Long- and short-term photoacclimation in epipsammon from non-tidal coastal shallows compared to epipelon from intertidal mudflat

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\textbf{ABSTRACT}

Long- and short-term photoacclimation and their interaction were determined in two types of microphytobenthos assemblages, i.e. epipelon from an intertidal mudflat and epipsammon from non-tidal sandy coastal shallows collected during summer and autumn months. Microphytobenthos photophysiology was assessed from steady-state light curve (SSLC) and rapid light-response curves (RLC) of variable chlorophyll fluorescence. The epipelon was low light acclimated, whereas in the epipsammon high light acclimation was observed. The epipelon turned out to be more susceptible to high light and in autumn a clear down turn in the relative electron transport rates was recorded. Long-term photoacclimation strongly affected both microphytobenthos types’ short-term light responses. The epipelon acclimated to high ambient light intensities through the decreased light absorption and energy dissipation. The epipsammon, on the other hand, developed physiological flexibility allowing efficient use of the absorbed light and thus providing protection against higher irradiance.

1. Introduction

Microphytobenthos is an ecological formation comprising photosynthetic organisms, including mainly: diatoms, cyanobacteria, euglenophytes, green algae etc., growing on various substrata in shallow aquatic ecosystems (Round, 1971). Microphytobenthos plays a key role as a significant primary producer (MacIntyre et al., 1996). It is an important source of food for benthic and pelagic organisms as well (Lucas et al., 2001). It stabilizes sediment surface through the mucilage production (Sutherland et al., 1998) and mediates numerous geochemical processes by regulating nutrients turnover in the sediment and their fluxes at the water-sediment interface (e.g. Sundbäck et al., 2006).

The biomass and functioning of microalgae in marine coastal ecosystems is controlled by various environmental factors, including: light, temperature, nutrients, tides and sediment type which appear to affect the presence of cyanobacteria and certain microalgal groups and thus the structure of the assemblage (MacIntyre et al., 1996). Consequently, microphytobenthos inhabiting soft sediments is traditionally separated into two groups, i.e. epipelon typical of muddy sediments, usually dominated by free-living, large, biraphid diatoms, and epipsammon present in sandy sediments, consisting of small-sized diatoms with a substantial contribution of other algal groups such as coccolid chlorophytes, euglenophytes etc., and cyanobacteria (Round, 1971). Sediment also determines light regime within, which depends on its granulometry (Kühel et al., 1994). This further affects microphytobenthos species composition, photosynthetic pigments, biomass distribution and primary productivity (e.g. Barranguet et al., 1998; Jesus et al., 2009).

Being exposed to highly dynamic environments, microphytobenthos optimizes its photosynthetic rates through photoacclimation, i.e. mechanisms allowing microalgae to efficiently use available light and protect themselves from excessive irradiance. Photoacclimation results from biophysical changes in photosynthetic systems and operates within constraints set by evolutionary adaptations (Falkowski and Raven, 1997; Talmy et al., 2013). At the community level, photosynthetic responses are dependent on the microphytobenthos type and its inherent features. Epipelon photo-regulates using physiological as well as behavioral mechanisms. Its migratory behavior is considered to be a key feature guaranteeing epipelon’s maximum productivity. Epipsammon, on the other hand, exclusively uses physiological mechanisms to optimize its photosynthesis and photoprotection, expressing no vertical migration rhythms (Jesus et al., 2009; Cartaxana et al., 2011; Pniewski et al., 2015). The photophysiology of diatom-dominated epipelon has been well recognized, whereas a gap exists in the understanding of photoacclimation in taxonomically diverse epipsammon. Therefore, further in-depth studies on its photophysiology are
necessary (Jesus et al., 2009).

Pulse Amplitude Modulated (PAM) fluorometry has been widely applied to study microphytobenthos (e.g. Serôdio et al., 1997; Jesus et al., 2006; Lefebvre et al., 2011). It proved to be a fast, sensitive and non-invasive tool lending an insight into the microalgal biochemical processes (Mouget and Tremblin, 2002) by providing information on the photochemical efficiency of photosystem II (PSII) (ΔF/Φm, the effective quantum yield of PSII; Gentyl et al., 1989). ΔF/Φm reflects the proportion of absorbed light used for photochemistry therefore, it can give a measure of the relative linear electron transport rate (rETR; Maxwell and Johnson, 2000) which plotted against irradiance allows for construction of fluorescence light-response curves (LCs). The fluorescence LCs are often used as a proxy for traditional photosynthesis-irradiance (P-E) curves, in which algal material is allowed to acclimate to a series of relevant irradiances when the measurements of steady-state photosynthetic rates are taken (SSLCs). SSLCs reflect algal potential photosynthetic activity and are used to characterize long-term photoacclimation status by means of the light saturation index Eᵦ (Henley, 1993; Ralph and Gademann, 2005). Contrary to SSLCs, rapid light curves (RLCs) use very short light steps (10–30 s) and they can be completed in < 2 min preserving photophysiological characteristics of microphytobenthos (Serôdio et al., 2006). RLCs depend on immediate light history showing light acclimation state developed over past few minutes, which is confounded by microphytobenthos’ long-term prehistory (Ralph and Gademann, 2005). A relationship between SSLCs and RLCs was previously studied in epipelon (Serôdio et al., 2006). No such attempt, however, was made to characterize the variability of long and short-term photosynthetic activity in epipsammon.

