Journal of
Plankton Research

Photophysiology of *Bolidomonas pacifica*

SARA L. THOMAS[1,2] AND DOUGLAS A. CAMPBELL[1]*

[1]DEPARTMENT OF BIOLOGY, MOUNT ALLISON UNIVERSITY, 63B YORK ST., SACKVILLE, NB, CANADA E4L 1G7 AND [2]DEPARTMENT OF BIOLOGY, ACADIA UNIVERSITY, 33 WESTWOOD AVENUE, WOLFVILLE, NS, CANADA B4P 2R6

*CORRESPONDING AUTHOR: dcampbell@mta.ca

Bolidomonas pacifica is a member of the Bolidophytes, chlorophyll *a/c* phytoplankters that lack siliceous frustules, in contrast to their sister lineage the diatoms. Under nutrient repletion, *B. pacifica* achieved a maximum growth rate of $\sim$0.51 day^{-1} at a growth light of 224 μmol photons m^{-2} s^{-1}, with a light compensation point for growth slightly below 30 μmol photons m^{-2} s^{-1}. The effective absorbance cross section for blue light for Photosystem II (PSII) for cells growing under low light was 1071 ($\pm$ 64) A^2 quanta^{-1}, with a modest decrease in cells growing under higher lights. Upon upward shifts in light, *B. pacifica* induced PSII repair within 15 min to stabilize PSII function in the face of accelerated photoinactivation, and the cells then achieved significant light acclimation within 1 h. When PSII repair was blocked, the cells suffered progressive loss of PSII function with a target size for susceptibility to photoinactivation of 7×10^{-5} ($\pm 0.5 \times 10^{-5}$) A^2 quanta^{-1}, across a range of growth lights. In comparison with similarly sized marine centric diatoms, *B. pacifica* shows larger absorbance cross sections serving PSII photochemistry but relies upon PSII repair to counter a higher intrinsic susceptibility to photoinactivation.

KEYWORDS: *Bolidomonas*; Electron transport; Light acclimation; Photoinactivation; Photosystem II

INTRODUCTION

Bolidomonas pacifica is a eukaryotic picophytoplankter with a chlorophyll *a/c* light capture system, originally isolated in 1994 from the equatorial Pacific Ocean (Guillou *et al.*, 1999a, b). The picoplanktonic flagellate class Bolidophyceae to which it belongs currently includes just one other known con-generic species,

Bolidomonas mediterranea, which was discovered in the Mediterranean Sea (Guillou *et al.*, 1999a, b). Both known species are round or heart-shaped cells measuring between 1.2 (Guillou *et al.*, 1999a, b) and 3 [The Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP)] μm in diameter.

Bolidomonas pacifica is motile and bears two ventrally inserted flagella, which differ in orientation between the species (Guillou *et al.*, 1999a). In *B. pacifica*, the two flagella are found at an angle of 90° and comprise one longer (4–7 μm) and one shorter (0.9–2.2 μm) appendages (Guillou *et al.*, 1999a, b). Tubular hairs are found on the surface of the long flagellum, whereas the short flagellum is naked (Guillou *et al.*, 1999a). The long flagellum occurring in *Bolidomonas* spp. is an unusual feature for a picoeukaryote as it is four times longer than the cell diameter of the organism. Rapid swimming is driven by the wave-like pattern of the long flagellum, but swimming motions differ between the two species, providing a distinguishing characteristic (Guillou *et al.*, 1999a). Both the size of the flagella and the rapid swimming are important distinguishing features of *Bolidomonas* that separate it from other picoeukaryote phytoplankters (Guillou *et al.*, 1999a, b). The presence of two flagella of unequal length, a mitochondrion that contains a tubular cristae and the presence of a nucleoplastidial complex are all characteristics that place the Bolidophyceae within the Heterokont lineage (Guillou *et al.*, 1999a; Daugbjerg and Guillou, 2001).

Chlorophylls *c1*, *c2* and *c3* are found in *B. pacifica*, along with the major carotenoid fucoxanthin (Guillou *et al.*, 1999a). The diadinoxanthin–diatoxanthin carotenoid couples, which are associated with photoprotection in characterized chlorophyll *a/c* phytoplankters, are present in *B. pacifica* and contribute to induction of non-photochemical quenching of excitation (Dimier *et al.*, 2009).

Within the Heterokont radiation, *Bolidomonas* is most closely related to diatoms based on both pigment analysis as well as the SSU rDNA and rbcL sequences (Guillou *et al.*, 1999a; Daugbjerg and Guillou, 2001), even though *Bolidomonas* retains two flagella and lacks the siliceous frustule which is characteristic of diatoms (Guillou *et al.*, 1999a, b). *Bolidomonas* spp. in the equatorial Pacific Ocean contribute <4% of the total chlorophyll *a*, but they contribute a significant fraction of the fucoxanthin pool in transition waters (10°S) (Guillou *et al.*, 1999b), showing that they are strong competitors to the similarly pigmented diatoms in at least some habitats. Furthermore, *Bolidomonas* cells have been found in mixed cultures derived from both the Mediterranean and the Pacific, where they outgrew other picoplankton, demonstrating that they are good competitors at least under culture conditions (Guillou *et al.*, 1999b).

