HPLC were cut into slices using a freezing microtome (1320, Leitz, Germany). The sediment was sectioned into the following depth intervals: 0–200, 200–400, 400–600, 600–800, and 800–1000 μm. The sections were placed in 1.5 mL Eppendorfs (pre-weighed), freeze-dried and weighed prior to analysis.

Freeze-dried sediment was extracted in 95% cold buffered methanol (2% ammonium acetate) for 15 min at −20 °C, in the dark. Samples were sonicated (1210, Bransonic,USA) for 30 s at the beginning of the extraction period. Extracts were filtered (Fluoropore PTFE filter membranes, 0.2 μm pore size) and immediately injected in a HPLC system (LC10ADVP, Shimadzu, Japan) with photodiode array and fluorescence (Ex. 430 nm; Em. 670 nm) detectors (Cartaxana and Brotas, 2003). Chromatographic separation was carried out using a C18 column for reverse phase chromatography (Supelcosil; 25 cm length; 4.6 mm diameter; 5 μm particles) and a 35 min elution program. The solvent gradient followed Kraay et al. (1992) with a flow rate of 0.6 mL min^{-1} and an injection volume of 100 μL. Pigments were identified from absorbance spectra and retention times and concentrations calculated from the signals in the photodiode array or fluorescence detectors. Calibration of the HPLC peaks was performed using commercial standards from DHI (Institute for Water and Environment, Denmark). Samples were analyzed for Chlorophyll *a* (Chl *a*) and the xanthophyll pigments diadinoxanthin (DD) and diatoxanthin (DT). The de-epoxidation state (DPS) was calculated as DT/(DD + DT).

2.5. Low Temperature Scanning Electron Microscopy (LTSEM)

Sediments were visualized using the Low Temperature Scanning Electron Microscopy (LTSEM) method described by Paterson (1995). Observations were made using a Cryotrans System (CT 1500, Oxford, U.K.) attached to a Scanning Electron Microscope (35CF, Jeol, Japan).

Photographs were taken from the sediment surface and from vertical profiles allowing the observation of biofilm changes on and within the sediment matrix.

2.6. Statistical analysis

The existence of significant differences on Chl *a* concentrations in sediment profiles under low light was tested using two-way analysis of variance (ANOVA) for effects of sediment type and depth. Student's *t*-test was done to evaluate significant differences in light attenuation coefficients between mud and sand. The existence of significant differences in Chl *a* and DPS was tested using two-way ANOVA for effects of depth and light/chemical treatment. One-way ANOVA was used to evaluate the effect of light/chemical treatment on RLC parameters. ANOVA assumptions were verified and data transformed whenever necessary using a logarithmic transformation. Multiple comparisons among pairs of means were performed using Tukey's HSD (Honestly Significant Difference). All statistical analyses were carried out using Statistica 9.0 (StatSoft Inc., USA).

3. Results

Measurements of F_0 on undisturbed sediment cores kept in the dark (Fig. 1) confirmed the presence of endogenous rhythms in the epipelic biofilms of muddy sediments and the absence of these rhythms in the epipsammic communities. Relative fluorescence increased up to a factor of 2.6 in the mud and 1.17 in the sand (Fig. 1). In the mud, relative fluorescence started to increase *ca.* 2 h before the beginning of the period of emersion at the sampling site, reaching the maximum value half-way through low tide.

The chlorophyll *a* (Chl *a*) profiles of sediments exposed to low light (15 μmol photons m^{-2} s^{-1}) are shown in Fig. 2. There were significant effects of sediment type ($F_{1,20} = 267.9$, $p<0.001$) and depth ($F_{4,20} = 4.615$, $p<0.01$) on Chl *a* concentrations. Although Chl *a* was highly variable due to sediment patchiness, concentrations were higher in mud showing consistently decreasing concentrations with depth. In the sand concentrations were lower and homogeneous throughout the sediment profile (Fig. 2).

