THE EFFECTS OF COCCOLITHS ON PHOTOSYNTHESIS IN *EMILIANA HUXLEYI*

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ABSTRACT OF THE THESIS

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Coccoliths are calcium carbonate exoskeletons produced by some unicellular marine algae referred to as coccolithophores. Large coccolithophore blooms play a significant role in the Earth's biogeochemical cycling and these blooms can often be seen from space. Despite numerous studies on coccoliths we still lack a complete understanding of their function. To test whether coccoliths enhance photosynthesis by amplifying incident radiation I measured bio-optical properties of the common coccolithophore *Emiliana huxleyi*. Variable fluorescence and absorption spectroscopy measurements of *E. huxleyi* cells with and without coccoliths (removed by bubbling CO_2 into the media) suggest that coccoliths do not enhance the light within cells, and if anything, shade them slightly due to increased scattering. This indicates that calcification does not provide any optical advantage for individual cells. Bubbling *E. huxleyi* with CO_2 to remove the coccoliths led to acidification of the media and stimulated ΔpH-related non-photochemical quenching (NPQ) and reduced net photosynthesis. The reason for the reduction in net

photosynthesis is unclear, as it does not appear to be entirely a consequence of stimulated NPQ. My results suggest that there is no photonic effect of coccoliths that has a biological function.

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Introduction

Emiliana huxleyi is an abundant marine phytoplankton found worldwide except the polar oceans (Brand, 1994). It forms blooms extending over a quarter of a million square kilometers that are visible from space as milky, turquoise blotches on the ocean (Balch, Kilpatrick, Holligan, & Harbour, 1996). It frequently grows to concentrations of 10 million cells per liter of seawater but in ideal conditions has reached cell densities 10-fold higher (Brand, 1994). *E. huxleyi* plays an important yet complex role in Earth's biogeochemistry. Its contribution to the biological carbon pump is moderated through the process of calcification, which acts as a counter pump, and calcification may increase the ocean's acidity (Thierstein & Young, 2004). But it also produces dimethyl sulfide, a cloud forming compound, which increases albedo and helps to cool the planet (Malin & Steinke, 2004). Thus understanding the physiology and responses of *E. huxleyi* is important to understand its complicated role in regulating Earth's biogeochemistry.

A fundamental feature of *E. huxleyi* and other coccolithophores is the intricate calcite scales that typically cover the entire cell. These scales are called coccoliths; the entire covering is called the coccosphere. *E. huxleyi* cells exist in three forms: calcified cells (C cells) that are coccolith-forming, naked cells (N cells) which do not produce coccoliths, and flagellate scaly cells (S cells) which form organic plates (Klaveness & Paasche, 1971). The coccoliths are formed inside the cell in a separate compartment named the coccolith vesicle and extruded to the cell exterior (Paasche, 2001).

Despite numerous studies on coccoliths we still lack a complete understanding of their function and what evolutionary advantage they confer. Several theories have been proposed including that the coccosphere could protect the integrity of the cell and maintain a suitable microenvironment around the cell surface (Young, 1994), shield against virus infection (Bratbak, Wilson, & Heldal, 1996), reduce grazing rates (Sikes & Wilbur, 1982), permit cells to regulate their buoyancy (Young, 1994), function as carbonate 'trash cans' in bicarbonate-base photosynthesis (Young, 1994), serve as an energy dissipating mechanism under high irradiances (Paasche, 2001), or modify the light environment of the cell (Young, 1994). These hypotheses have been tested in a variety of studies through there is no clear evidence supporting any one theory.

In this study I examined the hypothesis that coccoliths enhance the intracellular light environment by redirecting photons into the cell and increasing absorption. To test this hypothesis I analyzed several photosynthetic parameters by measuring variable fluorescence of calcified cells and cells whose liths were removed by lowering the pH of the medium for a few minutes. This approach employed the closure of photosystem II (PSII) reaction centers (RC) as internal cellular light meters. I found that the coccoliths did not substantially change the light environment in the cell. Though I did observe changes in the photosynthesis of cells whose liths were removed that are consistent with the role of calcification as a means of inorganic carbon acquisition.

Materials and Methods

Culture conditions – A highly calcifying (607) and naked (374) strain of *E. huxleyi* were grown in dilute batch cultures in .45μm filtered, autoclave-sterilized natural seawater enriched with f/2 vitamins and trace metals without silica (SiO_3). Cultures were grown in polystyrene culture flasks at a constant temperature of 18°C and illuminated with white fluorescent tubes in a light : dark cycle of 16h : 8h at a photon flux density (PFD) of 300 μmol photons m^{-2} s^{-1}.

Spectroscopic Measurements – Attenuation and absorption of untreated, treated and "free" liths were measured to estimate scattering. Attenuation (c) is the sum of absorbance (a) and scattering (b), such that $c = a + b$. Cell suspensions of *E. huxleyi* are a highly scattering so the coefficient actually measured (x) is intermediate between the absorption coefficient and the attenuation coefficient. It can be expressed by $x = a + (1 - \epsilon)b$, where ϵ represents the fraction of scattered light entering the detector (Bricaud, Bédhomme, & Morel, 1988). If all the scattered light were included ($\epsilon = 1, x = a$), then the device would be a perfect absorption meter whereas if all the scattered light is excluded ($\epsilon = 0, x = c$) it would be a perfect attenuation meter. Estimates of absorbance and attenuation were measured with an Olis-modernized Aminco DW-2000 spectrophotometer. The spectrophotometer was configured as outlined in Fig 2-1a to measure absorbance and Fig 2-1b to measure attenuation. Cell cultures were measured in 1-cm-path-length quartz cuvettes against a reference of f/2 media. Untreated and treated cell cultures were diluted to the same concentration prior to spectral analysis as

measured by a Beckman Multisizer3™ Coulter Counter® and cell suspensions were confirmed to be dilute enough that multiple scattering and absorption did not significantly affect the *a* or *c* values. Free coccoliths were obtained by dissolving *E. huxleyi* cells in 90% acetone overnight. Then the coccoliths were concentrated by centrifugation and resuspended in .45μm-filtered seawater.

Coccolith removal – Throughout the paper the term "treated" cells refers to samples that underwent the coccolith removal protocol whether they were naked or calcified. Samples of culture were diluted with fresh f/2 media in a ratio of 3:1 (f/2 : sample) due to the sensitivity of our fluorometer. Samples were bubbled gently with CO_2 until media acidified between pH 5.85-6.30. The pH was verified with a pH meter and coccolith removal was verified via light microscopy and absorption spectroscopy. Naked cells received the same treatment as calcified cells to serve as a control. After completing the coccolith removal the treated cells were concentrated by spinning in a centrifuge for 3min at 3000RPM. The acidified supernatant media was discarded and the cells were resuspended in fresh f/2 kept at 18°C. Both the untreated and treated samples were incubated in low light (<5 μmol photons m^{-2} s^{-1}) for 30-60min to dark-adapt and allow for recovery of treated cells.

Fluorescence measurements – Chlorophyll fluorescence and photosynthetic parameters were measured in a custom built Fluorescence Induction and Relaxation (FIRe) system (Gorbunov & Falkowski, 2005). Measurements were taken employing the Single Turnover Flash (STF) protocol. The FIRe system applies a strong short pulse (70-100μs) of light (peak 450nm, bandwidth 20nm) to cumulatively saturate

photosystem II (PSII) and measure the fluorescence induction from minimum fluorescence (F_o) to maximum fluorescence (F_m). The maximum quantum yield of photochemistry in PSII was determined from a dark adapted state as the ratio $F_v/F_m = (F_m - F_o)/F_m$ (Gorbunov, Kolber, & Lesser, 2001). In addition, analysis of fluorescence induction during a STF provided the functional absorption cross-section of PSII (σ_{PSII}).