The Bolidophytes present an intriguing ecophysiological contrast to the diatoms, with both groups bearing similar light capture systems, housed in different cellular structures. The marine centric diatoms we have characterized (Key *et al.*, 2010; Wu *et al.*, 2011) show smaller functional absorbance cross sections serving Photosystem II (PSII), in comparison with other taxa, but also show low intrinsic susceptibilities to photoinactivation of PSII. Since PSII photoinactivation must be countered by metabolically expensive PSII repair (Raven, 2011), this low susceptibility to photoinactivation may in part underlie the competitive success of diatoms in fluctuating environments of variable light (MacIntyre *et al.*, 2000; Key *et al.*, 2010). As part of our cross-taxon comparisons of phytoplankton photophysiologies (Six *et al.*, 2007, 2009; Key *et al.*, 2010; Loebl *et al.*, 2010; Wu *et al.*, 2011; McCarthy *et al.*, 2012), we therefore sought to characterize the photophysiology of *B. pacifica*, in particular their ability to acclimate to different growth lights and their ability to withstand and exploit moderate light fluctuations.

METHODS

Strain and culture growth conditions, cell counting

Bolidomonas CCMP *pacifica* (1866) was obtained from the CCMP. CCMP 1866 was in turn derived from the synonymous parent strain RCC 205 originally isolated in the South Pacific Ocean (05.5000°S, 105.0000°W) at a depth of 15 m (Guillou *et al.*, 1999a). *Bolidomonas pacifica* was grown in incubators at 20°C with a 12:12 light:dark cycle (Guillou *et al.*, 1999b) with no ramping, at an initial maintenance light level of 100 μmol photons $m^{-2} s^{-1}$ provided by day light fluorescent tubes. Cultures were initially maintained in both K media (Guillou *et al.*, 1999b) and Pro 99 media (CCMP), in both cases in Corning rectangular plastic flasks with ventilation caps. Although we achieved cell growth in both media, the cultures grew faster with shorter lag phase upon inoculation into K media, so we performed the current experiments on cultures grown in K media. Cultures were grown in semi-continuous culture under 30, 50, 150, 250 and 450 μmol photons $m^{-2} s^{-1}$ for at least eight cellular generations, and over at least four rounds of media dilution, to determine growth rates. We did not use data from the first round of culture media dilution to estimate growth rates. If growth rates changed thereafter over time within a semi-continuous culture, we waited until growth rates stabilized before using the cell count data to estimate growth rates. Light

within the culture volume was measured with a Walz micro-spherical PAR quantum sensor connected to an LiCor 250 meter, and was within $\pm\,10\%$ of the stated levels throughout the culture volume, with the culture flasks maintained within growth chambers with reflective white interior surfaces so that light reached the cultures from all directions. Note that for 30 μmol photons m^{-2} s^{-1} we include data only from cultures that actually grew; some attempts to grow cultures at this light level failed and so 30 μmol photons m^{-2} s^{-1} is near the lower limit for culture growth for *Bolidomonas* under a 12:12 light−dark cycle.

Bolidomonas cultures were counted every 1−3 days using a Beckman Multisizer 3 Coulter Counter. Culture was diluted in Artificial Sea Water (ASW) medium at a ratio of 4:14, 2:14 or 1:10 (culture:ASW) depending on density of the culture. The resulting cell suspension was then counted using a 17 μm aperture through which a 0.5 mL volume was run. The counter simultaneously determines the average spherical equivalent diameters of the counted sample particles.

Cell specific growth rate (μ, day^{-1}) was plotted versus growth irradiance (E, μmol photons m^{-2} s^{-1}), and fit with a second-order polynomial (Eilers and Peeters, 1988; Peeters and Eilers, 1978):

$$\mu = \frac{E}{aE^2 + bE + c}.$$

Chlorophyll fluorometry and data analysis

We measured the functional absorbance cross sections of PSII, σ_{PSII} (A^2 photon^{-1}), for both blue and green light using a Fluorescence Induction and Relaxation (FIRe) fluorometer (Satlantic, Halifax, NS, USA). We applied a saturating, single turnover flash (80 μs) of either blue (455 nm, 60 nm bandwidth) or green (540 nm, 60 nm bandwidth) light that saturates PSII photochemistry, to generate a fluorescence induction curve, detected at a wavelength of 680 nm, used to estimate F_O, F_M and σ_{PSII} using the FIReWORX script (Audrey Barnett, http://sourceforge.net/projects/fireworx/) and the flash lamp calibration factors provided by Satlantic. Fluorometry measurements were taken from cells that were dark-acclimated for 10 min, to determine $\sigma_{\mathrm{PSII\ blue}}$ and $\sigma_{\mathrm{PSII\ green}}$, F_O, and F_M. σ_{PSII} is a target size formulation for the effective absorbance cross section serving PSII photochemistry (Gorbunov *et al.*, 1999) derived from the exponential rise of fluorescence during a single turnover, saturating flash of light to deliver photons to the PSII antenna, driving the PSII centers to closure and thus driving an

increase in fluorescence yield (Falkowski and Raven, 2007). We used both blue and green excitation to track whether chlorophyll absorbance (blue) serving PSII, or ancillary carotenoid absorbance (green) serving PSII, changed differentially with light acclimation or during short-term shifts to higher light. We did pilot experiments and found that a 10 min dark acclimation period was sufficient for relaxation of non-photochemical influences on F_O, F_M and σ_{PSII}. We also measured σ_{PSII}, σ_{PSII}', F_O, F_S, F_M' and F_M (van Kooten and Snel, 1990) from culture samples taken during light shift experiments, with measurements from both dark-acclimated cells (F_O, F_M, σ_{PSII}) and from cells after 1 min under an increasing range of actinic light levels that spanned the light levels we applied to the culture during the light challenge experiments (F_S, F_M', σ_{PSII}'). Since we used a single-turnover saturating flash, our F_V/F_M estimates, even after dark acclimation, are lower than that would be expected from measurements using a multiple-turnover flash (Falkowski and Raven, 2007).