Microscopic examination by LTSEM revealed that muddy sediment had extremely dense and thick surface diatom biofilms (Fig. 3A, C and E). These benthic diatom communities were composed of motile pennate diatoms, particularly naviculoids (Fig. 3 C). Several species of *Gyrosigma* (*G. limosum*, *G. fasciola* and *G. acuminatum*), *Nitzschia* sp., *Surirella* sp. and *S. curvifacies*, *Diploneis didyma* and *Plagiotropis* sp. were also present

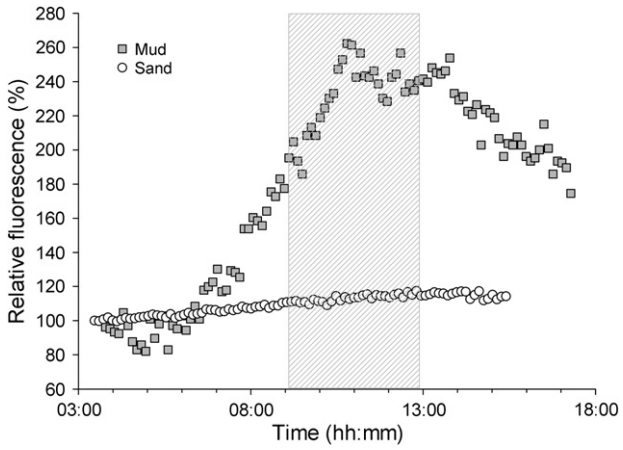

Fig. 1. Relative fluorescence (%) change with time for intertidal muddy and sandy sediments kept in the dark. Shaded area represents the estimated period that the mud would be emerged in the sampling site.

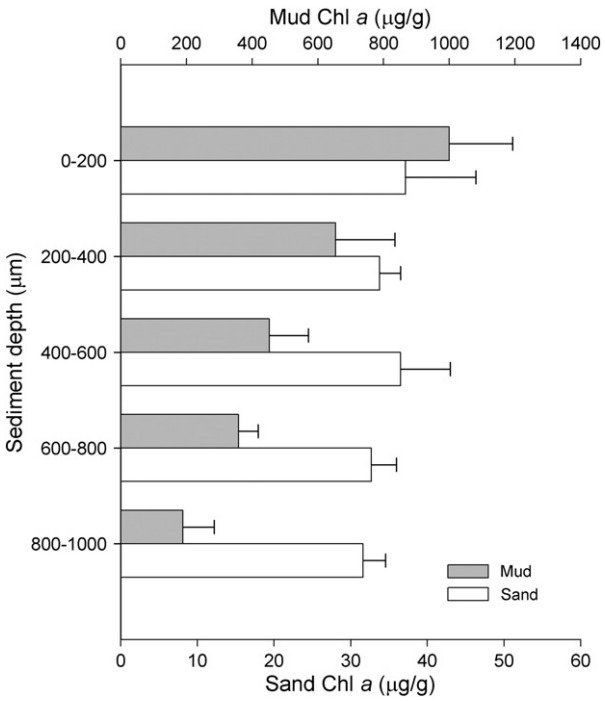

Fig. 2. Chlorophyll *a* (Chl *a*, μg/g dw, mean ± standard error) sediment depth profiles under low light for intertidal muddy and sandy sediments.

in the epipelic community (see also Fig. 6). Sandy sediments showed much lower cell density and the diatom community was mainly composed by small sessile diatoms attached to sand grains (Fig. 3B and D). However, large epipelic diatoms were occasionally found in the sand (Fig. 3F).

Light availability in the sediment profiles was considerably different between mud and sand. Although in both sediments surface irradiance decreased with depth (Fig. 4), light attenuation coefficients were significantly (*t*-test, $p<0.001$) higher in the mud ($k = 8.6 \pm 0.9$ mm^{-1}) than in the sand ($k = 1.6 \pm 0.5$ mm^{-1}). Light availability in the mud decreased to approximately 33 and 7% at depths of 200 and 400 μm, respectively. At these depths, light availability in the sand was 80 and 55%, respectively. At a depth of 3 mm, 2% of light in the sand was still available, whereas in the mud microalgae cells below 600 μm were exposed to less than 1% of incident light (Fig. 4).

Fig. 5 depicts the Chl *a* profiles in intertidal muddy and sandy sediments for different light and chemical treatments. In the mud, there was a significant ($F_{4,30} = 15.78$, $p<0.001$) effect of depth in the Chl *a* concentration but no significant treatment effect. Nevertheless, there was clearly a decrease in surface (0–200 μm) Chl *a* for the high light control sediments (Cont HL) when compared to low light (LL) and high light Lat A treated sediments (Lat A HL; Fig. 5A). In LTSEM micrographs it was clear that surface cell density was lower in Cont HL (Fig. 6A) than in Lat A HL (Fig. 6B) or LL (Fig. 3A, C and E). This indicates that cells migrated downwards in response to high light, as shown by the increase of Chl *a* in Cont HL observed at the 200–400 μm depth layer (Fig. 5A). This photophobic migration did not occur on Lat A HL sediments due to the presence of the motility inhibitor (Figs. 5A and 6B). The differences in surface cell density between Cont and Lat A treated sediments were particularly obvious in LTSEM micrographs after 2 h of darkness, with much denser biofilms in Lat A (Fig. 6D) than in Cont samples (Fig. 6C).