Measurements of fluorescence were also taken under illumination with an actinic light source to evaluate steady-state (F_s) and maximum yields (F'_m) of chlorophyll fluorescence in a light-acclimated state. From these measurements the fate of absorbed light energy was estimated according to Hendrickson (2004). Briefly, the quantum efficiencies of photochemistry (Φ_{PSII}), Δph- and xanthophyll regulated non-photochemical quenching (Φ_{NPQ}), fluorescence (Φ_f), and non-regulated heat dissipation (Φ_D) are given below:

$$\Phi_{PSII} = 1 - \frac{F_s}{F'_m} \quad (1)$$

$$\Phi_{f,D} = \Phi_f + \Phi_D = \frac{F_s}{F_m} \quad (2)$$

$$\Phi_{NPQ} = \frac{F_s}{F'_m} - \frac{F_s}{F_m} \quad (3)$$

Measurement of light saturation parameter (E_k) – Two neutral filters were placed in front of the actinic light source to reduce the light intensity. Fluorescence parameters were measured as a function of light intensity from 0-110 μmol photon m^{-2} s^{-1} in increments of ~3μmol photon m^{-2} s^{-1}. The F_v/F_m was plotted as a function

of background light providing information on the fraction of open and closed PSII reaction centers. As the light intensity increases a plateau corresponding to the region of irradiance when "all" the reaction centers are "open" is evident (Falkowski & Raven, 2007). This is analogous to the initial slope of an O_2 evolution vs. irradiance curve. When plotted on a log scale the E_k can be estimated by the intersection of lines extending the "plateau" and the decrease in fluorescent yield (see Fig 2-2 for an example).

Photosynthesis vs. irradiance curves – Multiplying the quantum yield of PSII photochemistry, Φ_{PSII}, by absorbed light, can approximate linear electron transport rate and overall photosynthetic capacity. Since absorbed light was unknown I multiplied Φ_{PSII} by irradiance to make relative comparisons of the PE curves (see Fig 2-3 for an example). A justification of this estimation is in the discussion. Maximum net photosynthesis (P_{max}) and E_k were found by fitting the PE curves to equation (4) using MATLAB®. To differentiate the E_k values obtained via the PE curves I will refer to them as E_{kPE}:

$$P = P_{max} \times \tanh(E/E_{kPE}) \quad (4)$$

Results

To determine the effect of coccoliths on light quality in *E. huxleyi* I determined the absorption and scattering of treated and untreated cell suspensions. The difference in absorption of untreated and treated cells indicates that even though I attempted to limit scattering by placing the sample close to the detector and using a diffusing plate (Fig 2-1a), scattering is still significant (Fig 3-1). I found that scattering is greater in cells with coccoliths at all wavelengths but increases at shorter wavelengths. This suggests that there may be less blue light within cells encased in coccospheres (Fig 3-2). There is no substantial change to the shape of the scattering function except for wavelengths below 450nm (Fig 3-2). This indicates that coccoliths will probably have minimal effect on the light within the cells except at wavelengths shorter than 450nm, which corresponds to violet and longest ultraviolet wavelengths.

To examine any intrinsic optical effects of the coccoliths themselves I isolated coccoliths (*in vitro*) and measured their absorption and scattering. I compared these to the estimated absorption and scattering of coccoliths on the cells. The absorption and scattering of coccoliths on the cells are determined by subtracting the absorption and scattering of untreated and treated cells respectively. The absorbance of free coccoliths and coccoliths on the cells are fairly featureless but also indicate that substantial scattering is still being measured as absorbance (Fig 3-3). The scattering of coccoliths on the cells deviates mildly from the scattering of free liths (Fig 3-4). This could indicate that there is a difference in the scattering

when coccoliths are arranged in a coccosphere as opposed to free coccoliths. Though it is more likely due to the presence of organic molecules that were not subtracted out because of the error in matching the concentration of treated and untreated cells.

If coccoliths enhance photosynthetically active photon flux within a cell it will increase the probability of photon absorption by the light-harvesting complex. To test whether coccoliths enhance light within the cell I measured the functional absorption cross section of dark-adapted treated and untreated *E. huxleyi* cells. The results suggest that there is no substantial modification to the light environment at photosynthetically active wavelengths as there is no significant difference between the functional absorption cross-section of treated and untreated cells in the experimental or control strain (Table 3-1).

Furthermore, any optical effect of coccoliths should manifest as a change in E_k as this is the state when photon absorption balances electron flow. I estimated the value of E_k by measuring the changes in variable fluorescence as the background light intensity was increased in small increments (Falkowski, Fujita, Ley, & Mauzerall, 1986). An advantage of this approach is that an optical enhancement due to coccoliths will be important under low light conditions and by increasing the background light in small increments any optical effect should be revealed. The results indicate there is no significant effects on E_k by removing the coccoliths (Table 3-2) hence coccoliths are not substantially affecting the photosynthetically active light environment of the cell.

Although this approach to estimating E_k has some advantages, it had the disadvantage of being dependent on estimating a best-fit line by hand and so is subject to bias. For this reason I also calculated relative E_{kPE} values as derived from fitting photosynthesis vs. irradiance curves with Eqn (4) (Table 3.3). These E_k values are not comparable to those in Table 3-2 because in calculating the photosynthesis vs. irradiance curves I multiplied Φ_{PSII} by incident light and not *absorbed* light. So the absolute value of E_k is not predicted but relative changes in E_k between treated and untreated samples can be examined (Maxwell & Johnson, 2000). Similar to the results above, the data suggests there is no significant change in E_k between treated and untreated cells for both the experimental and control group, implying there is no meaningful difference in the amount of photosynthetically active light entering the cell.

To determine if the removal of liths has any effect on photosynthesis I calculated the relative photosynthetic rate at saturation levels, P_{max}, by fitting the photosynthesis vs irradiance curves with Eqn (4). I found that calcified cells did show a reduction in P_{max} by 19%, indicating that removing the liths, or the process of removing the liths, affects their net photosynthesis. Naked cells did not show a change in P_{max} by the lith removal treatment implying the process of removing liths did not affect their photosynthesis (Table 3-4).

To investigate the affect of coccolith removal on calcified cells I evaluated the fate of absorbed light energy in calcified and naked cells (Figs 3-1 and 3-2). The calcified cells showed a sharp increase in the quantum yield of ΔpH- and

xanthophyll-regulated thermal energy dissipation (Φ_{NPQ}) compared to naked cells. Φ_{NPQ} rose in both the experimental and control group though the process of removing the coccoliths disproportionately affected the calcified cells. This corroborates the decrease in P_{max} because energy that treated cells cannot use in photosynthesis is dissipated via NPQ and reveals that lith removal by CO_2 noticeably reduces their net photosynthesis.

Discussion

My findings suggest that there is no biologically important photonic effect of coccoliths in *E. huxleyi*. Using the closure of PSII reaction centers as intracellular light sensors I have shown there is no significant change in functional absorption cross-section (σ_{PSII}) or the light saturation parameter (E_k) of cells with or without liths. The data does not predict that there is no *physical* optical effect, in fact, my spectroscopic measurements and the lower mean value of E_k in treated cells ($E_{k\ treated}$ = 37 umol quanta $m^{-2}\ s^{-1}$ vs $E_{k\ untreated}$ = 58 umol quanta $m^{-2}\ s^{-1}$) suggests there could be a physical effect. Both imply the coccosphere shades the cell slightly since it scatters strongly, especially at shorter wavelengths (Fig 3-1, 2). The statistical insignificance of the differences between treated and untreated cells emphasize the point that any *physical* effect of coccospheres is outweighed by biological variation. The coccosphere may induce a small *physical* change in the optics of the cell without an important *biological* effect. At very low irradiances the coccoliths may disadvantage cells but blooms of *E. huxleyi* are found in zones of high light intensity (Nanninga & Tyrrell, 1996). Thus I conclude that the coccospheres do not have any optical effect of biological importance.