Therefore, the main goal of this study was to compare and contrast photosynthetic activity in two types of microphytobenthos assemblages, i.e. epipelon from an intertidal mudflat (Aiguillon Bay, the Atlantic, France) and epipsammon from non-tidal sandy coastal shallows (Puck Bay, the Baltic, Poland), emphasizing differences between them. In the study, measurements were conducted on microphytobenthos communities collected in summer and autumn. The long-term photoacclimation status of microphytobenthos was assessed by means of steady-state light response curves of chlorophyll variable fluorescence, i.e. rETR vs. E (SSLCs) and NPQ (non-photochemical quenching) vs. E curves, variations of the RLC patterns with changing ambient light conditions were described and the influence of long-term photoacclimation status on the short-term photosynthetic activity of microphytobenthos communities was evaluated.

2. Materials and methods

2.1. Research areas and sampling

Samples were collected in two different areas. The first station was Espanades in Aiguillon Bay located along the French Atlantic coast (47° 00′ N, 1° 05′ W). The bay is dominated by intertidal mudflats composed of fine mud with a mean size grain ranging from 7 to 13 μm (Guarini et al., 2004). The maximum tidal range reaches ca. 6 m. Low water spring tides tide occurs at midday resulting in a long diurnal emersion period. During neap tides, high tide occurs in the middle of the day causing two short diurnal low tides in the morning and in the late afternoon (Herlory et al., 2005). The second station was Władysławowo in Puck Bay (54° 43′ N, 18° 34′ E) which is a subregion of the western part of the Gulf of Gdańsk (The Baltic Sea, Poland). Bottom sediments are dominated by fine sands with 69% of particles between 250 and 500 μm (Jankowska and Łęczyński, 1993). Sampling was carried out during two seasons, in summer and autumn 2007. At the intertidal site, samples were taken during two summer and two autumn months, i.e. June/July and November/December, respectively. At the non-tidal site, the sampling was carried out in August (summer) and October (autumn). At both sites, samples were collected 5 times each season (ones every 5 to 7 days). Sampling usually took place before noon and during each sampling day, light intensity and temperature values were measured at the sediment surface using LI-189 and LI-1400 Datalogger (Li-Cor, Lincoln, Nebraska, USA) with a cosine collector LI-192 and Multiparameter WTW meter (WTW GmbH, Weilheim, Germany), respectively. On the intertidal mudflat the observed maximum light intensity reached ca. 2700 and ca. 560 μmol m⁻² s⁻¹ in summer and autumn, respectively. Whereas at the non-tidal site light intensity at the sediment surface was lower, up to ca. 1220 μmol m⁻² s⁻¹ in summer and ca. 260 μmol m⁻² s⁻¹ in autumn. Despite different sampling months, average seasonal temperatures for both sites matched; average temperature reached ca. 20°C in summer (in Aiguillon Bay it was 20.2 ± 0.8°C whereas in Puck Bay 18.7 ± 1.6°C) and ca. 10°C in autumn (7.6 ± 2.7°C and 10.6 ± 0.8°C in Aiguillon Bay and Puck Bay, respectively). The measured temperatures and light values reflected their seasonal variation patterns (the Baltic site, e.g. Kaczmarek and Dera, 1998, Rak and Wiczkorek, 2012; the Atlantic site, unpubl data).

2.2. Algal material preparation

In Aiguillon Bay samples were collected as described in Herlory et al. (2007). During low tide the upper ca. 2 mm of the sediment were scrapped from the randomly chosen area. To collect microphytobenthos samples, the method of Eaton and Moss (1966) based on the natural ability of microalgae to move was used. The method allows collecting motile cells actively forming biofilms at the mud surface (Haubois et al., 2005). Sediment samples were placed in trays and covered with 2 nylon nets with mesh of 100 μm, and left till the beginning of the low tide the next day. When algae appeared on the net surface they were washed with filtered sea water of salinity 35. In Puck Bay sediment cores (10 cm in diameter) were taken as presented in Dahl and Blanck (1996). The top centimeter of the collected sediment cores was cut off and placed in filtered sea water of salinity 6.7. Subsequently, an epipsammon assemblage was separated from the sediment as described by Pniewski et al. (2015). First, sediment samples were mixed and sonicated for 5 min to detach and resuspend algal cells adhering to sediment particles. The cell suspension was separated from the sediment and filtered through planktonic net (100 μm) in order to remove as many small invertebrates as possible.