No significant effects of depth or treatment were observed for Chl *a* in the sand (Fig. 5B). Chl *a* concentrations in the sand were homogeneous throughout the sediment profile. No differences between light and chemical treatments were visible in LTSEM micrographs of sandy sediments (data not shown).

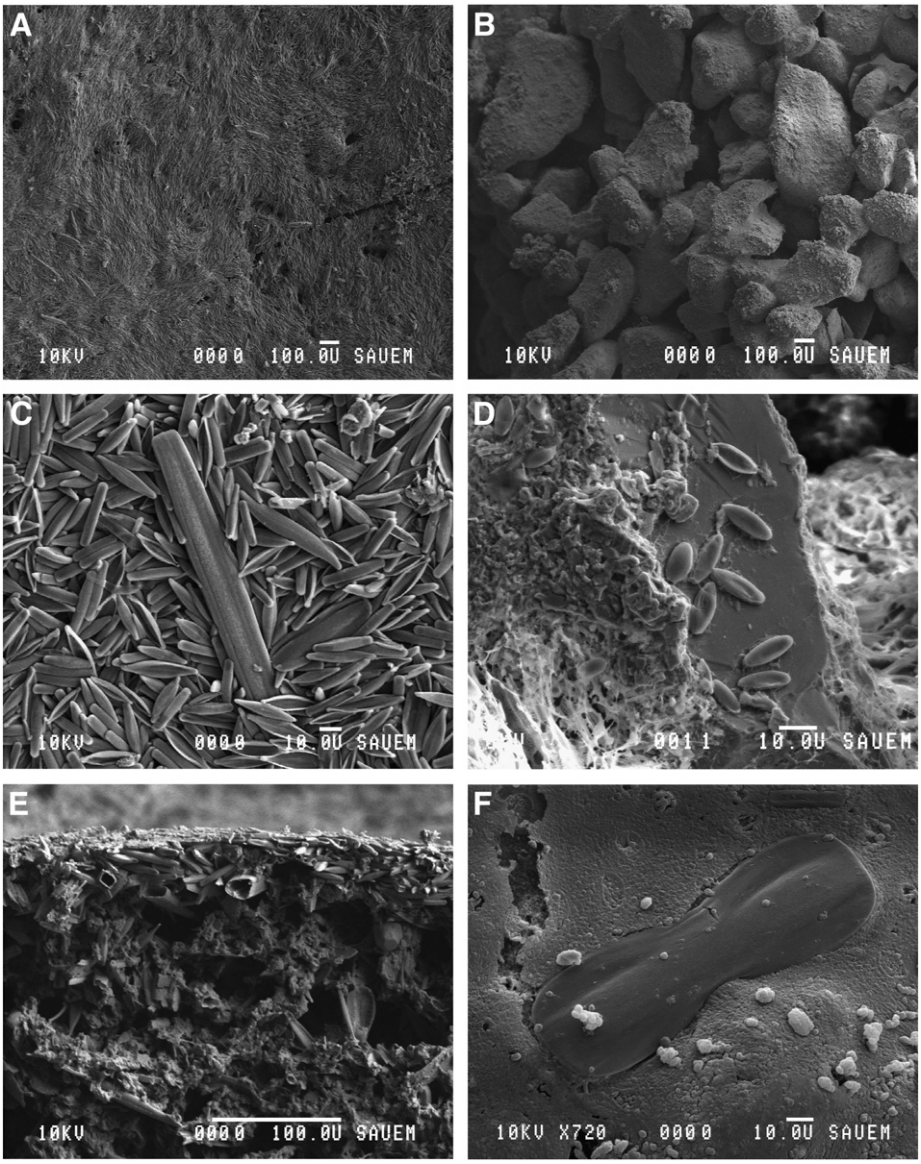

Fig. 3. Low-temperature scanning electron micrographs of the intertidal sediment. (A) General view of the muddy sediment showing a compact surface with an extremely dense diatom biofilm; (B) General view of the porous sandy sediment with sand grains; (C) Higher magnification of the surface of the muddy sediment showing a benthic community dominated by naviculoid diatoms; (D) Higher magnification of the surface of the sandy sediment showing a few small diatom cells attached to sand grains; (E) Fracture face of a muddy sediment showing an extremely dense surface biofilm (*ca.* 50 μm depth) and few diatoms deeper in the profile, probably migrating upwards; (F) *Plagiotropis lepidoptera* cell at the surface of the sandy sediment.

