Photosynthesis Research (2019) 139:553–567
https://doi.org/10.1007/s11120-018-0526-8

ORIGINAL ARTICLE

Modulation in light utilization by a microalga *Asteracys* sp. under mixotrophic growth regimes

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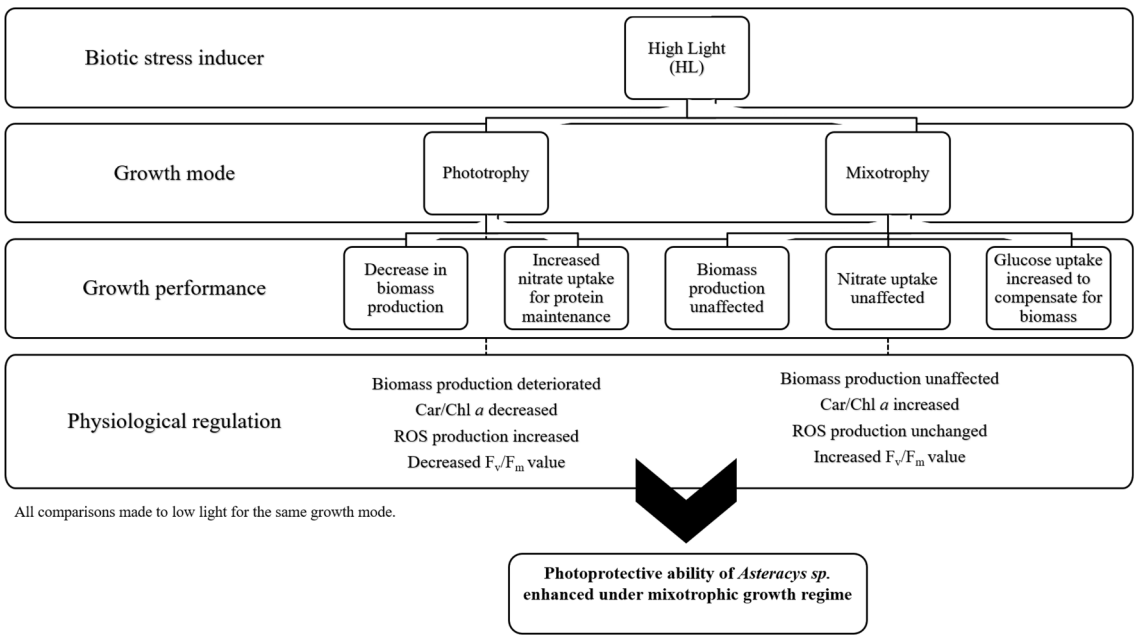
Received: 8 March 2018 / Accepted: 28 May 2018 / Published online: 2 June 2018

Abstract

This study is the first to explore the influence of incident light intensity on the photosynthetic responses under mixotrophic growth of microalga *Asteracys* sp. When grown mixotrophically, there was an enhanced regulation of non-photochemical quenching (NPQ) of the excited state of chlorophyll (Chl) *a* within the cells in response to white cool fluorescent high light (HL; 600 µmol photons m^{-2} s^{-1}). Simultaneous measurement of reactive oxygen species (ROS) production as malondialdehyde (MDA) and ascorbate peroxidase (APX), an ROS scavenger, showed improved management of stress within mixotrophic cells under HL. Despite the observed decrease in quantum yield of photosynthesis measured through the Chl *a* fluorescence transient, no reduction in biomass accumulation was observed under HL for mixotrophy. However, biomass loss owing to photoinhibition was observed in cells grown phototrophically under the same irradiance. The measurements of dark recovery of NPQ suggested that "state transitions" may be partly responsible for regulating overall photosynthesis in *Asteracys* sp. The partitioning of photochemical and non-photochemical processes to sustain HL stress was analysed. Collectively, this study proposes that mixotrophy using glucose leads to a change in the photosynthetic abilities of *Asteracys* sp. while enhancing the adaptability of the alga to high irradiances.

Graphical Abstract



Extended author information available on the last page of the article

Keywords Mixotrophy · Chlorophyll fluorescence · Photoinhibition · Light intensity · Microalgae

Abbreviations

APX	Ascorbate peroxidase
CEF	Cyclic electron flow
Car	Carotenoids
Chl	Chlorophyll
DCW	Dry cell weight
EOL	Enhancement effect of light
HL	High light
LL	Low light
MDA	Malondialdehyde
MHL	Mixotrophy under high light
MLL	Mixotrophy under low light
OD	Optical density
OJIP	Chl *a* fluorescence transient wherein O refers to the minimum fluorescence, J and I for inflections and P for peak
PHL	Phototrophy under high light
PLL	Phototrophy under low light
PS	Photosystem
ROS	Reactive oxygen species
WW	Wet weight

Introduction

Phototrophic organisms have optimized strategies to harvest sunlight for growth in varying environments. Despite a theoretical solar-to-biomass conversion efficiency of 9–10%, microalgal yields have attained a maximum conversion of 3% (Dubinsky et al. 1978; Ben-Amotz and Avron 1990; Melis 2009; Béchet et al. 2013) implying that a large proportion of the incident energy goes unutilized. In geographical regions exposed to high light (HL) intensities, not only the excess light energy is wasted, but also it causes damage within the algal cells. Under optimum light intensities, a linear electron flow (LEF) navigates electrons through the photosystems (PSI and PSII), thereby oxidizing water to oxygen and reducing NADP to NADPH, as well as synthesizing ATP for cell metabolic functions (Munekage et al. 2004; Blankenship 2014; Shevela et al. 2018). High irradiance saturates photosynthetic machinery and can cause photoinhibition of PSII leading to decreased quantum yield and photosynthetic rates (Kasajima et al. 2009; Zhao et al. 2017). More importantly, an excess of singlet excited state chlorophyll (Chl) is generated which reacts with oxygen to form reactive oxygen species (ROS) within the cells (Simionato et al. 2013). High levels of ROS generation are attributed to delayed PSII repair processes and therefore a detoxification of these ROS becomes crucial for cell survival (Takahashi and Badger 2011; Cruces et al. 2017). Hence, the production of ROS is counteracted through the production of ROS scavengers, which allows the maintenance of cellular homeostasis for successful photoprotection under HL (Zhao et al. 2017).

Non-photochemical quenching (NPQ) of the excited state of chlorophyll, which leads to harmless conversion of excitation energy into thermal energy, is another coping mechanism adopted by plants, algae and cyanobacteria (Kasajima et al. 2011; Demmig-Adams et al. 2014). The NPQ is known to increase the dissipation of excitation energy through non-radiative processes (heat, as implied above) within the pigment matrices (the antenna) of PSII, consequently decreasing the efficiency of energy transfer towards PSII photochemistry (Genty et al. 1989). Hence, to enable optimal photosynthesis under HL, the excitation energy absorbed by the photosystems requires careful regulation for subsequent biomass production.

In addition to its necessary role in photosynthesis, light also affects the chemical composition of algal cells through regulation of metabolic pathways (Norici et al. 2011; He et al. 2015b). The interdependence of photosynthesis on photorespiration, photoprotection, biomass generation and lipid accumulation has been widely studied (He et al. 2015a; Grama et al. 2016; Zhang et al. 2017). Photoinhibition, under photodamaging irradiance, has been addressed through various genetic modifications leading to reduction in the antenna size and pigment content (Lichtenthaler and Burkart 1999; Murchie et al. 2005; Zhou et al. 2015; Patil et al. 2017). In the current research, we are interested in investigating the inherent capacity of an oleaginous microalga, *Asteracys* sp., in order to be able to modulate its machinery to adapt to excessive incident light under mixotrophic growth. This microalga has been explored by researchers for its bioproduct formation and fatty acid composition (Sathya and Srisudha 2013; Kattarath and Ramani 2017). However, the photosynthetic capabilities of this microalga remain to be studied.

Mixotrophy has been explored, in this paper, to understand its effect, if any, on the overall photoprotective machinery within the cells. Mixotrophic growth involves an interdependent carbon metabolism, both photosynthetic and non-photosynthetic (Smith et al. 2015; Zhang et al. 2017). This metabolic flexibility makes the mixotrophic growth mode a dynamic system to study under variable environmental conditions. Further, mixotrophy is a well-established mode to improve algal feedstock. It enables biomass production through synergistic energy input from phototrophic growth and readily available carbon sources provided in the media (Baldisserotto et al. 2016; Subramanian et al. 2016; Sarnaik et al. 2017; Zhang et al. 2017). To further utilize this mode for feedstock production, several studies have explored the

uptake of industrial wastes as a carbon source for mixotrophy (Andrade and Costa 2007; Mitra et al. 2012). Through our study, we have probed, for the first time, the natural capacity of *Asteracys* sp. to enhance its photoprotective mechanisms through adoption of mixotrophic growth modes. We expect that a solution to HL stress through carbon uptake in mixotrophic microalga will provide a means of waste utilization along with biomass production towards industrial applications. Our study demonstrates the impact of light intensity on glucose uptake, thereby affecting the species response to HL. Despite the available knowledge on the influence of light on phototrophic growth (Schulze et al. 2014; Vadiveloo et al. 2017), studies pertaining to the effect of light on mixotrophy have remained elusive. With the emerging preference, at the present time, for mixotrophic growth mode and the known significance of light on algal physiology, it is now essential to understand the photo-physiology and growth responses of algae under the above-mentioned regime. In a scenario of enhanced photoprotection under mixotrophic mode, we expect to find altered ROS production, variations in NPQ mechanisms and subtle changes in overall cell physiology.

Thus, we began this study with the investigation of the influence of light intensity on *Asteracys* sp. growth under both phototrophic and mixotrophic modes. First, we carried out experiments to understand the enhancement effect of light (EOL) in mixotrophic batches through quantification of biomass and nutrient assimilation profiles. Then, to understand the extent of photodamage within cells, we measured ROS and scavenging enzymes. Further, to obtain detailed information on the various photosynthetic processes, we used Chl *a* fluorescence as a tool to monitor the overall response of the differently treated cells under incremental photosynthetically active radiations (PAR). In summary, the current work has explored the possibility of mixotrophic regimes under HL stress to reduce photodamage within the cells, thereby resulting in maximum biomass production. Our study is the first to demonstrate that mixotrophy using glucose alters the photosynthetic abilities of *Asteracys* sp. while enhancing the adaptability of the alga to high irradiances.

