The role of coccoliths in protecting *Emiliania huxleyi* against stressful light and UV radiation

Running Title: Photoprotective role of coccoliths in *Emiliania huxleyi*

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Abstract

Coccolithophores are a group of phytoplankton species which cover themselves with small scales (coccoliths) made of calcium carbonate ($CaCO_3$). The reason why coccolithophores form these calcite platelets has been a matter of debate for decades but has remained elusive so far. One hypothesis is that they serve a role in light/UV protection, especially in surface dwelling species like *Emiliania huxleyi* which can tolerate exceptionally high levels of solar radiation. In this study, we tested this hypothesis by culturing a calcified and a naked strain under different light conditions with and without UV radiation. The coccoliths of *E. huxleyi* reduced the transmission of visible radiation (400-700 nm) by 7.5%, UV-A (315-400 nm) by 14.1% and UVB (280-315 nm) by 18.4%. Growth rates of the calcified strain (PML B92/11) were about 2 times higher than those of the naked strain (CCMP 2090) under indoor constant light levels in the absence of UV radiation. When exposed to outdoor conditions (fluctuating sunlight with UV radiation), growth rates of calcified cells were almost 3.5 times higher compared to naked cells. Furthermore, relative electron transport rate was 114% higher and non-photochemical quenching (NPQ) 281% higher in the calcified compared to the naked strain, implying higher energy transfer associated with higher NPQ in the presence of calcification. When exposed to natural solar radiation including UV radiation, maximal quantum yield of photosystem II was only slightly reduced in the calcifid but strongly reduced in the naked strain. Our results reveal an important role of coccoliths in mitigating light and UV stress in *E. huxleyi*.

Key words: coccoliths, *Emiliania huxleyi*, light protection, growth, photosynthetic performance, UV radiation

1 Introduction

Coccolithophores are a group of marine phytoplankton species which are able to precipitate $CaCO_3$ in the form of small calcitic scales (coccoliths) surrounding the organic part of the cell. They contribute about 1-10% to marine primary production (Poulton et al., 2007) and approximately 50% to pelagic deep ocean $CaCO_3$ sediments (Broecker and Clark, 2009). Blooms of coccolithophores can cover up to 8 million km^2 of the Earth's surface (Moore et al., 2012), and are considered to be important drivers of biogeochemical cycling (Rost and Riebesell, 2004).

Despite intense research on coccolithophore calcification and its biogeochemical relevance during the last decade, it is still an unresolved question why coccolithophores calcify (Young, 1994; Raven and Crawfurd, 2012). One hypothesis is that the layer of coccoliths surrounding the cell (coccosphere) protects the organism from excess light and UV radiation. This notion is supported by the exceptionally high light tolerance of the surface layer dwelling species *Emiliania huxleyi* (Nanninga and Tyrell, 1996; Ragni et al., 2008; Gao et al., 2009; Loebl et al., 2010).

Physiological studies investigating the light tolerance of *E. huxleyi* showed that the radiation wavelength matters in this context. The coccosphere does not seem to constitute a protection against very high intensities of photosynthetically active radiation (PAR) since non-calcifying *E. huxleyi* cells are equally resistant to photoinhibition as their calcifying counterparts (Nanninga and Tyrrell, 1996). This is in clear contrast to the influence of stressful ultraviolet radiation (UVR) on the cells where results from different physiological experiments support a protective role of the coccoliths (Gao et al., 2009; Guan and Gao, 2010; Gao et al., 2012). Protection from UVR or high light exposures by coccoliths may either work by physically shading

intracellular organelles or by strongly scattering light which is certainly a feature of coccolithophore blooms (Balch et al., 1996; Voss et al., 1998). The underlying mechanisms, however, are not well understood and warrant further investigations.

UVR strongly contributes to photoinhibition of photosystem II (e.g. Hakala-Yatkin et al., 2010) and effectively inhibits repair processes (Ragni et al., 2008). Therefore, it is likely that the coccoliths protect PSII repair from UV inhibition. In this study we explore in more detail how different PAR and UV radiation (280-400 nm) treatments affect calcified and naked *E. huxleyi* cells. Specifically we address the question whether the coccosphere of *E. huxleyi* helps the cells to withstand stressful levels of PAR and/or UV radiation and whether calcification influences photochemical performance.