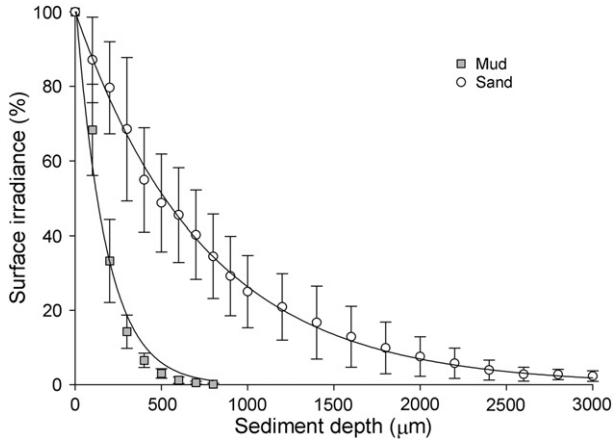

Fig. 4. Percentage of surface irradiance (mean ± standard deviation) along sediment depth profiles for intertidal muddy and sandy sediments.

Fig. 7 depicts the de-epoxidation state (DPS) profiles in intertidal muddy and sandy sediments for different light and chemical treatments. In the mud, there were significant effects of depth ($F_{4,30} = 4.579$, $p < 0.01$) and treatment ($F_{2,30} = 31.2$, $p < 0.001$) on DPS. Significantly (Tukey's, $p < 0.05$) lower DPS levels were found in LL than in Cont HL and Lat A HL for the 0–200 and 200–400 μm sediment layers (Fig. 7A). Although no significant differences were found in pair comparisons between Lat A HL and Cont HL, DPS values were consistently higher for Lat A HL than for Cont HL for the illuminated layers of the mud (0–600 μm; Fig. 7A). In the sand, there was a significant effect of treatment ($F_{2,30} = 10.55$, $p < 0.001$) on DPS and no significant effect of depth. Although no significant differences were found in pair comparisons, there was a consistent trend of lower DPS for LL, intermediate for Cont HL, and higher for Lat A HL throughout the sediment profile (Fig. 7B). DPS levels were also consistently higher in the sand than in the mud (Figs. 7A and B).

In Fig. 8 are depicted the main differences between the RLCs parameters $rETR_{max}$ (relative maximum electron transport rate), α (initial slope of the RLC) and E_k (light saturation coefficient) for the

