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Diurnal changes of photoadaptive pigments in microphytobenthos

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*Microphytobenthos need photoadaptive strategies to survive the highly dynamic light environment in which they reside. Xanthophyll pigments can provide photoprotection by cycling electrons between epoxide and de-epoxide forms, dissipating excess light energy as heat. This study examined the xanthophyll cycle in microphytobenthos on a tidally exposed substrate at Browns River, Tasmania. Fv/Fm decreased from 0.52 ± 0.01 to 0.47 ± 0.01 at noon in surface samples and a decrease in the diadinoxanthin:chlorophyll-*a *ratio from 0.022 ± 0.003 to 0.015 ± 0.005 also suggests that the microphytobenthos was under physiological stress at noon. The results indicate that the cells exposed to light at the surface migrated deeper into the sediments and replenished the epoxide form of their xanthophylls. The results suggest that micrphytobenthos utilizes both behavioural and physiological strategies to survive in the dynamic intertidal environment.*

Keywords: xanthophyll, microphytobenthos, non-photochemical quenching of energy (NPQ), diatom, Australia, diadinoxanthin, diatoxanthin

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INTRODUCTION

In intertidal environments many physical parameters can change over a range of time scales, from minutes (tidal current, wind wave and cloud cover) to seasons (temperature, light and sediment properties) (Koh *et al.*, 2007). Microphytobenthos (MPB) and other aquatic photoautotrophs are able to adjust their photosynthetic activity, in response to these changes in ambient light, through physiological regulation and behaviour (Serôdio *et al.*, 2006). Although this control of the amount of light is achieved by individual cells, in the MPB its effects are observed at the community level through variation in the biofilm biomass present in the photic zone of the benthos (Serôdio *et al.*, 2006). Behavioural regulation is the relationship between light and cell position, controlled by vertical migration, which optimizes light availability whilst avoiding damaging high irradiances (Jesus *et al.*, 2006). Physiological regulation includes non-photochemical quenching of energy (NPQ) by diversion of excess light energy away from photosystem reaction centres using processes such as the xanthophyll cycle (Jesus *et al.*, 2006).

Physiological regulation, through non-photochemical quenching by the dissipation of excess energy, is an important short term process for the photoprotection of photosystem II against light induced damage (Lavaud *et al.*, 2004). In diatoms NPQ includes the photoprotective operation of the xanthophyll cycle (qE, energy dependent quenching) and photoinhibitory damage to the photosynthetic apparatus (qI, photoinhibitory quenching) (Cruz & Serôdio, 2008). The xanthophyll cycle occurs in the thylakoid membranes of all higher plants, ferns, mosses and several algal groups (Eskling *et al.*, 1997). There are two variants, the violaxanthin cycle, which is more commonly found in higher plants, and the diadinoxanthin (DD) cycle found in some algal groups (Eskling *et al.*, 1997). Xanthophylls are carotenoids containing one or more oxygen radicals and are essential for survival and ecological success by providing photoprotection by quenching the excited states of chlorophylls and by harvesting and efficiently transferring light energy to chlorophylls (Lohr & Wilhelm, 2001). Some of the other methods used to safely dissipate excess light energy via non-photosynthetic quenching include: differential excitation of photosystem I (PSI) versus photosystem II (PSII), ratio of cyclic to non-cyclic electron transport and state transitions, migration of chlorophyll-*a* (Krause & Weiss, 1991), PS II reaction centre quenching (Ivanov *et al.*, 2003), chloroplast shading (Jeong *et al.*, 2002), nitrate reduction and nitrite excretion (hypothesized as very significant for polar diatoms) (Lomas & Gilbert, 1999), and photorespiration and reduced carbon (often glycolate) excretion (Wingler *et al.*, 2000).

The xanthophyll pigments DD and diatoxanthin (DT) are found in many groups of microalgae especially the Bacillariophyceae (diatoms), Chrysophyceae, Xanthophyceae and Dinophyceae (Brown *et al.*, 1999). The DD cycle found in diatoms, involves a rapid and reversible conversion from DD (one epoxide form) to DT (de-epoxidated form) (Muller *et al.*, 2001). DD de-epoxidation begins rapidly after the onset of high light and this rate is light intensity dependent (Lavaud *et al.*, 2004). The epoxide to de-epoxide cycle can dissipate excess energy through non-radiative pathways, decreasing the transfer of captured excitation energy to the PSII reaction centres and thus limiting the amount of photodamage to the photosynthetic apparatus (Serôdio *et al.*, 2005). The xanthophyll cycle is an effective quenching mechanism

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which does not affect the light harvesting efficiency and lessens the cost of synthesizing other carotenoids (Moisan *et al.*, 1998). The amount of DT synthesized via the DD cycle is correlated with the level of qE (Muller *et al.*, 2001). NPQ and DT are linearly related and if DT is not present, qE cannot occur (Lavaud *et al.*, 2004). Under more prolonged, severe light stress qE is replaced by a sustained slowly reversible component of NPQ called qI (Muller *et al.*, 2001). In this study we measured the content of DT and DD to estimate qE and measured photosynthetic parameters to estimate qI.