We estimated the maximum quantum yield for PSII from dark-acclimated cells as

$$\frac{F_V}{F_M} = \frac{F_M - F_O}{F_M}$$

We estimated the PSII-specific electron transport rate (Suggett *et al.*, 2003) with units of e-PSII^{-1} s^{-1} as:

$$\mathrm{ETR} = E \times \sigma_{\mathrm{PSII}} \times \Phi\mathrm{PSII}$$

where E is the actinic light level (μmol photons m^{-2} s^{-1}), σ_{PSII} is the effective absorbance cross section for PSII photochemistry in the dark acclimated state and ΦPSII is an estimate of the photochemical yield of PSII centers open and available for photochemistry at the given actinic light E (Genty *et al.*, 1989; van Kooten and Snel, 1990):

$$\Phi\mathrm{PSII} = (F_M\prime - F_s)F_M\prime.$$

The ETR estimate is based upon incident light transferred to PSII through the antenna capacity parameterized as σ_{PSII} and then converted to photochemistry with a yield specific to the given excitation level, parameterized as ΦPSII. Our fluorescence induction curves showed no evidence for lake-type connected antenna properties (Kramer *et al.*, 2004) in *Bolidomonas*. ΦPSII decreases as PSII centers close and as non-photochemical quenching is induced. We fit the plots of ETR versus light with a hyperbolic tangent model of photosynthesis versus irradiance (Jassby and Platt, 1976) to extract the light saturation irradiance E_K (μmol photons m^{-2} s^{-1}) which represents the transition from light limited to

light-saturated photosynthesis (Falkowski and Raven, 2007), and the light level at which the cells achieved maximal PSII electron transport.

Light shift challenge experiments

We took cultures from their growth light and shifted them up to a treatment light level in order to approximate an upward mixing event through the photic zone that accelerates the rate of photoinactivation and provokes photoacclimatory changes, and then returned the cultures to their growth light to detect any subsequent recovery (Key et al., 2010). Cultures grown at 30 μmol photons m^{-2} s^{-1} were shifted to 150 μmol photons m^{-2} s^{-1} for 60 min; cultures grown at 150 μmol photons m^{-2} s^{-1} were shifted to 450 μmol photons m^{-2} s^{-1} for 60 min. These light levels were chosen so that the response to the short-term shift from 30 to 150 μmol photons m^{-2} s^{-1} could be compared with the properties of the cultures after long-term grown under 150 μmol photons m^{-2} s^{-1}. The cultures grown at 250 μmol photons m^{-2} s^{-1} were left at 250 μmol photons m^{-2} s^{-1} for 90 min to analyze the response of cultures near the optimal light for growth, when PSII repair was blocked. For each experimental replicate, a culture was taken from the growth light and divided into two portions (with the exception of cultures grown at 250 μmol photons m^{-2} s^{-1} which had no control flask). We added lincomycin to one flask at a final concentration of 5 μg lincomycin mL^{-1}. We made up the lincomycin solution as a $\times$100 stock titrated to a pH of 8.2 to limit the pH difference between the culture media and the lincomycin solution (McCarthy et al., 2012). Control and lincomycin-treated flasks were pre-incubated in the dark for 10 min in order to let the lincomycin enter the cell and inhibit the function of chloroplastic ribosomes. Light response curves of the fluorescence measurements were taken at 0 min (just prior to addition of lincomycin and the 10 min dark pre-incubation), and then after 15, 30 and 60 and sometimes 90 min of exposure to higher light. The two flasks were then shifted back to their original growth light or down to low light of 30 μmol photons m^{-2} s^{-1} (for those grown at 250 μmol photons m^{-2} s^{-1}) with additional fluorescence measurements taken after 30 and sometimes 60 min (90 and 120 min from the start of the light shift). We plotted the F_V/F_M values for the culture flask treated with lincomycin and shifted to higher light against cumulative photons (photons m^{-2}) and fit the data with a single-phase exponential decay function to obtain the effective absorbance cross section provoking PSII photoinactivation, σ_i (A^2 photon^{-1})

(Oliver et al., 2003; Six et al., 2007; Campbell and Tyystjärvi, 2012).

For cultures grown at 30 and 250 μmol photons m^{-2} s^{-1}, a culture sample was also filtered onto 25 mm glass fiber filters (Millipore, APFB02500, 1 μm pore size) flash frozen with liquid nitrogen, and then stored at -80°C at each time point that fluorescence measurements were taken. The filters were then analyzed using quantitative immunoblotting to determine levels of PsbA, a key subunit of the PSII complex. Total protein was extracted using three cycles of 60 s at a speed of 6.5 m s^{-1} on a FastPrep®-24 with bead lysing matrix D (SKU 116913050), with the addition of 450 μL of 1× extraction buffer (0.1375 M Tris buffer, 0.075 M lithium dodecyl sulfate, 1.075 M glycerol, 0.5 mM Ethylenediaminetetraacetic acid, 0.1 mg mL^{-1} Pefabloc). The Bio-Rad protein assay kit (500-0116) was used to determine total protein in the extracted samples.