To my surprise I discovered that calcified cells without liths show a decreased P_{max} and an increase in ΔpH- and xanthophyll regulated non-photochemical quenching. It is important to note that the method of approximating the P vs E curve, by multiplying Φ_{PSII} by incident light, does not estimate the true value of P_{max} and can only provide meaningful comparisons if the absorption by the

two samples is similar. If the absorption of the samples differ substantially then the P vs E curves cannot be compared as calculated. I argue that the absorption of calcified and treated cells is similar enough, especially at high light, that the observed difference in P_{max} is real. The fact that neither σ_{PSII} nor E_k change with or without the coccosphere implies that light absorption by the photosynthetic pigments remain similar in both samples.

This leads to the conclusion that the acidification of the media through the addition of CO_2 stimulates ΔpH-related non-photochemical quenching and a concomitant reduction of energy leading to photosynthesis. This effect appears to be occurring as we see an increase in Φ_{NPQ} in both the calcified and naked cells (Fig 3-5, 3-6) but since the Φ_{NPQ} of naked cells does not rise as quickly as calcified cells and because naked cells do not experience a significant change in P_{max} it does not explain the whole effect. The drop in P_{max} of calcified cells appears to be due to congestion in electron flow from water to carbon fixation.

Coccolith production in *E. huxleyi* has long been shown to be light dependent (Nimer, Merrett, & Brownlee, 1996; Paasche, 2001) though the precise link between them is poorly understood. Investigations into the carbon sources for photosynthesis in marine phytoplankton suggest that while most phytoplankton are not limited by carbon, *E. huxleyi* and other coccolithophorids may be an exception (Falkowski & Raven, 2007). Sikes et al. (1980) proposed coupling photosynthesis and calcification through CO_2 according to the following reaction:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 \text{ (calcite)} + CO_2 \text{ (to photosynthesis)} + H_2O$$

The reaction may be separated between the coccolith vesicle and chloroplast in two reactions, though the mechanisms and transport routes of substrates are still unclear (Brownlee & Taylor, 2004):

$$HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+ \text{ (coccolith vesicle)}$$

$$HCO_3^- + H^+ \rightarrow CO_2 + H_2O \text{ (chloroplast or cytosol)}$$

(see Fig 4-5 for model)

Thus calcification might function as a carbon concentrating mechanism in reducing photorespiration by the oxygenase activity of ribulase-bisphosphate-carboxylase-oxygenase (Rubisco) (Buitenhuis, De Baar, & Veldhuis, 1999). Any disruption in that carbon concentrating mechanism would lead to a decrease in electron flow and net photosynthesis. The congestion of electrons in the linear electron transport chain would necessitate the dissipation of excess energy via other means, namely an increase in NPQ. This line of reasoning fits well with my results, though it is not clear how the addition of CO_2 would lead to a decrease in the availability of DIC or how it would disrupt coccolith production. Perhaps acidification of the media by CO_2 reduces Ca^{2+} uptake, which prevents calcification and inhibits use of bicarbonate in photosynthesis. Though recent studies have seriously questioned the role of calcification as a carbon concentrating mechanism (Kottmeier, Rokitta, Tortell, & Rost, 2014; Rokitta et al., 2012)

In addition to providing DIC, Paasche (2001) proposed that the remarkable lack of photoinhibition in *E. huxleyi* might be due to calcification as a means of

dissipating excess energy at high light intensity thus reducing photooxidative damage. It has long been observed that *E. huxleyi* is one of the few coccolithophorids that will "over calcify", i.e. continue to calcify after completing a full coccosphere, and this has been conjectured to contribute to its prevalence. The continued calcification could reduce photoinhibition and uniquely adapt *E. huxleyi* to high light, stratified surface waters. Importantly, the resistance of *E. huxleyi* to photoinhibition is *not* attributed to a shading effect of the coccoliths (Nanninga & Tyrrell, 1996), instead it is due to excess energy dissipation. My results support this conclusion, as any significant shading effect would result in a change of σ_{PSII} and E_k.

The over production of coccoliths could have two other important consequences contributing to its success. First, the increased scattering of light by large concentrations of coccoliths will increase light attenuation leading to an increased and more stable stratification due to a decrease in heating of deeper water compared to surface waters (Balch, Holligan, Ackleson, & Voss, 1991). Second, the rapid attenuation of light may prevent the establishment of competing phytoplankton in the thermocline which may reduce the flux of nutrients to the surface (Nanninga & Tyrrell, 1996).

Evidence supporting calcification as an energy dissipating hypothesis includes observations that after an abrupt increase in irradiance *E. huxleyi* increased the organic carbon fixation 5-fold while the calcification rate increased almost 11-fold (Barcelos e Ramos, Schulz, Febiri, & Riebesell, 2012). My results also support the notion that calcification is a means of excess energy dissipation though the

mechanism is unclear. NAD^+ and NADP have been implicated in several pathways and signaling mechanisms beyond metabolism including calcium homeostasis (Ying, 2008). This may provide a connection between calcification and photosynthesis but does not suggest any role for calcification to dissipate excess energy. Enhanced cytosolic levels of NADPH may lead to a down-regulation of NADPH production in the light reactions (Rokitta et al., 2012) which could lead to increased NPQ but it is unclear why this would disproportionately occur in calcified cells. The added NADPH may elicit increased calcification as a side-product, not a primary coping mechanism of increased light.

Studies by Trimborn (2007) and Herfort (2002) contradict the hypothesis of a link between calcification and photosynthesis via inorganic carbon acquisition or as an alternate energy dissipation mechanism. They grew *E. huxleyi* under decreasing Ca^{2+} concentrations; such that under very low concentrations coccoliths were not produced. Then they measured photosynthesis and particulate inorganic and organic carbon production. Trimborn (2007) concluded that calcification does not provide a means of energy dissipation because increased photoinhibition of noncalcifying cells was not observed. She also found that noncalcifying cells could photosynthesize as efficiently as calcifying ones while both used bicarbonate as the main carbon source, implying no linkage between photosynthesis and calcification. Barcelos e Ramos et al. (2012) rationalized the conflicting results by noting that the absence of extracellular coccoliths does not mean that intracellular calcification was not occurring. The growth medium at low Ca^{2+} concentrations was very corrosive to

$CaCO_3$ and the coccoliths may have dissolved, so calcification could still assist photosynthesis.

I propose that the conflicting results may also be explained by the long period of acclimation of the cells to low Ca^{2+}, which was at least 10 days. Naked cells do not calcify and consequently do not use calcification for carbon assimilation or energy dissipation, which suggests that *E. huxleyi* has a great deal of plasticity in DIC uptake mechanisms and energy dissipation strategies. The spontaneous change of calcified cells to naked cells is a well known and often unwelcome phenomenon, frequently occurring in senescent, stationary-phase stock cultures (Paasche, 2001). Indeed, such a change occurred in the course of my experiment, much to my dismay. Furthermore, high-calcifying cultures of *E. huxleyi* change their predominant pathway of inorganic carbon uptake from bicarbonate during exponential growth to CO_2 in stationary phase (Nimer et al., 1996). This indicates that cell stress will elicit a change in carbon acquisition or metabolism. Some evidence suggests that naked cell strains are obligate CO_2 users (Dong, Nimer, Okus, & Merrett, 1993; Dong, Merrett, & Chao-yuan, 1999).Thus *E. huxleyi* appears to have considerable plasticity in phenotypic expression of carbon acquisition, and presumably energy dissipation. This plasticity is further evidenced in that photosynthesis and calcification do not *have* to be coupled, as some calcification occurs in the dark, albeit at a much reduced rate (Paasche, 2001). So the long acclimation phase used by Trimborn (2007) and Herfort (2002) may have enabled their cultures to adjust carbon uptake and energy dissipation strategies before experimentation began. This plasticity complicates comparing studies as a different strain is used in almost every study.