Materials and methods

Strain and culture conditions

The oleaginous microalga *Asteracys* sp. was a generous gift from the Institute of bioresources and sustainable development (IBSD), Manipur, India; it was cultured in modified BG-11 media (Andersen 2005) on a 12:12-h light:dark cycle at 25 °C. Cells were cultured in 250-ml Erlenmeyer flasks with continuous shaking and aseptic conditions and were illuminated with white cool fluorescent light [from 100 (LL) to 600 µmol photons m^{-2} s^{-1} (HL)]. The batch cultures grown phototrophically in LL and HL were named PLL and PHL, while the cultures grown mixotrophically were named MLL and MHL, respectively. For mixotrophic treatment, the culture was supplied with 25 mM glucose (previously optimized concentration for the growth of *Asteracys* sp.). The inoculum was acclimated to the light intensities and glucose by repeated passages until no significant difference in growth rate was observed in sequential cultures. After acclimation, exponentially growing cells were inoculated to obtain an optical density (OD) of ≈0.5 at 750 nm. Cell concentration within each flask was monitored by measuring ODs using a UV–Visible spectrophotometer (PerkinElmer, LAMBDA 35) after appropriate dilution to maintain the absorbance range of 0.05–0.9 (Ho et al. 2012). A heterotrophic treatment independent of light (HNL) was set up for comparison of biomass accumulation in the absence of light. At the end of the exponential phase of growth, dry cell weight (DCW) was obtained upon drying biomass for 4 days at 60 °C (Berteotti et al. 2016). The enhancement effect of light (EOL) was calculated as a modification to the approach adopted in previous studies (Dring and Lüning 1985b; Zhang et al. 2017). It was defined as the ratio between light-influenced mixotrophic DCW (DCW under mixotrophic growth minus DCW under heterotrophic growth) and the DCW accumulated under phototrophic growth at the respective light intensities (Eq. 1).

$$\mathrm{EOL(fold)} = \frac{(\text{DCW under mixotrophic growth}) - (\text{DCW under heterotrophic growth})}{(\text{DCW under phototrophic growth})} \quad (1)$$

Analysis of nutrient consumption

Residual nitrates from the media were analysed as described previously (Collos et al. 1999). Briefly, filtered liquid sample from each flask was taken on alternate days and diluted appropriately with deionized water to measure the OD at 220 nm. The concentration of residual nitrate in the medium was calculated in µg/ml. The amount of glucose remaining in the mixotrophic and heterotrophic media was measured using the Eco-Pak Glucose kit (*Accurex*) to quantify glucose uptake efficiency (Buch et al. 2010). The uptake of nutrients was calculated as shown in Eqs. 2 and 3:

$$\text{Nitrate uptake}(\%) = \left(\frac{N_{\text{initial}} - N_{\text{final}}}{N_{\text{initial}}}\right) \times 100 \quad (2)$$

$$\text{Glucose uptake}(\%) = \left(\frac{G_{\text{initial}} - G_{\text{final}}}{G_{\text{initial}}}\right) \times 100 \quad (3)$$

where $N_{initial}$ and N_{final} are the nitrate concentrations (µg/ml) on the initial and final days of growth, while $G_{initial}$ and G_{final} are the glucose concentrations (mg/ml) in the media on the initial and final days of growth, respectively.

Estimation of pigment content

Total chlorophyll (Chl) and total carotenoid (Car) contents were measured after the microalgal cells were pelleted down and desalted followed by pigment extraction using methanol. The pellets were incubated in the dark at 50 °C for 30 min followed by centrifugation at 10,000 rpm for 5 min. The ODs of the supernatant containing the extracted pigments were read at 652, 665 and 480 nm, using an UV–Visible spectrophotometer (PerkinElmer, LAMBDA 35). Calculations to quantify Chl *a, b* and carotenoid concentrations were made, as described previously (Lichtenthaler and Wellburn 1983). Quantification of carotenoids was carried out according to the protocol described by Graham and Bryant (2008). Filtered pigment extracts were analysed on an Agilent C1 RP column. Peaks were compared with standards as described by Sarnaik et al. (2017).

Detection of ROS

Algal cell pellets corresponding to 10^7 cells were used to quantify malondialdehyde (MDA) equivalents to signal oxidative damage in membranes (Salama and Pearce 1993; Wang et al. 2012; Ozkaleli and Erdem 2018). The molar wavelength-dependent extinction coefficient of 1.57×10^5 M^{-1} cm^{-1} was used to calculate the amount of MDA at 532 nm (Albro et al. 1986). Results are expressed in terms of nmol MDA/10^7 cells. Ascorbate peroxidase (APX; EC 1.11.1.11) was extracted in phosphate buffer and the enzyme activity was initiated with the addition of ascorbate and the monitoring of the decrease in absorbance at 290 nm for 90 s (Cruces et al. 2017). APX activities were calculated using an extinction coefficient for ascorbate of 2.8 mM^{-1} cm^{-1} (Nakano and Asada 1981). One unit of APX (U) was defined as the activity capable of oxidizing 1 nmol of ascorbic acid at 1 min and 25 °C (Bonnecarrère et al. 2011). Results are expressed as APX activity (U)/10^7 cells.

Measurements of Chl *a* fluorescence as a proxy for photosynthetic activity

Chl *a* fluorescence transient measurement

Chl *a* fluorescence signals were recorded at room temperature using the Dual-PAM-100 fluorometer (Heinz Walz, Effeltrich, Germany). Measurements were made at the end of the exponential phase of the growth of *Asteracys* sp. to understand the light stress response in both the phototrophic and the mixotrophic cells. In the OJIP transient, O denotes the minimum fluorescence measured on the first exposure of light and J and I denote two inflections, while P is the maximum fluorescence measured (for details, and relation to photosynthesis, see Stirbet and Govindjee 2011, 2012). In order to compare measurements made on different samples, all values of fluorescence were double normalized to obtain the relative variable Chl *a* fluorescence as described by Kalaji et al. (2014):

$$\text{Relative variable fluorescence } \left(V_t\right) = \frac{F_t - F_0}{F_m - F_0} \quad (4)$$

where fluorescence at time *t* is denoted as F_t. The fluorescence at 0.02 ms (20 µs) and maximum fluorescence are denoted as F_0 (the O level) and F_m (the P level), respectively. Three culture replicates of each treatment were used and three separate measurements per sample were used for obtaining the Chl *a* transients ($n = 9$). The maximum quantum efficiency of PSII photochemistry was inferred from the ratio F_v/F_m, where F_v is the variable fluorescence defined as the difference between the maximum (F_m) and minimum (F_0) fluorescence (Baker 2008; Zhou et al. 2015; for early work and assumptions, see Govindjee 1995, 2004).

Non-photochemical quenching (NPQ) of excited state of chlorophyll and partitioning of incident light

Cells of *Asteracys* sp. were dark adapted for 15 min, following which the F_0 measurements were made. A saturation light pulse (6000 µmol photons $m^{-2}s^{-1}$; 300 ms; $\lambda = 660$ nm) was used to determine the F_m value. After ~ 300 s, the actinic light was turned off to allow the quenching of excited chlorophyll wherein the saturating pulses were applied for another ~ 200 s to measure the dark recovery of NPQ. NPQ was calculated as described by Zhou et al. (2015). NPQ_{max} was calculated as the NPQ at the end of illumination period (~ 300 s) as described by Berteotti et al. (2016). The quantum yields of photochemical quenching, Y(II), non-photochemical quenching, Y(NPQ), and the energy dissipated as heat or fluorescence, Y(NO), were calculated from the fluorescence obtained at incremental PAR to estimate the partitioning of light energy of PLL, MLL, PHL and MHL cultures when exposed to high irradiances. For every result set, we had three biological samples ($n = 3$); in all cases, we had a Chl concentration of 40 µg ml^{-1} before the measurements.

Statistical analysis

Data visualization and statistical investigation were done using the Daniel's toolbox add-in for Excel (Kraus 2014). Means and standard deviations were calculated from at least

three independent samples per treatment ($n \geq 3$). A single-factor analysis of variance (ANOVA) was used to compare the data. Differences observed within the treatments were analysed using the Tukey's honest significant difference (HSD) at a significance level of 5%.

Results and discussion

Effect of light on growth and nutrient assimilation

The global average solar irradiances range from 450 to 1350 µmol photons m^{-2} s^{-1} (lighting energy conversion 2014; Meteonorm 2016). Utilization of these irradiances for algal cultivation is a sustainable and cost-efficient method of biomass generation. Hence, studies pertaining to successful cultivation of microalga under HL were incorporated in the current study. Phototrophic cultivation of *Asteracys* sp. under HL produced striking variations in biomass production when compared to LL (Fig. 1a). The DCW for phototrophic growth under HL (1340 ± 9.89 mg L^{-1}) was significantly ($p < 0.05$) reduced as compared to LL (1600 ± 35.5 mg L^{-1}). These observations are in accordance to studies with *Chlamydomonas reinhardtii* wherein a decrease in biomass was observed with increasing light intensities, from 200 to 800 µmol photons m^{-2} s^{-1} (Berteotti et al. 2016). While phototrophic growth was significantly affected by light (16.25% decrease in HL), mixotrophic growth did not display attenuation of biomass under HL (2620 ± 4.95 mg L^{-1}) when compared to LL (2580 ± 14.85 mg L^{-1}). Additionally, cell numbers were compared for all samples (data not shown) and a similar conclusion was drawn pertaining to mixotrophic responses to light variation. Mixotrophy is not entirely dependent on light for biomass generation; it represents a fine synergy between organic and inorganic carbon assimilation within the cells (Chen et al. 2011). This could be the reason for MHL not displaying a decrease in biomass as seen in PHL (Fig. 1a).

The growth profiles of *Asteracys* cultures, obtained under varying light intensities, were further probed for the EOL, for which a heterotrophic treatment (HNL) was set up as a glucose control. In view of the well-known phenomenon of the "Emerson enhancement effect" that led to the concept of two light reactions and two photosystems (Rabinowitch and Govindjee 1969; Blankenship 2014; Dring and Lüning 1985a, b), the term enhancement effect of light or EOL in our study was defined as "the ratio of light driven biomass accumulation in mixotrophy over that under phototrophy". While the EOL under LL was 0.44-fold, it was 0.55-fold under HL, which is significantly higher ($p < 0.05$). Further, the results presented in our paper here, using *Asteracys* sp., clearly show that mixotrophic growth has an advantage in terms of biomass maintenance over its phototrophic counterpart, when grown under HL.

