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Photo-regulation in microphytobenthos from intertidal mudflats and non-tidal coastal shallows

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Abstract

The study investigated seasonal changes in the photo-regulatory mechanisms of microphytobenthos found in intertidal mudflats (Aiguillon Bay, the Atlantic, France) and non-tidal sandy coastal shallows (Puck Bay, the Baltic, Poland) based on photosynthetic pigment characteristics and the estimates of photosynthetic parameters obtained through oxygen evolution measurements. The intertidal communities consisted of motile diatom species typical of epipelon. The non-tidal microphytobenthos was composed of epipsammic species mostly belonging to four taxonomic groups chiefly contributing to the assemblage biomass, namely cyanobacteria, euglenophytes, green algae and diatoms (comprising mainly small-sized species). The epipelon was low light acclimated as shown by the lower values of photoprotective/photosynthetic (PPC/PSC) carotenoids and diatoxanthin/diadinoxanthin (Dt/Dd) ratios. In contrast, the epipsammon exhibited features of high light acclimation (high PPC/PSC and Dt/Dd ratios). In both microphytobenthos types, the photosynthetic capacity (P_m) showed the same seasonal variation pattern and there were no statistically significant differences between the investigated sites in corresponding seasons ($P > 0.05$). In both assemblage types, the photosynthetic efficiency at limiting irradiance (α) decreased over time. The epipelon had higher α compared to the epipsammon. Seasonal changes of the photoacclimation index (E_k) estimated for the epipellic communities reflected variations observed in P_m , whereas in the epipsammon an increasing trend in E_k values was observed. E_k was always higher for the epipsammon when comparing analogous seasons, which further corroborated low and high light acclimation in the epipellic and epipsammic communities, respectively. The presence of the photoinhibition parameter (β) in the epipelon and the lack of it in the epipsammon suggested that the latter was resistant to high irradiance and the physiological mechanisms were sufficient to protect it from photoinhibition. In the epipelon, a downturn in photosynthetic rates showed that it was susceptible to high light intensities, suggesting that physiological photoprotective mechanisms must be supported by behavioural photoacclimation in order to avoid damaging light influence.

Keywords: microphytobenthos; photoacclimation; photosynthesis; photosynthetic pigments; Aiguillon Bay; Baltic Sea

1 Introduction

Microphytobenthos is an ecological formation grouping various photosynthetic organisms, i.e. cyanobacteria and algae (e.g. euglenophytes, diatoms, green algae etc.) growing on a variety of substrata in shallow aquatic environments. Microphytobenthos inhabiting sediments [are](#) [is](#) traditionally divided into two groups depending on the nature of the sediment, i.e. free-living epipelon and epipsammon attached to the sediment particles (Round, 1971). Epipelon is usually dominated by large, biraphid diatoms, whereas epipsammon consists of small-sized diatoms with a substantial contribution of other algal groups, including cyanobacteria and coccoid chlorophytes (Round, 1981). Microphytobenthos is responsible for a significant proportion of estuarine primary production, especially in intertidal mudflats which are devoid of higher plants and macroalgae (Blanchard and Cariou-Le Gall, 1994; Underwood and Kromkamp, 1999). In addition, benthic microalgae can be a substantial source of food, not only to benthic but also pelagic food webs due to the resuspension in the water column during the high tide (Lucas et al., 2001). They mediate a number of geochemical processes,

including nutrients turnover in the sediment and their fluxes at the water–sediment interface (e.g. Sundbäck, 1994; Sundbäck et al., 2006). They can also stabilize sediment surface through mucilage production (Sutherland et al., 1998).

The presence and functioning of microalgae in marine sediments is controlled by various environmental factors (e.g. temperature, nutrients, tides, grazing) (Barranguet et al., 1998; Consalvy et al., 2004 and references therein) with light availability being the major ecological variable affecting microphytobenthos biomass, species composition and photosynthetic pigments, as well as driving its photosynthetic reactions (e.g. Hartwig, 1978; MacIntyre et al., 1996; Light and Beardall, 2001; Underwood et al., 2005). The irradiance must be strong enough to exceed respiration losses and enable net photosynthesis. However, light is quickly attenuated in the sediment, which depends on its granulometry. Consequently, in muddy sediments light is limited to the top approx. millimetre, whereas in sandy sediments it can penetrate deeper, down to a few millimetres (Kühl et al., 1994; MacIntyre et al., 1996; Kromkamp et al., 1998; Cartaxana et al., 2011). In addition, light intensity varies within an extremely wide range and it is the fastest changing factor (Kühl et al., 1994). Therefore, it requires from microphytobenthos a complex adaptive response.

Photoacclimation, which is a dynamic process occurring on different spatio-temporal scales, allows algae to efficiently use available irradiance if limited, and protect themselves from excessive light (Copertino et al., 2009). Epipellic microalgae from intertidal mudflats developed not only physiological acclimation mechanisms but also, due to their motility, a behavioural photoacclimation strategy allowing them to position themselves in the sediment within an optimal light regime (e.g. Kromkamp et al., 1998; Seródio et al., 2006). Epipsammic microalgae have received relatively less attention in this aspect, having no or strongly limited ability to move they have to rely on their physiology when acclimating to changing light conditions. Moreover, photoacclimation at the community level may also depend on species composition, abundance and behaviour (Underwood et al., 2005). Therefore, it has been assumed that epipellic and epipsammic assemblages exhibit different photo-regulatory and adaptive strategies (Jesus et al., 2009).

The main objective of this study was to assess and compare photosynthetic activity and photo-regulation mechanisms in two types of microphytobenthos assemblages: epipellic from an intertidal mudflat (Aiguillon Bay, the Atlantic, France) and epipsammic from non-tidal sandy coastal shallows (Puck Bay, the Baltic, Poland) across the seasons.

2 Materials and methods

2.1 Research areas, sampling and algal suspension preparation

Sampling was carried out in two different areas. The first location was Esnandes in Aiguillon Bay located on the west coast of France (the Atlantic, 47°00' N, 1°05' W). The sampling site consists of mud with a mean grain size ranging from 7 to 13 µm (Lorin, 1968; Guarini et al., 2004). The second location was Puck Bay (the Baltic, 54°43' N, 18°34' E), which is a subregion of the western part of the Gulf of Gdańsk (Poland). The sampling site – Władysławowo – is located in the western-internal part of Puck Bay and the bottom sediments of this part of the bay are dominated by fine sands with a narrow zone of medium sands along the coast (Jankowska and Łęczyński, 1993). At the sampling site 69% particles were between 250 and 500 µm.

Sediment samples were taken during three seasons: spring, summer and autumn 2007. At the intertidal site, samples were collected in March (spring) and during two summer and two autumn months, June/July and November/December, respectively. At the non-tidal site, the sampling was carried out in May (spring), August (summer) and October (autumn). At both sites, sampling took place 3 to 5 times each season. During sampling days temperature and light measurements were carried out using Multi-parameter WTW meter (WTW GmbH, Weilheim, Germany), and LI-189 and LI-1400 Datalogger (Li-Cor, Lincoln, Nebraska, USA), respectively. In Aiguillon Bay average sediment temperatures varied within the range of 12.6–16.0 °C in spring, 19.2–21.2 °C in summer and 4.1–11.0 °C in autumn. In Puck Bay, the sediment temperatures changed within similar ranges of 11.9–13.5 °C in spring, 16.8–20.6 °C in summer and 9.6–11.6 °C in autumn. On the intertidal mudflat the maximum solar radiation input was up to approx. 1030, 2700, 560 µmol m⁻² s⁻¹ in spring, summer and autumn, respectively. Maximum light intensity at the sediment surface at the non-tidal site was lower and reached 516 µmol m⁻² s⁻¹ in spring, 1220 µmol m⁻² s⁻¹ in summer and 260 µmol m⁻² s⁻¹ in autumn. The measured temperatures and light values reflected their seasonal variation patterns.