In summary, my work reveals no support of the hypothesis that coccoliths enhance light within cells of *E. huxleyi*. None of the photosynthetic parameters measured, the functional cross-section, σ_{PSII}, nor the light saturation parameter, E_k, indicated any increase in photosynthetically active light. An increase in photon flux due to the coccoliths should have resulted in a larger σ_{PSII} and lower E_k for untreated cells, which was not observed. Since there was no change in σ_{PSII} or E_k, I conclude that there is no biologically important photonic effect on the light within a cell from the coccosphere. I did observe a lower net photosynthesis, P_{max}, and higher quantum yield of ΔpH- and xanthophyll regulated non-photochemical quenching (Φ_{NPQ}) in treated cells. I attribute these differences to a direct manipulation of ΔpH regulated NPQ by acidification of the medium and a disruption of linear electron transport. It is unclear how electron transport is disrupted, but could be connected to a reduction of calcification leading to a decrease in inorganic carbon availability or a general stress response of the cell. One complicating factor in determining the reason for the reduction in net photosynthesis is that *E. huxleyi* is flexible in calcification and energy dissipation strategies and the internal cell signaling pathways for modulating those pathways are not known.

The discrepancies revealed invite further investigation into the role and function of coccoliths, the link between photosynthesis and calcification, the mechanisms of high light tolerance, and the phenotypic plasticity of the cosmopolitan coccolithophore *E. huxleyi.* Increased understanding of its ecology and physiology is needed to accurately predict its responses to elevated atmospheric

CO_2 and the fate of this abundant species may have reveberating consequences to the biogeochemical cycling of carbon on a global scale.

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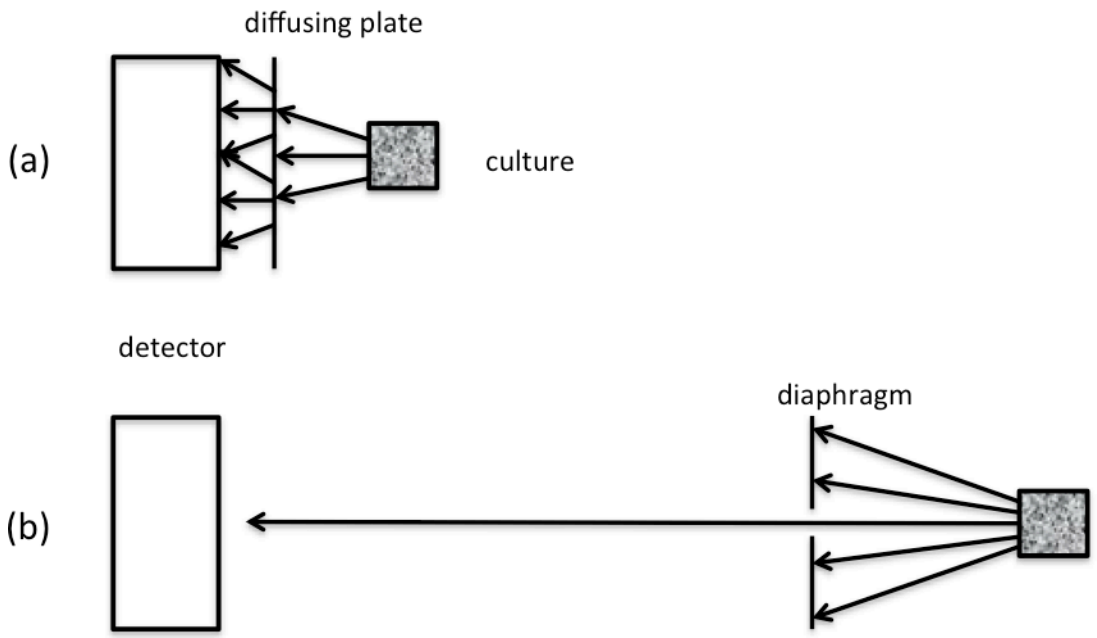

Fig 2-1 The relative positions of culture sample and detector in the spectrophotometer for measuring absorption (a) and attenuation (b)

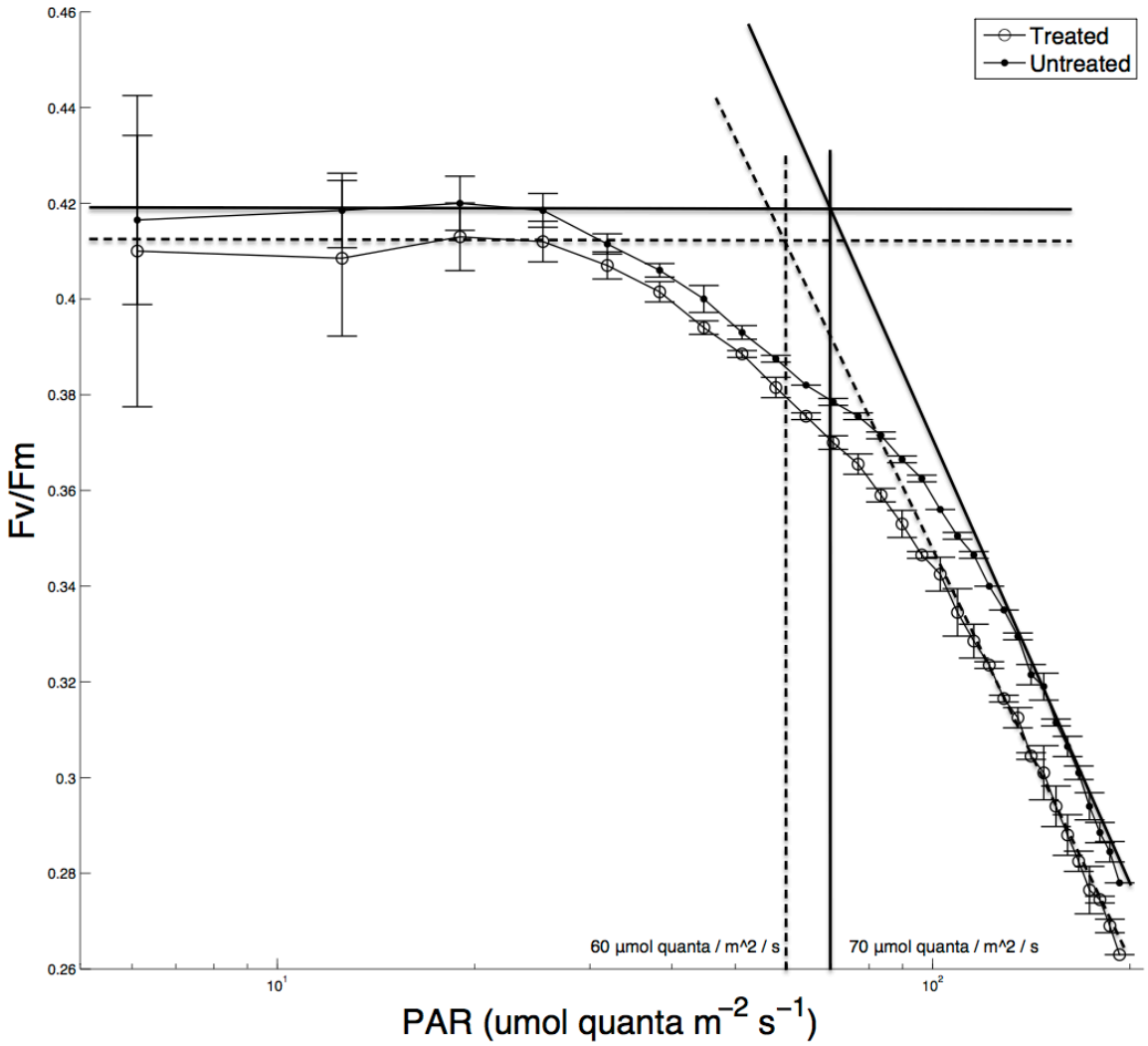

Figure 2-2 Changes in variable fluorescence of Ehux 374 (control) as a function of background irradiance (PAR) provided by an actinic light source provide an estimation of E_k values of treated (⊖) and untreated cells (-●-). The plateau at low irradiance results when most reaction centers are open and functional (Falkowski & Raven, 2007). Each point is the mean ± SE (error bars) of 2 samples.