Very little work has been done on microphytobenthos in intertidal areas of the southern hemisphere. Previous work has questioned the possible use of migration as a behavioural response to changing light conditions at Browns River (Jordan *et al.*, 2008). In that study a seasonal pattern in chlorophyll-*a* and a diurnal pattern of F_v/F_m values were observed, however, the benthic microalgal community at Browns River did not clearly demonstrate vertical migration through the sediment. In response to these findings the goal of this current work was to examine to what extent microphytobenthos at Browns River use the xanthophyll cycle throughout the day as a physiological defence against photoinhibition.

MATERIALS AND METHODS

Samples were collected at sunrise (6:37 am), noon (12:00 pm and sunset (17:45 pm) on 2 September 2007. During each sampling period seven 45 mm diameter sediment cores were taken from an artificially shaded (1 m^2, 50% decrease in irradiance) and non-shaded site. Three cores were taken for fluorescence analysis, three for pigment analysis and one for taxonomic purposes. The core was manually pushed into the sediment and stoppered using a rubber bung. The top 2 mm of the 45 mm diameter cores was removed with a knife and saved; the remaining core was then sectioned at 5 mm intervals. The core samples for pigment analysis were immediately frozen in liquid nitrogen and returned to the laboratory where they were stored at -80°C freezer until analysis.

Pigments were extracted twice in 100% acetone. Samples were vortexed for 30 seconds and then sonicated (Bransonic) at 0°C for 15 minutes in the dark. The samples were then kept in the dark at 4°C for 15 hours. After this time the tubes were centrifuged and the supernatant removed. The second extraction was only 3 hours at 4°C. The final extract mixture was adjusted to 90:10 acetone: water (vol: vol) and filtered through a 0.2 μm membrane filter (Whatman, anatope) prior to analysis by high performance liquid chromatography (HPLC). A Waters-Alliance HPLC system was used, comprising a 2695XE separations module with column heater and refrigerated autosampler, a 2996 photo-diode array detector and methodology after Wright *et al.* (1991). Concentrations of chlorophyll-*a*, chlorophyll-*b* and β,β-carotene in sample chromatograms were determined from Sigma standards while all other pigment concentrations were determined from DHI standards (Denmark). Pigment concentrations were calculated as pigment per wet weight of sediment.

Filtered seawater (0.22 μm membrane filter; Pall Supor, New York) was added to the top 2 mm samples taken from the 3 replicate cores for fluorescence analysis. A pulse amplitude modulated (PAM) fluorometer (Water PAM, Walz, Effeltrich) was used to determine the chlorophyll fluorescence. To calculate the maximum PSII quantum yield (F_v/F_m) immediately after sampling the samples were dark adapted in the field for 15 minutes by wrapping the jars in foil and placing them in a dark container. Rapid light curves (RLC) were taken under software control (Wincontrol, Walz) to obtain values for F_v/F_m, NPQ, and E_k (Ralph & Gademann, 2005). Red light emitting diodes (LED) provided the actinic light used in the RLC at levels of 0, 58, 88, 131, 204, 304, 435, 608 and 1012 μmol photons $m^{-2}\,s^{-1}$. The saturation pulse was at 3000 μmol photons $m^{-2}\,s^{-1}$ for a period of 0.8 seconds. Samples were exposed to each light level for 10 seconds. Photomultiplier gain settings were between 7 and 10 (Wincontrol, Walz). E_k, the light saturation parameter, was calculated from the intercept between the maximum relative electron transport rate (rETR) and α, the photosynthetic efficiency (Falkowski & Raven, 2007). The rETR was calculated by multiplying the irradiance by the quantum yield measured at the end of that interval (Genty *et al.*, 1989). PAR versus rETR curves were described using the model of Jassby & Platt (1976) using multiple non-linear regression curve fitting protocols on Systat software (v5.2 for Macintosh Systat Inc.).

The xanthophyll cycle pigments DD and DT were normalized against chlorophyll-*a* to improve the discrimination of cycling by removing the bias caused by variation in amount of MPB biomass (Claustre *et al.*, 1994). The xanthophyll pool was calculated as diadinoxanthin + diatoxanthin (DD + DT) and normalized to chlorophyll-*a* (Chl*a*) following Brown *et al.* (1999). The pigment concentrations and fluorescence parameters were analysed for significant change with a three-way ANOVA using SigmaStat (Systat Software Inc. Chicago, USA) after checking for normality and homoscedasticity. The null hypothesis of no difference was considered disproven if the probability was >0.05. The level of variability around the mean values is represented by the standard error.

Taxonomic composition was assessed both microscopically (Zeiss Axioskop, Germany, 400 × objective) and using pigment data with the interpretative software CHEMTAX (Mackey *et al.*, 1996). The input matrix of pigment ratios was derived from the published literature (Mackey *et al.*, 1996).

RESULTS

Several strategies potentially used by microphytobenthos to survive in the changing light environment throughout a day were examined in the predominately muddy sediment (34% sand, 66% mud/silt) of Browns River (42°96′S 147°51′E) in the greater Hobart area on 2 September 2007. Low tides occurred at 5.45 am and 7.00 pm with a high tide at 11.50 am and the sampling site was continuously covered with water throughout the day. At sunrise water depth was ∼20, at noon it was ∼110 cm and at sunset it was ∼30 cm. The maximum midday surface irradiance on 2 September was approximately 1350 μmol photons $m^{-2}\,s^{-1}$. Irradiance at sunrise was ∼35 μmol photons $m^{-2}\,s^{-1}$ and was ∼45 μmol $m^{-2}\,s^{-1}$ photons at sunset.