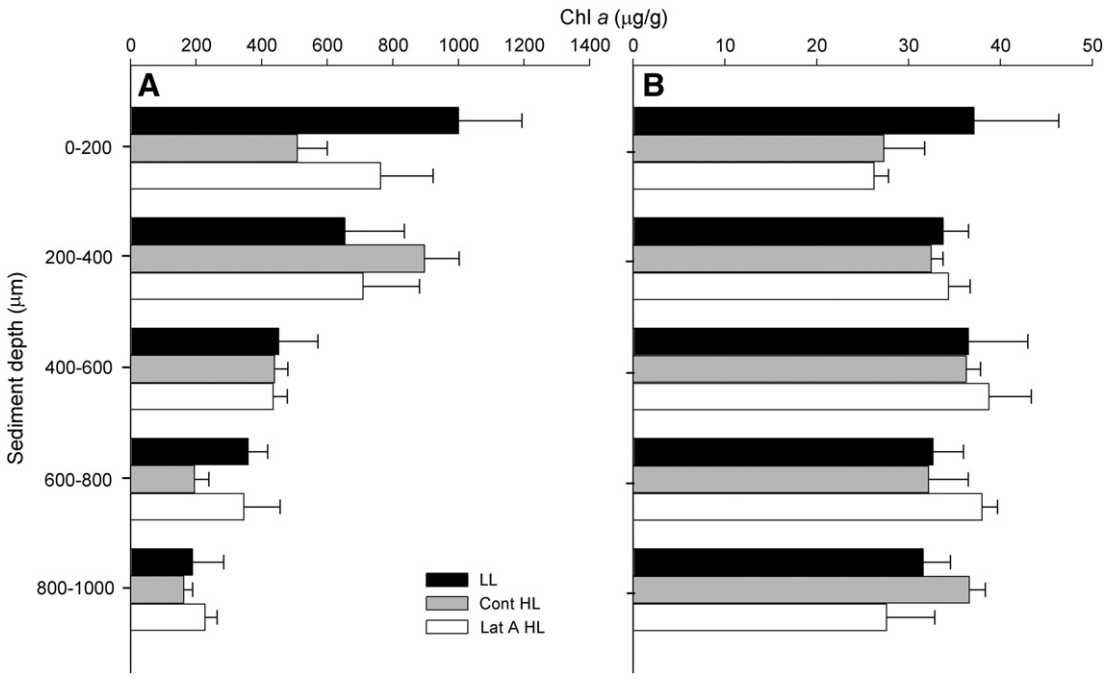

Fig. 5. Chlorophyll *a* (Chl *a*, μg/g dw, mean ± standard error) sediment depth profiles for intertidal muddy (A) and sandy (B) sediments. LL: Low Light; Cont HL: Control High Light; Lat A HL: Latrunculin A High Light.

light/chemical treatments and sediment types. In the mud, there were significant effects of treatment on all three parameters: $rETR_{max}$ ($F_{2,6} = 30.49$, $p<0.001$), α ($F_{2,6} = 91.33$, $p<0.001$), and E_k ($F_{2,6} = 10.336$, $p<0.05$). Lat A HL treated sediments showed significantly (Tukey's, $p<0.01$) lower $rETR_{max}$ than LL and Cont HL sediments (Fig. 8A). The initial slope of the RLC, α, was significantly (Tukey's, $p<0.01$) higher at LL, intermediate at Cont HL, and lower at Lat A HL (Fig. 8B), whereas E_k was significantly (Tukey's, $p<0.01$) higher at Cont HL than at Lat A HL (Fig. 8C). All three RLC parameters were significantly lower for Lat A HL than for Cont HL.

In the sand, there were significant effects of treatment on both $rETR_{max}$ ($F_{2,6} = 8.885$, $p<0.05$) and α ($F_{2,6} = 15.167$, $p<0.01$), but no

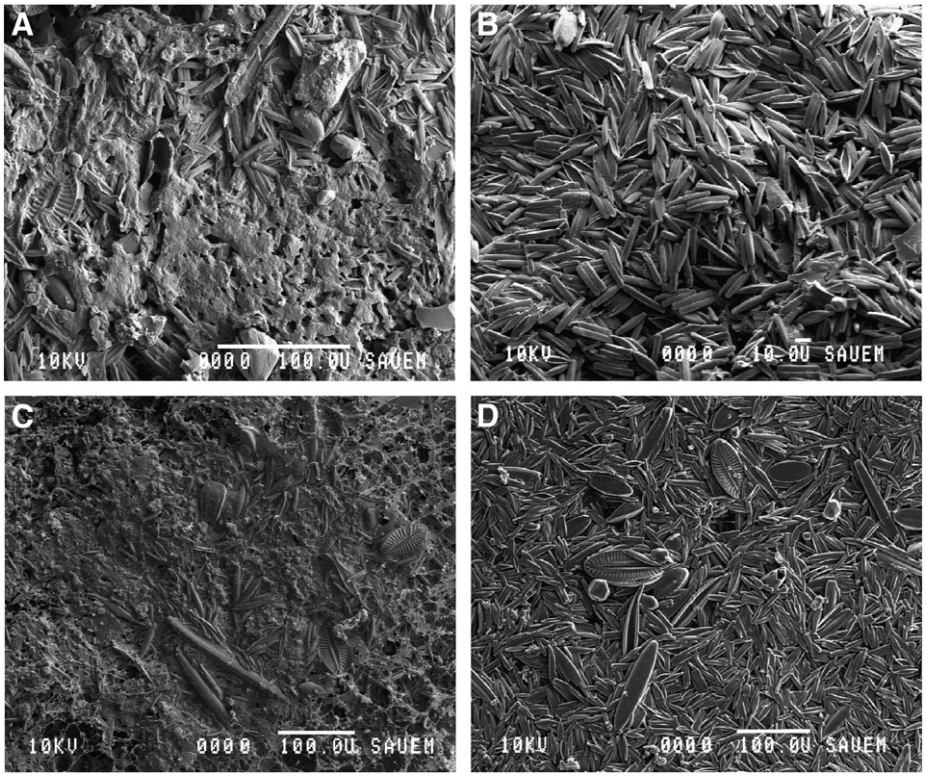

Fig. 6. Low-temperature scanning electron micrographs of the muddy intertidal sediment. Micrographs show lower cell density at the surface in Control High Light (A) than in Latrunculin A High Light (B), and in Control Dark (C) than in Latrunculin A Dark (D).