Microphytobenthos species composition from both studied sampling sites, described in detailed elsewhere (Pniewski et al., 2015, 2017), differed significantly. Epipelon samples were exclusively composed of diatoms. In summer the assemblages were dominated by Navicula sp. (> 85% of the total cell count), while in autumn four species prevailed, i.e. Plagiotropis tayrecta T. B. B. Paddock, Plagiotropis sp., Pleurosigma aestivalii (Brebisson ex Kützing) W. Smith and Gyrosigma sp., constituting ca. 91% of all cells. The epipsammon assemblages were dominated by diatoms which constituted 51 and 59% of the community biomass in summer and autumn, respectively. The same five diatom species dominated throughout the year (> 57% of the total cell count), i.e.: Halamphora coffeiformis (C. Agardh) Levkov, Fragilaria sopotospis Witkowski and Lange-Bertalot, Nitzschia aurariae Cholnoky, Opephora mutabilis (Grunow) Sabbe and Wyverman and Planothidium delicatulum (Kützing) Round and L. Bukhitjaryova. Other taxonomic groups which significantly contributed to the assemblage biomass were green algae, euglenophytes and cyanobacteria.

Prepared suspensions (ca. 300 ml) were kept overnight and during the next day in a growth chamber at constant light and temperature conditions; low light intensity of ca. 50 μmol m⁻² s⁻¹ with 14:10 L:D photoperiod and temperatures corresponding to the seasonal means observed in the environment, i.e. 10°C in autumn and 20°C in summer.

2.3. Fluorescence measurements

Measurements of chlorophyll a fluorescence were carried out three times a day using a computer-operated Diving-PAM (Walz, Effeltrich,
Germany). The device employs a red light emitting diode (LED) with emission maximum at 650 nm to excite fluorescence and a PIN-phodiode (type BPY 12, Siemens) at wavelengths beyond 700 nm (as defined by a long-pass filter, type RG 9, Schott) to detect it. An integral halogen lamp (8V/20W type Bellaphot, Osram, Munich, Germany) provides actinic as well as saturating irradiance. Light was measured with a Fiber Quantum Sensor provided together with the fluorometer and calibrated against Li-Cor Data Logger-1000. All measurements were made with 5.5-mm-diameter Fiberoptic DIVING-F kept perpendicularly to the biofilm at the constant distance of 4 mm.

To measure microphytobenthos fluorescence, 5 ml aliquots of algal suspension were taken and placed in small plastic tubes. The suspension was allowed to sediment to form an artificial non-migratory biofilm. The tube was then placed within a small glass coat filled with water. The glass water coat was connected to a circulating water bath and all the measurements were made at stable temperature, 20 °C and 10 °C in summer and autumn, respectively. The preparation procedure was carried out prior to each measurement and after that the samples were adapted to darkness for 30 min (e.g. Mouget et al., 1999). Steady-state and rapid light curves were constructed according to the protocol of Serôdio et al. (2006). After dark adaptation, $F_o$ and $F_m$ (the minimum and maximum fluorescence of a dark-adapted state, respectively) were monitored for 10 min by applying saturating pulses ($> 3500 \mu\text{mol} \text{m}^{-2} \text{s}^{-1}$ for 400 ms) every 120 s and $F_o/F_m$ ($= F_m - F_o$/ $F_m$; the maximum quantum yield of PSII) was calculated. The samples were then exposed to 11 increasing levels of light. The range of light intensity applied for microphytobenthos was 12–2000 $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$.

At each light level, $F$ and $F_m'$ (the steady-state and maximum fluorescence of a light-adapted state, respectively) were determined every 90 s until steady-state was achieved (usually within 4.5–15 min) and $\Delta F/F_m'$ ($= F_m' - F/F_m'$), $r\text{ETR}$ ($= \Delta F/F_m' \times E$) as well as NPQ ($= F_{m,m}' - F_m'$/ $F_{m,m}'$; $F_{m,m}'$– the maximum $F_m'$ value measured during the LC; Serôdio et al., 2005b) were calculated. RLC was constructed after each SSLC light step, when the sample reached steady-state – constant $\Delta F/F_m'$. RLCs were constructed by exposing the sample to the same 11 light levels of 10 s duration (Ralph and Gademann, 2005) and calculating $r\text{ETR}$. After the completion of the RLC, the sample was subjected to the next SSLC light level and allowed to reach a new steady-state. It was proved that the RLC constructed after each light level does not affect $r\text{ETR}$ and NPQ values measured during the next steady-state (Serôdio et al., 2006).

To quantitatively compare the SSLC and RLC curves empirical data were mathematically fitted to the model of Platt et al. (1980) and photosynthetic parameters, i.e. the maximum relative electron yield of PSII $F_{m,m}'$. 