2. Materials and Methods

2.1 Materials and pre-culture conditions

Calcified *E. huxleyi* (PML B92/11 isolated in the Raunefjord area, Bergen, Norway) and naked cells (CCMP 2090 isolated in the South Pacific) were used in the experiments. Both strains were grown in triplicate cultures (300 ml square glass bottles) at 15^{o}C in 0.2 µm filtered natural seawater (gathered from the Gulf of Biscay) at a photon flux density of 500 µmol photons m^{-2} s^{-1} on a 16/8 light/dark cycle. The natural seawater medium was enriched with 64 µmol L^{-1} nitrate, 4 µmol L^{-1} phosphate, f/8 concentrations of a trace metal and vitamin mixture (Guillard & Ryther 1962), and 10 nmol kg^{-1} selenium. Pre-cultures and experimental incubations in semi-continuously diluted batch cultures (>8 generations) ensured exponential growth throughout the experiment.

2.2 Experimental setup

2.2.1 Indoor growth experiments

After pre-culture for at least 8 generations, the cells of calcified and naked strains were inoculated in the same glass bottles of 300 ml and cultured under the same condition as pre-cultures, maintaining the cell concentrations at exponential growth within a range of 3-10*10^4 cells/ml.

2.2.2 Outdoor growth experiments

Following the indoor growth experiment, the cells were transferred into quartz tubes (100 ml) for the outdoor growth experiment and were exposed to natural solar radiation at the institution's pier. The cultures were maintained outside in a flow-through water tank, where the seawater temperature was maintained within a range of 14-16^{o}C. After the cells had acclimated for 7 days under the solar radiation, aliquots of the cell cultures were transferred to new quartz tubes filled with fresh medium before measurements were taken. For the outdoor cultures, the cells received 60% full spectrum solar radiation (the quartz tubes wrapped with neutral density screens). The daytime average intensities (from 7:00 am to 5:00 pm) of PAR, UV-A and UV-B which the cells received during the outdoor experiment were about 260 µmol photons m^{-2} s^{-1} (about 53 W m^{-2}), 12.4 and 0.34 W m^{-2}, respectively.

2.2.3 Short-term incubation experiments

Short-term incubation experiments were carried out to test UV effects around noon time on a cloudy day and sunny day, respectively. Three different radiation treatments were implemented as follows: 1) Cells in uncovered quartz tubes, receiving the full spectrum of solar radiation (above 280 nm, PAB treatment); 2) cells in quartz tubes covered with Folex 320 (Montagefolie, Nr. 10155099, Folex, Dreieich, Germany), exposed to UV-A and PAR (above 320 nm, PA treatment); and 3) cells receiving only PAR (P treatment) in quartz tubes covered with Ultraphan film 395 (UV Opak,

Digefra, Munich, Germany). The transmission spectra of the quartz tubes and the cut-off foils are given by Zheng and Gao (2009). A time-course experiment was also conducted around noon under full solar spectrum conditions.

2.3 Absorptivity of coccoliths

We examined absorption spectra of the cells with or without coccoliths to get an indication on how much light and/or UV are blocked by the coccosphere. Therefore, calcified cells, de-calcified cells and cells of the naked strain were filtered onto Whatman GF/F glass fiber filters (25 mm), and then were subsequently placed at the window near the detector of a double beam UV-VIS-NIR spectrophotometer (PerkinElmer, Lambda950, USA) which can obtain the absolute absorbance of coccoliths based on the recaptured scattered light. The absorption of the GF/F filter was corrected with a control filter which was soaked with particle free culture medium (Kishino et al., 1985).

2.4 Growth measurement

Cell densities were measured during a period of 7 days with a particle counter (Coulter Z1, Beckman). The specific growth rate was calculated as: μ (d^{-1}) = ($\ln N_t$-$\ln N_0$)/t, where N_0 and N_t represent the cell concentrations at the beginning and the end of the incubations and t is the incubation time in days.

2.5 Chlorophyll fluorescence measurement

Parameters of in vivo induced chlorophyll a fluorescence of photosystem II were estimated by a phyto-pulse amplitude modulated fluorometer (Phyto-PAM, Walz). The maximum quantum yield of PSII (Fv/Fm) was calculated as: Fv/Fm=(Fm-Fo)/Fm; where Fo is the basal fluorescence under measuring light of 0.2 μmol photons m^{-2} s^{-1} and Fm the maximal fluorescence measured with a saturating light pulse of 5000 μmol photons m^{-2} s^{-1} (0.8 s) in dark-adapted (15 min) cells.