CHEMTAX

Pigment analysis using CHEMTAX confirmed the taxonomic composition observed through microscopy (Table 1). CHEMTAX estimated that MPB at Browns River was

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Table 1. The taxonomic composition (CHEMTAX; Mackey *et al.*, 1996) of surface sediment from Browns River on the 2 September 2007.

	Chlorophyte	Chrysophyte	Cryptophyte	Cyanobacteria	Diatoms	Dinoflagellate	Haptophyte	Prasinophyte
Total pigment (%)	21.7%	2.8%	14.4%	0.12%	57.9%	0.37%	2.4%	0.35%
SD	4.23%	0.81%	8.60%	0.17%	5.55%	0.11%	0.48%	0.92%

SD, standard deviation.

dominated by diatoms with 57.9% of the overall community composition. The major diatom genera present, determined by microscopy, included *Navicula, Cocconeis, Nitzschia, Amphora* and *Pleurosigma* species.

Chlorophyll

Chlorophyll-*a* (chl*a*) in the surface 2 mm was between 9.02 ± 0.56 and 14.12 ± 0.52 mg chl*a* m^{-2} and between 3.77 ± 0.46 and 10.27 mg chl*a* m^{-2} at 5 mm depth (Table 2).

At both the shaded and unshaded sites the surface total chl*a* was significantly greater than that at 5 mm depth (df = 1, F = 13.8, $P = 0.001$) and the chl*a* concentrations at the non-shaded site were significantly greater than in the artificially shaded site (df = 1, F = 5.4, $P = 0.029$).

There was a significantly greater percentage of chlorophyll-*a* found at the surface relative to the percentage at depth (df = 1, F = 54.5, $P < 0.001$) at both sites. The percentage of chlorophyll in the surface 2 mm changed throughout the day with a significant decrease at noon compared to sunrise and sunset with an inverse relationship with 5 mm depth (df = 2, F = 5.14, $P = 0.014$) (Figure 1).

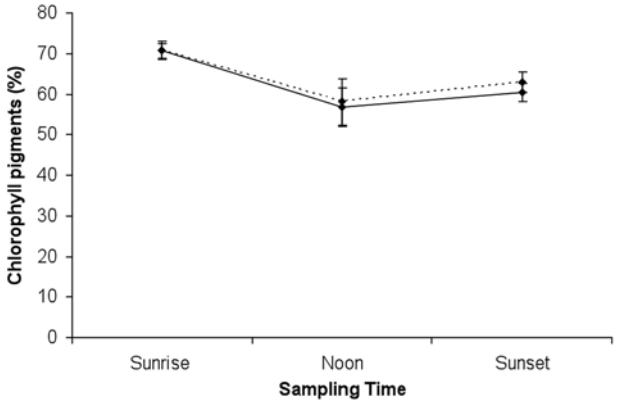

Fig. 1. Percentage of chlorophyll pigments observed at the surface throughout the day on the 2 September 2007 at Browns River in a non-shaded (solid) and artificially shaded (broken) site. Values are means ± standard error.

Fluorescence

The maximum PSII quantum yield of chlorophyll fluorescence (F_v/F_m) of the MPBs from the surface 2 mm was between 0.52 ± 0.01 and 0.35 ± 0.06 and between 0.46 ± 0.01 and 0.26 ± 0.04 at 5 mm depth (Figure 2).

F_V/F_M values changed significantly throughout the day (df = 2, F = 4.691, $P = 0.02$). Both the shaded and non-shaded sites demonstrated a significant decrease in F_V/F_M at noon compared with sunrise and sunset. The differences between F_V/F_M at the surface and 5 mm depth were also significant (df = 1, F = 17.3, $P < 0.001$) with the surface values consistently greater. There was a significant interaction between the time of day and F_V/F_M when comparing the shaded and non-shaded sites (df = 2, F = 3.8, $P = 0.038$).

E_k values varied between 181 ± 10 and 428 ± 47 μmol photons m^{-2} s^{-1} (Table 2; Figure 3). There was a significant time of day effect with E_k values at sunset greater than sunrise and noon (df = 2, F = 3.43, $P = 0.04$). The values at 5 mm depth were consistently greater than the surface 2 mm values. In contrast with the shaded site the E_k values were not significantly different with depth or time of day but ranged from 231 ± 16 μmol photons m^{-2} s^{-1} at the surface at noon to 305 ± 40 μmol photons m^{-2} s^{-1} at the surface at sunrise (Figure 3).

Non-photochemical quenching

Non-photochemical quenching (NPQ) by the benthic microalgae changed significantly throughout the day (df = 2, F = 5.8, $P = 0.008$; Table 2). The NPQ values at the non-shaded site ranged from 0.13 ± 0.02 at noon at 5 mm depth to 0.42 ± 0.05 at sunset at 5 mm depth (Figure 4A). In the

Table 2. Chlorophyll-*a* biomass, Fv/Fm, E_k, NPQ, diadinoxanthin:chlorophyll ratio and diatoxanthin:chlorophyll ratio.