Based on the protein assay, 0.7 μg or 1 μg of total protein was loaded into a 4–12% gradient Bis–Tris NuPAGE gel (Invitrogen) along with standards with known concentrations of PsbA protein (Agrisera). Separation of proteins was then achieved using electrophoresis for 40 min at 200 V and the gel was then transferred to a polyvinylidene difluoride membrane for 80 min at 30 V. Membranes were then placed in a 5% milk powder blocking reagent for 1 h, incubated in PsbA primary antibody (Agrisera; 1:25 000) for 1 h and incubated in goat anti-rabbit secondary antibody (Agrisera; 1:25 000) for 1 h. Rinses with tris buffered saline with 0.1% Tween were done between antibodies and before imaging.

A VersaDoc imager (Biorad) was used to image blots by chemiluminescence after incubation in ECL Select (GE Healthcare). Band densities were then measured with ImageLab 3.0 software (Biorad) and used to estimate PsbA contents of the culture samples.

RESULTS

Growth versus light

Figure 1 presents our estimates of growth rate versus growth irradiance for B. pacifica growing under nutrient repletion and 20°C. The polynomial (Eilers and Peeters, 1988) curve fit of growth rate (day^{-1}) versus growth irradiance (μmol photons m^{-2} s^{-1}) gave estimates for μ_{max} of 0.51 day^{-1} achieved at a light of 224 μmol photons m^{-2} s^{-1}, and a growth compensation light K_C of $\sim$0. Though K_C from the curve fit was $\sim$0, there were flasks that failed to grow at our lowest

Fig. 1. Response of *Bolidomonas pacifica* growth rate to increasing growth light. Maximum growth rate of 0.51 day^{-1} was attained at 224 μmol photons m^{-2} s^{-1}. $N = 4-26$ separate growth rate estimates from individual cultures for each growth light; SD as error bars. Curve fit according to Peeters and Eilers, 1978, dotted lines show 95% confidence range.

light level of 30 μmol photons m^{-2} s^{-1}, showing that 30 μmol photons m^{-2} s^{-1} is actually near the lower limit for reliable growth. Some cultures grown at 450 μmol photons m^{-2} s^{-1} showed inhibition of growth rates although at our level of replication the Eilers and Peeters curve fit did not detect significant inhibition. The growth versus light plot suggests a niche for *Bolidomonas* in the mid-photic zone where exponential attenuation has lowered the light level to ~250 μmol photons m^{-2} s^{-1}.

PSII functional absorbance cross section

To detect acclimatory responses of the functional antenna serving PSII, we plotted σ_{PSII} versus growth light. For *Bolidomonas* growing under nutrient repletion, late exponential phase, the $\sigma_{PSIIBlue}$, reflecting chlorophyll absorbance serving PSII (455 nm, 60 nm bandwidth) (Fig. 2A) was 950 ± 43 A^2 quanta^{-1} ($N = 12$, $\pm$ SD) for cells growing under 50 μmol photons m^{-2} s^{-1}, with a modest but significant decrease to 850 ± 72 A^2 quanta^{-1} ($N = 4$, $\pm$ SD) for cells growing under 450 μmol photons m^{-2} s^{-1}. For $\sigma_{PSIIGreen}$ reflecting primarily carotenoid pigment absorbance serving PSII (540 nm, 60 nm bandwidth) (Fig. 2B), cells growing under 50 μmol photons m^{-2} s^{-1} showed 644 ± 49 A^2 quanta^{-1} ($N = 12$, $\pm$ SD), again decreasing modestly to 576 ± 67 A^2 quanta^{-1} ($N = 4$, $\pm$ SD) for cells growing under 450 μmol photons m^{-2} s^{-1}. All of these measures were taken from cells held in the dark for 10 min, to allow relaxation of non-photochemical quenching. The σ_{PSII} measure reflects the total allocation of pigment absorbance capacity to serve each PSII

Fig. 2. Response of functional absorbance cross section for PSII photochemistry to increasing growth light. (**A**) $\sigma_{PSIIBlue}$ or (**B**) $\sigma_{PSIIGreen}$ versus growth light. Mean of $4-20$ culture replicates per growth light level, SD as error bars. Dotted lines show 95% confidence ranges for linear regression lines.

center, and its effective coupling to the reaction center in the dark acclimated state. Our measurements of cells from a wide range of growth light show only small changes in σ_{PSII} versus growth light, for both blue light, delivered through chlorophyll absorbance, and for green light delivered through carotenoid absorbance.

To detect short-term regulatory changes in the functional antenna serving PSII, we measured rapid light response curves of $\sigma_{PSIIBlue}'$ (A^2 quanta^{-1}) (Fig. 3A and B), by measuring $\sigma_{PSIIBlue}'$ from culture samples exposed to a series of light levels, with 1 min at each light level before the measurement of $\sigma_{PSIIBlue}'$. For cultures growing under 30 μmol photons m^{-2} s^{-1}, $\sigma_{PSIIBlue}$ decreased from 836 ± 125 A^2 quanta^{-1} ($N = 3$, $\pm$ SD) for dark-adapted cells measured under 0 μmol photons m^{-2} s^{-1}, sharply down to 435 ± 155 A^2 quanta^{-1} for $\sigma_{PSIIBlue}'$ ($N = 3$, $\pm$ SD) measured at the end of a brief 1 min exposure to 150 μmol photons m^{-2} s^{-1} After 1 h of incubation at high light (150 μmol photons m^{-2} s^{-1}), $\sigma_{PSIIBlue}'$ values measured under actinic light