In order to compare the transmission of the same strain with or without coccoliths and to relate this to that of the naked strain, the calcified strain was de-calcified with HCl (1 mol/L, the final concentration is 0.01 mol/L) for 10 s and subsequent recovery of the pH with equimolar amounts of NaOH. Photochemical performance was measured for dark-adapted (15 min) cells in calcified, de-calcified or naked cells. De-calcified cells revealed Fv/Fm values similar to those obtained prior to de-calcification. The actinic light levels were set at 533, 1077 and 2130 μmol photons m^{-2} s^{-1}, respectively (growth light, saturated light and over-saturated light). Non-photochemical quenching (NPQ) was calculated as: NPQ = $(F_m-F_m')/F_m'$, where F_m was the maximum fluorescence yield after dark adaptation and F_m' the maximum fluorescence yield under the actinic light levels.

To determine rapid light curves (RLCs, electron transport rate vs light), the cells were exposed to 10 different PAR levels in sequence (87, 140, 263, 382, 449, 611, 778, 993, 1195 and 1391 μmol photons m^{-2} s^{-1}), each of which lasted for 20 s. The relative electron transport rate (rETR) was assessed as: rETR = Yield × 0.5 × PFD, where the yield represents the effective quantum yield of PSII (F_v'/F_m'); the coefficient 0.5 takes into account that roughly 50% of all absorbed quanta reach PSII; and PFD is the photon flux density of the actinic light (μmol m^{-2} s^{-1}) (Genty et al., 1989).

To examine immediate photochemical responses of the cells to UV radiation, the cells were exposed to the three different solar radiations (see above) for 60 min during noontime under natural solar radiation. The effective quantum yield was calculated as: $F_v'/F_m' = (F_m' - Ft) / F_m'$, where F_m' and Ft are the maximal fluorescence and steady state fluorescence in the light adapted cells, respectively.

2.6 Measurement of solar irradiances

Solar PAR was measured using a Quantum Scalar Laboratory Irradiance Sensor (QSL-2100/ 2101, Biospherical Instruments, San Diego, USA). The measured values were recorded every 10 s and saved on a computer. Solar UV-A and UV-B radiation were measured with a radiometer (PMA 2100 Solar Light Co., Glenside, USA), the mean irradiances of solar UV-A and UV-B during the experimental periods were confirmed according to the ratios of UV-A /UV-B to PAR at the experimental location.

2.7 Statistics

The data were expressed as the means ± standard deviation (SD). Statistical significance of the data was tested with software of Origin 9.0 (one way ANOVA, Tukey's post-hoc test). A confidence level of 95% was used in all analyses.

3 Results

The coccolith layer of *E. huxleyi* absorbed both visible and UV radiation. It reduced the transmission of visible radiation (400-700 nm) by 7.5%, UV-A (315-400 nm) by 14.1% and UVB by 18.4% (280-315 nm) relative to de-calcified cells and 6.5% for PAR, 6.6% for UV-A and 5.1% for UV-B, relative to naked cells (Fig. 1). The specific growth rate of calcifying *E. huxleyi* strain (PML B92/11) was about 2 times higher than that of the naked strain (CCMP 2090) ($P < 0.05$) when grown at 500 μmol photons m^{-2} s^{-1} of PAR under indoor conditions (Fig. 2A). Growth rates of both strains were significantly ($P < 0.05$) reduced when the cells were transferred outdoor and exposed to natural solar radiation. However, under outdoor conditions, growth rates of calcified cells were 3.5 times higher than those of the naked cells, indicating that the latter was more harmed by the solar exposure than the former (Fig. 2A). The cell diameter was not significantly different in the calcified cells between the indoor

and outdoor conditions ($P > 0.05$), but an 18% increase was found in the naked cells after they had grown under the outdoor conditions for 7 days ($P < 0.05$) (Fig. 2B). The maximal quantum yield (Fv/Fm) decreased when the cells were transferred from indoor to the outdoor conditions, reflecting a harmful effect of solar radiation. The decrease of Fv/Fm, however, was much more pronounced in the naked cells (27%) compared to calcified cells (11%) (Fig. 2C).

Calcified cells had significantly higher apparent light use efficiency (α), maximal electron transport rate (rETRmax) and light saturation parameters (Ik) compared with naked cells. The de-calcified cells of the calcified strain showed a remarkable decrease of rETRmax ($P < 0.05$), and also alpha and Ik decreased, however statistically not significantly (Fig. 3, Table 1). Increased actinic light levels (acclimating light during the fluorescence measurement) led to higher NPQ in both the calcified and naked strain (Fig. 4). Furthermore, calcified cells showed higher NPQ values compared to naked cells ($p < 0.05$).