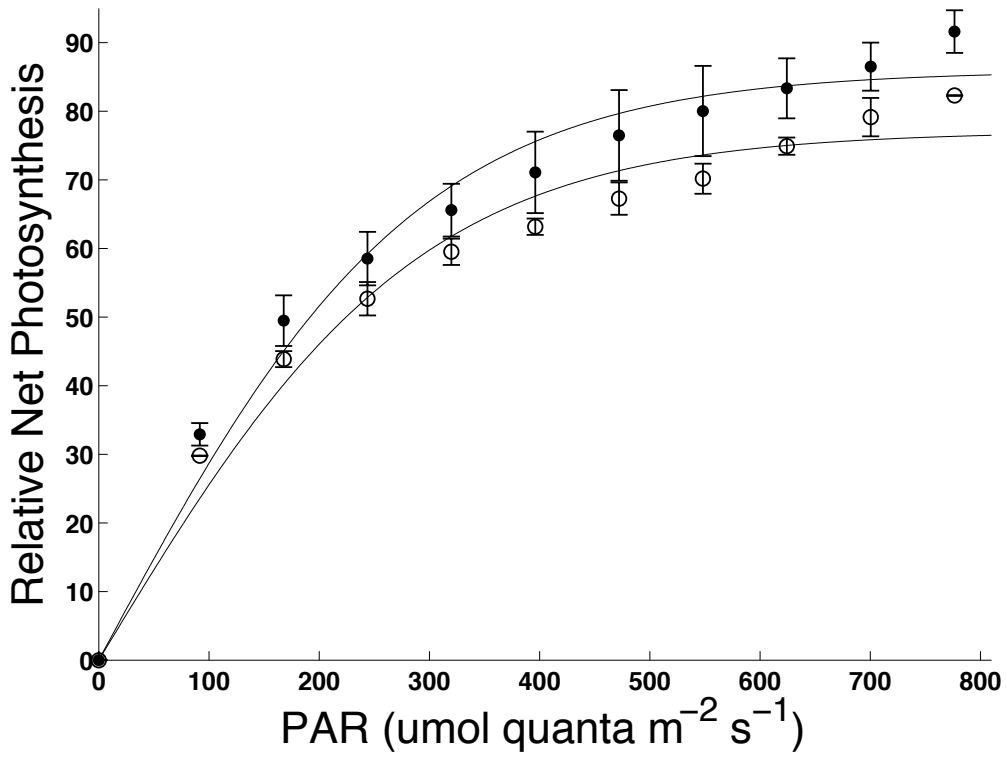

Figure 2-3 Relative net photosynthesis (estimated as Φ_{PSII} * irradiance) as a function of background irradiance of *E. huxleyi* (607) treated (⊖) and untreated (-●-) cultures. The curve is fitted to the data using Eqn (4) to estimate E_k and P_{max}. Each point is the mean ± SE (error bars) of 2 samples.

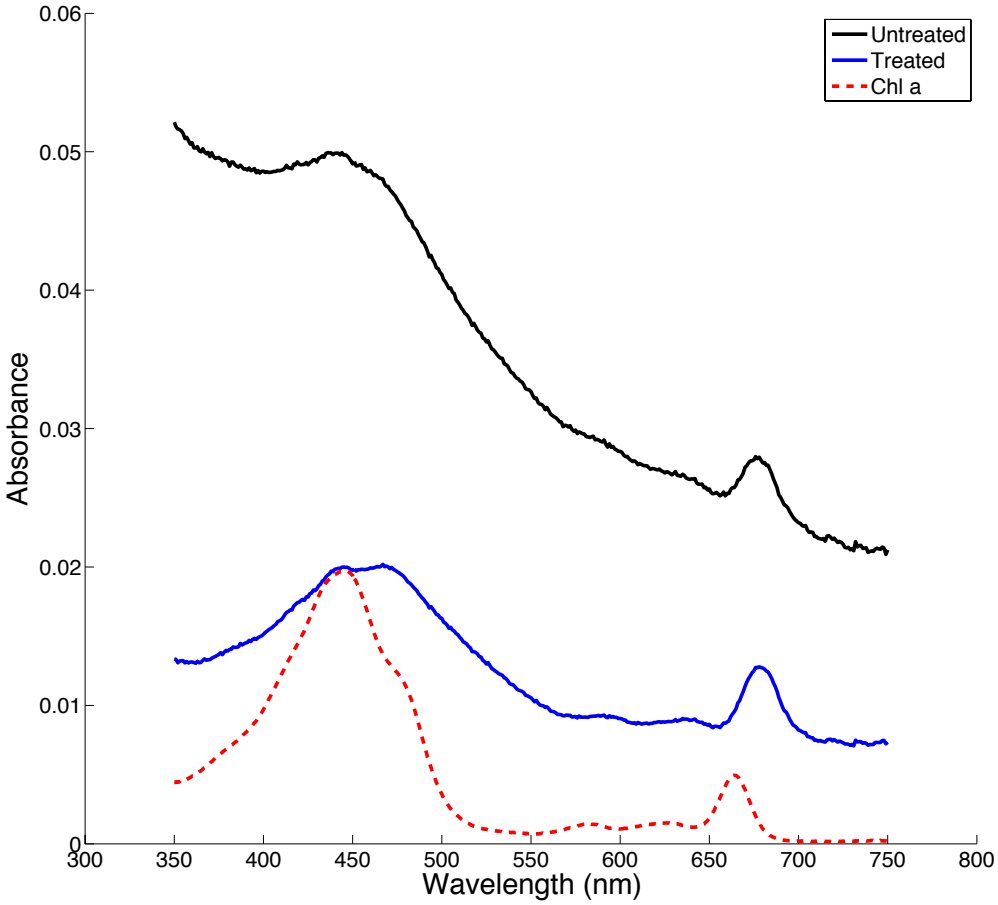

Fig 3-1 Apparent absorbance (absorbance and limited scattering) as a function of wavelength (nm) of *E. huxleyi* strain 607 before and after treatment at the same concentration. Absorbance of Chl a provided as reference.