In Aiguillon Bay samples were collected during low tide (scrapping the upper approx. 2 mm of the sediment randomly chosen area) as described in Herlory et al. (2007). To collect microphytobenthos samples the method based on the natural ability of microalgae to move was used (Eaton and Moss, 1966). Sediment samples were placed in trays, covered with 2 nylon nets (100 µm mesh) 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. The method was chosen as it allows selecting motile cells actively forming biofilms at the mud surface (Haubois et al., 2005). In Puck Bay sediment cores (10 cm in diameter) were taken as presented in Dahl and Blanck (1996). The top centimetre of the collected sediment cores was cut off and placed in filtered sea water of salinity 6.7. The sediment was then mixed and sonicated for 5 min to detach and resuspend algal cells adhered to sediment particles. Finally, the algal suspension was separated from the sediment and filtered through planktonic net (100 µm) in order to remove as many small invertebrates as possible. Prepared suspensions (approx. 300 ml) were kept overnight and during the next day in a growth chamber at constant light and temperature conditions; low light intensity of approx. 50 µmol m⁻² s⁻¹, 14:10 L:D photoperiod and temperatures corresponding to those observed in the environment, i.e. approx. 10 °C (autumn), 15 °C (spring) or 20 °C (summer).

2.2 Species composition

Species composition was analysed in replicated subsamples of prepared microalgal suspensions fixed with Lugol's solution. The material was studied under a Nikon 80i microscope using 40x objective in order to determine the relative abundance of cyanobacteria and microalgae by counting at least 300 cells. In order to perform a detailed analysis of diatom species composition, one of collected subsamples was treated with 10% hydrochloric acid, then rinsed a few times with distilled water, and then treated with hydrogen peroxide at 30–90 °C for 6–9 h. The diatom sample was mounted in Naphrax (Battarbee, 1986) and analysed with the same microscope under 100x oil immersion objective, counting at least 300 frustules. The biovolume of observed species was calculated according to Olenina et al. (2006). The biomass (wet weight) of each taxonomic group was derived based on an assumption of a plasma density of 1 g cm⁻³ across all taxa (HELCOM, 2013).

2.3 Photosynthetic pigment analyses

Five ml aliquots of microphytobenthos suspension were taken and filtered through GF/C Whatman glass filters (25 mm diameter) under low vacuum then frozen and stored at $-20\text{ }^{\circ}\text{C}$ until further processing. Pigments were extracted with 4 ml of cold 90% acetone (HPLC grade) at $-20\text{ }^{\circ}\text{C}$ for 4 h (Strickland and Parsons, 1972). After this time, extracts were centrifuged (7000 rpm, 10 min) and filtered through PTFE filter. Prepared extracts were analysed with KONTRON liquid chromatograph comprising triple system pump Kontron 422, Diode Array Detector 440 (set at 440 nm) and fluorescence detector SFM 25 KONTRON Analytical (Ex. 430 nm; Em. 670 nm). Pigment separation was performed using reverse phase chromatography (RP-HPLC) (250 mm \times 4.6 mm Allsphere [ALLTECH] column packed with Spherisorb ODS2 5 μm particle size) following optimized analytical gradient protocol provided by Wright et al. (1991). The HPLC system was calibrated using high purity pigment standards purchased from The International Agency for ^{14}C Determination DHI Institute for Water and Environment in Denmark. Pigments were identified from their absorbance spectra and retention times, and quantified following the procedure described by Mantoura and Repeta (1997).

2.4 Photosynthesis-irradiance (P-E) curves

Photosynthesis and respiration rates were determined using the micro-respirometric technique described by Zurzycki and Starzecki (1971) suitable to obtain reliable results from small biomass sample. The measurements were based on the volumetric principle, i.e. a change in gas volume under constant pressure.

The main part of the apparatus was a plate with micro-chambers (Fig. 1.), each connected to capillaries, which was placed inside a hermetically sealed compensation chamber. All measurements were carried out under the assumption that the plate with research material was kept at a constant temperature completely isolated from the external atmospheric conditions. In this study a plate with 5 micro-chambers was used. Four of them were employed for the gas exchange measurements and the last one was designed as a thermobarometer (the micro-chamber did not contain research material) which was used to eliminate all the possible fluctuations of temperature and pressure within the measuring system. The lighting system consisted of an OSRAM tungsten halogen lamp 20 W/8 V with dichroic reflector emitting white light, a condenser, collector lenses, iris diaphragm and water filter. The light intensity was measured separately for each micro-chamber using Fiber Quantum Sensor of DIVING-PAM (Walz, Effeltrich, Germany). The maximum irradiance level was $1330\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$. A set of neutral filters and diaphragms was used to achieve eleven light levels. A stable temperature was assured by connecting the micro-respirometer to a circulating water bath (Zurzycki and Starzecki, 1971). Gas exchange measurements were performed at temperatures of $20\text{ }^{\circ}\text{C}$, $15\text{ }^{\circ}\text{C}$ and $10\text{ }^{\circ}\text{C}$ for summer, spring and autumn benthic communities, respectively.

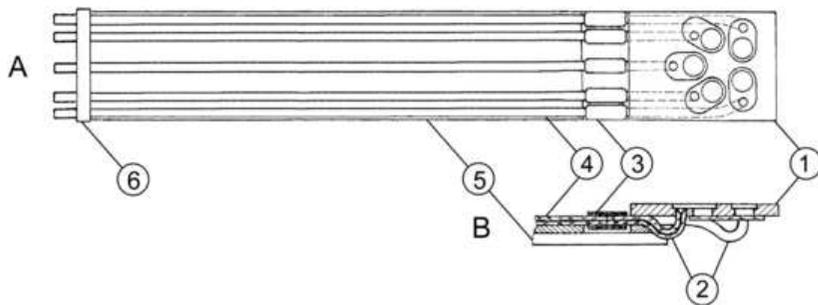


Fig. 1 Plate with micro-chamber system used in volumetric micro-respirometer; typical plate – view from above (A), cross-section (B); brass plate (1), capillaries (2), plastic tubes (3), measurement capillaries (4), mounting base (5), combs (6); [modified from Zurzycki and Starzecki \(1971\)](#), adapted from [Zurzycki J. and Starzecki W., Volumetric methods. In: Šesták Z., Čatský J., Jarvis P.G. \(Eds.\), Plant photosynthetic production. Manual of methods. 1971. Dr.W. Junk N.V. Publishers The Hague, pp. 261. © Springer Netherlands. With kind permission of Springer Science+Business Media.](#)

Three to five ml of microphytobenthos suspension aliquots were filtered onto a GF/C Whatman glass filter. After that, small pieces of filters were cut out and placed in micro-chambers moistened with the filtered sea water. The micro-chambers were then covered with a cover glass with a hanging drop of 0.2 M carbonate buffer and sealed with vaseline. The carbonate buffer served as the source of CO_2 for photosynthesis and being maintained at constant level in the chambers enabled gas volume change by oxygen evolution only. The respiration and photosynthesis rates were estimated by measuring the volume of produced or used oxygen in dark and each light level. The changes in oxygen volume were monitored through the movement of kerosene bars coloured with Sudan III and placed in capillaries. During the measurement, in darkness and at each light level, the samples were left for 10 min to adapt to measurement conditions. After the adaptation period, the readings of kerosene bars movement were taken at equal time intervals for the next 10 min (every 5 min) and 9 min (every 3 min) for respiration and photosynthesis, respectively. The final data were calculated according to the equation of Cunningham and Kirk (1940) simplified by Zurzycki and Starzecki (1971), $\Delta V = F(\Delta A - \Delta B) / l$, where: ΔV is the volume of the produced gas, F is the active capillary cross section, ΔA and ΔB are the displacements of the kerosene bars inside the measuring capillary and thermobarometer, respectively, and coefficient l is the correction for temperature and pressure changes. The obtained volume readings were converted to mass units using ρ values provided by Šesták et al. (1971): $\rho = 1.331\text{ kg m}^{-3}$ for summer measurements at $20\text{ }^{\circ}\text{C}$, $\rho = 1.355\text{ kg m}^{-3}$ for [summer/spring](#) measurements at $15\text{ }^{\circ}\text{C}$ and $\rho = 1.378\text{ kg m}^{-3}$ for autumn measurements at $10\text{ }^{\circ}\text{C}$. Calculated photosynthesis and respiration rates were then normalized per total chlorophyll *a* (Chl *a*) concentration.