When exposed to full spectrum solar radiation, the quantum yield of calcified cells showed no significant change during the first 30 min ($P > 0.05$). After 30 minutes, quantum yield quickly dropped from about 0.35 to 0.22 for ~20 min ($P < 0.05$) followed by a slight recovery in the last 25 minutes. A similar trend was observed in the de-calcified cells with the key difference that the sharp decrease already happened during the first 10 min. Quantum yield of the naked cells decreased constantly for the first 50 minutes and remained at the low level thereafter (Fig. 5).

No effect of the radiation treatment (P, PA and PAB radiation) on the quantum yield of calcified cells was observed after the cells grown under indoor condition were transferred to outdoor solar radiation for 1h exposure (very cloudy day, average PAR, UV-A and UV-B were 481µmol photons m^{-2} s^{-1}, 22.1 and 0.7 W m^{-2}, respectively) ($P >$

0.05). Quantum yield was significantly higher in the naked cells, however, when they were exposed to UVA radiation (PA vs. P treatment, $P < 0.05$ Fig. 6A).

Similar responses were observed when the same test was done on a sunny day with average PAR, UV-A and UV-B of 1605 µmol photons m^{-2} s^{-1}, 69 and 2.4 W m^{-2}, respectively. Here, the quantum yield of the calcified cells showed no significant difference between the different light treatments but it decreased significantly under PAB treatment compared to P treatments in the naked cells ($P < 0.05$) (Fig. 6B).

4 Discussion

Various hypotheses were proposed for the possible functions of coccoliths, but none of them is supported by sufficient evidence (Young, 1994; Raven and Crawfurd, 2012). One important function of coccoliths for surface-dwelling species such as *E. huxleyi* could be the protection against high photon flux densities, especially UV radiation (Berge, 1962; Young, 1994; Gao et al., 2009).

Some of our results support this hypothesis. The growth rate of the calcified cells of *E. huxleyi* grown under indoor conditions was about 2 times higher than that of naked cells. This difference came out even stronger, with growth rates 3.5 times higher in calcified versus naked cells, when the cells were exposed to full spectrum solar radiation (Fig. 2A). This could potentially be attributed to the screening of PAR, UV-A, and UV-B by coccoliths. Although the daytime PAR of solar radiation was reduced to about half of the light level of the indoor test, noon time PAR levels were higher than 500µmol photons m^{-2} s^{-1}, and the presence of UV could lead to more harms to the naked cells. Light protection by coccoliths is further supported by the Fv/Fm measurements. The maximum photochemical efficiency of PSII was only slightly reduced in calcified cells but significantly decreased in naked cells when they

were exposed to natural solar PAR and UV radiation (Fig. 2C). Furthermore, photochemical performance of de-calcified cells decreased significantly faster and stronger with time compared calcified cells (Fig. 5).

The diameter of calcified cells did not significantly change when they were exposed to the full spectrum of solar radiation. The diameter of the naked cells, however, increased significantly (Fig. 2B). Perhaps, the naked cells experienced more DNA damage and so did not enter the S phase regularly (Buma et al., 2000). Alternatively, it may reflect a strategy to acclimatize to stressful solar UV radiation since it is well known that smaller cells are usually more sensitive to UV than their larger counterparts (Garcia-Pichel, 1994; Laurion and Vincent, 1998). Some field and laboratory studies showed increased cell size with increased UV exposures (Buma et al., 2000), which can be interpreted as adaptive or acclimation mechanism for protecting the cells against UV radiation. Furthermore, the naked cells might also employ other strategies such as synthesizing UV screening compounds to ameliorate UV stress because the naked strain had a lower UV transmittance than the decalcified strain.

Several studies found that coccoliths do not protect *E. huxleyi* from excess PAR (Nanninga and Tyrrell, 1996; Houdan et al., 2005; Trimborn et al., 2007). However, UV radiation was not considered in these experiments. Our results showed that the naked cells were more sensitive to full spectrum solar radiation than calcified cells and even in the same strain, the photochemical performance of de-calcified cells decreased significantly when comparing the calcified cells. This suggests that coccoliths efficiently protect the cells from solar UV radiation.

