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Effects of short-term changes in sediment temperature on the photosynthesis of two intertidal microphytobenthos communities

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ABSTRACT

Intertidal microphytobenthos (MPB) has been found to maintain high productivity rates despite the variability in various key environmental parameters, namely rapid temperature changes during emersion. The effects of short-term (30 min and 2 h) changes in temperature (15, 25, 35 and 42 °C) on the photosynthetic activity of two intertidal MPB communities (Trancão and Alcochete) of the Tagus estuary were studied using imaging pulse amplitude modulated (Imaging-PAM) fluorometry. MPB communities differed in species composition and size-class distribution: Trancão was dominated by diatoms of the size-class 100–250 μm^3, particularly *Navicula* cf. *phyllepta*, whereas Alcochete had higher relative abundances for size-class 250–1000 μm^3, dominated by a mixture of diatom species of the genera *Navicula*, *Thalassiosira* and *Gyrosigma*. The Trancão MPB community had higher photosynthetic capacity (higher ETR_{max}), was photoacclimated to higher irradiances (higher E_k) and had lower efficiency at limiting irradiances (lower α). The different taxonomic composition and size-class distribution could explain the observed results, as small cells are usually more active due to larger surface to volume ratios. Photosynthetic capacities of the two studied MPB communities increased with temperature until 35 °C. Photosynthetic efficiencies were not affected by temperature in the 15–35 °C range and both ETR_{max} and α decreased at the extreme temperature of 42 °C. MPB communities were able to increase photosynthetic capacity and productivity under transient exposure to high sediment temperatures, similar to that observed during summer midday low tides.

1. Introduction

The intertidal flats of estuaries are characterized by the presence of microphytobenthos (MPB) communities dominated by diatoms, forming highly dense biofilms on the sediment surface. Intertidal MPB communities are responsible for a significant fraction of the total primary productivity of estuaries (Underwood and Kromkamp, 1999), despite the extreme variability in various key environmental parameters (e.g. irradiance, salinity or temperature).

Intertidal sediment temperature fluctuations occur on long (seasonal) and short (daily and hourly) time scales, depending on factors such as meteorological conditions, time of day and tidal inundation. In summer, intertidal sediment temperature can easily change 10–15 °C during an emersion period, reaching values as high as 35 °C at midday (Blanchard et al., 1997; Serôdio and Catarino, 1999). Short-term (hours) temperature changes, similar to those experienced by intertidal MPB communities during a tidal cycle, have been shown to have a significant effect on the photosynthesis of cultured benthic diatoms (Admiraal, 1984; Morris and Kromkamp, 2003; Salleh and McMinn, 2011) and suspensions of intertidal MPB (Blanchard et al., 1996, 1997). In these studies, the described relationship between maximum photosynthetic capacity (P_{max}) and temperature is a progressive increase of P_{max} with increasing temperature up to an optimum value, beyond which P_{max} declines rapidly (Blanchard et al., 1996). Although both approaches (cultures and suspensions) may provide valuable information regarding the potential photosynthetic responses of benthic diatoms to short-term changes in temperature, the results thus obtained may not accurately represent the photosynthesis of these organisms while part of an MPB biofilm.

The aim of this study was to characterize the effects of short-term temperature changes on the photosynthetic activity of two intertidal MPB communities of the Tagus estuary. Most studies on MPB ignore species composition and treat the assemblages as a black box (Underwood, 2005). In this study, we present a detailed

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description of the diatom taxonomic composition of the two studied MPB communities. A chlorophyll (Chl) *a* fluorescence imaging system was used to determine photosynthetic parameters, allowing the simultaneous analysis of several sediment samples. We hypothesize that community-level photosynthetic rates and productivity of intertidal MPB biofilms increase with transient high temperatures, similar to those observed during summer midday exposure.

2. Materials and methods

2.1. Sampling and sample preparation

Sediment samples were collected in two different intertidal flats of the Tagus estuary (Portugal) at Alcochete (38°44′45″N, 08°59′04″W) and Trancão (38°47′46″N, 09°05′33″W). Both sediments are fine mud with more than 97% particles <63 μm. Alcochete site is exposed for up to 3 h during low spring tides, being subtidal during neap tides. Trancão is a steep intertidal flat, exposed for periods of up to 6 h during low tide in both spring and neap tides. Sampling was carried out in June 2011 at spring tides when the intertidal flats were exposed. The surface layer of sediment (approximately the 0–1 cm) was collected using a spatula.