To quantitatively compare the *P-E* curves inferred from gas exchange measurements empirical data were mathematically fitted to the model of Platt et al. (1980) and photosynthetic parameters, i.e. the photosynthetic capacity (P_m), the maximum light utilization coefficient (α), the light saturation index ($E_{1/2}$), the light saturation point (E_m) and the photoinhibition parameter (β) were estimated (Sakshaug et al., 1997).

2.5 Statistical analyses

To compare the mean values of the analysed parameters analysis of variance (ANOVA) was used. If it was necessary, particular data sets were transformed (using natural logarithm). If the requirements of ANOVA were not met, non-parametric test, Kruskal–Wallis ANOVA by Ranks was used. Post-hoc comparisons were made with Tukey HSD test. To describe linear relationship between analysed variables, if present, the Pearson correlation coefficient was applied. All statistical analyses were performed using Statistica 10 (StatSoft Inc., USA).

3 Results

3.1 Microscopic observations

From all the samples collected at the intertidal site only 11 diatom species belonging to 6 genera were identified. Species richness varied within the range of 5–8 species per sample and during each season a different taxon dominated the assemblage (Fig. 2). During two seasons a strong domination of one species (over 85% of total cell count) was observed: *Pleurosigma aestuarii* (Brébisson ex Kützing) W. Smith and *Navicula* sp. in spring and summer, respectively. In autumn, 4 out of 9 identified species constituted 90.8% of all cells: *Plagiotropis tayrecta* T. B. B. Paddock (31.4%), *Plagiotropis* sp. (30.2%), *Pleurosigma aestuarii* (Brébisson ex Kützing) W. Smith (15.9%) and *Gyrosigma* sp. (13.3%). Other species were observed occasionally. Contrastingly, the non-tidal epipsammic communities exhibited more diversity, with four taxonomic groups, including: cyanobacteria, diatoms, euglenophytes and green algae, being most frequently observed. During all three seasons diatoms dominated the microphytobenthos (Fig. 3.) and contributed to the total community biomass in 50, 51 and 59% in spring, summer and autumn, respectively. In the samples, numerous small-sized diatoms were observed and the total number of 63 species was identified. In summer 50 species were recorded, whereas in spring and autumn it was 33 and 38, respectively. Throughout the year five species were most frequently observed (>57% of cell count), i.e. *Amphora coffeaeformis* (C. Agardh) Kützing, *Fragilaria sopotensis* Witkowski & Lange-Bertalot, *Nitzschia aurariae* Cholnoky, *Opephora mutabilis* (Grunow) Sabbe & Wyverman and *Planothidium delicatulum* (Kützing) Round & L. Bukhtiyarova (Fig. 2). The second most common group in spring was cyanobacteria with 38% input to the microphytobenthos biomass, which later strongly decreased (Fig. 3). In summer, cyanobacteria were replaced by green algae (25% of biomass) and euglenophytes (12% of biomass). Subsequently, the biomass of green algae decreased (down to 10%) in autumn, while further increase of euglenophytes biomass, up to 17%, was observed (Fig. 3).

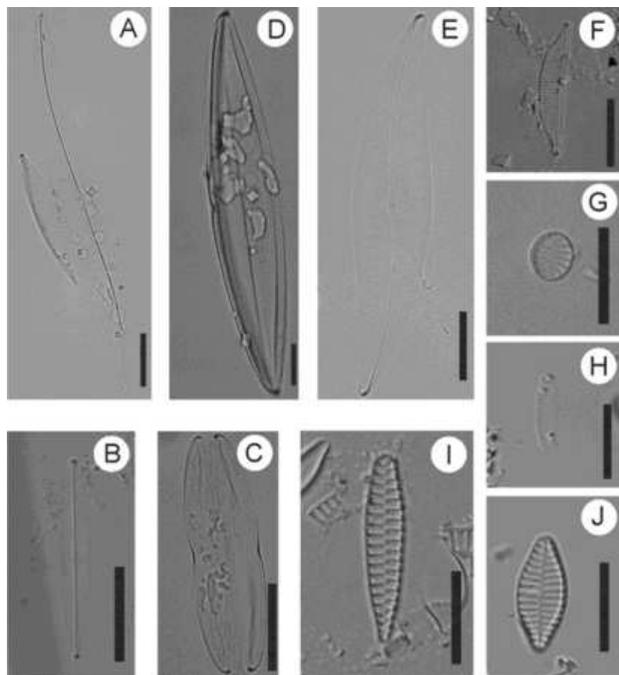


Fig. 2 Dominant diatom species at the intertidal site: (A) *Gyrosigma* sp., (B) *Navicula* sp., (C) *Plagiotropis tayrecta*, (D) *Plagiotropis* sp., (E) *Pleurosigma aestuarii*, scale bar = 20 µm; and the non-tidal site: (F) *Amphora coffeaeformis*, (G) *Fragilaria sopotensis*, (H) *Nitzschia aurariae*, (I) *Opephora mutabilis* and (J) *Planothidium delicatulum*, scale bar = 10 µm.

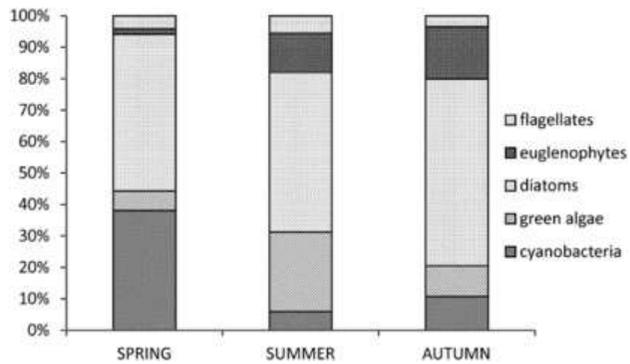


Fig. 3 Seasonal changes in the contribution of algal groups to the total community biomass in microphytobenthos from the coastal shallows of the Puck Bay.

3.2 Pigment analyses

The analyses of pigments specific to the observed dominant taxonomic groups showed that there were statistically significant effects of both variables site and season (ANOVA, $P < 0.05$). Fucoxanthin (fuco) was the most abundant carotenoid for both epipelonal and epipsammon (more than 70% and 50% of total amount of carotenoids identified in the microphytobenthos samples of the intertidal and non-tidal site, respectively). The comparison of the fuco/Chl a ratio calculated for both sampling sites showed that it was always significantly higher for microphytobenthos from the intertidal site (ANOVA, $P < 0.001$). In the non-tidal microphytobenthos this ratio reached values an order of magnitude higher when compared to the ratios of other pigments (Fig. 4). Additionally, it was confirmed that fucoxanthin significantly correlated with the biomass of diatoms ($r = 0.905$, $P < 0.001$). For both studied assemblage types, the highest values of fuco/Chl a were observed in autumn, although no clear seasonal pattern was found (Fig. 4A). The zeaxanthin/Chl a (zea/Chl a) ratio showed a steady increase throughout the year, also reaching the highest values in autumn (Fig. 4B). The highest lutein/Chl a (lut/Chl a) and Chl b /Chl a ratios were observed during the warmest period (Figs. 4C,D). The increased summer values of lut/Chl a remained the same next season (Fig. 4C), whereas the Chl b /Chl a ratio significantly declined (Fig. 4D). In addition, in autumn strong correlation between chlorophyll b and diatoxanthin was found ($r = 0.947$, $P < 0.001$).