Fig. 3. Short-term light response curves of σ_{PSII}' to increasing actinic light. $\sigma_{PSIIBlue}'$ (A^2 quanta^{-1}) versus actinic light for cultures grown at (**A**) 30 μmol photons m^{-2} s^{-1} or (**B**) 150 μmol photons m^{-2} s^{-1}. Open circles: control cultures taken direct from growth light, indicated by large open circle; closed circles: cultures after 1 h of incubation at 150 (A) or 450 (B) μmol photons m^{-2} s^{-1}, indicated by large closed circle. Dotted arrow indicates upward light shift; solid arrow indicates culture acclimation during 1 h of exposure to (A) 150 μmol photons m^{-2} s^{-1} or (B) 450 μmol photons m^{-2} s^{-1}. Mean of (A) 3 or (B) 5 replicate cultures, SD as error bars.

of 150 μmol photons m^{-2} s^{-1} had increased significantly to 627 ± 238 A^2 quanta^{-1} ($N = 3$, $\pm$SD). For cultures growing under 150 μmol photons m^{-2} s^{-1}, $\sigma_{PSIIBlue}$ decreased from 1015 ± 103 A^2 quanta^{-1} ($N = 5$, $\pm$SD) for dark-adapted cells measured under 0 μmol photons m^{-2} s^{-1}, down to 629 ± 58 A^2 quanta^{-1} for $\sigma_{PSIIBlue}'$ ($N = 5$, $\pm$SD) measured at the end of brief exposure to 200 μmol photons m^{-2} s^{-1}. Note that rapid downregulation of $\sigma_{PSIIBlue}'$ persisted after 1 h of incubation under higher light for both the low light (Fig. 3A) cultures and the higher light (Fig. 3B) cultures.

PSII electron transport light responses and acclimation

We estimated the electron transport rate (e-PSII^{-1} s^{-1}) and plotted it versus actinic light for cultures grown at 30 μmol photons m^{-2} s^{-1} (Fig. 4A, open symbols), and for the same cultures 1 h after a shift upward to 150 μmol photons m^{-2} s^{-1} (closed symbols). We fitted the data with a hyperbolic tangent model of photosynthesis versus irradiance (Jassby and Platt, 1976) to estimate the threshold irradiance, E_K, between light limitation and light saturation (Falkowski and Raven,

Fig. 4. Short-term light response curves of PSII electron transport. (**A**) Electron transport rate (e- PSII^{-1} s^{-1}) versus actinic light for cultures grown at 30 μmol photons m^{-2} s^{-1}. Open circles: control cultures taken direct from growth light of 30 μmol photons m^{-2} s^{-1}, with dotted line showing hyperbolic tangent regression fit. Closed circles: cultures after 1 h of incubation at 150 μmol photons m^{-2} s^{-1}, with solid line showing hyperbolic tangent regression fit. (**B**) Electron transport rate (e-PSII^{-1} s^{-1}) versus actinic light for cultures grown at 150 μmol photons m^{-2} s^{-1}. Open circles: control cultures measured directly from growth light of 150 μmol photons m^{-2} s^{-1}, with dotted line showing hyperbolic tangent regression fit. Closed circles: cultures measured after 1 h of incubation at 450 μmol photons m^{-2} s^{-1}, with solid line showing hyperbolic tangent regression fit. (A) Mean of four, (B) mean of five replicate cultures, SD as error bars. Thin dotted lines show 95% confidence intervals on the fits.

2007). For cells grown at 30 μmol photons m^{-2} s^{-1}, E_K for PSII electron transport was 58 μmol photons m^{-2} s^{-1} and did not increase within a 1 h upward light shift to 150 μmol photons m^{-2} s^{-1}. For cells grown at 150 μmol photons m^{-2} s^{-1}, E_K for PSII electron transport was 71 μmol photons m^{-2} s^{-1}, but within a 1 h upward light shift to 450 μmol photons m^{-2} s^{-1}. E_K increased to 172 μmol photons m^{-2} s^{-1}. For cells from both growth light levels, ETR$_{MAX}$ increased after the 1 h shift to higher light.

PSII function (F_V/F_M) during changing light levels

We shifted cultures to higher light to approximate mixing upward through the photic zone and then

shifted them back to their original growth light for recovery. For shift experiments done at 30 and 150 µmol photons $m^{-2}\,s^{-1}$, we divided the culture into a subculture with active chloroplastic protein synthesis to support repair of PSII, and a sub-culture in which the inhibitor of chloroplast protein synthesis lincomycin was used to inhibit PSII repair. For the experiment done at 250 µmol photons $m^{-2}\,s^{-1}$, only a lincomycin flask was used. Figure 5A shows PSII function (F_V/F_M) for cultures grown at 30 µmol photons $m^{-2}\,s^{-1}$, shifted upwards to 150 µmol photons $m^{-2}\,s^{-1}$, with subsequent recovery at 30 µmol photons $m^{-2}\,s^{-1}$. F_V/F_M was measured after 10 min of dark acclimation, using a PSI chlorophyll fluorometer operating in fast repetition rate mode to apply a single turnover saturating flash. Because we were performing kinetic shift experiments, we were not able to apply longer dark acclimation periods. In test experiments however (data not

presented), more prolonged dark incubations did not result in significant further increases in F_V/F_M. Cultures with active PSII repair (open symbols) were able to counteracclerated photoinactivation and stabilize PSII function within 900 s (15 min) of the upward shift, whereas cultures where PSII repair was blocked (closed symbols) suffered progressive loss of PSII function. The response patterns were similar for cultures grown at 150 (Fig. 5B) µmol photons $m^{-2}\,s^{-1}$ and shifted upwards to 450 µmol photons $m^{-2}\,s^{-1}$ and those grown at 250 (Fig. 6A) µmol photons $m^{-2}\,s^{-1}$, except that under the higher treatment light levels the progressive loss of PSII function was faster in the presence of lincomycin (closed symbols). The initial F_V/F_M measures from our cultures grown at 30 µmol photons $m^{-2}\,s^{-1}$ were 0.47 ± 0.01 ($n = 3$, $\pm$ SD) (Fig. 5A); from cultures grown at 150 µmol photons $m^{-2}\,s^{-1}$ (Fig. 5B) were 0.33 ± 0.04 ($n = 5$, $\pm$ SD) (Fig. 5B) and from cultures grown at 250 µmol photons $m^{-2}\,s^{-1}$ were 0.29 ± 0.9 ($n = 5$,

