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The potential of quantitative models to improve microalgae photosynthetic efficiency

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The massive increase in carbon dioxide concentration in the atmosphere driven by human activities is causing huge negative consequences and new sustainable sources of energy, food and materials are highly needed. Algae are unicellular photosynthetic microorganisms that can provide a highly strategic contribution to this challenge as alternative source of biomass to complement crops cultivation. Algae industrial cultures are commonly limited by light availability, and biomass accumulation is strongly dependent on their photon-to-biomass conversion efficiency. Investigation of algae photosynthetic metabolism is thus strategic for the generation of more efficient strains with higher productivity.
Algae are cultivated at industrial scale in conditions highly different from the natural niches they adapted to and strains development efforts must fully consider the seminal influence on productivity of regulatory mechanism of photosynthesis as well as of cultivation parameters like cells concentration, light distribution in the culture, mixing, nutrients and carbon dioxide availability. In this review we will focus in particular on how mathematical models can account for the complex influence of all environmental parameters and can be exploited for development of improved algae strains.

Abbreviations – ASII, antenna size of PSII; Chl, chlorophyll; $^3Chl^*$, triplet chlorophyll state; CpSRP, chloroplast signal recognition particle; GHG, greenhouse gases; LHC, light harvesting complexes; LTD, LHC translocation defect proteins; NPQ, non-photochemical quenching; $^1O_2$, singlet oxygen; PAM, pulse-amplitude modulation; PBCE, photon-to-biomass conversion efficiency; PBR, photobioreactor; PSII, photosystem II; PSI, photosystem I; PSU, photosynthetic units; RNAi, RNA interference; TLA, truncated light-harvesting antenna; VDE, violaxanthin de-epoxidase; ZE, zeaxanthin epoxidase.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ppl.12915

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Potential of microalgae as source of biomass

Current global economy is causing a massive release of greenhouse gases (GHG) and other pollutants in the atmosphere (Gies 2017) with heavy negative consequences (Walsh et al. 2015). Photosynthetic organisms use sunlight to fix CO_2 into biomass and their exploitation as source of energy, food and other bio-commodities can provide a strong contribution in mitigating GHG emissions. An increased demand for biomass, however, cannot be satisfied by plants alone (Rulli et al. 2016) motivating the search for alternative sources. Photosynthetic organisms like microalgae have an interesting potential and a strong complementarity with crops since they do not need arable land for their cultivation, they can use civil and industrial wastewaters as nutrient sources and recycle CO_2 released from industrial processes (Salih 2011).

Microalgae are a group of organisms with a large genetic and metabolic diversity and their biomass can represent a valuable feedstock for food/feed or biofuels production. They also contain many valuable molecules like pigments (i.e. carotenoids and keto-carotenoids) and fatty acids (i.e. omega-3 fatty acids), showing several applications already commercialized in nutraceutical and pharmaceutical markets (Bilal et al. 2017, Ng et al. 2017, Koutra et al. 2018, Maeda et al. 2018, Renuka et al. 2018). Algae-base products are however still not competitive in larger markets since costs for biomass production, harvesting and processing are still too high (Acién Fernández et al. 2012, Lam et al. 2018). One unavoidable challenge to make industrial production of algae biomass competitive in large markets and maximize their potential environmental benefits is to improve their productivity upon cultivation at large scale. Here, algae are very often limited by light availability, thus making photon-to-biomass conversion efficiency (PBCE) highly strategic for competitiveness of all algae-based products (Ruiz et al. 2016). Microalgae in lab scale conditions have a PBCE around 3%, higher than most crops in the field (i.e. 0.2-1 %; Melis 2009, De Vree et al. 2015). This is not due to a more efficient metabolism per se but to other factors like having the whole biomass photosynthetically active unlike plants. Moreover, crops are photosynthetically active only for few months in temperate climates, limiting the total biomass producible per year. Algae cultivated at large scale, however, are less performant than in lab with a lower PBCE (i.e. 0.5-1 %; De Vree 2015)) and the overall productivity is thus not sufficient to compensate for higher energetic and monetary costs for such large scale cultivation, thus limiting commercial competitiveness (Park et al. 2016, Carneiro et al. 2017). Research efforts in investigating algae photosynthetic metabolism, assessing how it responds to large-scale cultivation conditions and identifying strategies to improve PBCE are thus seminal to drive new applications of algae biomass to the market.

