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Photoacclimation state determines the photobehaviour of motile microalgae: The case of a benthic diatom

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ABSTRACT

High productivity in intertidal microphytobenthic communities is achieved despite exposure to extreme and dynamic conditions (e.g. light, salinity, temperature). As an adaptation to this hostile environment, most of the microalgae species inhabiting fine-sediment habitats are motile, being able to migrate vertically within the uppermost layers of the sediment and actively regulating their exposure to light. In this work we tested the hypothesis that the migratory photobehaviour of benthic diatoms, the dominant group in microphytobenthic assemblages, is conditioned by their photophysiological state (i.e. photoacclimation). Unialgal cultures of the motile diatom *Navicula* cf. *recens* were grown under contrasting light regimes (20 and 300 μmol quanta m^{-2} s^{-1}) to induce different photoacclimation states. The migratory response to light was characterized by studying the distribution of motile cells along a light gradient (photoaccumulation curve), using a custom-build photoaccumulation chamber. The photoaccumulation curves were constructed by measuring the accumulation of cells along the light gradient using a light transmission index. The variation of the photophysiological state of the cells along the light gradient was measured using Pulse Amplitude Modulation (PAM) fluorometry (maximum quantum yield and light response curves of the relative electron transport rate of photosystem II). The results showed clearly different photoaccumulation curves for low and high-light acclimated cells. Although in both cases, cells avoided extreme low and high light levels, maximum cell accumulation was reached at markedly different light intensities depending on growth light conditions and resulting photoacclimation state: 72 and 104 μmol quanta m^{-2} s^{-1} for the low and high light-acclimated cells, respectively. Strong relationships were found between photophysiological parameters characterizing photoacclimation or susceptibility to photoinhibition and migratory light response, supporting that this diatom uses motility to select the optimal light exposure according to its photophysiological preferences.

1. Introduction

Estuaries are among the most productive and valuable ecosystems on the planet (Costanza et al., 1997; Underwood and Kromkamp, 1999). This high biological productivity is mainly due to the photosynthetic activity of microalgae, constituents of both phytoplankton and microphytobenthos (MPB). The MPB communities themselves may be responsible for more than half of the total carbon fixation in these environments, also contributing to the water column production (MacIntyre et al., 1996; Phinney et al., 2004; Underwood and Kromkamp, 1999). In estuarine intertidal flats, MPB form extensive biofilms that are the basis of food webs and contribute to the stabilization of sediments (Dejong and Dejonge, 1995; Hart and Lovvorn, 2003; Heip et al., 1995; Nienhuis, 1993; Paterson et al., 1990, 2003). These biofilms are generally dominated by diatoms which may exhibit one of the highest photosynthetic rates found among aquatic photoautotrophs (Krause-Jensen and Sand-Jensen, 1998; Lavaud, 2007; MacIntyre et al., 1996; Underwood and Kromkamp, 1999).

It is known for a long time that benthic diatoms are able to migrate vertically within the upper layers of the sediment (Barranguet et al., 1998; Fauvel and Bohn, 1907; Round and Palmer, 1966) often following daily and tidal rhythms (Palmer and Round, 1967; Paterson, 1986; Serôdio et al., 1997). Vertical migratory behavior has also been shown to occur as a response to changes in light conditions, with low light levels inducing the upward migration and high light levels triggering a downward response (Admiraal, 1984; Coelho et al., 2011; Mitbavkar and Anil, 2004; Paterson et al., 1998; Perkins et al., 2002; Serôdio et al., 2006a; Underwood and Kromkamp, 1999).

This light-induced migratory behavior and the similar dimensions of the cells and of the sediment photic zone have led MPB pioneer researchers to put forward what became known as the 'behavioral

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photoprotection' hypothesis (Admiraal, 1984), stating that benthic diatoms may regulate light exposure by adjusting their position along the vertical light gradient of the photic zone of the sediment, thus (i) optimizing light exposure and photosynthesis under low light and (ii) minimizing light absorption under high, potentially damaging, light levels. This form of photoprotection would operate complementary to the physiological processes common to other photoautotrophs, namely the thermal dissipation of excessive light energy through the xanthophyll cycle (Latowski et al., 2011; Lavaud, 2007; Lavaud et al., 2004; Muller et al., 2001), and the scavenging of harmful ROS by antioxidants (Cartaxana et al., 2013; Edreva, 2005; Mittler, 2002; Niyogi, 1999).

In fine muddy sediments, motile epipelic diatoms have the possibility to behaviorally control their exposure to light as the photic zone is typically less than 250 μm-deep (Consalvey et al., 2004; Forster and Kromkamp, 2004; Kromkamp et al., 1998; Serôdio et al., 2001). This corresponds to a vertical distance that can be covered by diatom cells within a few minutes, considering their relatively fast speed (up to 6–9 μm s^{-1}; Consalvey et al. (2004); van Leeuwe et al. (2008)). Due to its importance for understanding this unique situation among photoautotrophs, the high light-induced negative phototaxis of benthic diatoms has been extensively studied and recurrently interpreted as a form of behavioral photoprotection (Cartaxana et al., 2011; Jesus et al., 2006; Laviale et al., 2015; Mouget et al., 2008; Perkins et al., 2001, 2010; Serôdio et al., 2006a, 2012). However, no study has yet experimentally addressed the positioning and distribution of cells along the light gradient, and how this may optimize light exposure and minimize photoinhibition.This work was set out to experimentally test the 'behavioral photoprotection' hypothesis, by addressing the key assumption that the preference by a certain light level (depth) within the photic zone is related to the photoacclimation status and photoinhibition susceptibility of the microalgae, that is, that photobehaviour is in fact related to photophysiology. This was tested by using a 'photoaccumulation chamber', a custom-made device allowing to measure the accumulation of freely moving diatoms along a light intensity gradient and to simultaneously determine their photosynthetic activity under different light levels. This setup provided a way to test if (i) benthic diatoms in fact migrate and accumulate under a preferred light range in the presence of a light gradient, and (ii) if their light preference is determined by their photoacclimation status and susceptibility to photoinhibition. In order to compare the photobehaviour of cells with distinct tolerances to high light, cultures of a benthic diatom photoacclimated to different light intensities were compared.