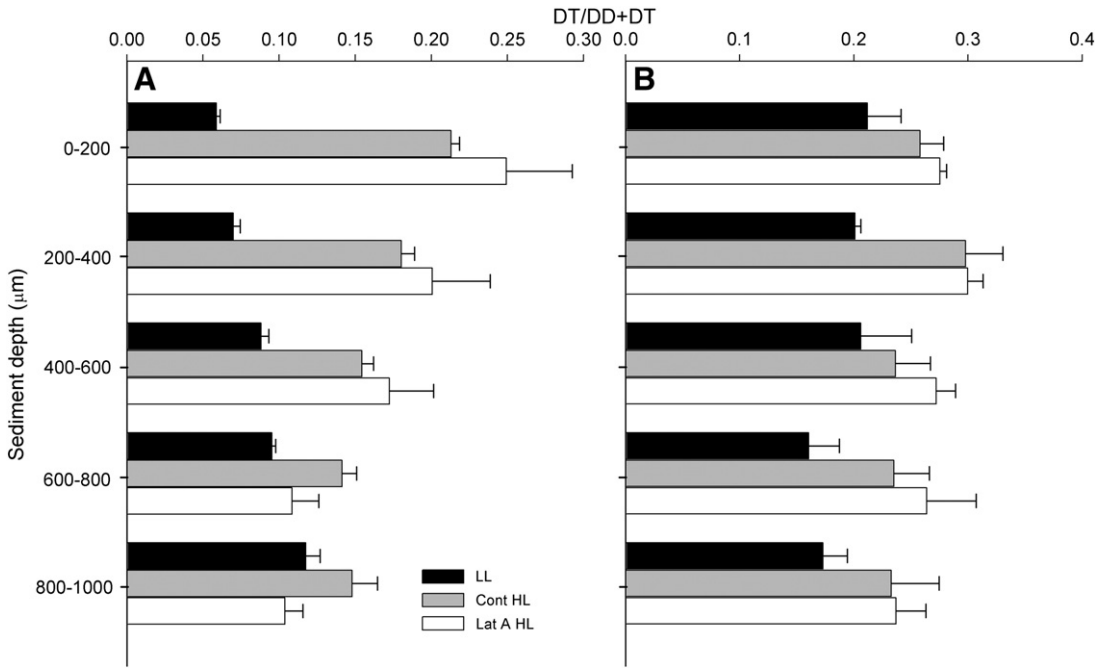

Fig. 7. De-epoxidation state (DT/DD + DT, mean ± standard error) in sediment depth profiles for intertidal muddy (A) and sandy (B) sediments. LL: Low Light; Cont HL: Control High Light; Lat A HL: Latrunculin A High Light.

significant effect on E_k. Lat A HL treated sediments showed significantly (Tukey's, $p<0.01$) lower $rETR_{max}$ than LL, but no significant differences were found between Lat A HL and Cont HL treatments (Fig. 8A). Low light (LL) sediments showed significantly (Tukey's, $p<0.05$) higher α values than Cont HL or Lat A HL (Fig. 8B). No significant differences were observed between Lat A HL and Cont HL for any of the three RLC parameters.

4. Discussion

Several differences were found between epipelic and epipsammic benthic diatom communities of the Tagus estuary. First, as expected, endogenous vertical migration was only observed in the epipelic community of muddy sediments. Curiously, accumulation of biomass at the sediment surface started *ca.* 2 h before the daytime emersion period, indicating that epipelic diatoms anticipate periods of favorable conditions for photosynthesis, as sun rises and tide ebbs. The predominance of epipelic taxa in muddy sediment and of epipsammic diatom cells in the sand was confirmed by LTSEM, as previously reported by other authors (e.g. Jesus et al., 2009; Saburova et al., 1995; Yallop et al., 1994). In addition, light attenuation was found to decrease with increasing sediment particle size, as shown by Kühl et al. (1994). Light attenuation was much stronger in the mud and light availability restricted to the upper 600 μm. Photic depths of 270 to 400 μm have been reported for muddy sediments (Baillie, 1987; Kromkamp et al., 1998; Serôdio et al., 1997). In the sand, photic depth extended below 3 mm. MPB biomass vertical distribution in intertidal mud and sandflats of the Tagus estuary was considerably different depending on the type of sediment. In the mud, a steep decrease in biomass accumulation from the surface down to 1 mm was observed, whereas in the sand Chl *a* concentrations were relatively stable throughout the profile. Similar biomass vertical distributions in sediments were observed by Wiltshire (2000) and Cartaxana et al. (2006).