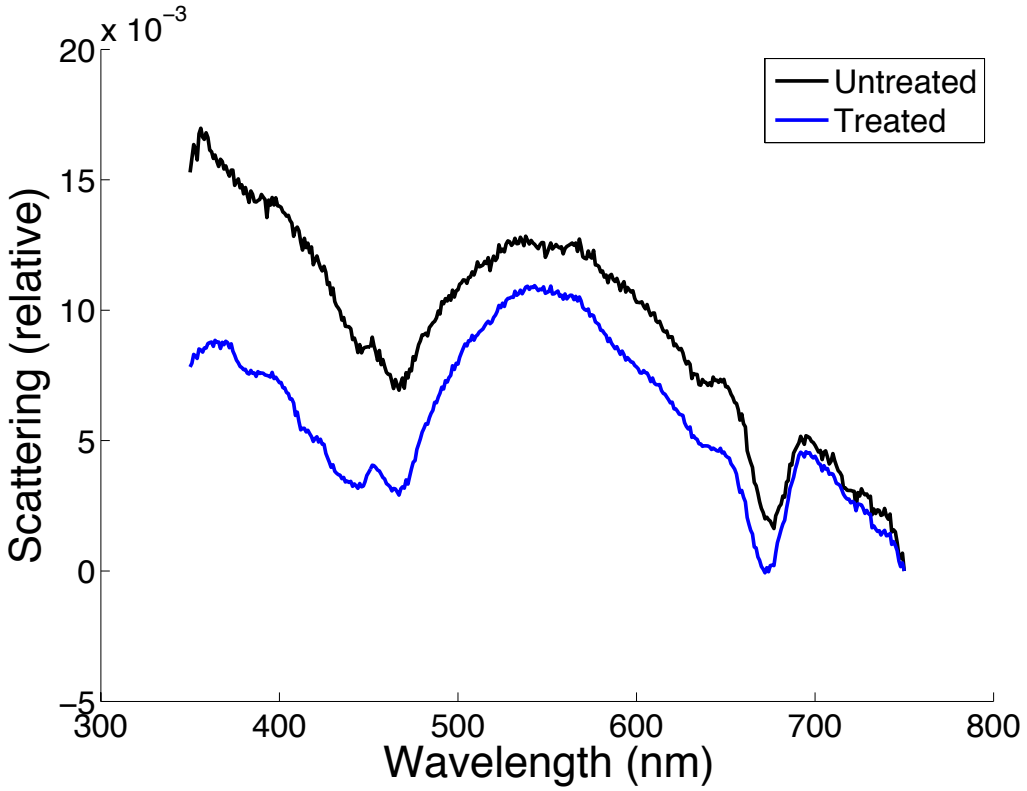

Fig 3-2 Estimated scattering (attenuation-absorbance) of treated and untreated *E. huxleyi* 607 cells, notice they are clearly depressed near the absorption bands. Spectra are normalized to 0 at 750nm to highlight the difference in scattering at short wavelengths.

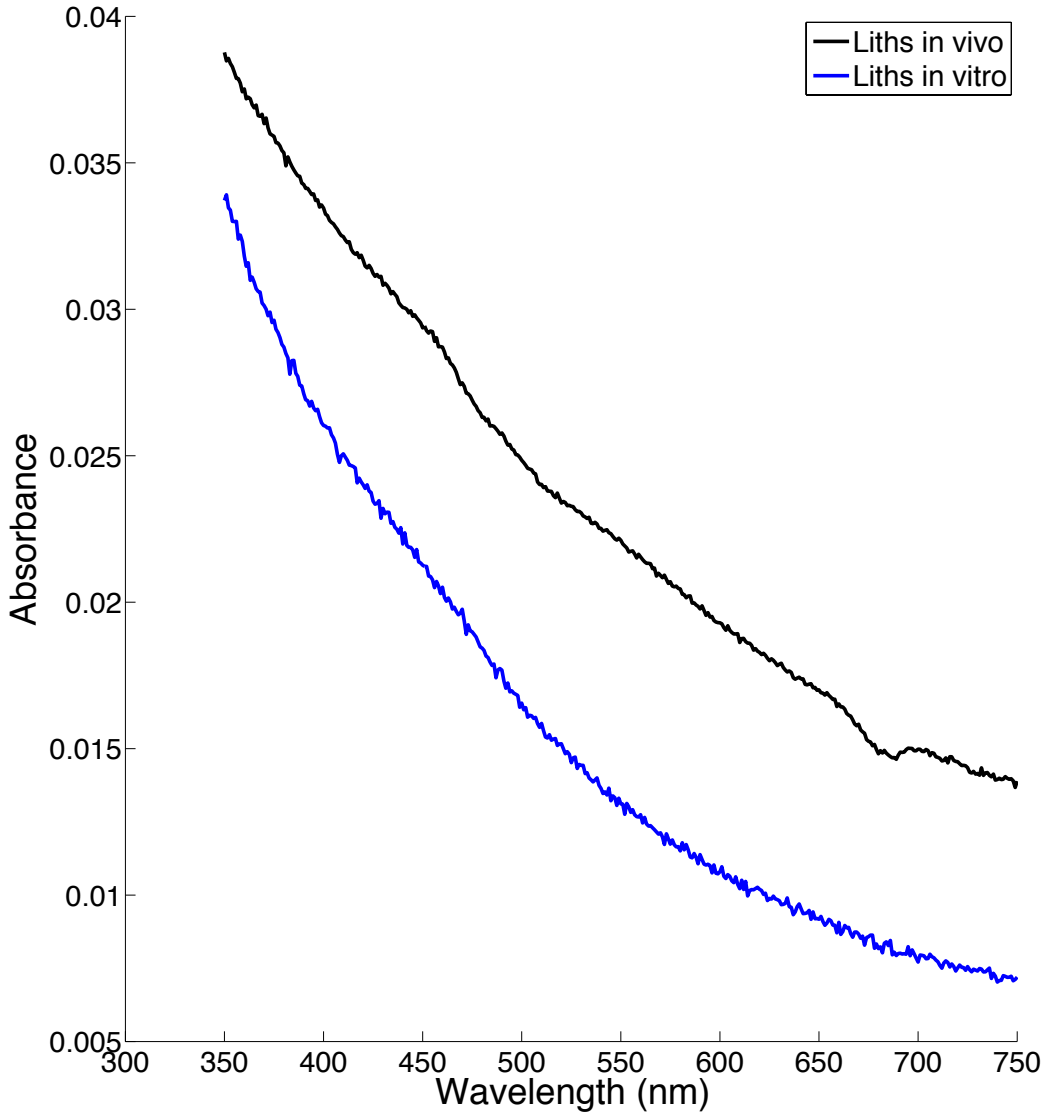

Fig 3-3 Apparent absorbance of free liths (*in vitro*) isolated by dissolving away cells with acetone, compared to the apparent absorbance of liths (*in vivo*) estimated by subtracting the absorbance of untreated (E607N) and treated (E607T) *E. huxleyi* 607.