	Depth	Treatment	Total chl pigments	Fv/Fm	E_k	NPQ	DD/chl	DT/chl
Dawn	2	Non-shaded	14.12 ± 0.3	0.52 ± 0.01	182 ± 13	0.20 ± 0.01	0.022 ± 0.003	0.053 ± 0.003
	5	Non-shaded	5.95 ± 0.54	0.37 ± 0.04	222 ± 34	0.21 ± 0.03	0.040 ± 0.004	0.044 ± 0.004
Dawn	2	Shaded	9.02 ± 0.65	0.45 ± 0.02	305 ± 40	0.31 ± 0.02	0.022 ± 0.002	0.052 ± 0.002
	5	Shaded	3.77 ± 0.46	0.36 ± 0.01	228 ± 36	0.32 ± 0.04	0.039 ± 0.005	0.038 ± 0.002
Midday	2	Non-shaded	11.69 ± 1.53	0.47 ± 0.01	181 ± 10	0.17 ± 0.02	0.015 ± 0.005	0.058 ± 0.005
	5	Non-shaded	10.27 ± 2.38	0.30 ± 0.03	242 ± 21	0.13 ± 0.02	0.030 ± 0.008	0.053 ± 0.003
Midday	2	Shaded	10.32 ± 2.39	0.35 ± 0.06	231 ± 16	0.17 ± 0.04	0.019 ± 0.010	0.062 ± 0.005
	5	Shaded	5.66 ± 0.71	0.26 ± 0.04	282 ± 45	0.22 ± 0.03	0.040 ± 0.003	0.048 ± 0.002
Sunset	2	Non-shaded	12.83 ± 1.76	0.45 ± 0.01	295 ± 19	0.25 ± 0.02	0.018 ± 0.004	0.055 ± 0.004
	5	Non-shaded	8.08 ± 0.69	0.34 ± 0.00	428 ± 57	0.42 ± 0.05	0.035 ± 0.001	0.045 ± 0.001
Sunset	2	Shaded	10.28 ± 0.59	0.52 ± 0.01	270 ± 26	0.21 ± 0.01	0.022 ± 0.005	0.056 ± 0.003
	5	Shaded	6.16 ± 0.61	0.46 ± 0.01	261 ± 25	0.22 ± 0.01	0.033 ± 0.001	0.045 ± 0.002

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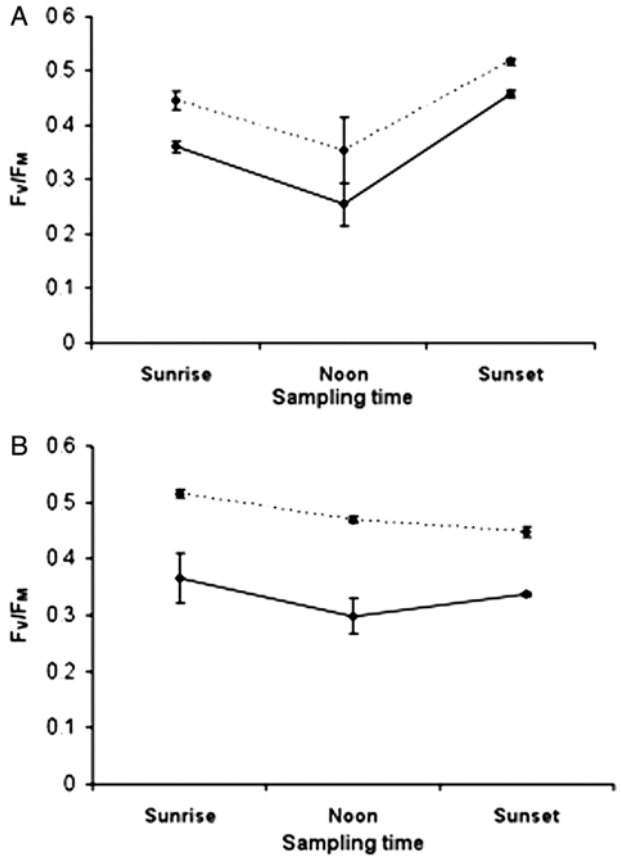

Fig. 2. Comparing F_V/F_M (broken, surface; solid, 5 mm depth) with time at Browns River 2 September 2007 in: (A) non-shaded area; (B) artificially shaded area. Values are means ± standard error.

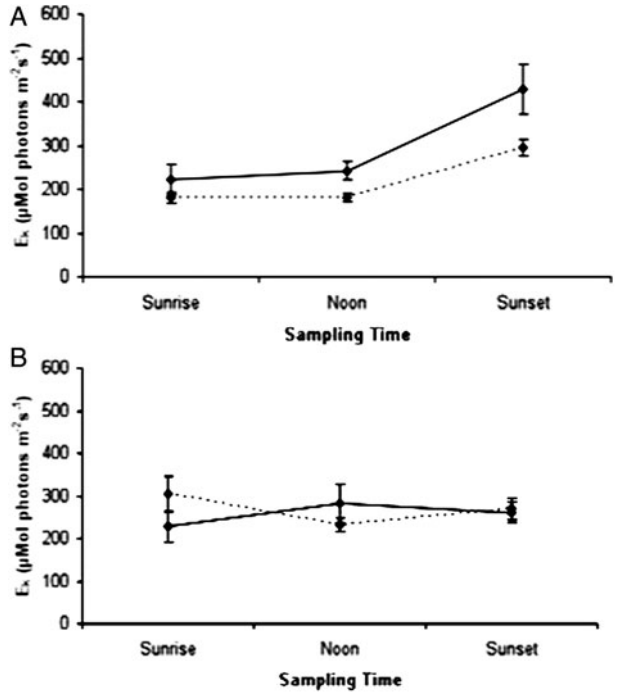

Fig. 3. Comparing E_k with depth (broken, surface; solid, 5 mm depth) and time at Browns River 2 September 2007 in: (A) non-shaded area; (B) artificially shaded area. Values are means ± standard error.