Analysis of residual nutrients in media showed that the uptake of glucose under mixotrophy was significantly higher under HL ($68 \pm 1.92\%$) as compared to that in LL ($40 \pm 2.01\%$) as shown in Fig. 1b. Thus, it is evident that mixotrophic growth is capable of maintaining its biomass production under HL through the regulation of glucose

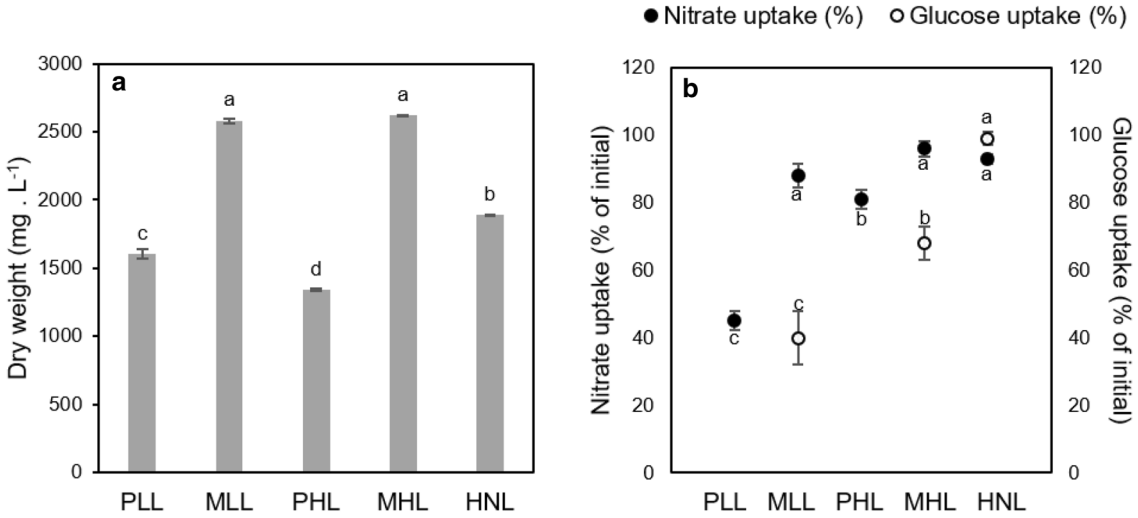


Fig. 1 Dry weight of biomass generated (**a**; left panel) and the assimilation of nutrients from the media (**b**; right panel) after the exponential growth phase under low and high light. In the right panel, nitrate uptake is indicated with filled circles and glucose uptake with open circles. Nitrate and glucose uptake was calculated as described in Eqs. 2 and 3. Black vertical bars represent the SD ($n = 3$). Letters *a, b, c* and *d* denote the significant difference between the treatments ($p < 0.05$, Tukey's HSD). *PLL* phototrophic growth under low light, *MLL* mixotrophic growth under low light, *PHL* phototrophic growth under high light, *MHL* mixotrophic growth under high light, *HNL* heterotrophic growth

uptake. Further, we probed the influence of light on nitrate uptake under mixotrophy. As seen in our experiments, nitrate uptake under mixotrophy was not influenced by light (Fig. 1b). Turpin et al. (1988) had established that nitrate assimilation is regulated through the tricarboxylic acid (TCA) cycle and that the glucose uptake is regulated by the mitochondrial electron transport chain, both of which are independent of each other. This explains why despite the increase in glucose assimilation under HL, no effect on nitrate uptake was observed in our experiments. In contrast to cells grown under mixotrophy, phototrophically grown cultures showed a significant variation ($p < 0.05$) in nitrate uptake from the media under LL (PLL) and HL (PHL). PHL cultures showed a 43% increase in its uptake as compared to the PLL cultures (see Fig. 1b). However, this increase was not reflected in the biomass generated by PHL as shown in Fig. 1a. Usually, nitrate uptake by the microalga is directed towards repair and maintenance of the protein machinery under HL stress (Greenberg et al. 1987). We note that biomass production in PHL was much less than that in PLL (16.25% decrease in HL); thus, HL may have induced an upsurge in nitrate uptake under phototrophic growth for the repair and maintenance of photodamaged proteins. Our observations on nitrate and glucose uptake profiles (see Fig. 1b) suggest a significant impact of light on the assimilation of these nutrients. While glucose assimilation is clearly enhanced under HL, uptake of nitrate is only influenced by HL under phototrophy. To our knowledge, this is the first study on the role of light intensity over physiological changes under mixotrophic growth. An understanding of this result requires a detailed probing of stress proteins to know the extent of stress which then needs to be related to the nitrate utilization by the cells. To understand the variation in glucose uptake by the cells, as seen in the current study (see Fig. 1b), our future plans are to measure the quantity of hexose transporters under LL and HL. It is obvious from our results on growth that the mixotrophic behaviour of *Asteracys* sp. is greatly influenced by the light intensity the culture is exposed to (see Fig. 1). However, this phenomenon could be specific to *Asteracys* sp. and requires to be established individually for each algal species.

Effect of light on photosynthetic pigments and ROS scavenging activity

Light intensities are known to profoundly affect the photosynthetic pigments (Pfündel et al. 2018) and hence we further investigated the influence of light on the concentrations of Chl and carotenoids. In our study, an increase in light intensity led to an elevated content of Chl *a* in phototrophic cells at the end of exponential growth (see Table 1; compare data on PHL with PLL cells). PHL contained 29.29 ± 0.820 µg mg^{-1} DCW of Chl *a*, while PLL had a much lower content (11.02 ± 0.256 µg mg^{-1} DCW). In comparison with phototrophically grown cells (PHL), mixotrophic cells (MHL) had a Chl *a* content of 16.54 ± 0.017 µg mg^{-1} DCW, 43.5% lower than PHL. The presence of external carbon sources has been observed to decrease the content of photosynthetic pigments (Cheirsilp and Torpee 2012). In agreement with earlier studies on mixotrophic cultures of *Scenedesmus obliquus* and *Dactylococcus* sp. (Yang et al. 2014; Grama et al. 2016), the Chl *a* production in MHL was significantly lower than that in PHL (Table 1) despite the high biomass production attained under this growth mode (Fig. 1a).

Similar to the Chl content, the carotenoid content was also higher in PHL cells over PLL cells; it was more than two times higher in PHL cells. As seen under phototrophy, a significant increase in carotenoid content was also observed under the mixotrophic growth mode (Table 1). Zhekisheva et al. (2002) and Solovchenko et al. (2008) have reported that an increase in carotenoids under high irradiances is mainly to help in the protection of PSII against photoinhibition (Frank et al. 1999). Overall, under phototrophic growth, an increase in Chl *a* content along with increased carotenoid content was seen under high irradiance (PHL). In contrast, mixotrophically grown cells had decreased Chl *a* content possibly to truncate the light harvesting when exposed to

Table 1 Effect of light on chlorophyll and carotenoid contents (in µg pigment mg^{-1} DCW) of *Asteracys* cells under phototrophic and mixotrophic growth

	PLL	MLL	PHL	MHL
Chl *a*	11.01 ± 0.256d	20.03 ± 1.093b	29.29 ± 0.820a	16.54 ± 0.016c
Chl *b*	15.16 ± 0.468d	27.97 ± 1.422b	39.19 ± 0.100a	22.65 ± 0.297c
Total Car	2.11 ± 0.018d	3.26 ± 0.027b	5.10 ± 0.011a	3.67 ± 0.013c
Lutein	0.29 ± 0.018c	0.47 ± 0.057b	0.29 ± 0.079c	1.22 ± 0.093a
β-carotene	ND	0.10 ± 0.009b	0.02 ± 0.002c	0.29 ± 0.025a
Car/Chl *a*	0.19 ± 0.002b	0.16 ± 0.004d	0.17 ± 0.001c	0.22 ± 0.008a

Chlorophyll (Chl) and carotenoid (Car) contents were determined at the end of exponential growth phase (average values ± SD for three independent replicates). Letters a, b, c and d denote the significant difference between treatments per row ($p < 0.05$, Tukey's HSD)

ND not detected, *PLL* phototrophic growth under low light, *MLL* mixotrophic growth under low light, *PHL* phototrophic growth under high light, *MHL* mixotrophic growth under high light, *DCW* dry cell weight

higher irradiance (Table 1). When we calculated the ratios of Car/Chl *a* for phototrophically grown cells, we observed a decrease from 0.19 ± 0.002 to 0.17 ± 0.001 from LL to HL ($p < 0.05$). Interestingly, in mixotrophically grown cells, while MLL displayed a Car/Chl *a* value of 0.16 ± 0.004, MHL displayed a 37.5% increased value of 0.22 ± 0.008 (Table 1). The low Chl *a* content in MHL can also be attributed to the higher biomass production under this growth mode as compared to PHL (Fig. 1a), thus indicating that mixotrophic growth was not affected by light intensity. Carotenoid content is reported to increase to protect the photosynthetic machinery from photoinhibition induced by high irradiances (Zhekisheva et al. 2002; Solovchenko et al. 2008; Singh et al. 2010). He et al. (2015a) have reported an overall increase in the ratio of Car/Chl *a* in *Chlorella* sp. and *Monoraphidium* sp. indicating a downregulation of the photosynthetic activity and an upregulation of the photoprotective mechanisms. This increase in Car/Chl *a* value is seen in our study with MHL cells. Our results pertaining to PHL are in agreement with an earlier theory by Murchie et al. (2005) that leaves may sometimes have higher light harvesting capacity (indicated through high Chl *a*) but low photosynthetic and photoprotective capabilities (see Chl *a* and Car values in Table 1). A detailed estimate of individual carotenoids produced under each treatment of our study revealed significantly higher amount of photoprotective pigments in MHL as compared to PHL (Table 1). Lutein content in MHL was fourfold higher (1.22 ± 0.093 µg mg^{-1} DCW) as compared to PHL (0.29 ± 0.079 µg mg^{-1} DCW), while β-carotene content was tenfold higher (0.29 ± 0.025 µg mg^{-1} DCW in MHL and 0.02 ± 0.002 µg mg^{-1} DCW in PHL). Wei et al. (2008) have reported an increase in lutein yield in heterotrophically grown *Chlorella* sp. through induction with hydrogen peroxide and sodium hypochlorite (ROS). An increase in ROS induces the cells to generate antioxidant pigments to prevent cell damage. However, the study also reports a decrease in lutein content at very high levels of ROS (Wei et al. 2008). Our findings of the low lutein content in PHL and high lutein in MHL are in tune with the findings of this study. In the current study, PHL displayed very high ROS as shown in Fig. 2a. This accumulation of ROS in PHL may have resulted in a decrease in lutein content despite the overall increase in carotenoids in the cells (see Fig. 2a; Table 1). Further, the lower content of β-carotene in PHL when compared to MHL agrees with the finding of Mojaat et al. (2008). Their study reports an influence of organic carbon sources on carotenoid ratios and increased β-carotene contents in photoheterotrophy when compared to the autotrophic counterparts. However, the mechanism of this influence remains to be studied through labelling of molecules to understand the pathways and assimilation mechanism. Demmig-Adams (1990) and Johnson et al. (2008) have reported the vital roles of lutein and β-carotene in regulating the switch between light harvesting and photoprotective modes of the photosynthetic machinery. Our results (as shown in Table 1) thus display an apparent upregulation in photoprotective mechanisms under mixotrophy when *Asteracys* sp. was subjected to HL stress.