How microalgae photosynthetic metabolism is influenced by intensive cultivation

Microalgae industrial cultivation is performed in open or closed systems, called respectively ponds and photobioreactors (PBR; De Vree 2015), where they are exposed to full sunlight and cultivated at

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high concentrations to maximize biomass productivity. Consequently, cultures have a high optical density that causes an inhomogeneous distribution of the light, with cells at the surface absorbing most incident radiation, leaving inner layers in light limitation (Fig. 1A). Such an inhomogeneous distribution has major consequences on the photosynthetic metabolism. If light is limiting, cells do not have enough energy to synthesize ATP/NADPH, feed the Calvin-Benson cycle, and sustain high biomass growth rate. The light intensity can even go below the compensation point where energy used for cell maintenance is higher than that available for CO_2 fixation (Fig. 1B). The need to avoid extreme light-limitation and to prevent part of the cells with a null or even negative productivity limits the cultures depth for algae large scale cultivation. Indeed, although specific limits depends on biomass concentration and cultivation conditions, culture depth normally do not go beyond 20 cm (Chisti 2016).

On the other extreme, cells exposed to full sunlight very often experience excess illumination (Fig. 1B). As an example, in the eukaryotic alga *Nannochloropsis*, photosynthesis is saturated at ≈ 150 µmol photons m^{-2} s^{-1} (Sforza et al. 2012), a value well below intensities reached by full sunlight (500-2000 µmol photons m^{-2} s^{-1} in temperate climates depending on the season). Even if saturating, sunlight is still efficiently absorbed by cells, where it generates excited chlorophyll states (triplet state chlorophyll, $^3Chl^*$) that are not usable for photochemical reactions. Accumulation of excited chlorophyll (Chl) leads to the formation of reactive oxygen species (such as singlet oxygen, $^1O_2$), harmful molecules that can oxidizes pigments, proteins and lipids, with D1 subunit of photosystem II (PSII) being a major target (Szabó et al. 2005, Murata et al. 2007; Fig. 1B).

Microalgae, as all photosynthetic organisms, evolved multiple mechanisms to cope with different light regimes to ensure their survival in natural environments where illumination is highly variable. Damaged protein D1 is constantly degraded and re-synthesized to restore photosynthetic activity (Kato et al. 2012). Antioxidant molecules, such as carotenoids or tocopherol, are also accumulated to scavenge reactive molecules like $^3Chl^*$ or $^1O_2$ (Stahl et al. 2003). Another protection mechanism, called non-photochemical quenching (NPQ), drives the non-radiative de-excitation of excited chlorophyll states, resulting in a dissipation of excess energy as heat. Carotenoids are important for detoxification of reactive species but are also involved in NPQ activation in algae, with zeaxanthin playing a major role (Kuczynska et al. 2015). Zeaxanthin is synthesized upon exposition to strong light from violaxanthin by Violaxanthin De-Epoxidase (VDE). Once cells return in limiting conditions, zeaxanthin is converted back to violaxanthin thanks to the activity of the enzyme Zeaxanthin Epoxidase (ZE), closing the so-called xanthophyll cycle.

Regulatory mechanisms of photosynthesis have a fundamental biological role but also impact PBCE. PSII repair requires the investment of energy for protein synthesis and can represent a major sink of nutrients for algae (Melis et al. 2000). Photoprotection mechanisms like NPQ are important to reduce photo-damage, but they achieve this objective by dissipating excitation energy as heat and thus cause a decrease in PBCE (Fig. 1B). If the objective is to maximize PBCE and biomass productivity, thus, the

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optimal balance between efficiency and protection from eventual light excess for the specific growth conditions must be found.

Algae cultures in large scale outdoor cultivations are actively mixed to ensure gas exchanges and avoid CO_2 limitations or oxygen accumulation (Brennan et al. 2010). Mixing kinetics are normally faster in closed PBR (i.e. ms/seconds time-scale) than in open ponds (i.e. seconds / minutes time scale; (Molina et al. 2001, Carvalho et al. 2011) but the precise values are strongly dependent from the specific design of the cultivation system. A consequence of mixing in all systems is that cells can abruptly move between different layers of the culture and be exposed to drastically different light conditions without having sufficient time to activate regulatory mechanisms (Eberhard et al. 2008). As example, zeaxanthin accumulation requires a few minutes of exposition to intense illumination (Nilkens et al. 2010, Murchie et al. 2011) and if cells are mixed faster, those in limiting light will not have time to activate photoprotection before being exposed to saturating conditions, leading to photoinhibition and a decrease in productivity. On the other hand, zeaxanthin epoxidation is even slower and takes several minutes to be completed (Goss et al. 2010). If cells exposed to excess light accumulate zeaxanthin when they move to a deeper part of the PBR, where light is limiting, they will still be in a photoprotective state, dissipating a significant fraction of the absorbed light as heat and thus losing efficiency.