On the other hand, *E. huxleyi* appears to be more sensitive to UV-B irradiances than other phytoplankton species, and its growth rate and physiological performances were

highly inhibited by UV radiation (Peletier et al., 1996; Buma et al., 2000; Xu et al., 2011). However, competition tests for community changes are rare, and longer-term experiments with less extreme UVR would be more ecologically and evolutionarily relevant (Raven and Crawfurd, 2012). In our work, UVR had no significant effect on the quantum yield of calcified cells regardless of high or low light condition but it showed inhibition in naked cells when they were exposed to high solar light (Fig. 6A, B). This provides further evidence for protection by coccoliths against UV radiation.

On the cloudy day, no significant difference was observed among the treatments for the calcified cells; on the sunny day, under the fluctuating light (data not shown) calcified cells manage to refurbish damage to their photosynthetic apparatus by balancing damage and repair (Gao et al., 2007; Ragni et al., 2008; Loebl et al., 2010). For the naked cells, on the other hand, UV damage was not effectively repaired, leading to the observed negative effect on photosynthetic performance.

It has to be noted that our experimental data is based on only two strains of a naked and calcified *E. huxleyi*. However, similar trends in photophysiology between naked and decalcified in comparison to calcified cells suggest that coccoliths of *E. huxleyi* play an important role in protecting this species against harmful solar radiation especially UV-A and UV-B. Furthermore, the reported absence of photoinhibition in this alga at high light levels also appears to be connected to the coccosphere of *E. huxleyi* or its calcification process. In view of ongoing ocean change, the projected shoaling of the upper mixed layer (UML) caused by global warming and progressive ocean acidification that reduces the thickness or the number of coccoliths per cell (Gao et al., 2009; De Bodt et al., 2010) could reduce *E. huxleyi* growth rates within the UML due to increased UVR exposure.

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Figure captions

Figure 1. Transmission spectra of cells with (calcified strain) and without (calcified strain with coccoliths removed artificially, de-calcified strain) coccolith cover and naked cells of *Emiliania huxleyi*.

Figure 2. The specific growth rate (μ) (A), diameter (B) and maximum quantum yield (C) of PSII (Fv/Fm) of the calcified and naked cells of *E. huxleyi* grown in indoor and outdoor conditions. Different letters represent significant difference between the indoor and outdoor experiments. Different horizontal lines represent significant difference between the different strains.

Figure 3. The relative electron rate (rETR) of calcified, de-calcified and naked cells of *E. huxleyi* grown under indoor conditions as function of PAR. The cells had been grown for 12-22 generations under 500 μmol photons m^{-2} s^{-1} of PAR.

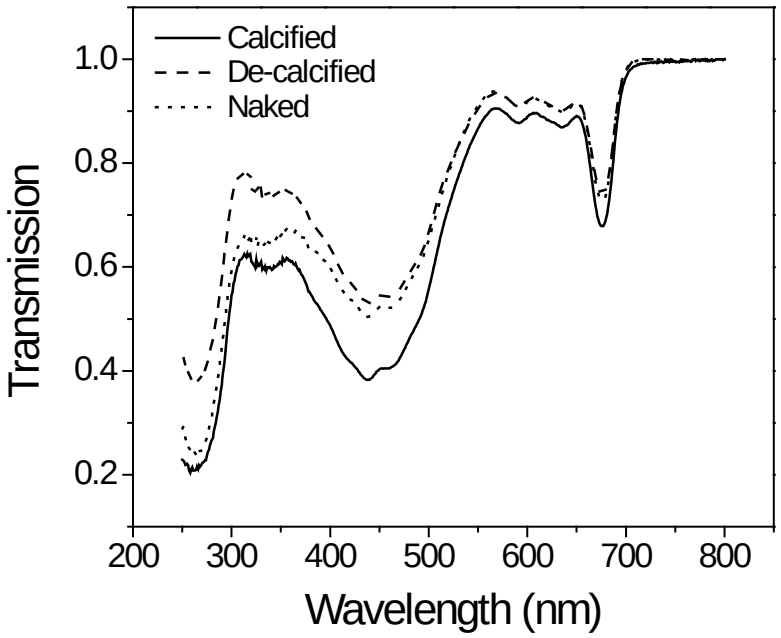
Figure 4. The non-photochemical quenching (NPQ) of calcified and naked cells of *E. huxleyi* grown under indoor conditions. Different letters represent significant difference among the light levels. Different horizontal lines represent significant difference among the different type cells.