To quantitatively analyse this photoprotective ability, the lipid peroxidation (measured in terms of MDA) and the antioxidant ability of the cells (measured as APX activity U per 10^7 cells) was evaluated (Fig. 2a, b). Under LL, the MDA

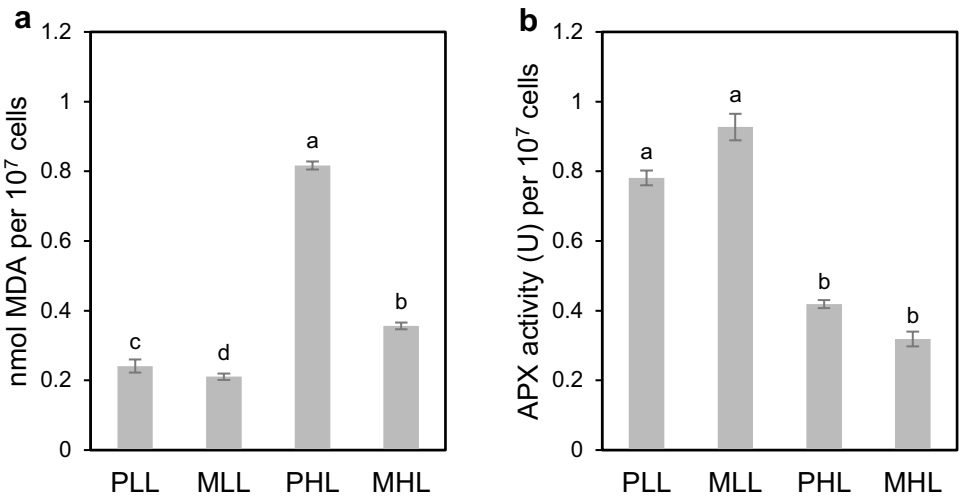


Fig. 2 Changes in the content of malondialdehyde (MDA) concentration (**a**) and the difference in antioxidant enzyme activity ascorbate peroxidase (APX) (**b**) in the cells of *Asteracys* under low and high light. Vertical bars represent the SD ($n = 3$). Letters *a, b, c* and *d* denote the significant difference between the treatments ($p < 0.05$, Tukey's HSD). *PLL* phototrophic growth under low light, *MLL* mixotrophic growth under low light, *PHL* phototrophic growth under high light, *MHL* mixotrophic growth under high light. MDA and APX concentrations were calculated as described by Wang et al. (2012)

production ranged from 0.24 ± 0.019 nmol per 10^7 cells in phototrophy to 0.21 ± 0.009 nmol per 10^7 cells in mixotrophy ($p < 0.05$). An increase in ROS levels, under abiotic as well as biotic stress, has been reported by several researchers (Apel and Hirt 2004; Suzuki et al. 2012; Cruces et al. 2017; Zhao et al. 2017); these redox species are known to contribute towards the damage of cell organelles and proteins under stress conditions. Our results showed an increase in MDA production under phototrophic growth (0.82 ± 0.012 nmol per 10^7 cells) in PHL. However, MHL produced significantly lower amounts of MDA (0.36 ± 0.009 nmol per 10^7 cells), when compared to PHL, indicating lower chances of cell damage under HL. While the natural response of *Asteracys* sp. towards HL was an increase in ROS accumulation in PHL cultures, it is evident through the MDA levels (Fig. 2a) that a modulation in this natural response was seen under mixotrophy.

A high degree of high-light-induced damage is caused by the production of free radicals in the chloroplast and the mitochondria and hence requires efficient ROS scavenging mechanisms. This defence and regulatory system is highly dynamic and balances the levels of free radicals within the cells (Singh et al. 2010; Sharma et al. 2012; Gwak et al. 2014). In our experiments, PLL- and MLL-grown *Asteracys* cells demonstrated a similar scavenging capability measured in terms of APX activity by the cells (Fig. 2b). APX activity under LL was limited to 0.78 ± 0.021 U per 10^7 cells (PLL) to 0.927 ± 0.038 U per 10^7 cells (MLL). Under HL, APX activity was similar with PHL producing 0.42 ± 0.012 U per 10^7 cells and MHL producing 0.32 ± 0.021 U per 10^7 cells. Despite the increased production of free radicals under PHL, an inability to naturally scavenge the excess free radicals was observed in these cells. Our results on PHL demonstrate an increase in both Chl *a* and lipid peroxidation (measured as MDA) by over 60% as compared to PLL production. These findings agree with those of Zhao et al. (2017) who reported a 20% increase in MDA levels in their high-chlorophyll content genotype of rice. Further, Zhao et al. (2017) reported an inability for scavenging the increased ROS which is the case seen in our results with *Asteracys* cells. Thus, it seems to us that the pigment content may play a crucial role in our understanding of the mitigation of HL stress in *Asteracys* sp. He et al. (2015a) have reported that β-carotene can act as a molecule that assists in scavenging ROS; our results on MHL cells display a significantly higher generation of this pigment (Table 1). Hence, in addition to the lower lipid peroxidation seen by MHL cells, there is an additional HL stress management through the regulation of pigment content under mixotrophy (Table 1). Our study shows the naturally enhanced capability of *Asteracys* sp. to photoprotect its cells (as shown in Fig. 2a, b; Table 1) under a mixotrophic growth mode.

Effect of light on photosynthetic activity

In photosynthetic organisms, ROS are constantly formed by the leakage of electrons to the molecular oxygen from the electron transport within the chloroplast and mitochondria (Foyer et al. 1997; Asada 1999; Deblois et al. 2013; Rochaix 2016). Under high light, the removal of hydrogen peroxide by the scavenging peroxidases is essential within the chloroplast to avoid the inhibition of the Calvin–Benson cycle enzymes (Tanaka et al. 1982). The pathways regulating ROS production are regulated by NPQ of the excited state of chlorophyll within the thylakoid membranes to alleviate photodamage under stress conditions (Demmig-Adams et al. 2014; Zhao et al. 2017). Hence, a variation in ROS production or scavenging is expected to affect the photosynthetic machinery of the cells.

Non-photochemical quenching as an indicator of photoprotection ability

A major regulator of over-excitation is a set of inducible mechanisms referred to as NPQ that induce lumen acidification under excess light thereby triggering qE, the most rapid component of NPQ (Walters and Horton 1991; Demmig-Adams et al. 2014). This involves excitation energy transfer to zeaxanthin or lutein, two pigments crucial to NPQ (Dominici et al. 2002; Wilk et al. 2013); the excess energy is lost as heat. However, in our experiments, despite the increased lutein content under MHL, the NPQ recovery curves did not show any significant variation through lumen acidification (Rohacek et al. 2014). As shown in Fig. 3a, all dark recovery curves of NPQ were characterized by a fast rise followed by no change within ~ 100 s of light exposure. The NPQ_{max}, the maximum level of NPQ reached after ~ 300 s of light treatment was compared as described by Berteotti et al. (2016). Even though the trend of NPQ recovery was similar in all samples, NPQ_{max} in MHL was higher than that in PHL by 18% (Fig. 3b). High NPQ capacity is known to decrease the excitation pressure on PSII under excess light by increased heat loss, and thus reduction in energy funnelled towards photochemistry (Deblois et al. 2013; Demmig-Adams et al. 2014). This mechanism also minimizes ROS formation (MacIntyre et al. 2002), observed in our study for MHL cells (Fig. 2a). Moreover, the NPQ continued to rise in all the samples even after the actinic light was turned off at ~ 300 s. The continued rise in NPQ after 10 min of dark recovery suggests the induction of other components of NPQ, namely, qT (state I to state II) and qI (photoinhibition), which require longer time to relax, as has been described in other microalgae and dinoflagellates (Allorent et al. 2013; Rochaix 2016; Ruban

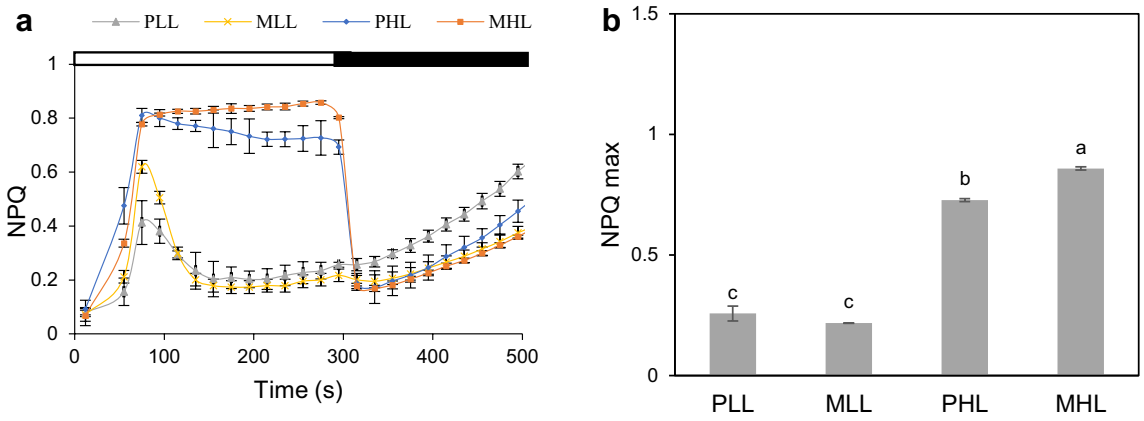


Fig. 3 Non-photochemical quenching (NPQ) kinetics of phototrophic and mixotrophic cells grown for several generations in low and high light (**a**; left panel) and the NPQ_{max} measured at the end of the illumination period (**b**; right panel). The white bar (in the left panel) indicates the illumination period, while the black bar indicates the dark period. Data reported are the mean of three independent replicates for each treatment ($n = 3$) with the vertical bars indicating the SD. Letters *a, b* and *c* in the right panel denote the significant difference between treatments ($p < 0.05$, Tukey's HSD). *PLL* phototrophic growth under low light, *MLL* mixotrophic growth under low light, *PHL* phototrophic growth under high light, *MHL* mixotrophic growth under high light. NPQ was calculated as described by Zhou et al. (2015). NPQ_{max} was calculated as described by Berteotti et al. (2016)

2016; Cui et al. 2017). In mixotrophic cells, along with the variations in the photoprotective pigments and ROS generation, we have observed higher NPQ ability.