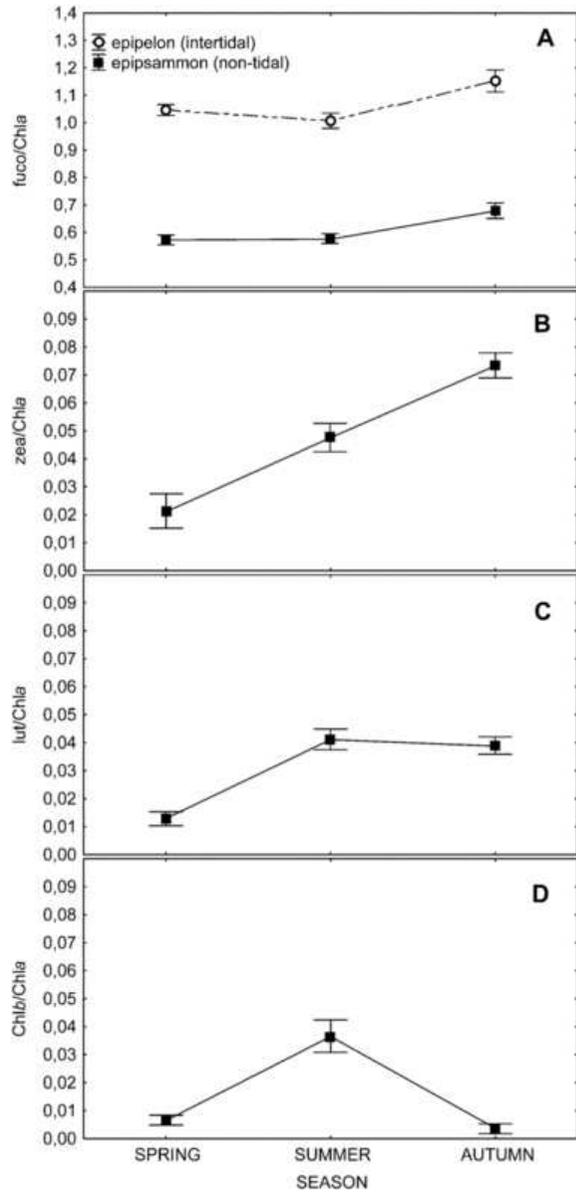


Fig. 4 Seasonal changes in photosynthetic pigment ratios ($n = 9-15$, mean \pm standard error). (A) fucoxanthin/chlorophyll *a* in microphytobenthos from the intertidal and non-tidal site; (B) zeaxanthin/chlorophyll *a*; (C) lutein/chlorophyll *a*; (D) chlorophyll *b*/chlorophyll *a* in microphytobenthos from the non-tidal site.

The diatoxanthin/diadinoxanthin (Dt/Dd) ratio values changed across the seasons at both sampling sites revealing different variation patterns (Fig. 5B). In the intertidal assemblages the Dt/Dd ratio varied across the year within a very narrow range (min÷max = 0.03–0.17) with the highest values observed in summer (Fig. 5A). Mean Dt/Dd values for summer and autumn were significantly different (Tukey HSD test, $P < 0.05$). In contrast, in the non-tidal communities the ratio values were significantly higher (ANOVA, $P < 0.001$) and their large, but statistically insignificant, variations were observed (min÷max = 0.35–0.75; Tukey HSD test, $P > 0.05$; Fig. 5A).

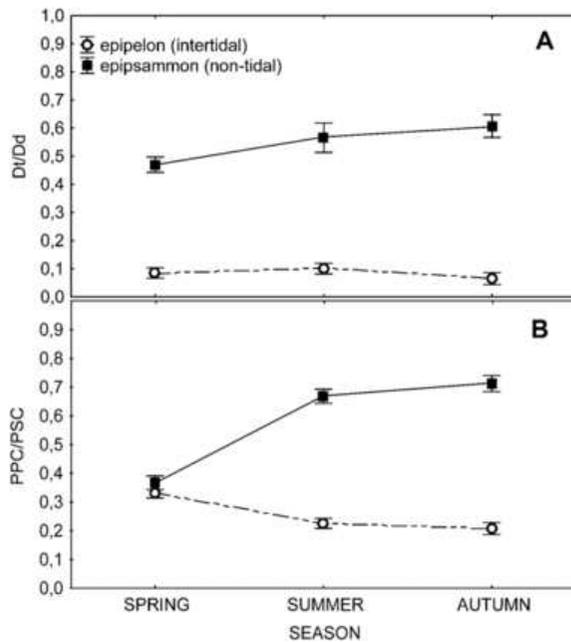


Fig. 5 Seasonal changes in photosynthetic and photoprotective pigments in microphytobenthos from the intertidal and non-tidal site ($n = 9-15$, mean \pm standard error). (A) Diatoxanthin/diadinoxanthin ratio; (B) photoprotective/photosynthetic carotenoids ratio.

Identified carotenoids were separated into two groups, namely (i) photosynthetic (PSC; fucoxanthin, peridinin, prasinoxanthin) and (ii) photoprotective (PPC; alloxanthin, β -carotene, diadinoxanthin, diatoxanthin, lutein, neoxanthin, violaxanthin, zeaxanthin) carotenoids (following Stoř and Kosakowska, 2000), and subsequently their relationships were studied. In the epipelon, the PPC/PSC ratio decreased in summer, while in the epipsammon conversely, an increasing pattern was observed (Fig. 5B). Moreover, there were no statistically significant differences between summer and autumn in the mean ratio values for both microphytobenthos types (Tukey HSD test, $P > 0.05$).

3.3 Photosynthetic parameters

The adjustment of the mathematical model (Platt et al., 1980) was always good and R^2 always exceeded the value of 0.9 (Fig. 6). The photosynthetic response of microphytobenthos varied significantly between seasons and sites (ANOVA, $P < 0.05$). In both assemblage types the highest P_m values were observed in summer, whereas the lowest in autumn. There were no differences between microphytobenthos types within each particular season (Tukey HSD test, $P > 0.05$). The highest initial slope (α) was measured in spring and no statistically significant difference between intertidal and non-tidal communities was observed (Tukey HSD test, $P > 0.05$). In summer and autumn, however, α values measured for the non-tidal communities were statistically lower comparing to the corresponding values from the intertidal assemblages (Tukey HSD test, $P < 0.001$). There were no significant differences in summer and autumn α values in the epipelon (Tukey HSD test, $P > 0.05$, Fig. 7A), while in the epipsammon photosynthetic efficiency (α) showed a significant decrease over time (Tukey HSD test, $P < 0.05$, Fig. 7B). The saturation index (E_k) measured for the epipelon and epipsammon exhibited different seasonal variations (Fig. 7C). E_k of the intertidal communities varied similarly to P_m with the highest value during the warmest season. In the microphytobenthos from non-tidal site, a significantly lower mean E_k was found in spring (Tukey HSD test, $P < 0.05$), and the parameter subsequently increased reaching its maximum in autumn. During summer and autumn, P_m and α were changing concomitantly ($y = 0.00459x + 0.00851$, $r = 0.814$, $P < 0.001$), therefore no difference between summer and autumn mean E_k values was observed.