Fig. 5. Response of PSII maximum quantum yield to changing light levels. (**A**) Cultures shifted from growth light of 30 to 150 µmol photons $m^{-2}\,s^{-1}$ with subsequent recovery at 30 µmol photons $m^{-2}\,s^{-1}$. Mean of three replicate cultures, SD as error bars. (**B**) Cultures shifted from growth light of 150 to 450 µmol photons $m^{-2}\,s^{-1}$ with subsequent recovery at 150 µmol photons $m^{-2}\,s^{-1}$. Mean of five replicate cultures, SD as error bars. (A and B) Open symbols: control cultures with active PSII repair cycles; closed symbols: cultures treated with lincomycin to inactivate the PSII repair cycle.

Fig. 6. Response of PSII maximum quantum yield and PsbA levels to loss of PSII repair. Culture replicates were grown at 250 µmol photons $m^{-2}\,s^{-1}$ and treated with addition of lincomycin to inhibit photosystem II repair. (**A**) PSII maximum quantum yield (F_V/F_M). (**B**) Change in PsbA protein content. Mean of five replicate cultures, SD as error bars. Solid line shows a single-phase exponential decay, with thin, dotted lines showing 95% confidence intervals on the fit.

$\pm$SD) (Fig. 6A) progressively lower as growth light increased, so that the ratio of active to inactive PSII decreased as growth light increased. Our culture F_V/F_M estimates are lower than those of Dimier *et al.* (Dimier *et al.*, 2009), so either our cultures were growing with PSII in a downregulated state or our single turnover flash fluorometer generated lower F_V/F_M estimates.

Along with PSII function (F_V/F_M), we also tracked PsbA content for cultures grown at 250 (Fig. 6B) and 30 (data not shown) μmol photons $m^{-2}\,s^{-1}$. Figure 6 depicts changes in both F_V/F_M (A) and PsbA (B) for cultures grown at 250 μmol photons $m^{-2}\,s^{-1}$, throughout the course of a lincomycin treatment shift. The rate constant for inhibition of F_V/F_M, 1.4×10^{-4} s^{-1} ($\pm 0.3 \times 10^{-4}$), was not significantly different from the rate constant for removal of PsbA protein, 1.7×10^{-4} ($\pm 0.6 \times 10^{-4}$), showing that clearance of PsbA was keeping pace with the photoinactivation of PSII. For cultures grown at 30 μmol photons $m^{-2}\,s^{-1}$, PsbA clearance was sufficiently slow and scattered that we were unable to determine a rate constant, but it lagged behind the drop in F_V/F_M.

To parameterize the cellular susceptibility to photoinactivation of PSII (Oliver *et al.*, 2003; Campbell and Tyystjärvi, 2012), we plotted the loss of PSII function (F_V/F_M) versus cumulative incident photons (photons m^{-2}) (data not shown) for cells treated with lincomycin to block PSII repair, and shifted from 30 to 150 μmol photons $m^{-2}\,s^{-1}$; from 150 to 450 μmol photons $m^{-2}\,s^{-1}$ or from 250 to 250 μmol photons $m^{-2}\,s^{-1}$. We estimated the effective absorbance cross section for photoinactivation, σ_I (A^2 photons^{-1}) by fitting the plot of F_V/F_M versus cumulative incident photons with a single-phase exponential decay function. σ_I was $7.0 \times 10^{-5}\,A^2$ quanta^{-1} ($\pm 0.7 \times 10^{-5}\,A^2$ quanta^{-1}), with no significant difference in susceptibility to photoinactivation of PSII among cultures grown across the range of growth light levels. σ_I is a target size parameterization with the same units as σ_{PSII} (m^2 photons^{-1}).

DISCUSSION

Under nutrient replete conditions, *B. pacifica* achieved maximum growth rates at a light level of 224 μmol $m^{-2}\,s^{-1}$ (Fig. 1) equivalent to the mid-range of the photic zone. The light compensation point for growth, at which *Bolidomonas* can just balance cell maintenance with growth, was just below $\sim$30 μmol $m^{-2}\,s^{-1}$. These estimates were generated for cells growing under a 12 h:12 h light:dark cycle, and so reflect light input sufficient to drive enough net photosynthesis to counter nighttime maintenance respiration, while supporting the net growth.

Bolidomonas acclimation to increasing growth light levels involved only modest decreases in $\sigma_{PSIIBlue}$ and $\sigma_{PSIIGreen}$ (Fig. 2). Thus decreasing the maximal antennae size is not the primary mechanism for light acclimation in *Bolidomonas*. Furthermore, the ratio between $\sigma_{PSIIGreen}$, reflecting light delivered to PSII through carotenoids, and $\sigma_{PSIIBlue}$, reflecting light delivered to PSII through chlorophyll, did not change with increasing growth light (data not shown), showing that there was no qualitative acclimation in the spectral profile, and thus the pigment composition, of the antennae serving PSII with increasing growth light. This suggests that long-term acclimation to light in *Bolidomonas* is controlled by changes in the content of active PSII centers (Falkowski and Owens, 1980), as indicated by the decline in F_V/F_M in cells growing under higher light, as opposed to changes in the effective absorbance cross section of PSII.