non-shaded site the NPQ values were greater at sunset with those from the 5 mm depth particularly high. At the shaded site the NPQ values ranged from 0.17 ± 0.04 at noon at the surface to 0.32 ± 0.04 at depth at sunrise (Figure 4B). NPQ at the shaded site was greatest at sunrise than noon or sunset. There was no significant difference between the values of NPQ at the shaded and non-shaded sites.

Xanthophyll cycle

Pigment concentration in the xanthophyll pool normalized to the total chl*a* (DD + DT/Chl*a*) ranged from a minimum of 0.072 ± 0.001 µg:µg at the surface at sunset to 0.084 ± 0.005 µg:µg at sunrise at 5 mm depth (Figure 5). The xanthophyll pool was significantly greater at 5 mm depth than at the surface throughout the day ($df = 1$, $F = 5.70$, $P = 0.025$). There were no significant differences between sunrise, noon and sunset values or between the shaded and non-shaded sites.

Diadinoxanthin normalized to chl*a* (DD/Chl*a*) values ranged from 0.052 ± 0.002 µg:µg to 0.062 ± 0.005 µg:µg at the surface and between 0.038 ± 0.002 to 0.053 ± 0.003 at 5 mm depth (Table 2; Figure 5). There was a significantly greater mean value of DD/chl*a* at the surface than at depth ($df = 1$, $F = 9.648$, $P = 0.005$). There was also a strong trend of decreasing DD/chl*a* at noon compared to sunrise and sunset ($df = 2$, $F = 3.37$, $P = 0.051$).

Diatoxanthin normalized to chlorophyll-*a* (DT/chl*a*) values ranged from 0.015 ± 0.005 to 0.022 ± 0.003 at the surface and between 0.040 ± 0.004 and 0.030 ± 0.008 at 5 mm depth (Table 2; Figure 5). There was consistently a greater amount of DT at 5 mm depth than at the surface

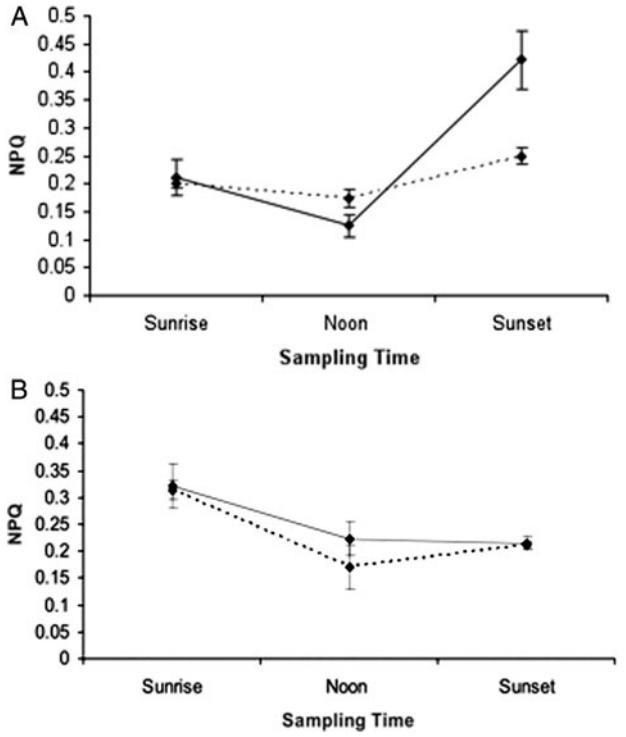

Fig. 4. Non-photochemical quenching of energy (NPQ) as a function of depth (broken, surface; solid, 5 mm depth) and time at Browns River 2 September 2007 in: (A) non-shaded area; (B) artificially shaded area. Values are means ± standard error.