Increasing amount of evidences showed that fluctuating light indeed has a strong impact on algae performances (Matthijs et al. 1996, Nedbal et al. 1996, Vejrazka et al. 2011, Sforza 2012) but still there is not enough information on how algae respond and perform in a fluctuating light environment to understand which mixing timescales are optimal to maximize productivity.

Genetic engineering to improve algal photosynthesis

Considerations above suggest the optimization of photosynthetic metabolism to the artificial environmental conditions could provide a significant enhancement of algae biomass productivity. In this case the priority should be overall population growth rather than the survival of single individuals, thus with a distinct difference from natural conditions, where selection pressure would prioritize the latter. In balancing photoprotection and efficiency, in fact, a unicellular organism should maximize survival chances even at the expense of the efficiency or growth rate. During industrial cultivation instead, strains should be optimized to have the maximal efficiency, even if this implies additional risk of exposition to strong stress for a fraction of the culture. Genetic engineering represents a valuable strategy to domesticate microalgae photosynthetic metabolism, modifying traits positively selected in the natural environment for the survival of the fittest, but detrimental for maximal productivity in the specific artificial PBR conditions.

One example of such approach is the light harvesting apparatus. In a natural environment, algae need to harvest as much light as possible and there is no selective advantage in leaving energy available for other cells. Algae in fact have large macromolecular complexes comprising different pigments binding

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proteins, called antenna or light harvesting complexes (LHC), which serve the biological functions of harvesting photons and transfer their energy to the reaction centres of both photosystems (Kirk 1994). On the contrary, in intensive cultivation conditions each cell should ideally only harvest enough energy to efficiently run photochemistry and the reduction in size of antenna complexes has indeed been proposed as strategy to reduce culture optical density without affecting the photosystems ability to perform photochemistry (Fig. 2). The expected benefits of this approach should be twofold: first, cells at the PBR surface should saturate photosynthesis at higher irradiances, reducing energy losses due to damage and heat dissipation and second, cells populating deeper layers should improve their productivity due to a more uniform light distribution in the mass culture (Simionato et al. 2013).

Efforts to improve light distribution identified also the reduction of Chl content per cell as a valuable strategy to improve light penetration while maintaining biomass growth (Bonente et al. 2011, Perin et al. 2015). Most efforts, however, aimed at generating microalgae strains with reduced antenna size (called truncated light-harvesting antenna (TLA) mutants) targeting LHC encoding genes with insertional mutagenesis and RNAi approaches (Melis et al. 1998, Nakajima et al. 2000, Nakajima et al. 2001, Nakajima et al. 2003, Polle et al. 2003, Bonente 2011, Perrine et al. 2012, Cazzaniga et al. 2014, Perin 2015). More recent efforts proved that also molecular factors involved in either the co-translational or post-translational regulation of antenna proteins (e.g. NAB1 repressor; Beckmann et al. 2009, Wobbe et al. 2009), genes involved in Chl biosynthetic pathway (Polle et al. 2000), chloroplast signal recognition particles (CpSRPs; Kirst et al. 2012b, Kirst et al. 2012a, Kirst et al. 2014) and LHC translocation defect proteins (LTD; Jeong et al. 2018), represent valuable targets to generate a TLA phenotype by indirectly controlling LHC expression and assembly (Formighieri et al. 2014). Mutants isolated so far proved that TLA strains have increased photosynthetic activity and reach saturation of photosynthesis at higher irradiances than parental ones (Beckmann 2009), with beneficial effects on growth demonstrated in small-scale experiments. This approach has been largely pursued with the model organism *C. reinhardtii* but it is expanding in other species with larger industrial potential (Cazzaniga 2014, Perin 2015), also thanks to recent developments in genome engineering methods (Wang et al. 2016, Poliner et al. 2018).