In contrast, cultures grown at 30 μmol $m^{-2}\,s^{-1}$ demonstrated a sharp decrease in $\sigma_{PSIIBlue}$ from measurements on dark acclimated cells to $\sigma_{PSII}'_{Blue}$ measured from cells under a series of 1 min exposures to increasing levels actinic light (Fig. 3A). A similar downregulation of $\sigma_{PSII}'_{Blue}$ with increasing actinic irradiance was shown by cells grown at a level of 150 μmol $m^{-2}\,s^{-1}$ (Fig. 3B). This rapid downregulation of σ_{PSII}' upon a brief exposure to higher light shows an induction of non-photochemical quenching mechanisms (Dimier *et al.*, 2009) to dissipate excitation from the antenna (Falkowski and Raven, 2007) in order to downregulate the short-term function of the antennae serving PSII. Instead of decreasing the total antennae size by re-allocating pigment and protein resources as growth irradiance increases, the *Bolidomonas* antennae release excess light energy as heat instead of passing it on to the reaction centers. Thus *Bolidomonas* uses short-term physiological regulation of effective antenna size to accommodate changes in growth light, retaining the flexibility to rapidly exploit upward or downward changes in growth light. We speculate that the cell motility of *Bolidomonas* may interact with its use of short-term antenna size regulation.

Plots of PSII function (F_V/F_M) were used to examine repair and photoinactivation in *Bolidomonas* under a high light phase and recovery. Under a moderate upward shift in light, photoinactivation was countered by PSII repair. Cultures from higher growth light levels experienced more photoinactivation because they were started at light levels where they were already photoinhibited and then treated under higher light, thus their metabolic cost for ongoing PSII repair is greater than those grown at lower light. For cultures grown at 250 μmol $m^{-2}\,s^{-1}$ (Fig. 6B), in the presence of lincomycin both F_V/F_M and PsbA content declined exponentially at similar rates, showing that clearance of

PsbA kept pace with PSII inactivation. Note that this balanced situation corresponds to the optimal growth light for the cultures. In contrast, for the cells growing at 30 μmol m^{-2} s^{-1}, clearance of PsbA protein lagged behind PSII photoinactivation during a shift to moderately higher light. Thus, although the low light cells were able to counter PSII photoinactivation through PSII repair over the first 60 min of increased light (Fig. 6A), their short-term capacity to clear photoinactivated protein was delayed.

The cellular susceptibility to photoinactivation of PSII was determined by plotting the loss of PSII function against the cumulative incident photons (photons m^{-2}) for cells grown at 30, 150 and 250 μmol m^{-2} s^{-1} and treated with lincomycin to block PSII repair. For these treatments under moderate levels of visible light, the effective absorbance cross section provoking photoinactivation (σ_I) was 7.0×10^{-25} m^2 quanta^{-1}, with no significant difference among cultures across the range of growth lights, even though the cultures were maintained for many cellular generations at the relevant growth light before the light shift treatments were imposed. In contrast, under treatments with light that included UV, in both cyanobacteria (MacDonald *et al.*, 2003) and diatoms (Guan and Gao, 2008), the growth light regime affects the susceptibility of phytoplankton to increased light. Either *Bolidomonas* is functionally distinct, or potentiation of susceptibility to increased light by previous growth light depends upon the presence or absence of UV in the treatments. *Bolidomonas* shows a higher susceptibility to photoinactivation of PSII compared with small marine centric diatoms, which have σ_I in the range of 0.3 to 0.4×10^{-24} m^2 quanta^{-1} (Key *et al.*, 2010; Wu *et al.*, 2011; McCarthy *et al.*, 2012). The rate constant for photoinactivation of PSII equals σ_I multiplied by the incident photons m^{-2} s^{-1} (Campbell and Tyystjärvi, 2012) and, to maintain photosynthesis, each PSII photoinactivation event must be countered by a metabolically expensive (Raven, 2011) and relatively slow PSII repair process. Therefore, the higher susceptibility to photoinactivation for *Bolidomonas* represents a significant competitive disadvantage in comparison with the small marine centric diatoms that incur lower PSII repair costs at a given incident light level, and which suffer less net photoinactivation during transient exposure to higher light during mixing. On the other hand, *Bolidomonas* enjoys a larger functional absorbance cross section for PSII photochemistry than do small centric diatoms we have grown under comparable conditions (Key *et al.*, 2010; Wu *et al.*, 2011; McCarthy *et al.*, 2012; Wu et al., 2012). The return-on-investment per PSII synthesized is therefore similar for the two groups, with ~1 photoinactivation round for every 12 000 000

photons delivered to PSII, under low light. Nevertheless, their strategies are different, with *B. pacifica* running a more metabolically active system of rapid light capture, rapid modulation of antenna function and repair of PSII, compared with the slower, lower cost strategy of the diatoms with a smaller absorbance cross section serving PSII but also slower photoinactivation.

CONFLICT OF INTEREST

D.A.C. is a minority shareholder and consultant with Environmental Proteomics, which produced the protein quantitation standard used for analyses of PsbA protein. He does not receive a financial return from use of the standard.

REFERENCES

Campbell, D. A. and Tyystjärvi, E. (2012) Parameterization of photosystem II photoinactivation and repair. *Biochim. Biophys. Acta*, **1817**, 258–265.