![Fig. 1. Steady-state light response curves of $\Delta F/F_m'$ (a, b), $r\text{ETR}$ (c, d) and NPQ (e, f) in epipelon and epipsammon collected in summer (open symbols) and autumn (closed symbols). Data show mean ± SE (n = 15).](image-url)
transport rate \( (r_{ETR_{max}}) \), the initial slope of the \( r_{ETR} \) vs. \( E \) response curve \( (\alpha) \), the light saturation index \( (E_{0}) \), the light saturation point \( (E_{ss}) \) and the photoinhibition parameter \( (\beta) \) were estimated \( (Sakshaug et al., 1997) \). Additionally, to describe steady-state light-response curves of NPQ the model of Serôdio and Lavaud \( (2011) \) was applied. Subsequently, the following parameters: the maximum NPQ value \( (NPQ_{max,LC}) \), the irradiance level corresponding to 50% of \( NPQ_{max,LC} \) \( (E_{50}) \) and the sigmoidicity coefficient \( (\gamma) \) were calculated.

2.4. Statistical analyses

Student-\( t \)-test and analysis of variance (ANOVA) were used to test significance of differences between mean values of studied parameters. Data distribution was checked with Shapiro-Wilk’s \( W \) test and the homogeneity of variance was tested with Levene’s test. If it was necessary, particular data sets were transformed. To describe linear relationship between analyzed variables the Pearson correlation coefficient was applied. To test whether or not the regression coefficients were constant the test for homogeneity of slopes was used. All statistical analyses were performed using Statistica 10 \( (\text{StatSoft Inc., USA}) \).

3. Results

3.1. Steady-state light-response curves

SSLCs of \( \Delta F/Fm’ \), \( r_{ETR} \) and NPQ exhibited significant seasonal variability in microphytobenthos assemblages from both studied habitats \( (\text{Fig. 1}) \). In \( \Delta F/Fm’ \) vs. irradiance \( (E) \) curves, in both assemblage types, maximum values were observed at the lowest irradiance, usually close to \( Fv/Fm \), followed by a gradual decrease in \( \Delta F/Fm’ \) with increasing irradiance in summer, and a steeper decrease in autumn \( (\text{Fig. 1A, B}) \). Comparing to epipelon, in epipsammon \( \Delta F/Fm’ \) values observed at low light levels were lower, but at saturating irradiances due to their less pronounced decline they were higher. This variability was subsequently reflected in the variations of \( r_{ETR} \) vs. \( E \) curves \( (\text{Fig. 1C, D}) \). \( \Delta F/Fm’ \) values observed at lower irradiances were very similar regarding each season for a given microphytobenthos type, resulting in similar initial slopes of the curves \( (\alpha_{LC}) \). On the other hand, a wide range of variations in \( \Delta F/Fm’ \) at high light, induced changes in \( r_{ETR} \) values and by extension in the maximum relative electron transport rate \( (r_{ETR_{max,LC}}) \). Student-\( t \)-test showed that there were no statistically significant variations in \( \alpha_{LC} \) values for epipelon, whereas in epipsammon the summer mean was significantly higher than the autumn one \( (P < 0.001, \text{Table 1}) \). In both assemblage types, the highest \( r_{ETR_{max,LC}} \) values were always observed in summer and they were ca. 2-fold higher than in autumn \( (P < 0.001, \text{Table 1}) \). Similarly to \( r_{ETR_{max,LC}} \) comparing seasonal \( E_{4LLC} \) means 2-fold higher values were observed in summer for both assemblage types \( (\text{Student-\( t \)-test,} \quad P < 0.001, \text{Table 1}) \). It is worth mentioning that during both seasons \( E_{4LLC} \) values calculated for epipsammon were approximately 3-fold higher of that in epipelon. The presence of the \( \beta_{LC} \) parameter was generally typical feature of light response curves constructed for epipelon \( (\text{Fig. 1C}) \). The higher mean value was observed in autumn, although the differences were not statistically significant \( (\text{Student-\( t \)-test,} \quad P = 0.085) \). The analysis of the saturation point in epipelon \( (E_{ss,LC}) \) showed that it was ca. 2.5-fold higher in summer than in autumn \( (\text{Student-\( t \)-test,} \quad P < 0.0001, \text{Table 1}) \). In epipsammon, on the other hand, \( \beta_{LC} \) and by extension \( E_{ss,LC} \) were found only occasionally \( (\text{once in summer and 6 times in autumn}) \) \( (\text{Table 1}) \).