In the laboratory, the sediment was mixed and then evenly spread in trays to a depth of about 2 cm. The sediment was left overnight in the dark with a shallow depth of site water to mimic the tidal rhythm and avoid dessication. The following morning, at the start of the low tide emersion period predicted for the original sample site, the shallow layer of site water was removed. Portions of the surface layer of the sediment were transferred to 6-well plates using a small spatula, making sure the sediment reached the surface of the wells as the Imaging PAM is designed for measurements at a fixed working distance. The well plates were exposed to a homogeneous light field provided by a halogen lamp (Philips focusline, 250W) through fiberoptics 460-F (Walz GmbH, Effeltrich, Germany), delivering a constant irradiance of 60 μmol photons m^{-2} s^{-2} at the sample surface. Low light levels induced cell migration to the sediment surface and the formation of a biofilm. After 60 min of biofilm establishment, a total of eight 6-well plates were used: two sampling sites (Trancão and Alcochete) × four temperatures (15, 25, 35 and 42 °C). Temperature treatments were set using temperature-controlled water baths and sediment temperatures followed using a ScanTemp 410 infrared thermometer (Tematec GmbH, Hennef, Germany). Photosynthetic activity was assessed using non-invasive fluorescence analysis after 30 min and 2 h at each temperature.

2.2. Fluorescence analysis

Chlorophyll fluorescence was measured using an imaging-PAM fluorometer (Mini PAM M-Series, Walz GmbH) with an IMAGE-K5 1/2″ CCD camera (640 × 480 pixel resolution) equipped with a 16 mm objective. The 24 × 32 mm area imaged by the Mini version is illuminated by a powerful Luxeon LED array (460 nm) covering a 6-well plate, so that 6 sediment samples could be monitored simultaneously. The LED array provided the measuring beam, the actinic light and the saturation light pulses. The saturation pulse intensity was 8000 μmol photons m^{-2} s^{-1} for 0.8 s and the measuring pulse frequency was 10^{-3} kHz. Photosynthetic activity was assessed using rapid light curves (RLC). For the construction of RLC, the samples were exposed to 12 intensities of actinic light: 0, 3, 23, 43, 61, 111, 223, 320, 491, 624, 782 and 996 μmol photons m^{-2} s^{-1}. The duration of each irradiance step was 30 s. Numerical values and images of the chlorophyll fluorescence parameters were extracted from the digital images using analytical software (Imaging Win, Walz), selecting a priori areas of interest (AOI) that corresponded to the total sediment surface of each well. RLC were constructed by calculating, for each level of actinic light, the relative electron transport rate (r*ETR*) from the delivered actinic irradiance (E) and the effective quantum yield of PSII ($\Delta F/F'_m$) by $rETR = E \times \Delta F/F'_m$. The light response was characterized by fitting the model of Platt et al. (1980) to r*ETR* versus E curves and by estimating the initial slope of the light curve α (light utilization coefficient), ETR_{max} (maximum r*ETR*) and E_k (light saturation parameter), where $E_k = ETR_{max}/\alpha$.

2.3. MPB taxonomic composition

Sediment trays of both sampling sites were also subjected to low light levels (60 μmol photons m^{-2} s^{-2}) to induce cell migration to the sediment surface and the formation of a biofilm. Sediment samples were collected directly from the trays after 60 min of biofilm establishment by scraping the sediment surface with a scalpel (upper 2 mm). Approximately 3 mL of sediment per sample were collected and placed in disposable 5 mL polypropylene tubes, to which was added 1 mL of a 2.5% glutaraldehyde solution and stored at 4 °C for later processing. Cells were extracted from the sediment following an isopycnic separation technique using silica sol Ludox® HS-40 (Ribeiro, 2010) that separates the organic material from mineral particles and is, thus, able to remove both migratory and non-migratory fractions of the diatom assemblages, as well as cyanobacteria, euglenids and other microphytobenthic algal groups. However, optical microscope analysis of these extracts with an Olympus BX50 at a 400× magnification revealed that exclusively diatoms composed the MPB communities. Diatom analysis was conducted after cleaning the cells of organic material, by incinerating the extracts in a muffle oven during 2 h at 450 °C, leaving the diatom frustules intact. Permanent slides, mounted in Naphrax™, were made for each sample. Phase and differential interference contrast (DIC) optical microscopy were used to identify and count diatoms at a magnification of 1,000×. For each sample, a minimum of 400 frustules were counted and identified to the species level, following Ribeiro (2010) and references therein. Diatom taxa relative abundances were also allocated to four size-classes which comprised the average cell biovolumes of <100 μm^3, 100–250 μm^3, 250–1000 μm^3 and >1000 μm^3. Cell biovolume calculation was based on equations proposed by Hillebrand et al. (1999) and derived from biometric measurements made by Ribeiro (2010).

2.4. Statistical analysis

The data set was separated in two groups corresponding to the 30 min and 2 h measurements. The existence of significant differences was tested using two-way analysis of variance (ANOVA) for the effects of the independent variables temperature (15, 25, 35 and 42 °C) and sampling site (Alcochete and Trancão) on fluorescence RLC parameters (α, ETR_{max} and E_k). Data normality and homogeneity of variances were tested with Shapiro–Wilk and Bartlett tests, respectively. Data were transformed whenever necessary to comply with ANOVA assumptions. Multiple comparisons among pairs of means were performed using Tukey HSD. Statistical analyses were carried out using Statistica 10 (StatSoft Inc., USA).

3. Results

3.1. MPB taxonomic composition

A total of 42 diatom species were identified in the intertidal MPB communities of the two study sites of the Tagus estuary, 28 in