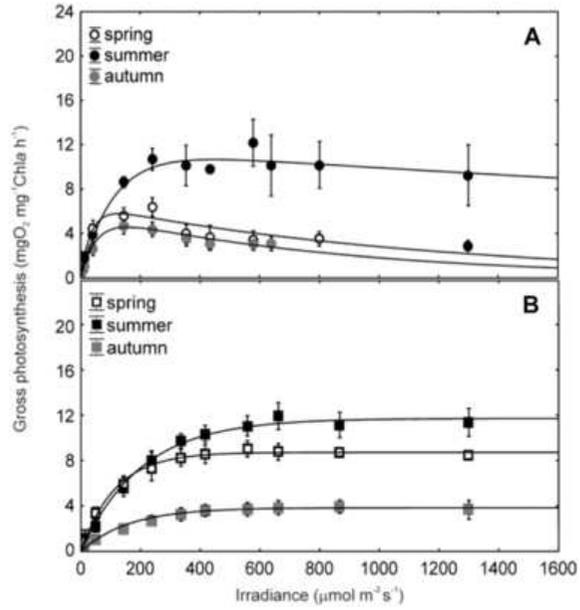
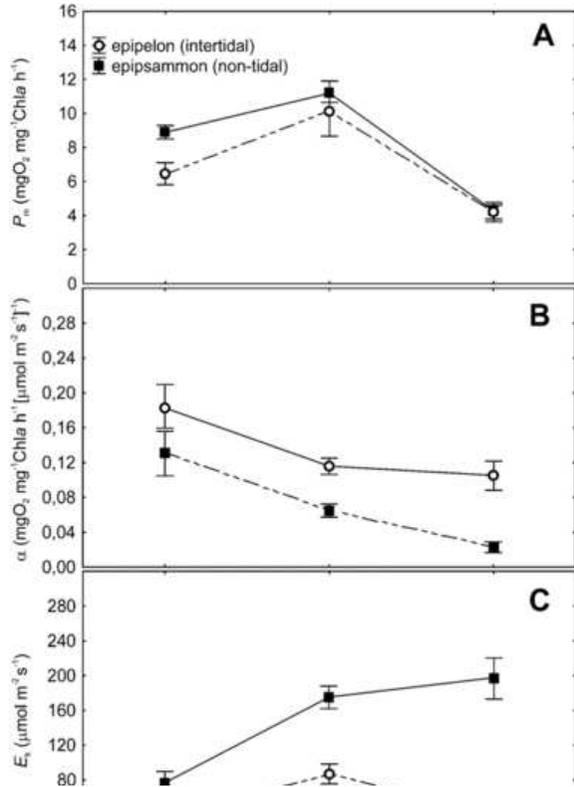


Fig. 6 Seasonal variation in the shape of P-E curves [gross photosynthesis ($n = 9-15$, mean \pm standard error) vs. light] in microphytobenthos from the intertidal (A) and non-tidal (B) site.



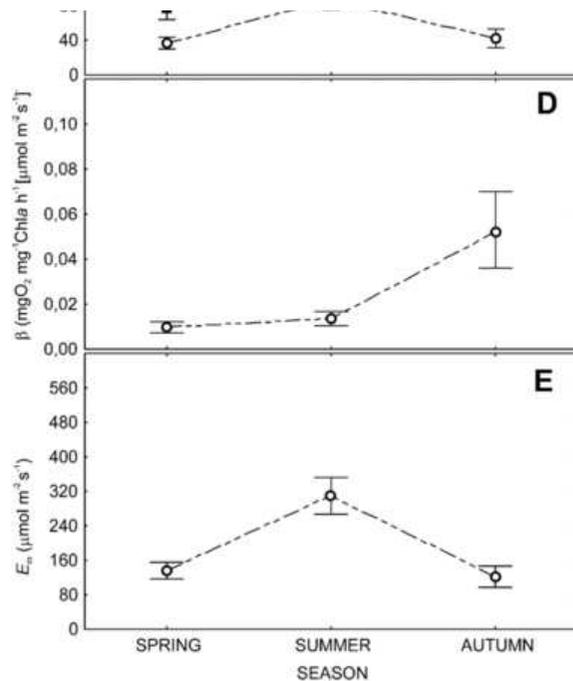


Fig. 7 Seasonal variations of photosynthetic parameters ($n = 9-15$, mean \pm standard error). (A) The photosynthetic capacity (P_m); (B) the maximum light utilization coefficient (α); (C) the light saturation index (E_k); (D) photoinhibition parameter (β); (E) the light saturation point (E_m) in microphytobenthos from the intertidal and non-tidal site.

One of the most apparent differences between P-E curves obtained for algae from the two sampling sites was the fact that in the non-tidal assemblages there was no downturn in photosynthetic rates when exposed to high light ($\beta = 0$). Contrastingly, in the intertidal samples β was always present. The autumn β value was significantly higher compared to spring and summer (Kruskal–Wallis test, $P < 0.05$, Fig. 7D). For light saturation point (E_m), maximum values were calculated for summer (Kruskal–Wallis test, $P < 0.05$), whereas they were lower for spring and autumn (Fig. 7E).

4 Discussion

Previous studies showed that microphytobenthos structure depends on the sediment type (Round, 1965, 1979). The species identified in the intertidal samples were typical of muddy sediments (e.g. Ribeiro et al., 2003; Haubois et al., 2005). The assemblages consisted of small and large taxa of diatoms, and across seasons a clear shift in dominance was observed. *Navicula* genus was the main component of the assemblage in summer, whereas larger diatoms dominated during cooler seasons. The biological activity of small and large diatoms is different, therefore they function within different ecological constraints (Snoeijs et al., 2002). Haubois et al., 2005 suggested that the high abundance of small species may be due to the resuspension/deposition cycle and redistribution of microphytobenthos during the high tide, which causes losses of biomass and in turn promotes smaller fast-growing taxa. However, although diatoms' growth rates depend on cell size (Williams, 1964), it is true at optimal temperatures, while within a low temperature range diatoms of different sizes grow at a similar rate (Admiraal, 1977). This could explain the strong dominance of a single species during warmer months and more species comparably contributing to the assemblage structure in autumn and winter seasons. The proportion between small and large diatoms may strongly affect the overall ecophysiological properties of the community. Thus the observed seasonal variability in species dominance may, at least partially explain variations in the photoacclimation of an assemblage.

Diatoms dominated microphytobenthos in Puck Bay considering both the number of species and their biomass, in congruence with previous results (e.g. Witkowski, 1993). This was also reflected in the pigments as fuco/Chl *a* ratio was the highest throughout the year. Microphytobenthos in non-tidal coastal shallows develops more complex structures lasting over longer periods of time as compared to the day-to-day formed biofilms on intertidal mudflats, leading to the higher diversity and evenness of species. Most of the species observed in the non-tidal microphytobenthos were smaller individuals ($\leq 15 \mu\text{m}$), which are typical of sandy sediments, as a limited colonization space and the environment dynamics favour the growth of small taxa (de Jonge, 1985; Sundbäck and Snoeijs, 1991). The dominant species were generally the same as reported in other studies (e.g. Witkowski, 1991; Zgrundo et al., 2009). Previous reports on microphytobenthos from the southern Baltic coast (e.g. Witkowski, 1993; Pliński and Kwiatkowski, 1996) showed that cyanobacteria and other algal groups are most abundant in summer, while in other seasons their number is strongly reduced. The results obtained in this study differ from previous observations showing that the input of non-diatom taxa to the micorphytobethos biomass was constant throughout the year, although with a seasonally changing dominant groups.

Diagnostic pigments confirmed the presence of particular taxonomic groups, however their concentration did not always correspond to algal groups' biomass, indicating that pigments had other sources or their concentrations were altered by photoacclimation. Strong variations in Chl**b**/Chl**a** and increased values of lutein/Chl**a** suggested that those pigments came also from phanerogam debris as the sampling site is located nearby underwater meadows formed by *Chara baltica* Bruzelius, *Myriophyllum Myriophyllum spicatum* L., *Ruppia maritima* L. and *Zostera marina* L., and is surrounded by *Phragmites australis* (Cav.) Trin. Moreover, a strong correlation between chl**b** and diatoxanthin in autumn pointed to euglenophytes as a significant source of the former.

Some authors speculated that microalgae from muddy sediments are usually acclimated to rather low light intensities, while the organisms of sandy sediments show features of high light acclimation (Barranguet et al., 1998; Barranguet and Kromkamp, 2000). This was later proved when Jesus et al. (2009) analysed the pigment composition of benthic communities inhabiting different sediment types and showed that benthic microalgae from mud revealed higher ratios of fuco/Chl**c** and fuco/Chl**a** and lower Dt/Dd ratios than those inhabiting sands. The same tendencies were recorded in the present study supporting the notion of the epipelonal assemblages' preference for low light, and the non-tidal epipsammon for higher irradiances.

Algae acclimated to low light conditions maximise light absorption through the increase in the number or/and size of photosynthetic units and variations in the proportion of photosynthetic and photoprotective pigment content. Conversely, under high light conditions algae limit light absorption and maximise the utilization of already absorbed photons by increasing the amount and activity of Calvin cycle enzymes (Falkowski and Raven, 1997; Copertino et al., 2009). Photoacclimation is reflected in *P-E* curve variations commonly involving increased value of the initial slope (α) and decreased P_m under low light leading to low E_k values. An inverse response is observed under high light resulting in high E_k . Other responses cannot be excluded however, as the behaviour and therefore interpretation of *P-E* curves depend on the unit of biomass to which photosynthesis rates are normalised (Richardson et al., 1983 and references therein). In ecological studies, photosynthetic rates are usually expressed on a chlorophyll *a* basis as it is the most common measure of algal biomass (MacIntyre et al., 2002).