Chl *a* fluorescence transient

A comparison of Chl *a* fluorescence transients provides a rapid, accurate and non-invasive way to decipher variations in photosynthetic activities (Govindjee et al. 1986; Govindjee 2004; Baker 2008; Kalaji et al. 2018). The JIP test is extensively used to understand the dynamics of the Chl fluorescence signals during the light-phase reactions of photosynthesis (Strasser et al. 2000, 2004). In dark-adapted photosynthetic samples, the first plastoquinone electron acceptor of PSII (Q_A) is fully oxidized at F_0 (the O level). The photochemical phase wherein Q_A is reduced to Q_A^- corresponds to the O–J fluorescence rise. The phase involving the reduction of the PQ pool and the electron acceptor side of PSI is represented by J–I and I–P (Munday Jr and Govindjee 1969; Stirbet and Govindjee 2011; Schansker et al. 2014; Kodru et al. 2015). Under saturating light, all electron carriers between the PSII reaction centres and NADP are reduced at the P step. In this paper, we have characterized the PSII performance of the microalga grown in PLL, MLL, PHL and MHL based on their OJIP curve responses.

When grown under LL, both phototrophically and mixotrophically grown cells display three characteristic steps in their fluorescence transient: O–J, J–I and I–P (Fig. 4a). Despite the similar transient curves under LL, differences were observed between MLL and PLL samples, the former showing a higher I level. Usually, the J–I phase is correlated with the PQ pool's redox status wherein electrons are shuttled between PSII and PSI through the cytochrome b6/f complex (Kirchhoff et al. 2000; Stirbet and Govindjee 2012). The rise in I level suggests a slower rate of plastoquinol (PQH_2) oxidation by PSI which could be due to decreased PSI/PSII ratio (Ceppi et al. 2012; Stirbet et al. 2014).

We speculate that mixotrophic cells may even have a differently regulated electron flow between the two photosystems, which requires further investigation under varying light intensities. However, it can be observed from Table 2 that the maximum fluorescence (F_m) was relatively similar in PLL (0.224 ± 0.027) and MLL (0.204 ± 0.012). A higher F_v/F_m ratio seen in PLL (+61.4%) over that on MLL would be usually interpreted to mean higher photosynthetic performance (Zhou et al. 2015). We note that mixotrophy in general would lead to a pool of reducing powers through photochemistry in addition to the reducing powers generated through respiratory routes leading to higher growth rates. Further, it can be observed from Fig. 4a, b that the cells exposed to HL displayed OJIP transients with much less pronounced steps with delayed J and I steps, a typical response seen in stress-treated cells of other organisms (see, e.g., Strasser et al. 2004).

While it is evident from Table 2 that HL in phototrophic growth, as compared to LL, decreased the F_m, F_v/F_m and F_v/F_0 values, mixotrophic growth did not show much variation in these parameters. The decrease in these parameters is usually correlated with photo-inhibition and lowered PSII excitation cross section (Strasser et al. 2000; Zhou et al. 2015). Further, PHL cells exhibited lower F_v/F_m (−68.8%) and F_v/F_0 values (−79.5%) when compared with MHL cells. Our results on Chl *a* fluorescence transients and calculations of parameters, which relate to photosynthesis, clearly

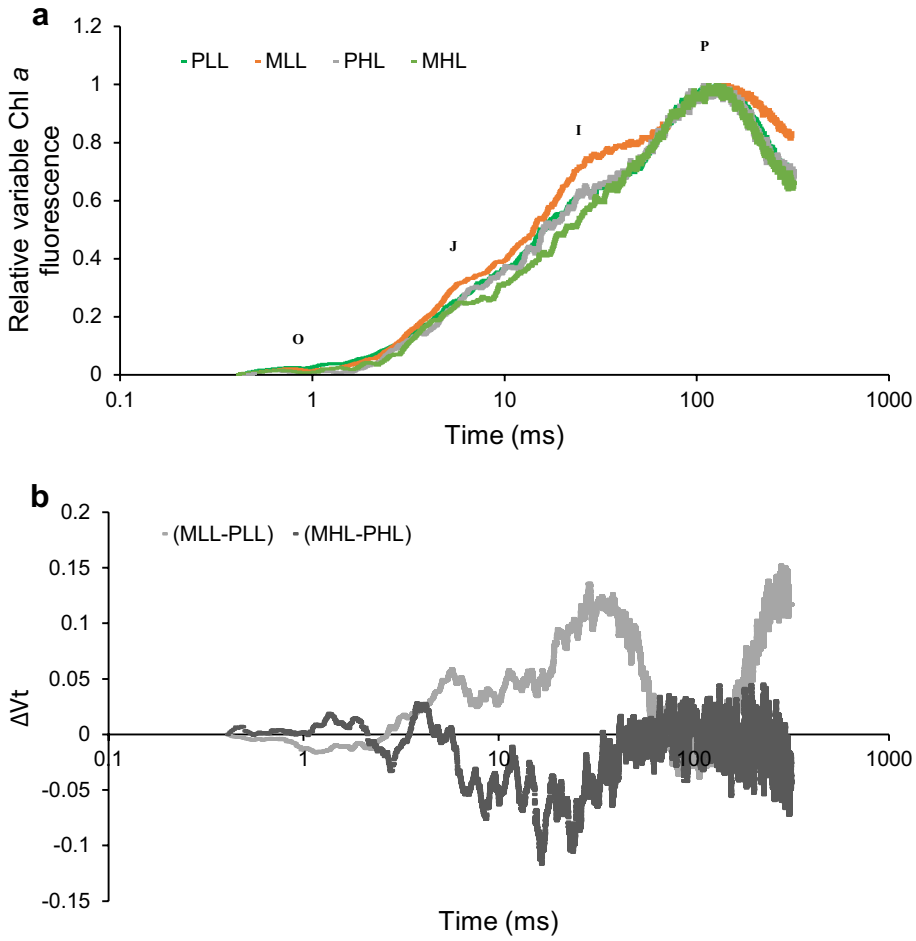


Fig. 4 Chl *a* fluorescence transient (up to 1 s) in phototrophic and mixotrophic growth under low and high light (**a**; top panel). Data are double normalized as described in "Materials and methods" and the transient is plotted on the logarithmic time scale range between 0 and 1 (= 1000 ms). Data points are an average of nine independent measurements. Difference curves of fluorescence for (MLL–PLL) [dark grey] and (MHL–PHL) [light grey] are plotted in the bottom panel (**b**). In the top panel, O refers to the minimal fluorescence and P to the maximum fluorescence, with J and I being the inflections between the two extremes. *PLL* phototrophic growth under low light, *MLL* mixotrophic growth under low light, *PHL* phototrophic growth under high light, *MHL* mixotrophic growth under high light

Table 2 Comparison of fluorescence parameters in *Asteracys* cells grown under low and high light

	PLL	MLL	PHL	MHL
F_m*	0.224 ± 0.027a	0.204 ± 0.012a	0.150 ± 0.018b	0.197 ± 0.019a
F_v/F_0*	1.752 ± 0.072a	0.652 ± 0.073c	0.153 ± 0.061d	0.746 ± 0.055b
F_v/F_m*	0.636 ± 0.081a	0.394 ± 0.058b	0.133 ± 0.077c	0.427 ± 0.061b

Values determined at the end of exponential growth phase (average values ± SD for three independent replicates). Letters a, b, c and d denote the significant difference between the treatments in rows ($p < 0.05$, Tukey's HSD)

PLL phototrophic growth under low light, *MLL* mixotrophic growth under low light, *PHL* phototrophic growth under high light, *MHL* mixotrophic growth under high light

*See list of abbreviations and the "Materials and methods" section

suggest that *Asteracys* cells undergo different physiological changes under phototrophy and mixotrophy and that the mixotrophic, as compared to phototrophic, growth has advantages under HL conditions.

Partitioning of incident light energy

Although OJIP measurements have provided important information on *Asteracys* cells, light intensity dependence of several parameters is a powerful means of understanding the overall flow of electrons within the cells. Although the quantum yields of photochemical (Y(II)) and non-photochemical (Y(NPQ)) quenching have been extensively used and discussed in the literature, insufficient information exists on the significance of Y(NO), which reflects the energy that is passively dissipated in the form of both heat and fluorescence when the PSII reaction centres are closed. This quantum yield demonstrates the partitioning of energy in PSII and allows for a deeper understanding of the organism's capacity to cope with excess light (Klughammer and Schreiber 2008a). Thus, a detailed analysis of the quenching of excess energy enables us to resolve the excitation energy

flux into the light-induced and non-light-induced quenching processes (Hendrickson et al. 2004). With the above understanding, we probed the capacity of phototrophically and mixotrophically grown cells to cope with high irradiances (see Fig. 5). Our analysis involved the resolution of the energy fluxes into the quantum yields of photochemical quenching, Y(II), non-photochemical quenching involving the light-induced process, Y(NPQ), and the non-light-induced energy dissipated as heat or fluorescence, Y(NO) at varying PAR (0–2000 µmol photons m^{-2} s^{-1}) as shown in Fig. 5.