Each season the shape of the NPQ vs. \( E \) curves for epipelon and epipsammon was quite similar \( (\text{Fig. 1E, F}) \). In autumn the NPQ values increased almost linearly with increasing light intensity as shown by sigmoidicity coefficient values \( (\gamma) \) closer to 1 \( (\text{Table 1}) \). In summer, on the other hand, a steeper increase of NPQ values was observed \( (n > 1.6) \) within a range of low to moderate irradiances. The sigmoidicity coefficient significantly varied between seasons for both epipelon and epipsammon as well \( (\text{Table 1}) \). The values of dark NPQ \( (NPQ_{0}) \) were usually very low \( (\text{usually below 0.5}) \) and thus they were considered to be negligible \( (\text{Fig. 1E, F}) \). The minimum NPQ \( (NPQ_{0} = 0) \) was usually observed at low light intensities ranging from 12 to 85 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) \( (E_{NPQ_{0}}) \). The highest estimated NPQ \( (NPQ_{max,LC}) \) values were always observed at the highest applied light intensity \( (i.e. 2000 \mu \text{mol m}^{-2} \text{s}^{-1}) \). In both assemblage types, no seasonal differences in \( NPQ_{max,LC} \) were observed \( (\text{Table 1}) \). Epipelon developed much higher \( NPQ_{max,LC} \) values which were ca. 2.5-fold higher than in epipsammon. For each microphytobenthos type no seasonal variations in \( E_{50} \) were found \( (\text{Table 1}) \). The values estimated for epipsammon were ca. 1.5 times higher as compared to epipelon.

3.2. Variation patterns in rapid light curves

Responses of RLCs to increasing light intensity were clearly marked regarding light-limited and light-saturated parts of the curve \( (\text{Figs. 2, 3}) \). The analysis of the changes in the initial slope of the RLCs showed that in autumn epipelon \( \alpha_{RLC} \) values decreased gradually with increasing light intensity, while in the rest of microphytobenthos communities a bi-phasic pattern of changes was observed; under low light conditions an increase in the \( \alpha_{RLC} \) values was observed until maximum became reached at the \( E_{NPQ_{0}} \), after which a decline was observed \( (\text{Fig. 3A, B}) \). In epipelon \( \alpha_{RLC} \) decreased for about 50% and 75% in summer and autumn, respectively. In epipsammon a descending trend was less pronounced and the values of \( \alpha_{RLC} \) decreased for about 25% in summer

### Table 1

|                         | Epipelon      |  | Epipsammon                          |  |
|-------------------------|---------------|  |-------------------------------------|  |
|                         | Summer        | Autumn | Summer                              | Autumn |
| \( r_{ETR_{max,LC}} \)  | 0.795 ± 0.021 | 0.805 ± 0.025<sup>⁎</sup> | 0.465 ± 0.010 | 0.381 ± 0.010<sup>⁎⁎</sup> |
| \( r_{ETR_{max,LC}} \)  | 148.4 ± 5.3   | 70.5 ± 4.7<sup>⁎</sup> | 205.4 ± 8.2 | 87.2 ± 4.7<sup>⁎</sup> |
| \( E_{ss,LC} \)         | 189.6 ± 9.8   | 90.1 ± 7.8<sup>⁎</sup> | 443.5 ± 17.9 | 230.6 ± 13.0<sup>⁎</sup> |
| \( \beta_{LC} \)        | 0.025 ± 0.009 | 0.033 ± 0.005<sup>⁎</sup> | 0.021 (n = 1) | 0.042 ± 0.010 (n = 6) |
| \( E_{ss,LC} \)         | 925.9 ± 92.1  | 356.0 ± 26.1<sup>⁎</sup> | 1577.8 (n = 1) | 399.7 ± 98.7 (n = 6) |
| \( NPQ_{max,LC} \)      | 7.1 ± 1.0     | 8.8 ± 0.8<sup>⁎</sup> | 2.9 ± 2 | 3.4 ± 0.3<sup>⁎</sup> |
| \( n \)                 | 1.6 ± 0.1     | 1.2 ± 0.1 | 1.8 ± 0.1 | 1.2 ± 0.1 |
| \( E_{50} \)            | 842.6 ± 110.1 | 719.8 ± 45.0<sup>⁎</sup> | 1112.4 ± 115.5 | 1110.8 ± 95.5<sup>⁎</sup> |

\( a = \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \), \( b = \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} \), n.s. = not significant.

<sup>⁎</sup> \( P < 0.05 \)

<sup>⁎⁎</sup> \( P < 0.001 \)
and 41% in autumn. Considerably different light response patterns were also observed for \( \text{rETR}_{\text{maxRLC}} \) (Fig. 3C, D). In epipelon, there were no statistically different mean \( \text{rETR}_{\text{maxRLC}} \) values estimated for different light intensities (1-way ANOVA, \( F_{10,151} = 1.862, P > 0.05 \)) in summer (Fig. 3C). In autumn, a decreasing trend was observed; the lowest \( \text{rETR}_{\text{maxRLC}} \) value observed under the highest irradiance was ca. 2-fold lower compared to the highest one observed at the light level of the minimum NPQ (\( E_{\text{NPQ}=0} \)). Only values estimated for the highest ambient lights were significantly different (Tukey HSD test, \( P < 0.01 \)). In epipsammon, on the other hand, in both seasons the saturation-like pattern was observed with summer values being constantly 2-fold higher compared to autumn (Fig. 3D). This pattern was later reflected...
increase in $F$ (12 $\mu$mol m$^{-2}$ s$^{-1}$) was observed; the range of rETR$_{\text{maxRLC}}$ variations increased with in-cular level of light were more diverse and a clear increasing tendency In epipsammon, on the other hand, the slopes of regression for parti-cles pooled together and described with one linear regression equation result, all data from summer and autumn obtained for epipelon were season has no signi-
cificant differences between linear re-
gressions for particular levels of light varied within limited range, 0.682–1.104 (Fig. 6A). Test for homogeneity of slopes, however, showed that they were statistically different ($F_{10,305} = 4.58; P < 0.0001$). In epipsammon, the range of changes in regression slopes was much wider, 0.196–0.980 and they significantly increased with increasing ambient light intensity approaching 1:1 ratio (test for homog-
enity of slopes, $F_{10,304} = 23.61; P < 0.0001$; Fig. 6B).