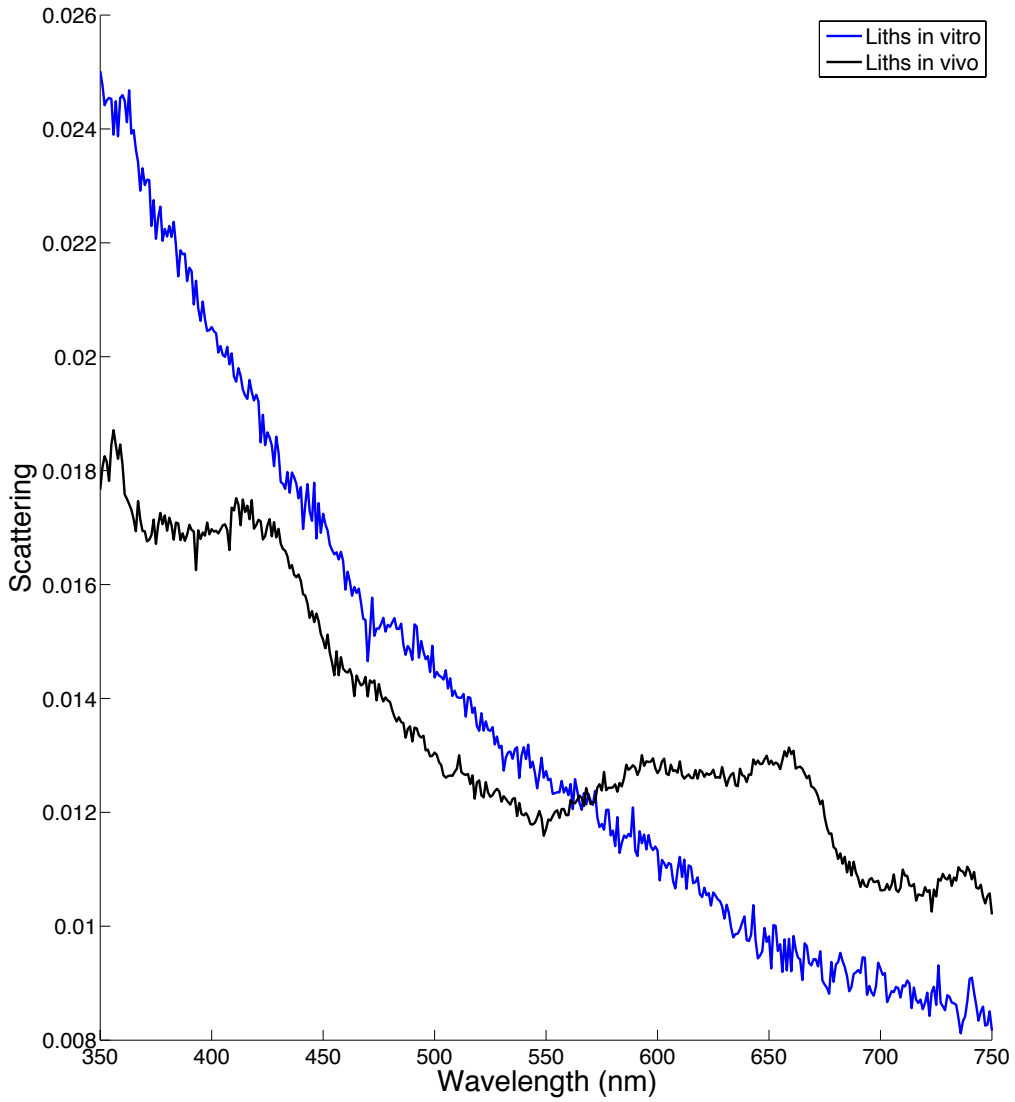

Fig 3-4 Estimated scattering (attenuation-absorbance) of coccoliths on *E. huxleyi* 607 (*in vivo*) and free coccoliths (*in vitro*), isolated by dissolving away cells with acetone.

Table 3-1 Functional absorption cross-section (a.u.) of PSII as measured from dark-adapted state in untreated and treated cells of *E. huxleyi*

Strain	Untreated	Treated
607 [1]	466±38	464±32
374 (control) [2]	499±43	494±41

[1] $t(38)=.53$, $p=.60$
[2] $t(25)=.90$, $p=.38$

Shown are the mean±SE and results of the paired T-test results, without any significant difference between treated and untreated samples.

Table 3-2 Estimated E_k (µmol quanta m^{-2} s^{-1}) values of treated and untreated *E. huxleyi* cells using low light method

Strain	Untreated	Treated
607 [1]	54±28	37±9
374 (control) [2]	43±15	42±20

[1] $t(4)=1.41$, $p=.3$
[2] $t(4)=.35$, $p=.76$
Shown are the mean±SE and results of the paired T-test results, without any significant difference between treated and untreated samples.

Table 3-3 Relative E_{kPE} (µmol quanta m^{-2} s^{-1}) values of treated and untreated *E. huxleyi* cells as derived from PE curve fitting.

Strain	Untreated	Treated
607 [1]	286±44	274±36
374 (control) [2]	186±62	226±52

[1] $t(6)=1.94$, $p=.11$
[2] $t(5)=1.36$, $p=.25$
Shown are the mean±SE and results of the paired T-test results, without any significant difference between treated and untreated samples.

Table 3-4 Relative P_{max} (a.u.) of untreated and treated *E. huxleyi* cells as derived from PE curve fitting.

Strain	Untreated	Treated
607 [1]	83±15	67±13
374 (control) [2]	52±13	51±15

[1] $t(6)=4.77$, $p=.005$
[2] $t(6)=.24$, $p=.82$

Shown are the mean±SE and results of the paired T-test results, with a significant difference between treated and untreated samples of calcified cells. No significant difference between treated and untreated samples of naked cells (control).

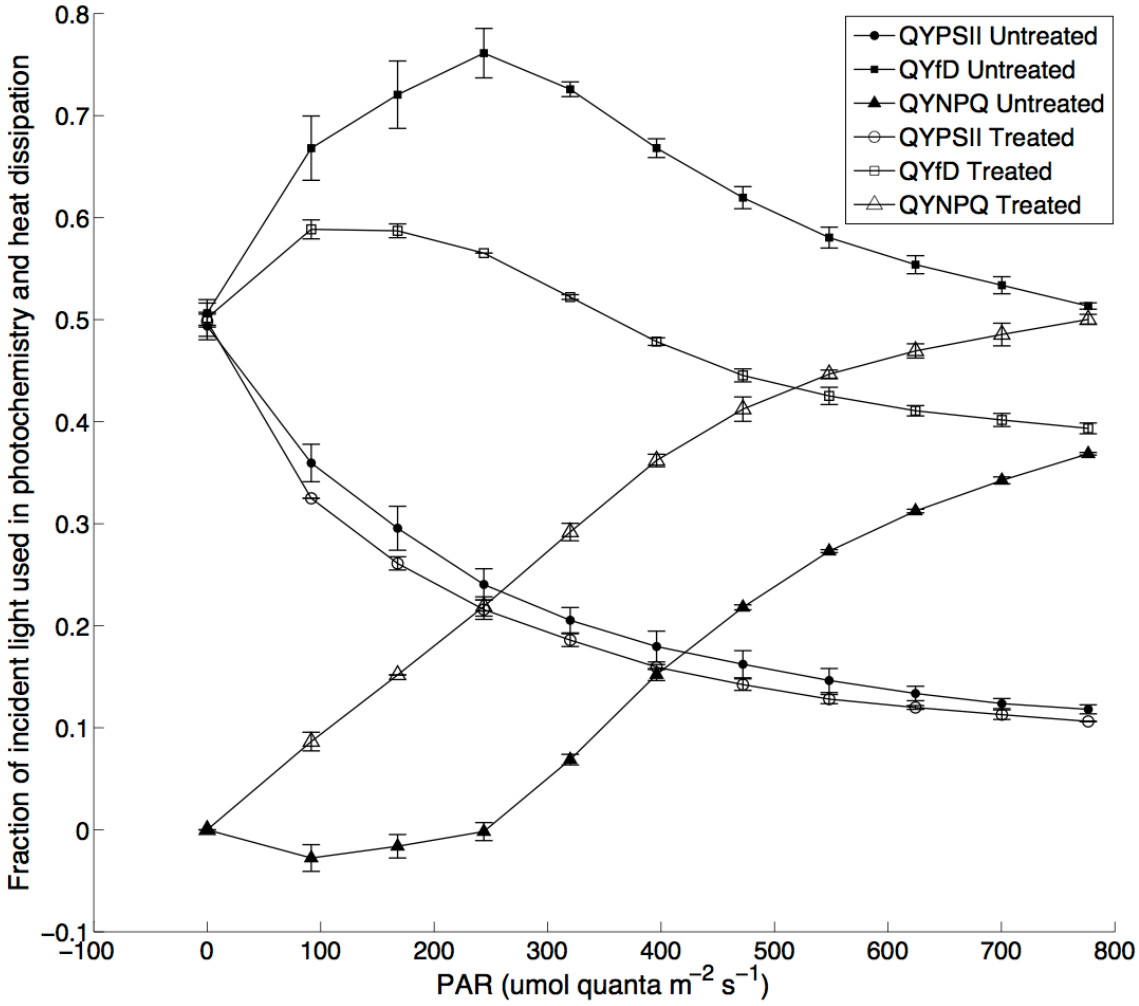

Figure 3-5 Estimated fraction of absorbed light used via photochemistry (Φ_{PSII}, QYPSII), fluorescence and constitutive thermal dissipation ($\Phi_{f,D}$, QYfD), and ΔpH- and xanthophyll-regulated non-photochemical quenching ($\Phi_{NPQ,}$ QYNPQ) in *E. huxleyi* strain 607 treated (⊖) and untreated (-●-) illuminated with an actinic light source at increasing light intensity, PAR. Each point is the mean ± SE (error bars) of 2 samples.