We base our analysis on the partitioning of light when cells were subjected to the highest PAR (~ 1980 µmol photons m^{-2} s^{-1}) and comparison of these results with their predicted behaviour under high irradiance. The quantum yield of PSII, Y(II), was significantly affected in PHL- and MHL-grown cells ($p < 0.05$), while no apparent effect was observed in LL-adapted cells (i.e., PLL and MLL). Absorption of light, under phototrophy, is higher than in cultures grown with glucose, since the former have higher Chl *a* content than the latter (Yang et al. 2000; also shown in Table 1). Liu et al. (2009) have further shown that mixotrophy changes the chloroplast ultrastructure, in *Phaeodactylum tricornutum*, in a manner that reduces light absorption by PSII, reducing the photosynthetic efficiency. Hence, we suggest that a further investigation of changes in chloroplast ultrastructure for MLL and MHL may provide further information on *Asteracys* cells, used in our research. Our Y(II) values for PHL suggest that *Asteracys* sp. is capable of adaptation under higher irradiances as reported in *Chlorella sorokiniana* (De-Bashan et al. 2008) and various benthic marine microalgae (King and Schramm 1976). However, this adaptation leads to lower microalgal biomass generation (Berteotti et al. 2016; shown in Fig. 1a) and hence any comment on phototrophic adaptation of *Asteracys* sp. to HL seems unsuitable at this time. Mixotrophy is a combination of carbon uptake through photosynthesis and glycolytic pathways (Smith et al. 2015; Zhang et al. 2017) and thus the Y(II) values cannot solely indicate biomass production tendency under this regime.

It can be observed from Fig. 5 that energy dissipation through Y(NO) is significantly different ($p < 0.05$) among PLL, PHL, MLL and MHL cells. PLL demonstrated high values of Y(NO) when subjected to ~ 1980 µmol photons m^{-2} s^{-1} (Fig. 5a), while significantly lower values were seen in MLL (Fig. 5b). This observation indicates a modulation within mixotrophic cells to function 'better' than phototrophic cells during a high-light exposure. Furthermore, as opposed to PLL cells, MLL cells are able to safely dissipate excess energy as attested by high values of Y(NPQ) (Lazár 2015). Y(NO) is often described as a simple indicator of the reduction state of plastoquinones within the photosynthetic membranes (Grieco et al. 2012). Under exposure to high irradiances, plants and algae are known to minimize

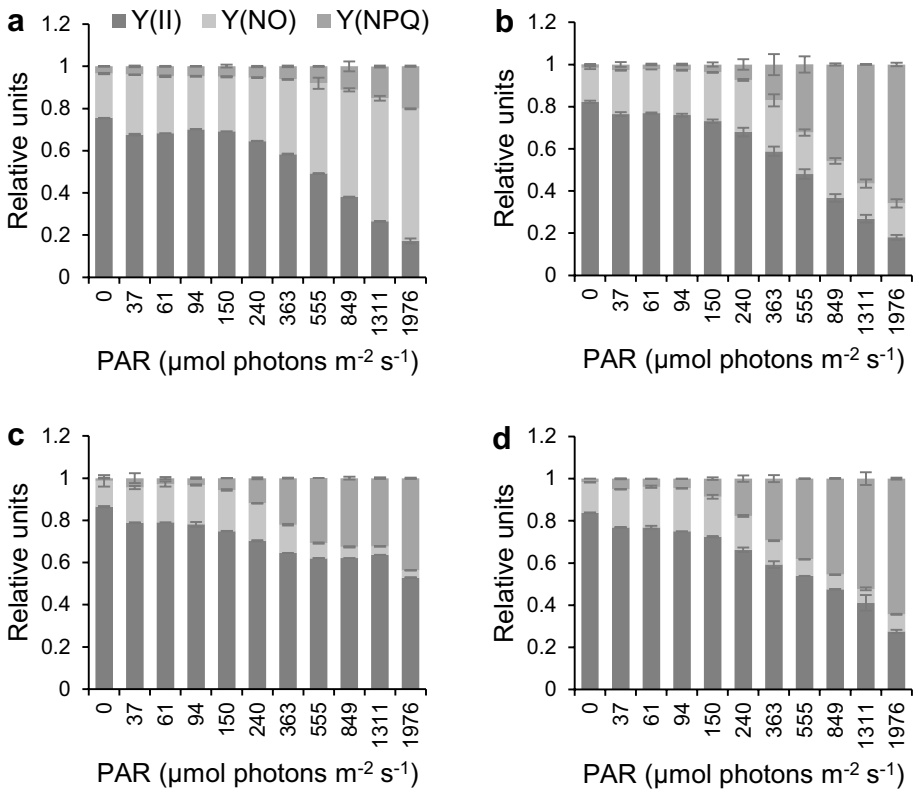


Fig. 5 Quantum yields of photochemical quenching (Y(II) represented in dark grey bars), non-photochemical quenching (Y(NPQ) represented in grey bars) and the non-light-induced energy dissipated as heat or fluorescence (Y(NO) represented in light grey bars) in PLL (**a**; top left panel), MLL (**b**; top right panel), PHL (**c**; bottom left panel) and MHL (**d**; bottom right panel). Data reported are the mean of three independent replicates for each treatment ($n = 3$) with the vertical bars indicating the SD. *PLL* phototrophic growth under low light, *MLL* mixotrophic growth under low light, *PHL* phototrophic growth under high light, *MHL* mixotrophic growth under high light

the reduction state of these plastoquinone (Klughammer and Schreiber 2008b). From this standpoint, mixotrophic cells successfully regulated the photosynthetic electron transport chain when compared to phototrophic cells.

As shown in Fig. 5, the extent of light-induced NPQ under high PAR was similar in both mixotrophy-adapted cells of MLL and MHL (~0.65), while a reduction was observed in PHL (0.44 ± 0.003) and PLL (0.205 ± 0.005). The activation of energy dissipation as Y(NPQ) in phototrophy (Fig. 5c) may be a survival strategy that results in a reduction in biomass under HL (*cf.* Berteotti et al. 2016). In agreement with observations made by Berteotti et al. (2016) for *Chlamydomonas reinhardtii*, our study on *Asteracys* sp. likewise suggests preference for photoprotection over productivity, when grown in HL (PHL). NPQ had been identified to have a close association with the generation and scavenging of ROS within cells (Munekage et al. 2004). Our research on *Asteracys* sp., presented in this paper, fully supports this concept. In our work, higher NPQ activation ability in MHL (Fig. 3b) resulted in low ROS production under HL (Fig. 2a). Additionally, the Car/Chl *a* ratio of MHL was indeed higher (Table 1), thereby suggesting an enhanced photoprotective capability. Hence, despite the reduction in photosynthetic efficiency, the addition of glucose in our study may have resulted in increased carbon fixation through "pressure" exerted by respiration as observed by Grama et al. (2016) (seen in our study as a reduction in Y(II) for MHL, compare Fig. 5c, d; and higher biomass for MHL as compared to PHL in Fig. 1a). Taking all our results together, we conclude that mixotrophy, using glucose, leads to enhanced biomass accumulation through increased tolerance of *Asteracys* sp. to high irradiance.

Concluding remarks

The role of light intensity in mixotrophy in the oleaginous microalga *Asteracys* sp. has been probed here under two irradiances: LL (100 µmol photons m^{-2} s^{-1}) and HL (600 µmol photons m^{-2} s^{-1}). Biomass generation and nutrient assimilation abilities of this microalga improved significantly under mixotrophy in comparison to its phototrophic counterpart (cf. Fig. 1). While phototrophic cells upregulated ROS generation under HL thereby leading to lower biomass production, the same was not true for mixotrophic growth. Despite the upregulation in photoprotective mechanisms under mixotrophy, no compromise on biomass was observed, perhaps due to the compensation of biomass through glucose assimilation. Further, an improved photoprotection efficiency (cf. in terms of MDA production Fig. 2a; pigments produced in Table 1; fluorescence parameters in Table 2 and Y(NPQ) in Fig. 5b, d) was observed under mixotrophy with regulated non-photochemical quenching of excess energy. This phenomenon was evidenced by the ROS production and scavenging capabilities of cells under HL. This feature of mixotrophy provides an advantageous tweak towards biomass generation wherein harsh natural light may be experienced due to topographical and seasonal changes. Additionally, our study provides a probable solution to the persistent impact of photoinhibition towards the decrease in biomass productivity for photosynthetic algal (or cyanobacterial) species under large-scale cultivation. Studies pertaining to the metabolic fluxes and the metabolites involved in this fascinating interdependent phenomenon of photosynthesis and carbon assimilation remain elusive and require further investigation.

Acknowledgements This research has been supported by the Department of Biotechnology, Ministry of Science and Technology, Govt. of India and University Grants Commission (UGC), India. The authors are grateful to Govindjee for his valuable comments that helped to improve the manuscript.