In the epipelonal α was quite high, compared to the epipsammon, which is in agreement with the low light photoacclimation model, and varied within a quite limited range, only slightly declining over the year. The pigment analyses revealed that the PPC/PSC ratio was very low in summer and autumn indicating an increased amount of photosynthetic carotenoids. Seasonal decrease in α coupled with the increase in PSC photosynthetic carotenoids/chlorophyll *a* (PSC/Chl *a* ratio; data not shown) is still consistent with the shade acclimation as light absorption is a self-limiting process and cell heavy pigmentation leads to the self-shading of pigment molecules, a decrease in optical absorption cross-section and consequently less efficient light harvesting (Dubinsky and Stambler, 2009). Photoacclimation in intertidal mudflats involves not only physiological adjustment of the photosynthetic apparatus but also behavioural photoacclimation (Serôdio et al., 2006; Mouget et al., 2008) connected to the diatoms ability to move. Serôdio et al. (2006) showed that the peak in biomass at the sediment surface was observed when light intensity was within the range of 100–250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and downward migration started at higher light intensities. Microalgae by moving position themselves within the most optimal light regime in the sediment (Blanchard and Cariou-Le Gall, 1994; Underwood, 2005). Migratory behaviour in epipelonal from Aiguillon Bay has already been well documented (Herlory et al., 2004), which together with present observations on limited α seasonal variations pointed to the vertical migration as its main photo-regulation mechanism, in agreement with Jesus et al. (2009).

In the epipsammon an approx. 6-fold decrease in α was recorded across the seasons. This was accompanied by a substantial increase in the xanthophylls pool in relation to chlorophyll *a*. Elevated levels of PPC/PSC ratio indicated an increase in photoprotective carotenoids (including a 3.5-fold increase in zeaxanthin/Chl *a*, Figs. 3B, and a 2-fold diatoxanthin + diadinoxanthin/chlorophyll *a* [Dt + Dd/Chl *a*], data not shown). Such pigments (e.g. zeaxanthin, diatoxanthin, β -caroten) do not transfer energy to the reaction centres, thus light absorption takes place with reduced efficiency (Falkowski and Raven, 1997) which allows a safe use of high irradiance. This suggests that adaptations to seasonally changing light regime in epipsammon are based on the physiological adjustment of photosynthetic systems and, as opposed to epipelonal, it lacks endogenous rhythms and does not display migratory behaviour (Raven and Geider, 2003; Jesus et al., 2009).

In both microphytobenthos types, the seasonal variations in P_m followed the same pattern with maximum during the warmest summer months which also corresponded with the period of the highest light intensity. This unimodal seasonality is consistent with previous findings (e.g. Light and Beardall, 2001). Increased P_m during summer months is in agreement with the photoacclimation theory indicating that microalgae maximize the use of absorbed light (Copertino et al., 2009), which is possible due to the increased content of electron transport chain and activity of enzymes involved in carbon metabolism, mainly RUBISCO (Davison, 1991).

The relationship between α and P_m is described by the E_k parameter which does not depend on biomass (Henley, 1993), and thus seems to be more useful in describing the photoacclimation status of algae (Falkowski and Raven, 1997; Light and Beardall, 2001).

In the epipelonal, the observed E_k values were quite low (37–87 $\mu\text{mol m}^{-2} \text{s}^{-1}$), confirming the hypothesis about its shade acclimation. The previously reported unimodal seasonality of E_k (e.g. Light and Beardall, 2001) was also observed in this study, with values highest in summer and lowest in autumn. The optimisation of P_m in relation to α suggests long-term photoacclimation allowing microalgae to use absorbed light efficiently throughout the year (Blanchard and Cariou-Le Gall, 1994; Light and Beardall, 2001). In the epipelonal, the photoacclimation process involved mainly changes in P_m as quite limited variation in α was observed indicating an " E_k -dependent" light-photosynthesis relationship (Behrenfeld et al., 2004). The significant correlation between E_k and P_m (linear correlation, $r = 0.811$, $P < 0.001$), and the lack of the correlation between E_k and α (linear correlation, $r = -0.285$, $P > 0.05$), implied that light intensity may not play such an important role in regulating productivity (Guarini et al., 2002). Relatively limited α variations in the intertidal epipelonal allow the assumption that the overall primary productivity is controlled mainly by temperature.

In comparison to the epipelon from intertidal mudflats, in the epipsammic assemblages E_k was higher (77–198 $\mu\text{mol m}^{-2} \text{s}^{-1}$), indicating high light acclimation. A significant change in E_k was observed during the transition from spring to summer months. No difference was found between summer and autumn values. The lack of E_k changes indicates that both contributing parameters i.e. P_m and α ($E_k = P_m/\alpha$; Talling, 1957) covary (linear correlation, $r = 0.814$, $P < 0.001$). The interpretation of data obtained for the non-tidal assemblages is difficult as no single mechanism exclusively responsible for the 'E_k-independent' variability was identified (see Behrenfeld et al., 2004).

Photoinhibition is a time-dependent, dynamic process, related to the light dose received and it was previously described as the negative slope of the light response curve (β) at supersaturating irradiance (e.g. Platt et al., 1980). Henley (1993) claimed that β indicates photoinhibition only during the incubation period and thus it does not reflect true photoinhibition that microalgae experience in their environment. In addition, a decrease in photosynthetic rates under high light may also result from a reversible down-regulation (e.g. xanthophyll cycle; Serôdio et al., 2005). The decrease in photosynthetic rates was only observed in the epipellic assemblages. In autumn β increased, and light saturation point ($E_m = 2.8E_k$) decreased significantly (Kruskal–Wallis test, $P < 0.05$, when E_m was expressed in terms of E_k) as compared to values observed in spring and summer (approx. $E_m = 3.7E_k$). Several studies have shown that at low temperature plants may increase the level of photoprotective pigments which makes them more resistant to photoinhibition (e.g. Maxwell et al., 1995). The same phenomenon was observed in the diatom *Fistulifera saprophila* (Biskup, unpublished data). A decreased pool of xanthophylls in the epipelon observed in this study (Figs. 5A,B) could have limited its photoprotective potential under lower temperatures leading to a photoinhibitory decrease in photosynthetic rates rather than photoprotective down-regulation. Laboratory experiments have shown that epipellic diatoms may experience photoinhibition when illuminated with high light for a longer time ($t > 90$ min; Blanchard et al., 2004), although it was rarely or never detected in the field (e.g. Blanchard and Montagna, 1992; Dodds et al., 1999). This was explained by epipelon photoprotective migratory behaviour and dense biofilm formation leading to self-shading, which limits damaging light exposure (Blanchard et al., 2004). Recent study of Serôdio et al. (2008), however, revealed that microphytobenthos may experience photoinhibition in its natural environment, and photoprotective mechanisms, both behavioural and physiological, may not be sufficient to prevent it. Whether it is due to the altered physiological state of cells, or structural changes within the biofilm it is still not known.

5 Conclusions

This study presents structure and photoacclimation mechanisms in two microphytobenthos communities from two different marine ecosystems, i.e., intertidal mudflats (Aiguillon Bay) and non-tidal sandy coastal shallows (Puck Bay). Photosynthetic activity of both microphytobenthos types changed seasonally indicating their good adjustment to varying environmental conditions. The composition and proportion of photosynthetic and photoprotective carotenoids confirmed low and high light acclimation in the epipelon and epipsammon, respectively. This was further supported by the variations observed in photosynthetic parameters estimated on *P-E* curves. The epipsammon turned out to be resistant to high irradiance, suggesting that physiological mechanisms were sufficient enough to prevent photoinhibition. In contrast, a downturn in photosynthetic rates was observed in the epipelon subjected to high light intensities revealing its susceptibility to higher irradiance. This fact reinforced the hypothesis that behavioral photoacclimation, accompanying physiological mechanisms, is an essential component of photosynthesis regulation in microphytobenthos from intertidal mudflats. Overall, the observed photosynthetic properties of studied microphytobenthos communities **seems seem** to be a resultant of autecological characteristics of species contributing to the assemblage and are governed by site specific environmental conditions.