Benthic diatom cells can respond to changing light environments, namely super-saturating light levels, through two mechanisms: i) physiological down regulation, often referred to as non-photochemical quenching (NPQ), involving de-epoxidation of DD to DT (e.g. Jesus et al., 2008; Serôdio et al., 2005) and/or ii) downward vertical cell movement within the sediment profile, referred to as behavioral photoprotection (Admiraal, 1984; Cartaxana and Serôdio, 2008; Jesus et al., 2006a; Kromkamp et al., 1998; Perkins et al., 2001; Round, 1979; Serôdio et al., 2006). To determine the relative importance of these two mechanisms a diatom motility inhibitor was used: Latrunculin A (Lat A). The effectiveness of Lat A in inhibiting diatom migration was shown by the different distribution of Chl *a* in sediment profiles and LTSEM micrographs of migratory control and Lat A treated sediments. This is in agreement with work by Cartaxana et al. (2008), Cartaxana and Serôdio (2008) and Perkins et al. (2010) using this highly specific diatom motility inhibitor.

In the mud, the decrease of Chl *a* in the sediment layer 0–200 μm (and the increase at 200–400 μm) for Cont HL as compared to LL or Lat A HL indicated that, when exposed to high light levels, epipelic diatoms responded with downward cell movement within the sediment profile. This was particularly clear in LTSEM micrographs, showing much lower cell densities in the sediment surface of Cont HL. The same was not observed for epipsammic diatoms of sandy sediments. Light-induced migration of diatom cells away from the sediment surface during periods of high irradiance was previously observed in epipelic benthic communities (Cartaxana and Serôdio, 2008; Kromkamp et al., 1998; Perkins et al., 2001; Perkins et al., 2010; Serôdio et al., 2006), but never demonstrated with this level of vertical resolution. Significant differences between Cont HL and Lat A HL in muddy sediments were also observed for the photosynthetic activity: after exposure to high light, non-migratory biofilms showed lower light use efficiency (lower α) and lower maximum photosynthetic capacity (lower $rETR_{max}$) than migratory control biofilms.

Biofilms exposed to high light (i.e. Cont HL and Lat A HL) showed higher DPS than sediment exposed to low light. In the mud, this was observed in the 0–200 and 200–400 μm sediment layers, whereas for the sand it was found throughout the sediment profile. Again, this was never demonstrated with this level of vertical resolution. In both mud and sand, differences were observed in both DT/DD + DT and DT/Chl *a* patterns, but not for DD + DT/Chl *a* (data not shown). This suggests that DT is formed from the de-epoxidation of DD through the xantophyll cycle and not by *de novo* synthesis. However, exposure of planktonic diatoms to excess light beyond 15–30 min has been shown