Daugbjerg, N. and Guillou, L. (2001) Phylogenetic analyses of Bolidophyceae (Heterokontophyta) using *rbcL* gene sequences support their sister group relationship to diatoms. *Phycologia*, **40**, 153–161.

Dimier, C., Giovanni, S., Ferdinando, T. *et al*. (2009) Comparative ecophysiology of the xanthophyll cycle in six marine phytoplanktonic species. *Protist*, **160**, 397–411.

Eilers, P. H. C. and Peeters, J. C. H. (1988) A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecol. Model.*, **42**, 199–215.

Falkowski, P. G., Dubinsky, Z. and Wyman, K. (1985) Growth-irradiance relationships in phytoplankton. *Limnol. Oceanogr.*, **30**, 311–321.

Falkowski, P. G. and Owens, T. G. (1980) Light—shade adaptation 1. *Plant Physiol.*, **66**, 592–595.

Falkowski, P. G. and Raven, J. A. (2007) *Aquatic Photosynthesis*, 2nd edn. Princeton University Press, Princeton.

Genty, B., Briantais, J.-M. and Baker, N. R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta*, **990**, 87–92.

Guan, W. and Gao, K. (2008) Light histories influence the impacts of solar ultraviolet radiation on photosynthesis and growth in a marine diatom, *Skeletonema costatum*. *J. Photochem. Photobiol. B Biol.*, **91**, 151–156.

Gorbunov, M. Y., Kolber, Z. S. and Falkowski, P. G. (1999) Measuring photosynthetic parameters in individual algal cells by fast repetition rate fluorometry. *Photosynth Res*, **62**, 141–153.

Guillou, L., Chretiennot-Dinet, M., Medlin, L. *et al.* (1999a) *Bolidomonas*: a new genus with two species belonging to a new algal class, the Bolidophyceae (Heterokonta). *J. Phycol.*, **35**, 368–381.

Guillou, L., Staay, S.-Y. M.-V. D., Claustre, H. *et al.* (1999b) Diversity and abundance of Bolidophyceae (Heterokonta) in two oceanic regions. *Appl. Environ. Microbiol.*, **65**, 4528–4536.

Jassby, A. D. and Platt, T. (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.*, **21**, 540–548.

Key, T., McCarthy, A., Campbell, D. *et al.* (2010) Cell size trade-offs govern light exploitation strategies in marine phytoplankton. *Environ. Microbiol.*, **12**, 95–104.

Kramer, D. M., Giles, J., Olavi, K. *et al.* (2004) New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. *Photosynth. Res.*, **79**, 209–218.

Loebl, M., Cockshutt, A. M., Campbell, D. A. *et al.* (2010) Physiological basis for high resistance to photoinhibition under nitrogen depletion in *Emiliania huxleyi*. *Limnol. Oceanogr.*, **55**, 2150–2160.

MacDonald, T. M., Dubois, L., Smith, L. C. *et al.* (2003) Sensitivity of cyanobacterial antenna, reaction centre and CO_2 assimilation transcripts and proteins to moderate UVB: light acclimation potentiates resistance to UVB. *J. Photochem. Photobiol. B Biol.*, **77**, 405–412.

MacIntyre, H. L., Kana, T. M. and Geider, R. J. (2000) The effect of water motion on short-term rates of photosynthesis by marine phytoplankton. *Trends Plant Sci.*, **5**, 12–17.

McCarthy, A., Rogers, S. P., Duffy, S. J. *et al.* (2012) Elevated carbon dioxide differentially alters the photophysiology of *Thalassiosira pseudonana* (Bacillariophyceae) and *Emiliana huxleyi* (Haptophyta). *J. Phycol.*, **48**, 635–646.

Oliver, R. L., Whittington, J., Lorenz, Z. *et al.* (2003) The influence of vertical mixing on the photoinhibition of variable chlorophyll a fluorescence and its inclusion in a model of phytoplankton photosynthesis. *J. Plankton Res.*, **25**, 1107–1129.

Peeters, J. C. H. and Eilers, P. (1978) The relationship between light intensity and photosynthesis—A simple mathematical model. *Hydrobiological Bulletin*, **12**, 134–136.

Raven, J. A. (2011) The cost of photoinhibition. *Physiol. Plant.*, **142**, 87–104.

Six, C., Finkel, Z. V., Irwin, A. J. *et al.* (2007) Light variability illuminates niche-partitioning among marine picocyanobacteria. *PLoS One*, **2**, e1341.

Six, C., Sherrard, R., Lionard, M. *et al.* (2009) Photosystem II and pigment dynamics among ecotypes of the green alga *Ostreococcus*. *Plant Physiol.*, **151**, 379–390.

Suggett, D. J., Oxborough, K., Baker, N. R. *et al.* (2003) Fast repetition rate and pulse amplitude modulation chlorophyll a fluorescence measurements for assessment of photosynthetic electron transport in marine phytoplankton. *Eur. J. Phycol.*, **38**, 371–384.

van Kooten, O. and Snel, J. F. H. (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth. Res.*, **25**, 147–150.

Wu, H., Cockshutt, A. M., McCarthy, A. *et al.* (2011) Distinctive Photosystem II photoinactivation and protein dynamics in marine diatoms. *Plant Physiol.*, **156**, 2184–2195.

Wu, H., Roy, S., Alami, M., Green, B. and Campbell, D. A. (2012) Photosystem II Photoinactivation, Repair and Protection in Marine Centric Diatoms. *Plant Physiol*, **160**, 464–476.