3.3. Steady-state light curves vs. rapid light curves

Comparing the initial slopes of RLCs ($\alpha_{\text{RLC}}$) obtained for particular levels of light to corresponding NPQ values an inverse pattern of changes was found (Fig. 1E, F, Fig. 3A, B). At very low irradiance, an increase in $\alpha_{\text{RLC}}$ and simultaneous decrease in NPQ was observed. Non-pho-
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in $E_{\text{RLC}}$ (Fig. 3F) and the same amplitude of changes was observed. In epipelon, during both seasons a similar ascending tendency of $E_{\text{RLC}}$ was present and its values increased 2.3 times comparing low and high light levels (Fig. 3E). A clear discrepancy was found under low light conditions, when in summer $E_{\text{RLC}}$ first decreased reaching minimum at $E_{\text{NPQ}=0}$ and then increased to its maximum, while in autumn a steady increase of $E_{\text{RLC}}$ was observed.

Fig. 3. Linear relationship between $\Delta \alpha_{\text{RLC}}$ and $\text{NPQ}$ for epipelon (a) and epipsammon (b) for ambient irradiances ranging from 12 to 2000 $\mu$mol m$^{-2}$ s$^{-1}$. In the panel a solid line represents linear regression for the combined seasonal data sets. In the panel b regression lines refer to 2000 $\mu$mol m$^{-2}$ s$^{-1}$ (solid line) and to 12 $\mu$mol m$^{-2}$ s$^{-1}$ (dashed line). Circles and rhombus denote summer and autumn samples, respectively.
Regression lines refer to ambient irradiances ranging from 12 to 2000 μmol m$^{-2}$ s$^{-1}$ (b) for ambient irradiances ranging from 12 to 2000 μmol m$^{-2}$ s$^{-1}$ in both panels regression lines refer to 2000 μmol m$^{-2}$ s$^{-1}$ (solid line) and dashed regression lines to 12 μmol m$^{-2}$ s$^{-1}$ (dashed line). Circles and rhombus denote summer and autumn samples, respectively.

4. Discussion

4.1. Long-term photoacclimation

The long-term photoacclimation strongly affects the variations of steady-state light-response curves (e.g. Perkins et al., 2006) and on that account distinctive SSLC variation patterns for epipellic and epipsammon were found. The observed photoresponses (Table 1), indicated good acclimation to low light conditions in epipellic and to higher light in epipsammon, in congruence with Pniewski et al. (2015). Furthermore, in epipellic a downturn of rETR values under high light was observed (the presence of the β parameter) which suggested that the communities were more sensitive to higher irradiance (Henley, 1993). This is typical of low light acclimated samples, and thus, it was rarely found in high light acclimated epipsammon. Low light acclimation in epipellic is consistent with the observation that in muddy sediment light is quickly attenuated and thus limited to the top millimeter (e.g. Cartaxana et al., 2011). Moreover, epipellic photo-regulates also through behavioral mechanisms, i.e. cells position themselves within optimal light regime due to the vertical migration limiting this way necessity for physiological photoacclimation (Laviale et al., 2015, 2016). No seasonal changes in α$_{LC}$ estimated for epipellic implied that the physiological regulation of light absorption played a rather limited role in long-term (seasonal) photoacclimation. Thus vertical migration was considered to be the primary light-response. High light acclimation in epipsammon of non-tidal sandy coastal shallows was expected as light penetrates deeper and thus, microphytobenthos is exposed to higher and highly fluctuating irradiances for prolonged periods of time (e.g. Kühl et al., 1994). Epipsammon lacking of or having strongly limited ability to move regulates light absorption exclusively based on physiological responses (Cartaxana et al., 2011). Therefore, seasonal changes in α$_{LC}$ exhibited necessary adjustments allowing the optimal use of available light and protection against excessive irradiance (Pniewski et al., 2015). Temperature also affects photosynthesis, but it usually has a greater impact on rETR$_{max}$ than α$_{LC}$ (Davison, 1991). The decrease of the rETR values in autumn (about 50% and 42% in epipellic and epipsammon, respectively) could be attributed to the activity of the Calvin-Benson cycle enzymes which work more slowly as temperature decreases (Kirk, 1996).