2. Materials and methods

2.1. Culture conditions

A monoclonal culture of the species *N.* cf. *recens* (Lange-Bertalot) Lange-Bertalot was established by single cell isolation from a microphytobenthic community sampled on intertidal muddy sediments at Vista Alegre, Ílhavo, Portugal (Serôdio et al., 2008). *N. recens* is an epipelic diatom species, commonly found in brackish-waters. It has elliptic prism geometry and our specimens had an average length and width of 22.17–25.19 μm and 6.39–7.83 μm, respectively (Ribeiro (2010) and references therein). This species is also capable of gliding at relatively high speeds on microscope slides (8.09–11.12 μm s^{-1}; personal observations). Non-axenic batch cultures were grown in 500 ml glass bottles, using f/2-enriched natural seawater medium (Guillard and Ryther, 1962) at a constant temperature (18 ± 2 °C) under a 12:12 light–dark cycle, in a Sanyo Growth Cabinet MLR 350H (Sanyo, Moriguchi, Japan). Illumination was provided by a combination of fluorescent tubes (Philips TL-D 36W/54-765, 6200 K, Cool White and Philips TL-D 36W/830, 3000 K, Warm White, Philips, Amsterdam, Netherlands) and LED projectors (Ledi9 10W Cool White, Ledi9, Portugal). Cultures were maintained in exponential growth phase at two photosynthetically active radiation (PAR) levels, 20–25 μmol quanta m^{-2} s^{-1} ('low light'; LL) and 300–350 μmol quanta m^{-2} s^{-1} ('high light'; HL), for more than three months prior to the experiment. PAR was measured with a quantum sensor equipped with a semi-spherical sensor (Model MQ-200, Apogee Instruments, Logan, USA).

2.2. Characterization of the photoacclimation status

The photoacclimation status of the LL- and HL-grown cultures was characterized by in vivo chlorophyll *a* (Chl *a*) fluorescence measurements (PAM fluorometry) and pigment content quantification from samples collected just before the start of the photoaccumulation experiment (see Section 2.3).

2.2.1. PAM fluorometry

Chl *a* fluorescence measurements were carried out using PAM fluorometer (Schreiber et al., 1986) comprising a computer-operated PAM-Control Unit (Walz, Effeltrich, Germany) and a WATER-EDF-universal emitter-detector unit (Gademann Instruments GmbH, Würzburg, Germany). Measuring, actinic and saturating lights were provided by a blue LED (detailed description of the PAM unit in Serôdio (2004)). Measurements were done on 1 ml cell suspensions in a fluorescence cuvette (KS-101, Walz) using a 6 mm diameter fluid light guide fiber optics. The fluorometer was zeroed before each measurement, using fresh f/2 medium. Each sample was first dark-acclimated during 10 min and F_v/F_m (Kitajima and Butler, 1975) was measured by:

$$F_v/F_m = \frac{F_m - F_0}{F_m} \quad (1)$$

where F_0 is the minimal level of fluorescence and F_m is the maximum level of fluorescence reached during a saturating light pulse.

After the measurement of F_v/F_m, samples were exposed to 72 μmol quanta m^{-2} s^{-1} for 5 min, in order to allow for light activation of the photosynthetic apparatus and avoid confounding interferences from the Kautsky induction (Kautsky and Hirsch, 1931). Samples were then exposed to 8 successive steps (10 s each) of increasing light level up to 920 μmol quanta m^{-2} s^{-1}. A saturating light pulse was applied at the end of each step and the light-adapted fluorescence (F_s) and maximum light-adapted fluorescence (F_m') were measured to calculate the relative PSII electron transport rate (rETR) for each light level (E), as:

$$rETR = E\frac{F'_m - F_s}{F'_m}. \quad (2)$$

rETR vs E curve parameters, α (initial slope), ETR_m (maximum rETR) and E_k (light-saturation parameter) were estimated by fitting the model of Eilers and Peeters (1988). Non-photochemical quenching (NPQ) was calculated as in Serôdio et al. (2005a):

$$NPQ = \frac{F'_{m,m} - F'_m}{F'_m} \quad (3)$$

where $F_m'_{,m}$ is the maximum F_m' value measured in the whole LC.

NPQ vs E curves were described by fitting the model of Serôdio and Lavaud (2011) and estimating the parameters NPQ_m (maximum NPQ), E_{50} (irradiance level for reaching 50% of NPQ_m) and n (sigmoidicity coefficient):

$$NPQ(E) = NPQ_m \frac{E^n}{E_{50}^n + E^n} \quad (4)$$

Both models were fitted using a procedure written in Microsoft Visual Basic and based on Microsoft Excel Solver. Model parameters were estimated iteratively by minimizing a least-squares function,