One complementary strategy to improve photosynthesis is to optimize photo-regulatory mechanisms (Cardona et al. 2018). In *C. reinhardtii*, it has been suggested that reducing NPQ capacity can improve light-use efficiency (Berteotti et al. 2016). A massive decrease in NPQ, however, could also have negative effects with cells unable to withstand intense illumination with an increased photoinhibition for more exposed layers. One promising possibility is to target not NPQ intensity but its induction/relaxation kinetics (Fig. 2), a strategy proven to enhance photosynthetic activity and biomass accumulation in tobacco plants on the field (Kromdijk et al. 2016). Such approach could help NPQ activation and deactivation to follow light dynamics, including those due to mixing, enabling cells to respond faster to light intensity changes, while maintaining their photoprotection ability.

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Efforts in improving photosynthetic efficiency have been so far concentrated on the regulation of light harvesting without considering electron transport reactions. However, a large set of recent evidences showed that mechanisms to modulate photosynthetic electron transport, like cyclic and pseudo-cyclic, have a major role in plants, eukaryotic algae and cyanobacteria response to light dynamics and particularly to fluctuating light conditions (Cardol et al. 2011). The interest of exploring such strategies is supported by the observation that when electron transport chain is over-saturated it damages PSI (Tiwari et al. 2016) that, unlike PSII, does not have an efficient repair mechanism and thus causes a major impact on growth (Larosa et al. 2018). Interestingly, regulatory mechanism for photosynthetic electron transport are found in all photosynthetic organisms but are not conserved (Alboresi et al. 2018), opening the possibility of modifications using synthetic biology approaches. It is thus possible to imagine that in a new artificial environment the modulation of electron transport could be optimized to improve efficiency (Fig. 2). As example, in cyanobacteria, some eukaryotic algae and plants, pseudo-cyclic electron transport is active and avoids over-reduction of the electron transport chain in fluctuating light conditions by redirecting electrons from NADPH to oxygen (Yamamoto et al. 2016, Alboresi 2018, Gómez et al. 2018). This activity could be beneficial to face the light fluctuations in PBR, improving cell tolerance to such conditions. Pseudo-cyclic electron transport by donating electron back to oxygen, however, represents a loss in efficiency and, as discussed above, the best balance between photo-protection and efficiency should be achieved.

Model-based approaches to generate strains with improved performances

The strategies discussed above aim at improving algae productivity in large scale cultivation by altering the composition and regulation of photosynthetic apparatus. The recent development of efficient genome editing approaches is opening the possibility to obtain any desired modification with high efficiency and precision (Nymark et al. 2016, Ferenczi et al. 2017, Jeong 2018, Poliner 2018). As discussed above, however, genetic modifications of photosynthesis can yield both positive and negative effects and to effectively improve algal PBCE in industrial conditions any increase in efficiency must outweigh all eventual decreases in photoprotection ability. Thus, to be effective, targeted genetic modifications need a preliminary knowledge on which genotype would allow achieving maximal productivity in the specific growing conditions. This is not a trivial task, considering the complexity of regulatory mechanisms of photosynthesis and their interaction with the environment described in Fig. 1. The cultivation conditions indeed have been shown to strongly influence biological properties and performances of genetic engineered microalgae strains, enhancing or masking the advantages of modifications isolated in the lab (Perin et al. 2017b). This observation helps understanding why promising strains showed contrasting results when cultivated at larger scale (Cazzaniga 2014, De Mooij et al. 2014).

Finding an improved balance between efficiency and photoprotection is clearly a complex problem where multiple parameters must simultaneously be accounted for. A further layer of complexity is that

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some of the most relevant phenomena affecting algae productivity are only measurable at a large scale and thus identifying the right genetic targets by a trial and error approach is ineffective and extremely time consuming.

Mathematical models can be very helpful to deal with this complexity by allowing the quantification of the impact of each parameter on productivity. Methods capable of quantifying the influence of environmental parameters on microalgae physiology could in fact be exploited to identify the genetic traits with the potential largest impact on productivity and use this information to identify the most promising targets for genetic modification.