Uncited reference

Behrenfeld et al., 1998, Woelfel et al., 2007.

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References

- Admiraal W., Influence of light and temperature on the growth rate of estuarine benthic diatoms in culture, *Mar. Biol.* **39**, 1977, 1–9.
- Barranguet C. and Kromkamp J., Estimating primary production rates from photosynthetic electron transport in estuarine microphytobenthos, *Mar. Ecol. Prog. Ser.* **204**, 2000, 39–52.
- Barranguet C., Kromkamp J. and Peene J., Factors controlling primary production and photosynthetic characteristics of intertidal microphytobenthos, *Mar. Ecol. Prog. Ser.* **173**, 1998, 117–126.
- Battarbee R.W., Diatom analysis, In: Berglund B.E., (Ed), *Handbook of Holocene Palaeoecology and Palaeohydrology*, 1986, Jhon Wiley & Sons Ltd., 527–570.
- Behrenfeld M.J., Prasil O., Babin M. and Bruyant F., In search of a physiological basis for covariations in light-limited and light-saturated photosynthesis, *J. Phycol.* **40**, 2004, 4–25.
- ~~Behrenfeld M.J., Prasil O., Kolber Z.S., Babin M. and Falkowski P.G., Compensatory changes in Photosystem II electron turnover rates protect photosynthesis from photoinhibition, *Photosynth. Res.* **58**, 1998, 259–268.~~
- Blanchard G.F. and Cariou-Le Gall V., Photosynthetic characteristic of microphytobenthos in Marennes-Oléron Bay, France: preliminary results, *J. Exp. Mar. Biol. Ecol.* **182**, 1994, 1–14.

- Blanchard G.F., Guarini J.-M., Dang C. and Richard P., Characterizing and quantifying photoinhibition in intertidal microphytobenthos, *J. Phycol.* **40**, 2004, 692–696.
- Blanchard G.F. and Montagna P.A., Photosynthetic response of natural assemblages of marine benthic microalgae to short- and long-term variations of incident irradiance in Baffin Bay, Texas, *J. Phycol.* **28**, 1992, 7–14.
- Cartaxana P., Ruivo M., Hubas C., Davidson I., Seródio J. and Jesus B., Physiological versus behavioral photoprotection in intertidal epipelagic and epipsammic benthic diatom communities, *J. Exp. Mar. Biol. Ecol.* **405**, 2011, 120–127.
- Consalvey M., Paterson D.M. and Underwood G.J.C., The ups and downs of life in a benthic biofilm: migration of benthic diatoms, *Diatom Res.* **19**, 2004, 181–202.
- Copertino M.S., Cheshire A. and Kildea T., Photophysiology of a turf algal community: integrated responses to ambient light and standing biomass, *J. Phycol.* **45**, 2009, 324–336.
- Cunningham B. and Kirk P.L., A new form of differential micro-respirometer, *J. Physiol.* **24**, 1940, 135–149.
- Dahl B. and Blanck H., Use of sand-living microalgal communities (epipsammon) in ecotoxicological testing, *Mar. Ecol. Prog. Ser.* **144**, 1996, 163–173.
- Davison I.R., Environmental effects on algal photosynthesis: temperature, *J. Phycol.* **27**, 1991, 2–8.
- de Jonge V.N., The occurrence of “epipsammic” diatom populations: a result of interaction between physical sorting of sediments and certain properties of diatom species, *Estuar. Coast. Shelf Sci.* **21**, 1985, 607–622.
- Dodds W.K., Biggs B.J.F. and Lowe R.L., Photosynthesis-irradiance patterns in benthic microalgae: variations as a function of assemblage thickness and community structure, *J. Phycol.* **35**, 1999, 42–53.
- Dubinsky Z. and Stambler N., Photoacclimation processes in phytoplankton: mechanisms, consequences, and applications, *Aquat. Microb. Ecol.* **56**, 2009, 163–176.
- Eaton J.W. and Moss B., The estimation of numbers and pigment contents in epipelagic algal populations, *Limnol. Oceanogr.* **11**, 1966, 584–595.
- Falkowski P.G. and Raven J.A., Aquatic Photosynthesis, 1997, Blackwell Science; Massachusetts, 375.
- Guarini J.-M., Cloern J.E., Edmunds J. and Gros P., Microphytobenthic potential productivity estimated in three tidal embayments of the San Francisco Bay: a comparative study, *Estuaries* **25** (3), 2002, 409–417.
- Guarini J.-M., Gros P., Blanchard G., Richard P. and Fillon A., Benthic contribution to pelagic microalgal communities in two semi-enclosed, European-type littoral ecosystems (Marennes-Oléron Bay and Aiguillon Bay, France), *J. Res. Res.* **52**, 2004, 241–258.
- Hartwig E.O., Factors affecting respiration and photosynthesis by the benthic community of a subtidal siliceous sediment, *Mar. Biol.* **46**, 1978, 283–293.
- Haubois A.-G., Sylvestre F., Guarini J.-M., Richard P. and Blanchard G.F., Spatio-temporal structure of the epipelagic diatom assemblage from an intertidal mudflat in Marennes-Oléron Bay, France, *Estuar. Coast. Shelf Sci.* **64**, 2005, 385–394.
- HELCOM, Manual for Marine Monitoring in the COMBINE Programme, 2013.
- Henley W.J., Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes, *J. Phycol.* **29**, 1993, 729–739.
- Herlory O., Guarini J.-M., Richard P. and Blanchard G.F., Microstructure of microphytobenthic biofilm and its spatio-temporal dynamic in an intertidal mudflat (Aiguillon Bay, France), *Mar. Ecol. Prog. Ser.* **282**, 2004, 33–44.
- Herlory O., Richard P. and Blanchard G.F., Methodology of light response curves: application of chlorophyll fluorescence to microphytobenthic biofilms, *Mar. Biol.* **153**, 2007, 91–101.
- Jankowska H. and Łęczyński L., Osady denne, In: Korzeniewski K., (Ed), *Zatoka Pucka*, 1993, Fundacja Rozwoju Uniwersytetu Gdańskiego; Gdańsk, 320–327.
- Jesus B., Brotas V., Ribeiro L., Mendes C.R., Cartaxana P. and Paterson D.M., Adaptations of microphytobenthos assemblages to sediment type and tidal position, *Cont. Shelf Res.* **29**, 2009, 1624–1634.
- Kromkamp J.C., Barranguet C. and Peene J., Determination of microphytobenthos PSII quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence, *Mar. Ecol. Prog. Ser.* **162**, 1998, 45–55.
- Kühl M., Lassen C. and Jørgensen B.B., Light penetration and light intensity in sand marine sediments measured with irradiance and scalar irradiance fiber-optic microprobes, *Mar. Ecol. Prog. Ser.* **105**, 1994, 139–148.
- Light B.R. and Beardall J., Photosynthetic characteristics of sub-tidal benthic microalgal populations from a temperate, shallow water marine ecosystem, *Aquat. Bot.* **70**, 2001, 9–27.
- Lorin J., Contribution à l'étude des transits sédimentaires dans la partie orientale du Pertuis Breton et la baie de l'Aiguillon, *Bull. l'Institut Géologie Bassin d'Aquitaine* **5**, 1968, 111–139.

Lucas C.H., Banham C. and Holligan P.M., Benthic-pelagic exchange of microalgae at a tidal flat. 2. Taxonomic analysis, *Mar. Ecol. Prog. Ser.* **212**, 2001, 39–52.

MacIntyre H.L., Geider R.J. and Miller D.C., Microphytobenthos: the ecological role of the “secret garden” of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production, *Estuaries* **19**, 1996, 186–201.