The variations in the initial slope and the maximum electron transport rate resulted in significant seasonal E$_{KLC}$ changes. Comparing the E$_{KLC}$ values between epipellic and epipsammonic assemblages, it was found that the photoacclimation index was substantially higher in epipsammon than in epipellic, confirming the hypothesis about their high and low light photoacclimation, respectively. The photoacclimation index was higher in summer than in autumn, which is in agreement with previous findings (e.g. Light and Beardall, 2001). The “E$_{K}$-dependent” photoacclimation model suggested independent changes in α$_{LC}$ and rETR$_{max}$ (Behrenfeld et al., 2004). In both assemblage types E$_{KLC}$ variability was strongly dependent on the rETR$_{max}$ variations, as shown by positive linear correlations (r > 0.96, P < 0.0001), which implied that photosynthesis was acclimated to environmental changes through the electron transport chain and/or carbon metabolism (Behrenfeld et al., 2004). A relationship between E$_{KLC}$ and α$_{LC}$ was only observed in epipsammon (r = 0.548, P = 0.0017). This confirmed that epipellic and epipsammon differently regulated their photoacclimation. In epipellic, the lack of correlation between E$_{KLC}$ and α$_{LC}$ together with the relatively limited variability of the latter, as previously mentioned, suggested minor role of physiological processes in light absorption regulation underpinning the importance of the vertical migration (Serôdio et al., 2001). In epipsammon, photosynthesis was controlled by both sets of physiological processes, as behavioral mechanisms were strongly limited.

In microalgae excess light energy can be safely dissipated as heat and this can be monitored by measuring non-photochemical quenching (NPQ) which is the indicator of the energy dissipation in the photosynthetic antenna (Ruban et al., 2004). NPQ can be divided into three different components: the energy-dependent quenching (qE), the quenching occurring due to the phenomenon of the state transition (qT) (not present in diatoms) and the one showing the slowest relaxation, i.e. the photoinhibitory quenching (qI component) (Müller et al., 2001). NPQ values observed for the epipellic assemblages reached values of ca. 8 and they were substantially higher than those in epipsammon (ca. 3.2) (Pniewski et al., 2017). The differences observed between studied assemblages could be caused by differences in species composition as the amplitude and regulation of energy dissipation is species-specific and depends on its growth form (Casper-Lindley and Björklund, 1998; Blommaert et al., 2017); in epipsammon heavily dominated by diatoms the NPQ values corresponded to those observed for non-motile diatom species (Barnett et al., 2015), whereas in epipellic they were higher and also coincided with previously reported values (Serôdio et al., 2005b, 2012). The ratio E$_{50}$/E$_{KLC}$ describing a relationship between a light response of NPQ and the saturation of photochemistry, showed that in epipellic the light of intensity 2.5E$_{KLC}$ and 4.9E$_{KLC}$ was necessary to induce half of NPQ$_{max}$ in summer and autumn, respectively. In epipellic, comparing respective seasons E$_{50}$/E$_{KLC}$ was ca. 1.5 times higher. Serôdio and Lavaud (2011) reported that usually E$_{50}$/E$_{KLC}$ equals ca. 3 assuming that NPQ consists mainly of qE. Here, however, the E$_{50}$/E$_{KLC}$ ratio values estimated for epipellic were higher suggesting that NPQ involved not only qE but also some sort of photoinhibitory quenching (qI component) (Serôdio et al., 2012; Pniewski et al., 2017). This observation together with other findings, i.e. increasing NPQ values throughout the illumination period and the lack of saturation of the NPQ vs. E curves, further adds to the conclusion that epipellic was sensitive to high light...
which eventually led to its limited photosynthetic performance (as seen from $\beta_{RLC}$).

4.2. Short-term photoacclimation

In both assemblage types, the initial increase of $\alpha_{RLC}$ at low ambient lights (usually 12–85 $\mu$mol m$^-2$ s$^-1$) was caused by the relaxation of the dark NPQ during the first SSLC light steps. Above the light intensity when NPQ = 0 a decline in $\alpha_{RLC}$ was observed due to the subsequent NPQ build-up. This decline was much steeper in epipelon, while in epipsammon $\alpha_{RLC}$ dropped gradually. NPQ needs some time to be relaxed therefore it strongly affects the constructed RLCs (Serôdio et al., 2006; Cruz and Serôdio, 2008).

$rETR_{maxRLC}$ varied differently in the studied communities. In epipsammon the observed variability corresponded to the results described by Serôdio et al. (2006). The pattern was characterized by a continuous increase of $rETR_{maxRLC}$ despite simultaneous NPQ build-up, due to compensatory processes including the increased electron turnover through the PSII and high-light induced Calvin-Benson cycle activation (Behrenfeld et al., 1998; Serôdio et al., 2005a). The second pattern of the $rETR_{maxRLC}$ variations typical of the epipelagic assemblages was characterized by the gradual decrease of $rETR_{maxRLC}$ values (strongly pronounced in autumn). As it was tentatively suggested the increasing irradiance during the constructing of SSLC could have photoinhibitory effect on the microphytobenthos assemblages and thus a decreasing $rETR_{maxRLC}$ values could be a consequence of the increasing percentage of the PSII reaction centers being deactivated due to the electron transport capacity exceeding carboxylation capacity (Henley et al., 1991).