Mathematical models have been developed to describe biological systems such as bacterial growth and optimize their productivity in industrial fermenters (Almquist et al. 2014, Esser et al. 2015). Describing photosynthetic organisms, however, requires the ability to also include light as a major parameter. Several modelling approaches have been proposed in the literature (for a detailed description see reviews by (Schirmer et al. 2016, Darvehei et al. 2018)). Some models aim at bulk description of microalgae growth in industrial systems taking into account the effects of all most relevant environmental parameters (e.g. Quinn et al. 2011, Bernard et al. 2012, Béchet et al. 2015). Other models focus on a more detailed description of specific parameters such as nutrients (e.g. Droop 1968, Flynn 2002), temperature (e.g. Rosso et al. 1993, Franz et al. 2012), and, most importantly, light (e.g. Grima et al. 1994, Rubio et al. 2003, Bernardi et al. 2014). Some models not only describe the static effect of light on growth, but aim at representing the dynamics of photosynthesis, accounting for regulation and inhibition (Eilers et al. 1988, Han et al. 2000). This idea was then further exploited and validated to model the dynamics of photo-inhibitory and photo-regulatory phenomena (Nikolaou et al. 2015, Bernardi et al. 2016), also including a description of some major photosynthetic features influencing microalgae growth such as pigments content, antenna size and NPQ.

The latter models, which are capable of capturing the key photosynthetic dynamics, can be exploited to estimate how photosynthetic activity changes in distinct parts of the culture, starting from the light distribution and the dependence of photosynthetic performances from illumination (Simionato et al. 2011, Alboresi et al. 2016, Meneghesso et al. 2016, Cordara et al. 2018; Fig. 3). Such approach allows estimating that cells exposed to full sunlight can experience a strong photo-inhibition, which can affect up to 60 % of photosynthetic units (PSU). However, such phenomenon is only relevant for the first centimetre of culture and cells beneath do not experience any substantial photo-damage (Fig. 3A). Most exposed cells are also predicted to activate photoprotection mechanisms to counteract photoinhibition (Fig. 3B). NPQ activation also only involves cells in the first centimetre of culture, while the rest can potentially use all the harvested energy for photochemistry with higher efficiency.

On the other hand, inner PBR layers are light-limited and, in the last two centimetres of culture, cells do not receive enough light to run photochemistry with respiration rate higher than photosynthetic oxygen evolution (grey area in Fig. 3C). These data allow estimating that in the specific conditions applied here, the first centimetre of culture absorbs approx. 60 % of the available light but also has

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limited efficiency because photosynthesis rate is saturated (Fig. 3C), and thus a large fraction of energy is lost due to photoinhibition and heat dissipation. Data presented are a specific example based on several assumptions on culture density, strains properties, illumination intensity and do not consider culture mixing, as detailed in Fig. 3. Nevertheless, they represent a clear example of how the photosynthetic metabolism is in significantly different states within the same culture.

Comprehensive models capable of including all major parameters influencing algae productivity are still missing since they need to account for many phenomena spanning numerous time-scales, from milliseconds in case of photochemical reactions, to days in modulation of photosynthetic apparatus composition during acclimation. Even if some major parameters influencing productivity at scale are not included, however, it is already possible to exploit available models to simulate in silico hypothetical genetic modifications and their impact on growth to obtain valuable predictions, such as the identification of Chl content per cell as a major parameter to be addressed by genetic modifications (Perin et al. 2017a).

In the example reported in Fig. 4, models describing biomass productivity for *Nannochloropsis gaditana* are exploited to predict the influence of different seasons, cultivation sites and PBR geometries. The model considered in this example combines a model describing photosynthetic productivity as function of the available light (Bernardi 2016) with the mathematical description of the light attenuation profile in a microalgae dense culture (Yun et al. 2001). In order to calculate the biomass concentration in the PBR, the volume has been divided in ten different sub-regions based on the distance from the illuminated surface. In each sub-region, the light was assumed to be constant and equal to the value in the median point to calculate the growth rate constant. The overall biomass concentration has then been calculated as the volumetric mean of the biomass concentration in the different sub-regions. The different PBR geometries considered are reflected in the volume of the sub-regions (Fig. 4A). This model represents a state-of-the-art model and its predictions have been validated against PAM fluorescence and growth experiments in lab-scale PBR irradiated with constant light showing excellent agreement with the experimental data (Perin 2017a).

It should be underlined that predicted productivities have a margin of uncertainty related to the parameter values, which are always known with a certain confidence level. Furthermore, the model considered in the example reported does not include the description of photo-acclimation, nutrient limitation or temperature effects. Clearly expanding available models to include more input variables will increase their value; for example, photo-acclimation modelling is likely to increase reliability for outdoor predictions (Bernardi et al. 2017).