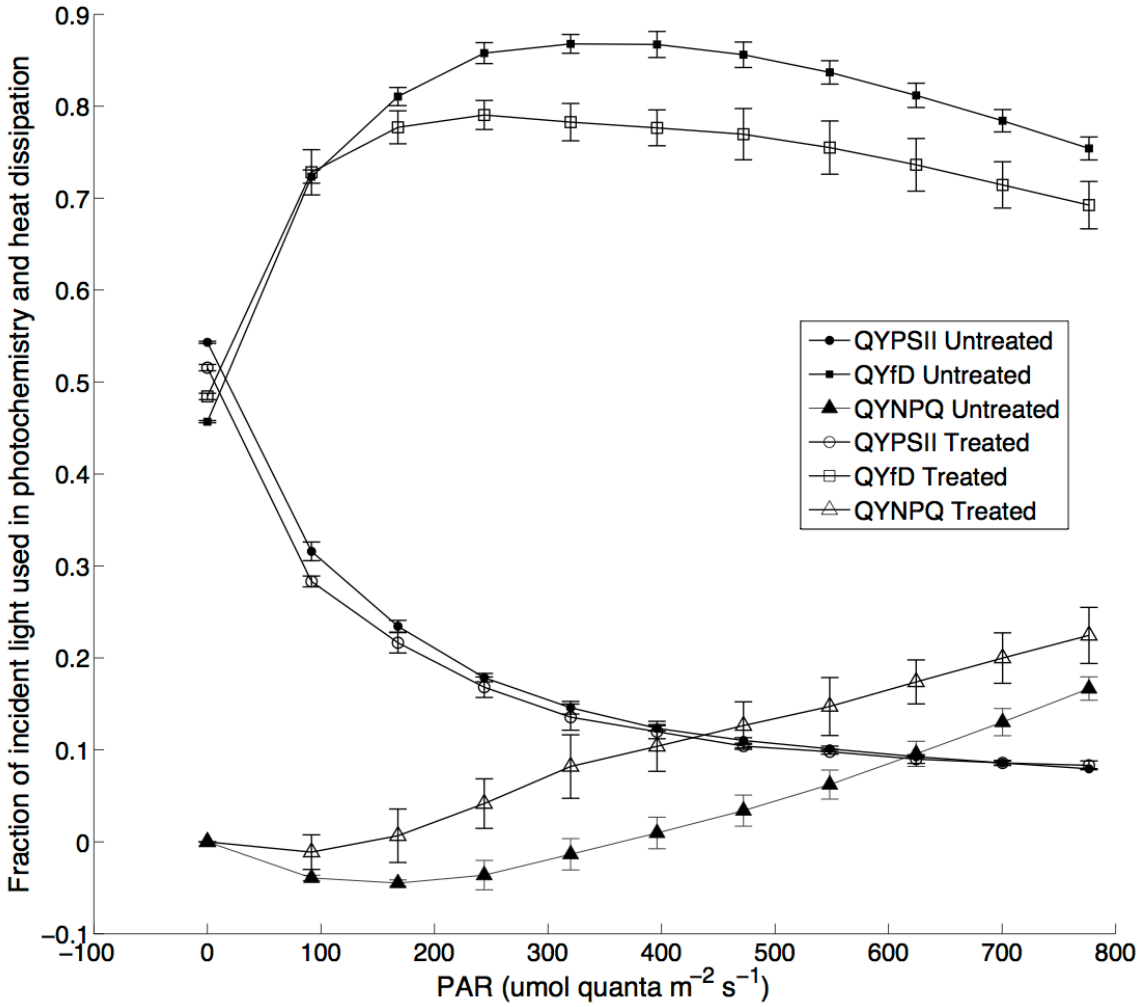

Figure 3-6 Estimated fraction of absorbed light used via photochemistry (Φ_{PSII}, QYPSII), sum of fluorescence and constitutive thermal dissipation ($\Phi_{f,D}$, QYfD), and ΔpH- and xanthophyll-regulated thermal dissipation (Φ_{NPQ}, QYNPQ) in the control strain of *E. huxleyi* 374 treated and untreated with CO_2 and illuminated with an actinic light source at increasing light intensity, PAR. Each point is the mean ± SE of 2 culture samples.

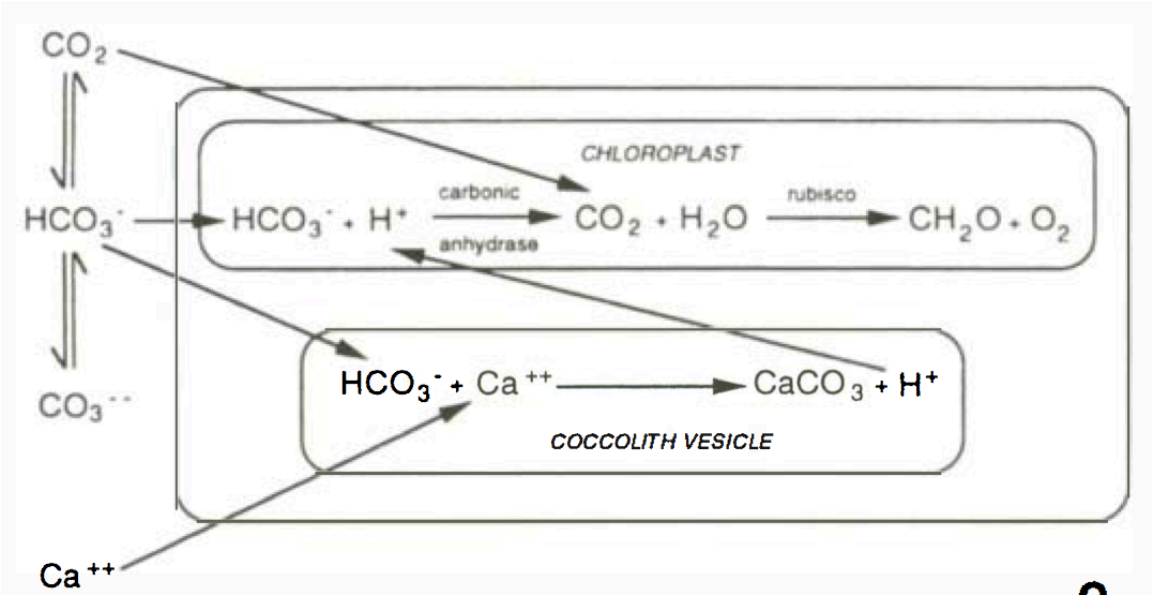

Fig 4-5. One representation of bicarbonate based photosynthesis model linked to calcification (Buitenhuis et al., 1999; Paasche, 2001).