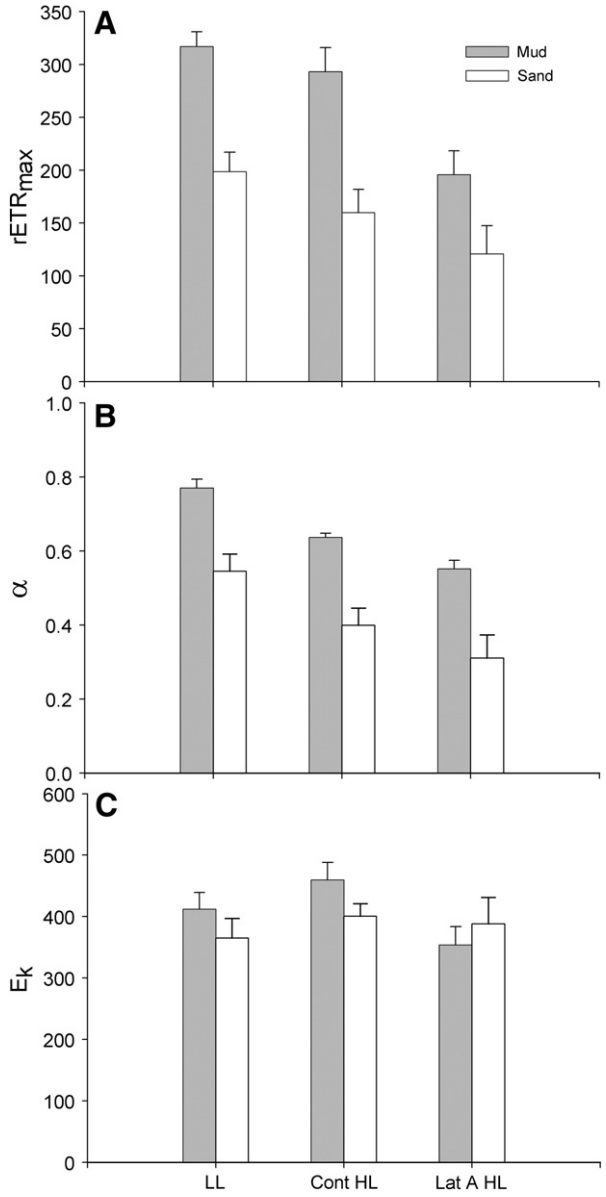

Fig. 8. Relative maximum electron transport rate ($rETR_{max}$, A), initial slope of the rapid light curves (α, B) and light saturation parameter (E_k, C) for intertidal muddy and sandy sediments (mean ± standard deviation). LL: Low Light; Cont HL: Control High Light; Lat A HL: Latrunculin A High Light.

to cause *de novo* synthesis of DT (Lavaud et al., 2004; Olaizola et al., 1994).

DPS levels were consistently higher in the sand, under all light levels, than in the mud, indicating that physiological processes of photoprotection were probably more important in epipsammic diatoms than in motile epipelic communities. Nevertheless, our results show that both epipelic and epipsammic microalgae benthic communities use the xanthophyll cycle as a photoprotection mechanism upon exposure to high light levels whether cells maintain their migratory capacity or not. Perkins et al. (2010) reported greater NPQ induction in non-migratory Lat A treated muddy sediments of the Tagus estuary and little or no NPQ induction in control sediments exposed to high light levels. Two differences between this study and that of Perkins et al. (2010) can explain these apparently contradictory results for epipelic communities. First, a high resolution sectioning technique was used in our study leading to the determination of DPS levels in 200 μm sediment layers, whereas in Perkins et al. (2010) DPS was integrated approximately over the surface 2000 μm. In the latter study, microspatial variation in pigment composition may have been lost due to "dilution" by pigments from deeper layers (Wiltshire, 2000). Alternatively, the high light dose was both stronger (occasionally over 2000 μmol m^{-2} s^{-1}) and more prolonged (up to 6 h) in Perkins et al. (2010) study, leading possibly to a different response of the MPB community, such as a much stronger photophobic downward migration by diatom cells of migratory biofilms. Chevalier et al. (2010) have shown higher DPS in the surface 1 mm sediment layer of intertidal mudflats during two consecutive daylight emersion periods.

DPS in epipelic benthic microalgae of muddy sediments exposed to low light were lower at the surface 0–200 μm and 200–400 μm, where light was available, than in the deeper layers. Jakob et al. (1999) observed decreased NPQ and DT content in a planktonic diatom upon exposure to low irradiance after prolonged darkness and other studies have reported formation of NPQ in the dark for diatoms (e.g. Jesus et al., 2006a; Mouget and Tremblin, 2002; Serôdio et al., 2005). This was hypothesized as an adaptation to prevent degradation of xanthophyll cycle pigments, providing readily functional photoprotection upon re-illumination by otherwise photodamaging light levels (Jakob et al., 1999). In MPB this could be particularly important as factors such as tidal and day/night cycles, migration, and occasional resuspension can cause rapid changes in light exposure.

In this study, the two diatom communities of the Tagus estuary showed different photoregulatory strategies: the epipelic community of muddy sediments used both physiological and behavioral photoprotective mechanisms; and the epipsammic community of sandy sediments, without the ability to migrate, used solely physiological mechanisms. We conclude that exposure to high light triggers the xantophyll cycle in both epipelic and epipsammic benthic natural communities. This response is restricted to an extremely thin sediment layer in muddy sediments and extends deeper in the sand due to differences in light penetration.

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