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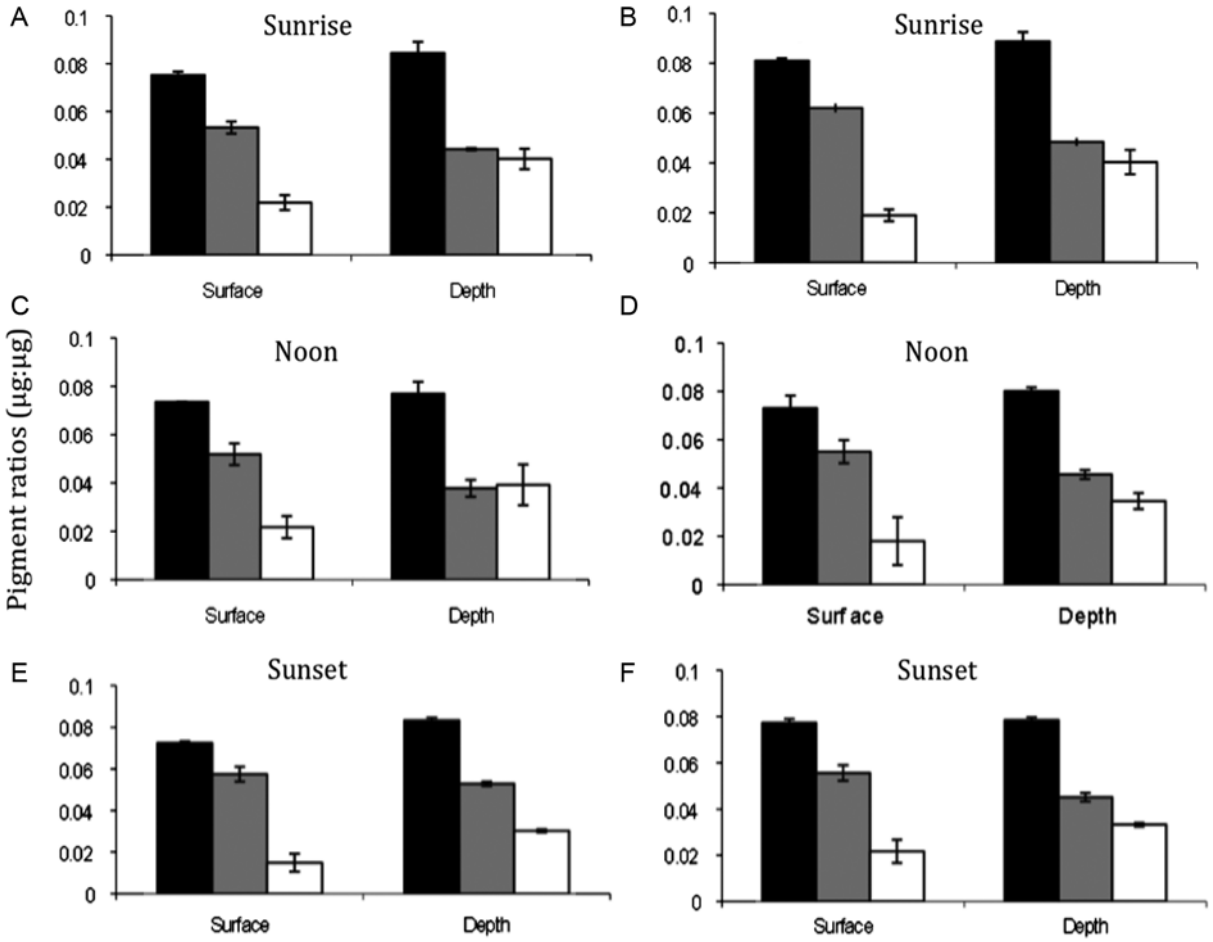

Fig. 5. Xanthophyll pigment ratios at Browns River on the 2 September 2007 measured in a non-shaded (A, C, E) and artificially shaded site (B, D, F). The xanthophyll pool, diadinoxanthin + diatoxanthin (DD + DT) normalized to chlorophyll-*a* (chl*a*) (black); DD/ch*la* (grey) and DT/ch*la* (white). Values are means ± standard error.

(df = 1, F = 9.91, $P = 0.004$). The amount of DT however did not change significantly with time of day or shading.

The DT/XP ratio ranged between 0.39 ± 0.01 and 0.056 ± 0.05 at the surface and between 00.22 ± 0.03 and 0.36 ± 0.08 (Figure 6). This ratio increased at noon compared to sunrise and sunset although this change was not statistically significant. In the shaded area the DT/XP ratios ranged from 0.46 ± 0.04 at 5 mm depth at sunset to 0.23 ± 0.03 at sunrise at the surface. The DT/XP ratio at 5 mm depth remained the same throughout the day in the shaded site but increased at noon at the surface. The DT/XP ratio was significantly greater at 5 mm depth than at the surface (df = 1, F = 12.1, $P = 0.002$).

DISCUSSION

Although the amplitude and regulation of energy dissipation in photosynthesis are species dependent, the following features have been found to be characteristics of most microalgae; a fast diadinoxanthin (DD) de-epoxidation and concomitant formation of non-photochemical quenching (NPQ) occurring within seconds, a direct linear relationship between diatoxanthin (DT) accumulation and NPQ development, a *de novo* synthesis of DT accounting for supplementary photoprotection under prolonged illumination and all parameters (especially those concerning the xanthophyll cycle) regulated by the light regime (Lavaud *et al.*, 2004). Serôdio *et al.* (2005) observed that in microphytobenthos variations in NPQ upon changes in irradiance were generally followed by proportional variations in DT content. This was observed under high light, during which the build up of NPQ was closely paralleled by a proportional increase in DT concentration. In diatoms, higher growth irradiances induced larger DD pools, increasing the production of energy dissipating DT under high light and enabling higher NPQ levels (Cruz & Serôdio, 2008). However, higher NPQ values may also be caused by an increase in photoinhibitory damage to the photosynthetic apparatus (qI) (Cruz & Serôdio, 2008).

A 15 minute dark adaption period is rarely sufficient to fully dissipate NPQ and so the measurements of Fv/Fm here are almost certainly lower than the equivalent, fully dark adapted values. However, in this study it is the changes in the photosynthetic parameters that are important rather than their absolute values and as all samples were given the same treatment, the documented changes will be consistent.