Author contributions The study was conceived and designed by AA, RP and AML. Experiments were performed by AA and KG. The data were analysed and interpreted by AA, KG and RP. Drafting and critical revision of manuscript were done by AA and RP. SP provided technical knowledge.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Albro PW, Corbett JT, Schroeder JL (1986) Application of the thiobarbiturate assay to the measurement of lipid peroxidation products in microsomes. J Biochem Biophys Methods 13:185–194. https://doi.org/10.1016/0165-022X(86)90092-8

Allorent G, Tokutsu R, Roach T et al (2013) A dual strategy to cope with high light in *Chlamydomonas reinhardtii*. Plant Cell 25:545–557. https://doi.org/10.1105/tpc.112.108274

Andersen RA (2005) Algal culturing techniques, 1st edn. Elsevier, China

Andrade MR, Costa JAV (2007) Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. Aquaculture 264:130–134. https://doi.org/10.1016/j.aquaculture.2006.11.021

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress and signal transduction. Annu Rev Plant Biol 55:373–399

Asada K (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Biol 50:601–639

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol 59:89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Baldisserotto C, Popovich C, Giovanardi M et al (2016) Photosynthetic aspects and lipid profiles in the mixotrophic alga *Neochloris oleoabundans* as useful parameters for biodiesel production. Algal Res 16:255–265

Béchet Q, Muñoz R, Shilton A, Guieysse B (2013) Outdoor cultivation of temperature-tolerant *Chlorella sorokiniana* in a column photobioreactor under low power-input. Biotechnol Bioeng 110:118–126

Ben-Amotz A, Avron M (1990) The biotechnology of cultivating the halotolerant alga *Dunaliella*. Trends Biotechnol 8:121–126

Berteotti S, Ballottari M, Bassi R (2016) Increased biomass productivity in green algae by tuning non-photochemical quenching. Sci Rep 6:21339. https://doi.org/10.1038/srep21339

Blankenship RE (2014) Molecular mechanisms of photosynthesis. Wiley-Blackwell, Hoboken

Bonnecarrère V, Borsani O, Díaz P et al (2011) Response to photoxidative stress induced by cold in *Japonica* rice is genotype dependent. Plant Sci 180:726–732

Buch A, Archana G, Naresh Kumar G (2010) Heterologous expression of phosphoenolpyruvate carboxylase enhances the phosphate solubilizing ability of fluorescent pseudomonads by altering the glucose catabolism to improve biomass yield. Bioresour Technol 101:679–687. https://doi.org/10.1016/j.biortech.2009.08.075

Ceppi MG, Oukarroum A, Çiçek N et al (2012) The IP amplitude of the fluorescence rise OJIP is sensitive to changes in the photosystem I content of leaves: a study on plants exposed to magnesium and sulfate deficiencies, drought stress and salt stress. Physiol Plant 144:277–288

Cheirsilp B, Torpee S (2012) Enhanced growth and lipid production of microalgae under mixotrophic culture condition: effect of light intensity, glucose concentration and fed-batch cultivation. Bioresour Technol 110:510–516

Chen C-Y, Yeh K-L, Aisyah R et al (2011) Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol 102:71–81

Collos Y, Mornet F, Sciandra A et al (1999) An optical method for the rapid measurement of micromolar concentrations of nitrate in marine phytoplankton cultures. J Appl Phycol 11:179–184. https://doi.org/10.1023/A:1008046023487

Conversion LR (2014) Environmental growth chambers. http://www.egc.com/useful_info_lighting.php

Cruces E, Rautenberger R, Rojas-Lillo Y et al (2017) Physiological acclimation of *Lessonia spicata* to diurnal changing PAR and UV radiation: differential regulation among down-regulation of photochemistry, ROS scavenging activity and phlorotannins as major photoprotective mechanisms. Photosynth Res 131:145–157. https://doi.org/10.1007/s11120-016-0304-4

Cui Y, Zhang H, Lin S (2017) Enhancement of non-photochemical quenching as an adaptive strategy under phosphorus deprivation in the dinoflagellate *Karlodinium veneficum*. 8:1–14. https://doi.org/10.3389/fmicb.2017.00404

De-Bashan LE, Trejo A, Huss VAR et al (2008) *Chlorella sorokiniana* UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater. Bioresour Technol 99:4980–4989

Deblois CP, Dufresne K, Juneau P (2013) Response to variable light intensity in photoacclimated algae and cyanobacteria exposed to atrazine. Aquat Toxicol 126:77–84. https://doi.org/10.1016/j.aquatox.2012.09.005

Demmig-Adams B (1990) Carotenoids and photoprotection in plants: a role for the xanthophyll zeaxanthin. Biochim Biophys Acta (BBA)-Bioenergetics 1020:1–24

Demmig-Adams B, Garab G, Adams III, Govindjee W (eds) (2014) Non-photochemical quenching and energy dissipation in plants, algae and cyanobacteria. In: Advances in photosynthesis and respiration. Springer, Dordrecht

Dominici P, Caffarri S, Armenante F et al (2002) Biochemical properties of the PsbS subunit of photosystem II either purified from chloroplast or recombinant. J Biol Chem 277:22750–22758

Dring MJ, Lüning K (1985a) Emerson enhancement effect and quantum yield of photosynthesis for marine macroalgae in simulated underwater light fields. Mar Biol 87:109–117. https://doi.org/10.1007/BF00539418

Dring MJ, Lüning K (1985b) Emerson enhancement effect and quantum yield of photosynthesis for marine macroalgae in simulated underwater light fields. Mar Biol 87:109–117

Dubinsky Z, Berner T, Aaronson S (1978) Potential of large-scale algal culture for biomass and lipid production in arid lands. In: Biotechnology and Bioengineering Symposium. City University of New York, Flushing

Foyer CH, Lopez-Delgado H, Dat JF, Scott IM (1997) Hydrogen peroxide-and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. Physiol Plant 100:241–254

Frank HA, Young AJ, Britton G, Cogdell RJ (eds) (1999) Incorporation of carotenoids into reaction center and light-harvesting pigment-protein complexes. In: The photochemistry of carotenoids. Springer, Dordrecht, pp 235–244

Genty B, Briantais J-M, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92. https://doi.org/10.1016/S0304-4165(89)80016-9

Govindjee (1995) Sixty-three years since Kautsky: chlorophylla fluorescence. Aust J Plant Physiol 22:131–160

Govindjee (2004) Chlorophyll a fluorescence: a bit of basics and history. In: Papageorgiou G, Govindjee (eds) Chlorophyll a fluorescence: a probe of photosynthesis. Springer, Dordrecht, pp 2–42

Govindjee, Amesz J, Fork DC (eds) (1986) Light emission by plants and bacteria. Academic Press, Orlando

Graham JE, Bryant DA (2008) The biosynthetic pathway for synechoxanthin, an aromatic carotenoid synthesized by the euryhaline, unicellular cyanobacterium *Synechococcus sp*. strain PCC 7002. J Bacteriol 190:7966–7974. https://doi.org/10.1128/JB.00985-08

Grama BS, Agathos SN, Jeffryes CS (2016) Balancing photosynthesis and respiration increases microalgal biomass productivity during photoheterotrophy on glycerol. ACS Sustain Chem Eng 4:1611–1618. https://doi.org/10.1021/acssuschemeng.5b01544

Greenberg BM, Gaba V, Mattoo AK, Edelman M (1987) Identification of a primary in vivo degradation product of the rapidly-turning-over 32 kd protein of photosystem II. EMBO J 6:2865–2869

Grieco M, Tikkanen M, Paakkarinen V et al (2012) Steady-state phosphorylation of light-harvesting complex II proteins preserves photosystem I under fluctuating white light. Plant Physiol 160:1896–1910

Gwak Y, Hwang Y, Wang B et al (2014) Comparative analysis of lipidomes and transcriptomes reveal a concerted action of multiple defensive systems against photooxidative stress in *Haematococcus pluvialis*. J Exp Bot 65:4317–4334

He Q, Yang H, Wu L, Hu C (2015a) Effect of light intensity on physiological changes, carbon allocation and neutral lipid accumulation in oleaginous microalgae. Bioresour Technol 191:219–228. https://doi.org/10.1016/j.biortech.2015.05.021

He Q, Yang H, Xu L et al (2015b) Sufficient utilization of natural fluctuating light intensity is an effective approach of promoting lipid productivity in oleaginous microalgal cultivation outdoors. Bioresour Technol 180:79–87. https://doi.org/10.1016/j.biortech.2014.12.088

Hendrickson L, Furbank RT, Chow WS (2004) A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. Photosynth Res. https://doi.org/10.1023/B:PRES.0000040446.87305.f4

Ho SH, Chen CY, Chang JS (2012) Effect of light intensity and nitrogen starvation on CO 2 fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. Bioresour Technol 113:244–252. https://doi.org/10.1016/j.biortech.2011.11.133

Johnson MP, Davison PA, Ruban AV, Horton P (2008) The xanthophyll cycle pool size controls the kinetics of non-photochemical quenching in *Arabidopsis thaliana*. FEBS Lett 582:262–266. https://doi.org/10.1016/j.febslet.2007.12.016

Kalaji HM, Schansker G, Ladle RJ et al (2014) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. Photosynth Res 122:121–158. https://doi.org/10.1007/s11120-014-0024-6

Kalaji HM, Rastogi A, Živčák M et al (2018) Prompt chlorophyll fluorescence as a tool for crop phenotyping: an example of barley landraces exposed to various abiotic stress factors. Photosynthetica. https://doi.org/10.1007/s11099-018-0766-z

Kasajima I, Takahara K, Kawai-Yamada M, Uchimiya H (2009) Estimation of the relative sizes of rate constants for chlorophyll de-excitation processes through comparison of inverse fluorescence intensities. Plant Cell Physiol 50:1600–1616

Kasajima I, Ebana K, Yamamoto T et al (2011) Molecular distinction in genetic regulation of nonphotochemical quenching in rice. Proc Natl Acad Sci 108:13835–13840

Kattarath SS, Ramani DG (2017) Bioproduction and characterization of silver nanoparticles from microalgae Asteracys quadricellulares, its antimicrobial and antibiofilm activities. Int J Pharma Biosci 8:714–725

King RJ, Schramm W (1976) Photosynthetic rates of benthic marine algae in relation to light intensity and seasonal variations. Mar Biol 37:215–222

Kirchhoff H, Horstmann S, Weis E (2000) Control of the photosynthetic electron transport by PQ diffusion microdomains in thylakoids of higher plants. Biochim Biophys Acta (BBA)-Bioenergetics 1459:148–168

Klughammer C, Schreiber U (2008a) Saturation pulse method for assessment of energy conversion in PS I. PAM Appl Notes 11–14

Klughammer C, Schreiber U (2008b) Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the saturation pulse method. PAM Appl Notes 1:201–247

Kodru S, Malavath T, Devadasu E et al (2015) The slow S to M rise of chlorophyll a fluorescence reflects transition from state 2 to state 1 in the green alga *Chlamydomonas reinhardtii*. Photosynth Res 125:219–231. https://doi.org/10.1007/s11120-015-0084-2

Kraus D (2014) Consolidated data analysis and presentation using an open-source add-in for the Microsoft Excel® spreadsheet software. Med Writ 23:25–28. https://doi.org/10.1179/2047480613Z.000000000181

Lazár D (2015) Parameters of photosynthetic energy partitioning. J Plant Physiol 175:131–147. https://doi.org/10.1016/j.jplph.2014.10.021

Lichtenthaler HK, Burkart S (1999) Photosynthesis and high light stress. Bulg J Plant Physiol 25:3–16