Bearing in mind such caveats, it is still highly interesting to observe that even if they are still incomplete, available models can be exploited to make valuable predictions. As example, estimation of productivities in different sites combined with the use of different PBR geometry can be highly valuable to plan large scale cultivation systems (e.g. deeper cultures show a lower productivity, even in lower latitudes and summer season; Fig. 4A).

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These models also include the description of several photosynthetic parameters and thus they can be exploited to predict the effect of different photosynthesis modifications, identifying the ones with the largest positive impact on productivity. Different mutant strains can be simulated by varying the model parameters related to chlorophyll content, antenna size of PSII (ASII) and NPQ (Fig. 4B). At least in lab scale experiments, thus in conditions minimizing the effects of parameters not included in the mathematical description like nutrients limitation and temperature changes, such models were shown to be able to predict the impact of genetic modifications with excellent agreement with the experimental data (Perin 2017a). In principle, it is also possible to include the effect of equipment design and operation parameters on a specific strain, looking for optimal conditions that yield the best increase in performances (Fig. 4B).

As said above, however, no comprehensive model is available and in order to further implement the potentiality of such quantitative methods to drive genetic engineering, current models focusing on specific parameters should be expanded with a proper multiscale representation of all major phenomena occurring in a complex large-scale environment. Models will need to be implemented to account for the effect of all the variables affecting algae performances beyond light availability, thus including CO_2/nutrients availability, temperature, and so on. For instance, to assess (and to optimize) the effect of light and dark cycles in industrial equipment, a detailed description of the fluid flow behaviour (e.g. see (Gao et al. 2018)) should be coupled to a representation of all key phenomena affecting microalgae photosynthetic response.

The definition of such comprehensive multiscale models poses however several challenges also in terms of experimental capabilities and reliable calibration. In fact, the effectiveness of such modelling approaches relies on a trade-off between the inclusion of the complexity of all physical-chemical-biological phenomena of interest (i.e. comprehensiveness), and the model mathematical identifiability, i.e. the possibility to estimate all the model parameters in a statistically meaningful way, thus retaining their physical significance. Physical significance in the model equations also ensures transferability, meaning that equations developed for a species can be exploited for others with an easy re-parametrization with species-specific experimental data.

Developing models with mathematical identifiability is one of the main challenges of biological phenomena, since the information on the underlying molecular mechanisms and the number of available experimental data points is usually limited. To address these challenges, the ability to design the experiments maximising the information that may be collected (Bernardi 2016), and to efficiently collect large experimental dataset testing the effect of multiple parameters will be strategic. In this perspective, the development of micro-scale devices (Kim et al. 2014, Luke et al. 2016, Perin et al. 2016) is opening the possibility to investigate the effect of multiple variables (e.g. light intensity, CO_2 and nutrients availability, temperature) on microalgae physiology, thus reducing experimental time and costs, and to rapidly tune models to different strains. These devices operating in a microliter-scale are the ideal systems to assess the influence of single parameters for instance allowing assessing light

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impact minimizing influence of cells shading. At the same time micro-scale devices are not able to represent other relevant phenomena such as hydrodynamic effects in a large culture that need to be assessed using complementary strategies.

Overall, developing comprehensive models capable of reliably describing the impact of all relevant parameter on algae productivity in large scale cultivation systems is a huge challenge considering the complexity of environmental conditions and the regulation of photosynthetic metabolism. These mathematical models, however, have a unique potential as a tool to optimize microalgae performances in an industrial cultivation and identify the most relevant targets for genetic engineering approaches. As exemplified in Fig. 4, even incomplete models can provide approximate estimations that can be extremely helpful in driving improvement in algae productivity.

Author contributions

G.P. and T.M. drafted the manuscript. G.P., A. Bellan and A. Bernardi contributed to figures preparation. All authors contributed to the writing of the manuscript and approved the final version.

Acknowledgments – TM acknowledges the financial support by the European Research Council (BIOLEAP grant no. 309485). The authors have no conflict of interest to declare.