In epipsammon, an increase of $E_{qRLC}$ with ambient light was observed which concurred with previously mentioned $E_k$-dependent photoacclimation suggesting shifts in $E_{qRLC}$ to match prevailing light conditions (Behrenfeld et al., 2004; Serôdio et al., 2005a). In epipelon, an ascending tendency of $E_{qRLC}$ with light was also observed which resulted from the much steeper decline of $\alpha_{qRLC}$ compared to $rETR_{maxRLC}$. Significant changes of RLCs' shape in the set of light curves appurtenant to each particular SSLC, described by constantly decreasing $\alpha_{RLC}$ and $rETR_{maxRLC}$ with cumulating light doses (with each next SSLC light step), indicated that epipelon underwent light stress (Henley, 1993) and an increasing contribution of $q_l$ could be further supported (as previously tentatively suggested from continually increasing NPQ during SSLC measurement).

4.3. Influence of long-term photoacclimation on short-term variability

Changes of RLCs as a result of ambient light variations showed that recent light history alters RLC shape and thus its characteristics (e.g. Perkins et al., 2006). In epipelon, $rETR_{maxRLC}$ and $E_k$ varied with ambient light within relatively limited ranges determined by the long-term photoacclimation status of the communities (Cruz and Serôdio, 2008) and their susceptibility to higher light (as shown by the decreasing $rETR_{maxRLC}$ values with increasing ambient light). In epipsammon, $rETR_{maxRLC}$ on $rETR_{maxRLC}$ values formed a pattern previously not reported; $rETR_{maxRLC}$ steeply increased with increasing ambient light intensity and the range of variations widened in samples acclimated to higher irradiances. This pattern was observed during each season, in summer and autumn as well, and when both data sets were combined it was enhanced even further. The observed variability was also reflected in the $E_k$ vs. $E_{qRLC}$ relationship. In both cases, with increasing ambient light RLC parameters approached steady-state values. Such increase in $rETR_{maxRLC}$ and $E_k$ values with ambient light, as previously mentioned, probably resulted from the compensatory electron turnover through the PSII and/or high-light-induced carbon metabolism (Behrenfeld et al., 1998; Serôdio et al., 2005a).

Long-term photoacclimation state clearly influences microphytobenthos short-term photosynthetic activity setting constraints for RLC changes. Epipelon being low light acclimated (which involves increased cellular content of light-harvesting pigments and large photosynthetic units) (Richardson et al., 1983), is still subjected to large and sudden irradiance fluctuations requiring rapid response mechanisms. In this study, the steep decrease in $\alpha_{RLC}$ followed by declining $rETR_{maxRLC}$ under high light conditions suggested that epipelon responded to increasing light intensity mainly by diminishing light absorption as absorbed photons could not be efficiently used. In epipelon cells photoacclimate through their vertical movement and physiological mechanisms plays rather supportive role (Perkins et al., 2010; Du et al., 2017). Processes such as the operational xanthophyll cycle (involving de-epoxidation of diadinoxanthin) and the de novo synthesis of diatoxanthin may help to prevent photodamage (Lavaud et al., 2004). However, the downward migratory movement and energy-dissipation processes, even acting together, may not be entirely sufficient to prevent photodamage (Serôdio et al., 2008). Some studies have in fact confirmed that epipelon may experience photo inhibition (e.g. Blanchard et al., 2004; Mouget et al., 2008; Serôdio et al., 2012). The phenotypical changes in rapid light response curves observed here were consistent with the pattern observed in photo inhibited samples (Henley, 1993). In epipsammon, increasing ambient light induced changes in photosynthetic activity which involved a decrease in $E_{qRLC}$ although to some limited degree as they were high light acclimated already, and much wider increase in $rETR_{maxRLC}$. Furthermore, higher temperatures extended the variation range magnifying the amplitude of the pattern. High light adaptation in microalgae involves a decrease in the number and/or size of photosynthetic units limiting this way their light harvesting and an increase in the carbon assimilation and utilization capacity through changes in the Calvin-Benson cycle activity (Raven and Geider, 2003). In epipsammon, the decreased light absorption (due to the long-term high light acclimation) was further down-regulated by the NPQ build-up during increasing incident irradiance which seemed to be compensated by the high light-induced increase in the carbon metabolism enzymes activity, most notably RU-BISCO. Such changes suggested that epipsammon was able to efficiently balance the light absorption and its utilization avoiding inhibition of photosynthetic rates. This also explained wider variation range in $rETR_{maxRLC}$ in summer. In general, epipsammon appeared to be acclimated to high and more fluctuating light, and having limited control over light exposer adjusted its photosynthesis to prevailing light conditions through the physiological processes.

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