The relationship between NPQ and DT accumulation at Browns River differed from previous findings. A linear relationship between NPQ and DT and an inverse relationship with F_V/F_M would have been expected, but this was not observed, as DT, NPQ and F_V/F_M all decreased at noon. An inverse relationship was seen, however, between F_V/F_M and

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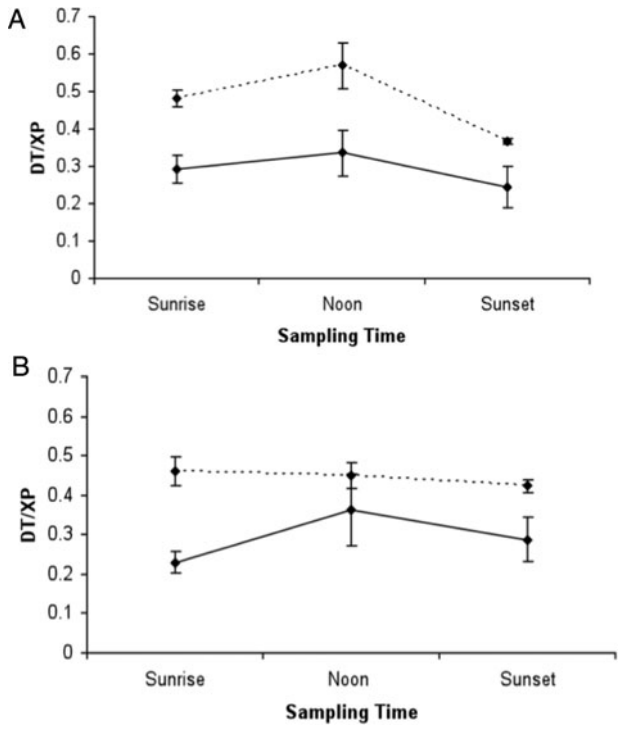

Fig. 6. The amount of DT in relation to the xanthophyll pool pigments (DD + DT/chl*a*) at different times of the day at Browns River on the 2 September 2007 measured in: (A) non-shaded area (B) artificially shaded area. Values are means ± standard error.

NPQ at sunset at the shaded site where F_V/F_M increased and NPQ decreased. This latter response is predictable as there was no need for excess light energy to be quenched. DT and NPQ were both greater at depth than at the surface except at noon in the non-shaded site. It is unclear why DT and NPQ would be greater at depth, where there is very little light compared to the surface. Serôdio *et al.* (2005), considered the development of NPQ capacity in the dark to be a form of maintenance to prevent degradation of xanthophyll cycle pigments during prolonged periods of darkness, and to provide functional photoprotection upon re-illumination. Formation of NPQ capability in the dark has previously been reported for diatoms and has been attributed to the conversion of DD to DT as a response to the establishment of a transthylakoidal pH- gradient, due to chlororespiration or reverse ATP synthase operation (Cruz & Serôdio, 2008). Furthermore, perhaps, as Fujiki *et al.* (2003) observed, the cells are responding to light exposure from the previous day as light history can also regulate the xanthophyll cycle. Further examination of the formation of NPQ capacity in the dark is clearly needed, as while it has been observed in some diatoms, it is unclear how many species undertake this maintenance. Although the majority of cells at Browns River were diatoms (58%) the other microalgal groups may exhibit alternative photoadaptive strategies.

Throughout the day at Browns River there was a greater amount of microalgal biomass (chlorophyll-*a* per unit wet weight of sediment) at the surface (0–2 mm) than at 5 mm below. The depth of light penetration and therefore the size of the euphoic zone are affected by the sediment type. In muddy sediments approximately 90% of the light is attenuated in the top 400 μm (Consalvey *et al.*, 2004). Thus at 5 mm depth at Browns River very little light would have penetrated and the microalgae found at this depth would not have been actively photosynthesizing. Although it is likely that bioturbation was responsible for a significant but unquantified proportion of the biomass found at depth, most of the cells that were there were likely to have either recently migrated there or were dead or dying. There was a significant decrease in biomass at the surface at noon compared to sunrise and sunset suggesting that the microalgal cells had migrated downward to avoid the potentially damaging high light levels. Cell migration is thought to provide advantages as it offers safety from tidal currents, reduces disturbance and grazing and increases nutrient availability (Decho, 2000; Perkins *et al.*, 2002).

There was a significant difference between the chlorophyll-*a* concentration at the shaded and non-shaded sites, with greater concentrations at the non-shaded site. However, it is probable that at this site the difference reflects the large natural variability of microalgal biomass in MPB communities. Artificially shading the surface of the sediment from 2–55% of ambient light during low tide has been shown elsewhere to increase upward migration of MPB to the surface, suggesting that bright sunlight can inhibit upward migration (Kingston, 1999). The F_V/F_M values were on average 25% greater at the surface than 5 mm below. The maximum quantum yield is obtained when all reaction centres are open and is proportional to the fraction of reaction centres capable of converting absorbed light to photochemical energy (Kolber & Falkowski, 1993). Thus a low F_V/F_M is potentially an indictor of stress. At Browns River the F_V/F_M had decreased by 20% at noon in both the surface and at 5 mm depth, indicating that the cells were less quenched during this time. This decrease corresponded with a drop in biomass at this time, indicating that the cells were probably 'stressed' and were moving away from the sunlight. Schofield *et al.* (1998) observed that during times of maximum solar irradiance F_V/F_M of macroalgae decreased by 60% of pre-dawn values but quickly recovered to the higher pre-dawn values within hours after sunset.