Figure 5. The time course of quantum yield of calcified, de-calcified and naked cells of *E. huxleyi* under full spectrum solar radiation (noontime, average PAR, UV-A and UV-B were 1082μmol photons m^{-2} s^{-1}, 48.1 and 1.6 W m^{-2}, respectively).

Figure 6. The change of quantum yield of the calcified and naked cells of *E. huxleyi* when transferred from indoor to outdoor conditions, being exposed to PAR alone (P), PAR+UVA(PA) and PAR+UVA+B(PAB) for 60 min at around noon time. A, measured under a cloudy day (average PAR, UV-A and UV-B were 481µmol photons m^{-2} s^{-1}, 22.1 and 0.7 W m^{-2}, respectively); B, measured under a sunny day (average PAR, UV-A and UV-B were 1605 µmol photons m^{-2} s^{-1}, 69 and 2.4 W m^{-2}). Different letters represent significant difference among the light treatments. Different horizontal lines represent significant difference between the different strains.

Table 1. Photosynthetic parameters of relative electron transport rate (Figure 3) as a function of PAR, different letters represent significant difference ($P<0.05$) among the calcified, de-calcified and naked cells.

	α	$rETR_{max}$	I_k
Calcified	0.23 ± 0.02^{a}	90.6 ± 9.0^{a}	1010.8 ± 95.0^{a}
De-calcified	0.20 ± 0.01^{a}	73.5 ± 3.5^{b}	986.3 ± 27.4^{a}
naked	0.17 ± 0.02^{b}	42.3 ± 8.5^{c}	621.8 ± 111.1^{b}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Wavelength (nm)

Fig. 1

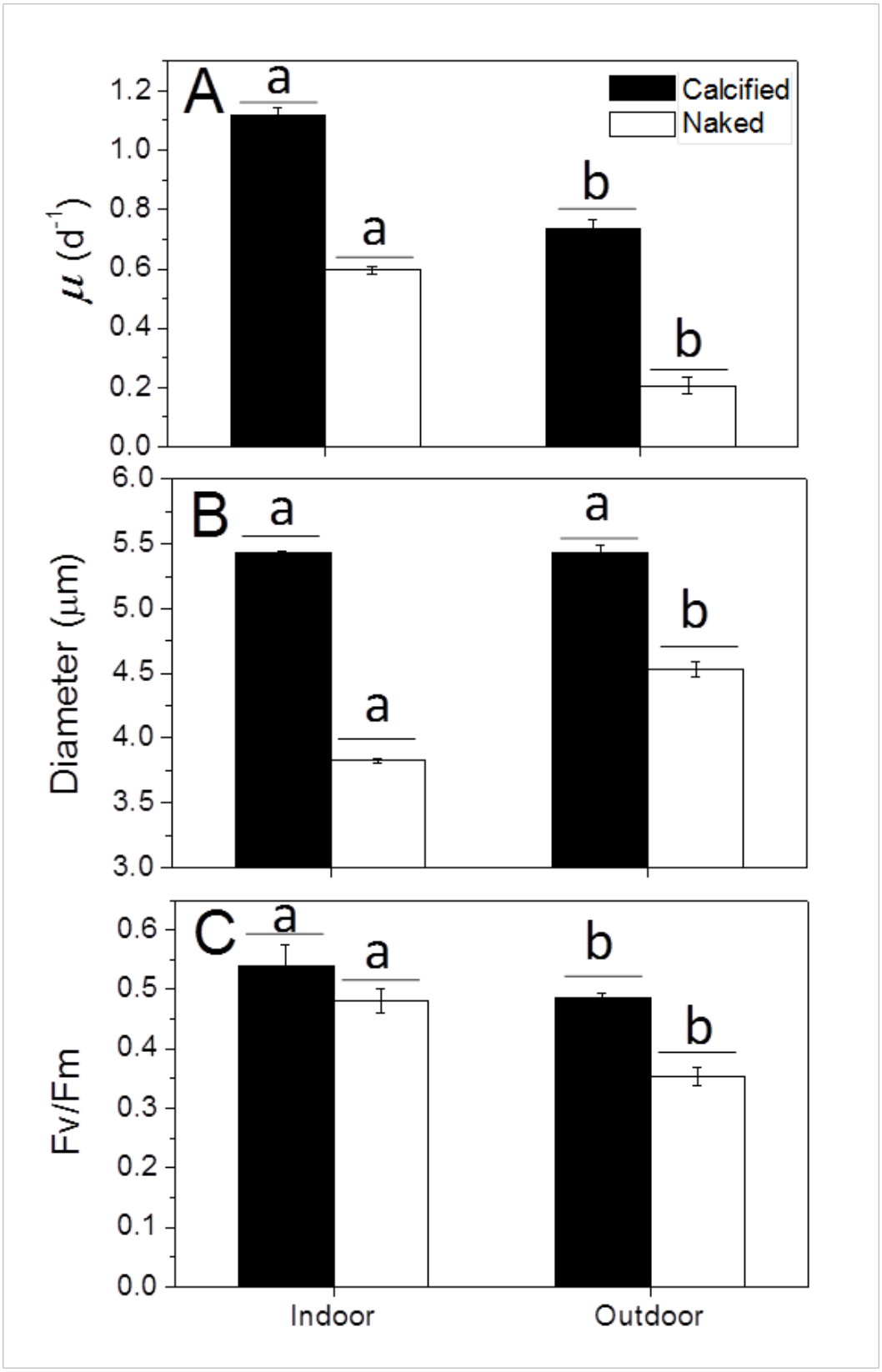
A
a
a
b
b
Calcified
Naked
μ (d-1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
B
a
a
b
b
Diameter (μm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
C
a
a
b
b
Fv/Fm
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Indoor
Outdoor