MacIntyre H.L., Kana T.M., Anning T. and Geider R.J., Photoacclimation of photosynthesis-irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria, *J. Phycol.* **38**, 2002, 17–38.

Mantoura R.F.C. and Repeta D.J., Calibration methods for HPLC, In: Jeffrey S.W., Mantoura R.F.C. and Wright S.W., (Eds.), *Phytoplankton Pigments in Oceanography: Guidelines to Modern Methods*, 1997, UNESCO Publishing; Paris, 407–428.

Maxwell D.P., Falk S. and Huner N.P.A., Photosystem II excitation pressure and development of resistance to photoinhibition, *Plant Physiol.* **107**, 1995, 687–694.

Mouget J.-L., Perkins R., Consalvey M. and Lefebvre S., Migration or photoacclimation to prevent high irradiance and UV-B damage in marine microphytobenthic communities, *Aquat. Microb. Ecol.* **52**, 2008, 223–232.

Olenina I., Hajdu S., Andersson A., Edler L., Wasmund N., Busch S., Göbel J., Gromisz S., Huseby S., Huttunen M., Jaanus A., Kokkonen P., Ledaine I. and Niemkiewicz E., Biovolumes and size-classes of phytoplankton in the Baltic Sea, *Balt. Sea Environ. Proc.* **106**, 2006, 144.

Platt T., Gallegos C.L. and Harrison W.G., Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton, *J. Mar. Res.* **38**, 1980, 387–701.

Pliński M. and Kwiatkowski J., Microphytobenthos of the shallow littoral of the southern Baltic, *Oceanol. Stud.* **4**, 1996, 65–79.

Raven J.A. and Geider R.J., Adaptation, acclimation and regulation in algal photosynthesis, In: Larkum A.W.D., Douglas S.E. and Raven J.A., (Eds.), *Photosynthesis in Algae*, 2003, Kluwer Academic Publisher; The Netherlands, 385–412.

Ribeiro L., Brotas V., Mascarell G. and Couté A., Taxonomic survey of the microphytobenthic communities of two Tagus estuary mudflats, *Acta Oecol.* **24**, 2003, S117–S123.

Richardson K., Beardall L. and Raven J.A., Adaptation of unicellular algae to irradiance: an analysis of strategies, *New. Phytol.* **93**, 1983, 157–191.

Round F.E., The epipsammon a relatively unknown freshwater algal association, *Br. Phycol. Bull.* **2** (6), 1965, 456–462.

Round F.E., Benthic Marine Diatoms, *Oceanography and Marine Biology. Annual Review* **vol. 9**, 1971, 83–139.

Round F.E., A diatom assemblage living below the surface of intertidal sand flats, *Mar. Biol.* **54**, 1979, 219–223.

Round F.E., *The Ecology of Algae*, 1981, Cambridge University Press; Cambridge, 653.

Sakshaug E., Bricaud A., Dandonneau Y., Falkowski P.G., Kiefer D.A., Legendre L.L., Morel A., Parslow J. and Takahashi M., Parameters of photosynthesis: definitions, theory and interpretation of results, *J. Plankton Res.* **19** (11), 1997, 1637–1670.

Seródio J., Cruz S., Vieira S. and Brotas V., Non-photochemical quenching of chlorophyll fluorescence and operation of the xanthophyll cycle in estuarine microphytobenthos, *J. Exp. Mar. Biol. Ecol.* **326**, 2005, 157–169.

Seródio J., Coelho H., Vieira S. and Cruz S., Microphytobenthos vertical migratory photoresponse as characterised by light-response curves of surface biomass, *Estuar. Coast. Shelf Sci.* **68**, 2006, 547–556.

Seródio J., Vieira S. and Cruz S., Photosynthetic activity, photoprotection and photoinhibition in intertidal microphytobenthos as studied *in situ* using variable chlorophyll fluorescence, *Cont. Shelf Res.* **28**, 2008, 1363–1375.

Šesták Z., Jarvis P.G. and Čatský J., Criteria for the selection of suitable methods, In: Šesták Z., Čatský J. and Jarvis P.G., (Eds.), *Plant Photosynthetic Production. Manual of Methods*, 1971, Publisher, Hague, 257–275.

Snoeijs P., Busse S. and Potapova M., The importance of diatom cell size in community analysis, *J. Phycol.* **38**, 2002, 265–272.

Stoń J. and Kosakowska A., Qualitative and quantitative analysis of Baltic phytoplankton pigments, *Oceanologia* **42** (4), 2000, 449–471.

Strickland I.D.H. and Parsons T.R., *A Practical Handbook of Seawater Analysis*, 1972, Fisheries Research Board of Canada; Ottawa, 310.

Sundbäck K., The response of shallow-water sediment communities to environmental changes, In: Krumbien W.E., Paterson D.M. and Stal L.J., (Eds.), *Biostabilization of Sediments*, 1994, Verlag; Oldenburg, 17–40.

Sundbäck K., Miles A. and Linares F., Nitrogen in nontidal littoral sediments: role of microphytobenthos and denitrification, *Estuaries Coasts* **29** (6B), 2006, 1196–1211.

Sundbäck K. and Snoeijs P., Effects of nutrient enrichment on microalgal community composition in a coastal shallow-water sediment system: an experimental study, *Bot. Mar.* **34**, 1991, 341–358.

Sutherland T.F., Grant J. and Amos C.L., The effect of carbohydrate production by the diatom *Nitzschia curvilineata* on the erodibility of sediment, *Limnol. Oceanogr.* **43**, 1998, 65–72.

Talling J.F., The phytoplankton population as a compound photosynthetic system, *New Phytol.* **56**, 1957, 133–149.

Underwood G.J.C., Microalgal (microphytobenthic) biofilms in shallow coastal waters: how important are species?, *Proceeding Calif. Acad. Sci.* **56** (15), 2005, 162–169.

Underwood G.J.C. and Kromkamp J., Primary production by phytoplankton and microphytobenthos in estuaries, *Adv. Ecol. Res.* **29**, 1999, 93–153.

Underwood G.J.C., Perkins R.G., Consalvey M.C., Hanlon A.R.M., Oxborough K., Baker N.R. and Paterson D.M., Patterns in microphytobenthic primary productivity: species-specific variation in migratory rhythms and photosynthetic efficiency in mixed-species biofilms, *Limnol. Oceanogr.* **50** (3), 2005, 755–767.

Williams R.B., Division rates of salt marsh diatoms in relation to salinity and cell size, *Ecology* **45** (4), 1964, 877–880.

Witkowski A., Diatoms of the Puck Bay coastal shallows (Poland, Southern Baltic), *Nordic J. Bot.* **11**, 1991, 689–701.

Witkowski A., Mikrofytobentos, In: Korzeniewski K., (Ed), *Zatoka Pucka*, 1993, Fundacja Rozwoju Uniwersytetu Gdańskiego; Gdańsk, 395–415.

~~Weelfel J., Schumann R., Adler S., Hübener T. and Karsten U., Diatoms inhabiting a wind flat of the Baltic Sea: species diversity and seasonal succession, *Estuar. Coast. Shelf Sci.* **75**, 2007, 296–307.~~

Wright S.W., Jeffrey S.W., Mantoura R.F.C., Llewellyn C.A., Bjørnland T., Repeta D. and Welschmeyer N., Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton, *Mar. Ecol. Prog. Ser.* **77**, 1991, 183–196.

Zgrundo A., Dziengo-Czaja M., Bubak I. and Bogaczewicz-Adamczak B., Studies on the biodiversity of contemporary diatom assemblages in the Gulf of Gdańsk, *Oceanol. Hydrobiological Stud.* **37**, 2009, 139–153.

Zurzycki J. and Starzecki W., Volumetric methods, In: Šesták Z., Čatsky J. and Jarvis P.G., (Eds.), *Plant Photosynthetic Production. Manual of Methods*, 1971, ~~Publisher, Hague~~ [Dr W. Junk N.V. Publishers The Hague](#), 257–275.

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