Lichtenthaler H, Wellburn A (1983) Determinations of total carotenoids and chlorophylls b of leaf extracts in different solvents. Biochem Soc Trans 11:591–592. https://doi.org/10.1042/bst0110591

Liu X, Duan S, Li A, Sun K (2009) Effects of glycerol on the fluorescence spectra and chloroplast ultrastructure of *Phaeodactylum tricornutum* (Bacillariophyta). J Integr Plant Biol 51:272–278

MacIntyre HL, Kana TM, Anning T, Geider RJ (2002) Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. J Phycol 38:17–38

Melis A (2009) Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant Sci 177:272–280

Meteonorm (2016) Meteonorm: irradiation data for every place on Earth. Meteonorm Softw. Weather Station ans Satell. NREL TMY Dataset Downloads. http://www.meteonorm.com/en/downloads/documents

Mitra D, van Leeuwen J (Hans), Lamsal B (2012) Heterotrophic/mixotrophic cultivation of oleaginous *Chlorella vulgaris* on industrial co-products. Algal Res 1:40–48

Mojaat M, Pruvost J, Foucault A, Legrand J (2008) Effect of organic carbon sources and Fe2+ ions on growth and β-carotene accumulation by *Dunaliella salina*. Biochem Eng J 39:177–184. https://doi.org/10.1016/j.bej.2007.09.009

Munday JC Jr, Govindjee (1969) Fluorescence transients in Chlorella: effects of supplementary light, anaerobiosis, and methyl viologen. Prog Photosynth Res 2:913–922

Munekage Y, Hashimoto M, Miyake C et al (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. Nature 429:579–582. https://doi.org/10.1038/nature02598

Murchie EH, Hubbart S, Peng S, Horton P (2005) Acclimation of photosynthesis to high irradiance in rice: gene expression and interactions with leaf development. J Exp Bot 56:449–460. https://doi.org/10.1093/jxb/eri100

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Norici A, BAZZONI A, Pugnetti A et al (2011) Impact of irradiance on the C allocation in the coastal marine diatom *Skeletonema marinoi* Sarno and Zingone. Plant Cell Environ 34:1666–1677

Ozkaleli M, Erdem A (2018) Biotoxicity of titanium dioxide nanoparticles on *Raphidocelis subcapitata* microalgae exemplified by membrane deformation. Int J Environ Res Public Health 15:22–26. https://doi.org/10.3390/ijerph15030416

Patil S, Pandit R, Lali A (2017) Responses of algae to high light exposure: prerequisite for species selection for outdoor cultivation. J Algal Biomass Util 8:75–83

Pfündel EE, Latouche G, Meister A, Cerovic ZG (2018) Linking chloroplast relocation to different responses of photosynthesis to blue and red radiation in low and high light-acclimated leaves of *Arabidopsis thaliana* (L.). Photosynth Res. https://doi.org/10.1007/s11120-018-0482-3

Rabinowitch E, Govindjee (1969) Photosynthesis. Wiley, New York

Rochaix J-D (2016) The dynamics of the photosynthetic apparatus in algae. In: Applied photosynthesis-new progress. InTech

Rohacek K, Bertrand M, Moreau B et al (2014) Relaxation of the non-photochemical chlorophyll fluorescence quenching in diatoms: kinetics, components and mechanisms. Philos Trans R Soc B Biol Sci. https://doi.org/10.1098/rstb.2013.0241

Ruban AV (2016) Nonphotochemical chlorophyll fluorescence quenching: mechanism and effectiveness in protecting plants from photodamage. Plant Physiol 170:1903–1916. https://doi.org/10.1104/pp.15.01935

Salama AM, Pearce RS (1993) Ageing of cucumber and onion seeds: Phospholipase d, lipoxygenase activity and changes in phospholipid content. J Exp Bot 44:1253–1265. https://doi.org/10.1093/jxb/44.8.1253

Sarnaik A, Pandit R, Lali A (2017) Growth engineering of *Synechococcus elongatus* PCC 7942 for mixotrophy under natural light conditions for improved feedstock production. Biotechnol Prog. https://doi.org/10.1002/btpr.2490

Sathya S, Srisudha S (2013) Fatty acid and hydrocarbon composition in Asteracys quadricellulare (Behre). Indian J Appl Res 3:10–12

Schansker G, Tóth SZ, Holzwarth AR, Garab G (2014) Chlorophyll a fluorescence: beyond the limits of the QA model. Photosynth Res 120:43–58. https://doi.org/10.1007/s11120-013-9806-5

Schulze PSC, Barreira LA, Pereira HGC et al (2014) Light emitting diodes (LEDs) applied to microalgal production. Trends Biotechnol 32:422–430. https://doi.org/10.1016/j.tibtech.2014.06.001

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot. https://doi.org/10.1155/2012/217037

Shevela D, Bjorn L, Govindjee (2018) Photosynthesis: solar energy for life. World Scientific, Singapore

Simionato D, Basso S, Giacometti GM, Morosinotto T (2013) Optimization of light use efficiency for biofuel production in algae. Biophys Chem 182:71–78. https://doi.org/10.1016/j.bpc.2013.06.017

Singh SP, Häder D-P, Sinha RP (2010) Cyanobacteria and ultraviolet radiation (UVR) stress: mitigation strategies. Ageing Res Rev 9:79–90

Smith RT, Bangert K, Wilkinson SJ, Gilmour DJ (2015) Synergistic carbon metabolism in a fast growing mixotrophic freshwater microalgal species *Micractinium inermum*. Biomass Bioenerg 82:73–86. https://doi.org/10.1016/j.biombioe.2015.04.023

Solovchenko AE, Khozin-Goldberg I, Didi-Cohen S et al (2008) Effects of light intensity and nitrogen starvation on growth, total fatty acids and arachidonic acid in the green microalga *Parietochloris incisa*. J Appl Phycol 20:245–251

Stirbet A, Govindjee (2011) On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and photosystem II: basics and applications of the OJIP fluorescence transient. J Photochem Photobiol B Biol 104:236–257

Stirbet A, Govindjee (2012) Chlorophyll a fluorescence induction: a personal perspective of the thermal phase, the J–I–P rise. Photosynth Res 113:15–61

Stirbet A, Riznichenko GY, Rubin AB (2014) Modeling chlorophyll a fluorescence transient: relation to photosynthesis. Biochem 79:291–323. https://doi.org/10.1134/S0006297914040014

Strasser RJ, Srivastava A, Tsimilli-Michael M (2000) The fluorescence transient as a tool to characterize and screen photosynthetic samples. Probing Photosynth 445–483

Strasser RJ, Tsimilli-Michael M, Srivastava A (2004) Analysis of the chlorophyll a fluorescence transient. In: Chlorophyll a fluorescence. Springer, The Netherlands, pp 321–362

Subramanian G, Yadav G, Sen R (2016) Rationally leveraging mixotrophic growth of microalgae in different photobioreactor configurations for reducing the carbon footprint of an algal biorefinery: a techno-economic perspective. RSC Adv 6:72897–72904. https://doi.org/10.1039/C6RA14611B

Suzuki N, Koussevitzky S, Mittler RON, Miller GAD (2012) ROS and redox signalling in the response of plants to abiotic stress. Plant Cell Environ 35:259–270

Takahashi S, Badger MR (2011) Photoprotection in plants: a new light on photosystem II damage. Trends Plant Sci 16:53–60

Tanaka K, Mitsuhashi H, Kondo N, Sugahara K (1982) Further evidence for inactivation of fructose-1, 6-bisphosphatase at the beginning of SO_2 fumigation. Increase in fructose-1, 6-bisphosphate and decrease in fructose-6-phosphate in SO_2-fumigated spinach leaves. Plant Cell Physiol 23:1467–1470

Turpin DH, Elrifi IR, Birch DG, Weger HG, Holmes JJ (1988) Interactions between photosynthesis, respiration and nitrogen assimilation in microalgae. Can J Bot 66:2083–2097

Vadiveloo A, Moheimani NR, Cosgrove JJ et al (2017) Effects of different light spectra on the growth, productivity and photosynthesis of two acclimated strains of *Nannochloropsis* sp. J Appl Phycol 1–10. https://doi.org/10.1007/s10811-017-1083-9

Walters RG, Horton P (1991) Resolution of components of non-photochemical chlorophyll fluorescence quenching in barley leaves. Photosynth Res 27:121–133

Wang Z-H, Nie X-P, Yue W-J, Li X (2012) Physiological responses of three marine microalgae exposed to cypermethrin. Environ Toxicol 27:567–572. https://doi.org/10.1002/tox

Wei D, Chen F, Chen G et al (2008) Enhanced production of lutein in heterotrophic *Chlorella protothecoides* by oxidative stress. Sci China Ser C Life Sci 51:1088–1093. https://doi.org/10.1007/s11427-008-0145-2

Wilk L, Grunwald M, Liao P-N et al (2013) Direct interaction of the major light-harvesting complex II and PsbS in nonphotochemical quenching. Proc Natl Acad Sci 110:5452–5456

Yang C, Hua Q, Shimizu K (2000) Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light-autotrophic/dark-heterotrophic conditions. Biochem Eng J 6:87–102

Yang S, Liu G, Meng Y et al (2014) Utilization of xylose as a carbon source for mixotrophic growth of *Scenedesmus obliquus*. Bioresour Technol 172:180–185

Zhang Z, Sun D, Wu T et al (2017) The synergistic energy and carbon metabolism under mixotrophic cultivation reveals the coordination between photosynthesis and aerobic respiration in *Chlorella zofingiensis*. Algal Res 25:109–116. https://doi.org/10.1016/j.algal.2017.05.007

Zhao X, Chen T, Feng B et al (2017) Non-photochemical quenching plays a key role in light acclimation of rice plants differing in leaf color. Front Plant Sci 7:1–17. https://doi.org/10.3389/fpls.2016.01968

Zhekisheva M, Boussiba S, Khozin-Goldberg I et al (2002) Accumulation of oleic acid in *Haematococcus pluvialis* (Chlorophyceae) under nitrogen starvation or high light is correlated with that of astaxanthin esters. J Phycol 331:325–331

Zhou Y, Schideman LC, Park DS et al (2015) Characterization of a *Chlamydomonas reinhardtii* mutant strain with improved biomass production under low light and mixotrophic conditions. Algal Res 11:134–147. https://doi.org/10.1016/j.algal.2015.06.001

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