Fig. 2

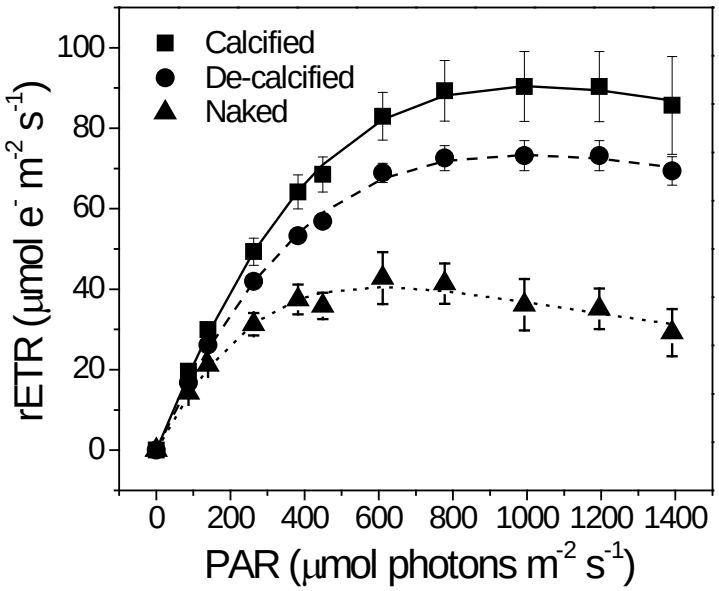
Calcified
De-calcified
Naked
rETR (µmol e⁻ m⁻² s⁻¹)
PAR (µmol photons m⁻² s⁻¹)
0
20
40
60
80
100
0
200
400
600
800
1000
1200
1400

Fig. 3

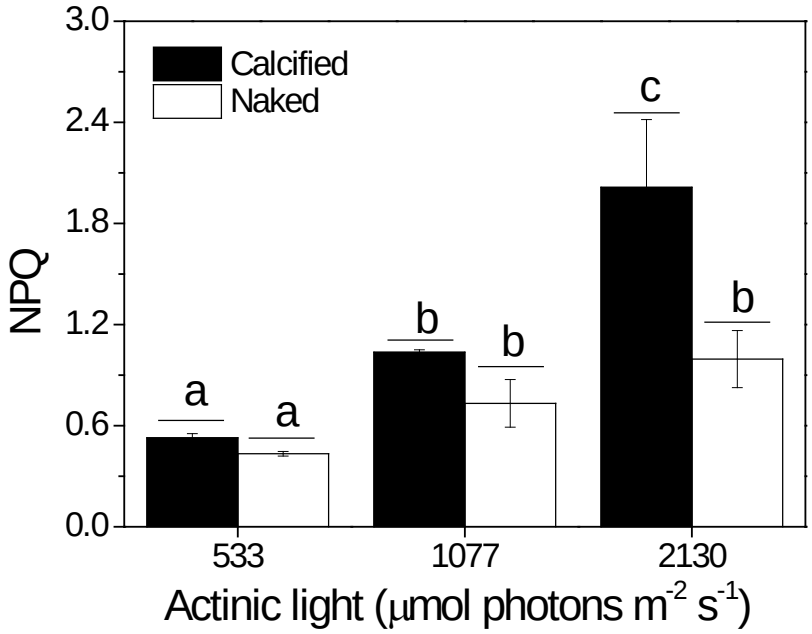
3.0
2.4
1.8
1.2
0.6
0.0
NPQ
Calcified
Naked
a
a
b
b
c
b
533
1077
2130
Actinic light (μmol photons m-2 s-1)