The present study was undertaken at the end of winter when water temperatures were still relatively cold at 13°C (range over 12 months is 9–21°C; Jordan *et al.*, 2008). Seasonal changes in NPQ capability may be expected in association with variation in the pool of xanthophyll cycle pigments, since it has been shown that the xanthophyll pool size increases as a response to lower growth temperatures in both diatoms and higher plants (Anning *et al.*, 2001). The xanthophyll cycle is an enzyme mediated reaction and therefore temperature will affect the turnover time of the DD ⇄ DT conversion (Fujiki *et al.*, 2003). Exposure to direct sunlight at low temperatures can be particularly damaging to the photosynthetic apparatus as low temperature will slow down the photoprotective response occurring at high irradiances (Anning *et al.*, 2001). An increase in the amount of xanthophyll cycle pigments in winter would thus be an advantage for MPB cells, enhancing photoprotection by allowing higher degrees of de-epoxidation at low temperatures (Anning *et al.*, 2001).

At Browns River the size of the xanthophyll pool, (DT + DD)/chl*a*, did not change during the day, although there were greater amounts at depth than at the surface. In phytoplankton cells, conversion between DD and DT occurs rapidly (seconds to minutes) but the sum of DD and DT remains unchanged on such small time scales (Fujiki *et al.*, 2003). Phytoplankton assemblages have been observed to adjust the size of their xanthophyll pool to variations in

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ambient irradiance on a time scale of days (Fujiki *et al.*, 2003). It is interesting to note that at Browns River the relative size of the xanthophyll pool at 5 mm depth was greater than at the surface, although very little light would be expected to penetrate that far. The reason for this is unclear but it is possible that photoprotective pigments are being stored in preparation for a sudden exposure to supra-optimal light. Alternatively, the cells may have moved down after a high light day the day before or the xanthophyll pool has been diminished on the surface. Fujiki *et al.* (2003) observed that phytoplankton assemblages regulated the xanthophyll cycle within a day depending on the irradiance of the previous day. It is also possible that the cells were migrating up and down within the sediment at a faster time scale than was being measured and therefore the data reflect an average distribution and not that of individual cells.

Although greater DT/chl*a* values were observed at 5 mm depth below the surface where there was little light penetration than at the surface, DD/chl*a* was always greater at the surface than at depth. When cells are given excess irradiance, such as a low to high light transition, DD is de-epoxidized into DT and DT accumulates during protracted high light conditions (Moisan *et al.*, 1998). In contrast, DT is epoxidized to DD during high to low light transitions and DD accumulates under low light conditions. Kashino & Kudoh, (2003) observed that when dark-adapted cells were exposed to high light (200 μmol photons m^{-2} s^{-1}), followed by exposure to dark, the DD-cycle pigments showed dynamic changes immediately after the change of light conditions, with an increase in DT and decrease in DD. As there was a difference in the DD/chl*a* and DT/chl*a* at Browns River at the surface and at 5 mm depth and as DT/chl*a* concentrations were greater at depth where there was very little light, xanthophyll cycling was occurring within the MPBs migrating up and down in these sediments. These results suggest that cells rapidly depart the surface when their supply of DD is exhausted and replenish this at depth before returning to the surface. At the surface the cells may use up all the DD pigment in the xanthophyll pool and then migrate to depth to replenish this pool. Moisan *et al.* (1998) found that rapid changes in DT:DD occurred within 5 minutes of the initial irradiance shift. They hypothesized that xanthophyll cycling may help to optimize photosynthesis in fluctuating light environments which change on minute to hourly time scales (Moisan *et al.*, 1998).

The rise in DT/XP at noon indicates that DD is being converted to DT during the highest irradiance of the day. As the cells are likely to be moving vertically as well as activating the DD/DT cycle, this may suggest that the rate of vertical movement is inadequate to provide the cells at the surface with sufficient DD for photoprotection. Brown *et al.* (1999) similarly found temporal fluctuations in the molar ratio of DT to the total xanthophyll pool in corals. The ratio was lowest at dusk and dawn and highest at midday. It was also observed that F_V/F_M decreased with increasing irradiance, reaching a minimum during the period of highest irradiance and subsequently recovering by the next morning (Brown *et al.*, 1999). The F_V/F_M values of MPB at Browns River followed this trend of lower values at midday along with greater values of DT/XP. Together these physiological acclimations indicate that the MPB at Browns River use the xanthophyll cycle throughout the day to mitigate excess irradiance and raise photosynthetic performance.

The significant decrease in chl*a* at the surface at noon compared to sunrise and sunset indicates that the microalgal cells have probably migrated downward to avoid the potentially damaging light. Therefore, MPB at Browns River are utilizing both behavioural and physiological strategies to survive in the dynamic changing intertidal environment. The data suggest that the cells exposed to light at the surface are migrating down to replenish the photoadaptive pigments of the xanthophyll pool. The diatoms may use this vertical migration to replenish DT and then remain longer at the surface undergoing high rates of photosynthesis. In combination with access to more nutrients at depth this vertical movement may significantly enhance growth relative to cells at either extreme.

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