Fig. 4

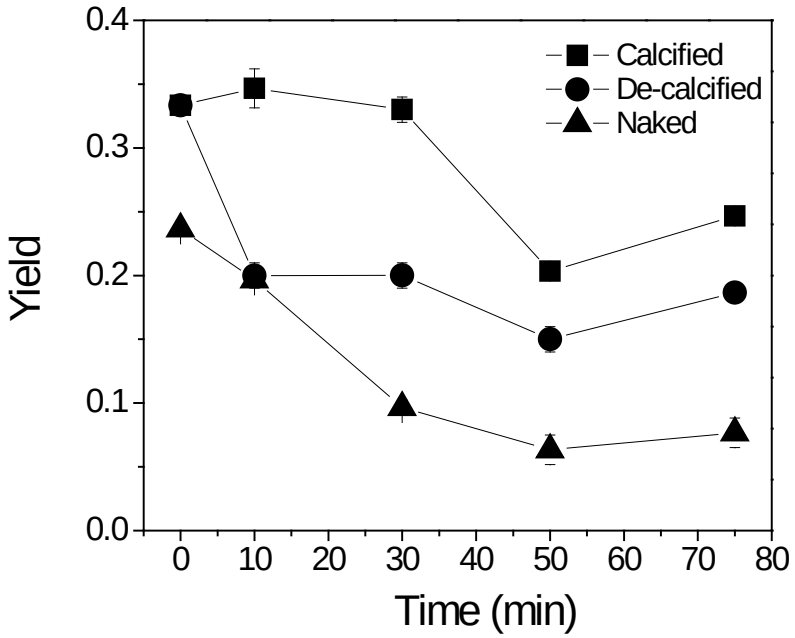
Calcified
De-calcified
Naked
Yield
Time (min)
0.0
0.1
0.2
0.3
0.4
0
10
20
30
40
50
60
70
80

Fig. 5

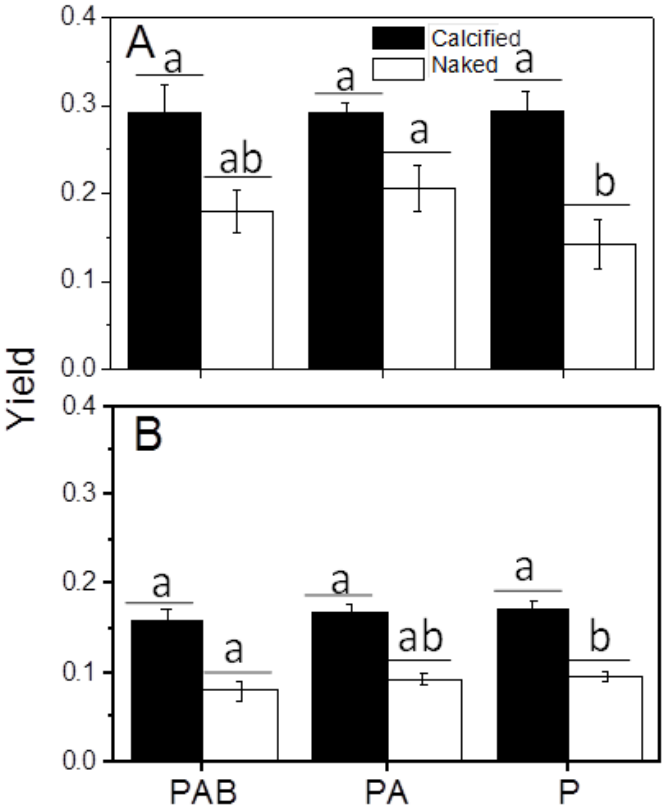
A
B
Calcified
Naked
Yield
0.0
0.1
0.2
0.3
0.4
a
ab
a
a
a
b
a
a
a
ab
a
b
PAB
PA
P

Fig. 6