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Figure Legends

Fig. 1. Schematic representation of the inhomogeneous light distribution in PBR and consequences on photosynthesis and photo-regulation. (A) High optical densities in PBR are responsible for an inhomogeneous light distribution in algae cultures. Cells on the surface are exposed to full illumination (represented by a paler colour) while the rest of the culture is in limiting light (darker culture). The light attenuation profile, expressed as transmittance (T; i.e. the ratio between transmitted and incident light intensities), is reported as a function of depth (in centimetres, cm), exploiting experimental data collected for *Nannochloropsis* cultures at 1 g l^{-1} concentration in cylindric PBR with 5 cm diameter (Perin 2017b). (B) The profile is expected to be affected by cells concentration and PBR geometry. Because of the inhomogeneous light distribution, some cells are exposed to full irradiation, perform CO_2 fixation at their maximal capacity but also waste part of the energy to repair damaged photosystems as well as in form of heat to reduce oxidative stress. Cells in inner layers are left in limiting light with reduced CO_2 fixation, potentially going below the compensation point where respiration and photosynthesis rates are equivalent.

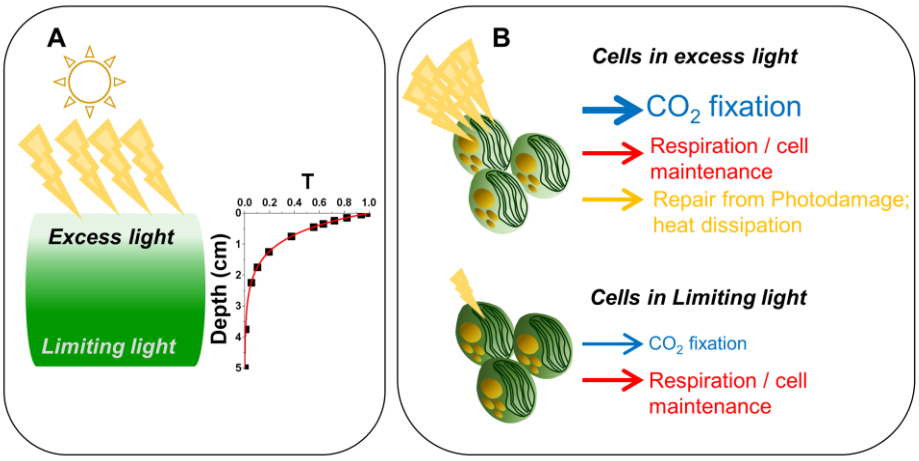


Fig. 2. Schematic representation of potential genetic targets to improve algae light-use efficiency. Photosynthesis components are here schematized as core complex and light-harvesting complexes (LHC). Red solid arrow indicates linear electron flow from photosystem II (PSII) to photosystem I (PSI), leading to the generation of reducing power (NADPH). Light-harvesting complexes stress related (LHCSR) are involved in NPQ in algae, dissipating excess energy as heat (red wave). The green dashed box includes the xanthophylls cycles (i.e. synthesis of zeaxanthin (Zx) in excess light catalysed by VIOLAXANTHIN DE-EPOXIDASE (VDE) and reconversion of Zx in violaxanthin (Vx) in limiting light catalysed by ZEAXANTHIN EPOXIDASE (ZE)) whose kinetics control NPQ induction/relaxation. Blue solid arrow indicates cyclic electron flow around PSI. Dashed blue arrow indicates pseudo-cyclic electron flow which redirects electrons downstream PSI to water. As detailed in the text, targets for improvement of algae photosynthetic efficiency proposed in the literature are:

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Chl content per cell, accumulation of LHC proteins, NPQ capacity, modulation of xanthophyll cycle /NPQ photoprotection induction/relaxation kinetics, optimization of electron flows.

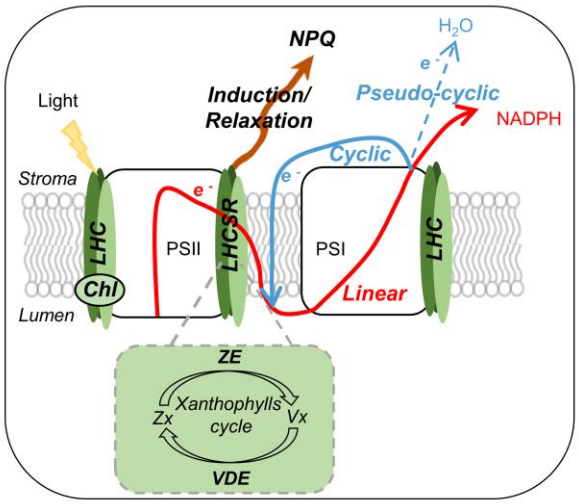
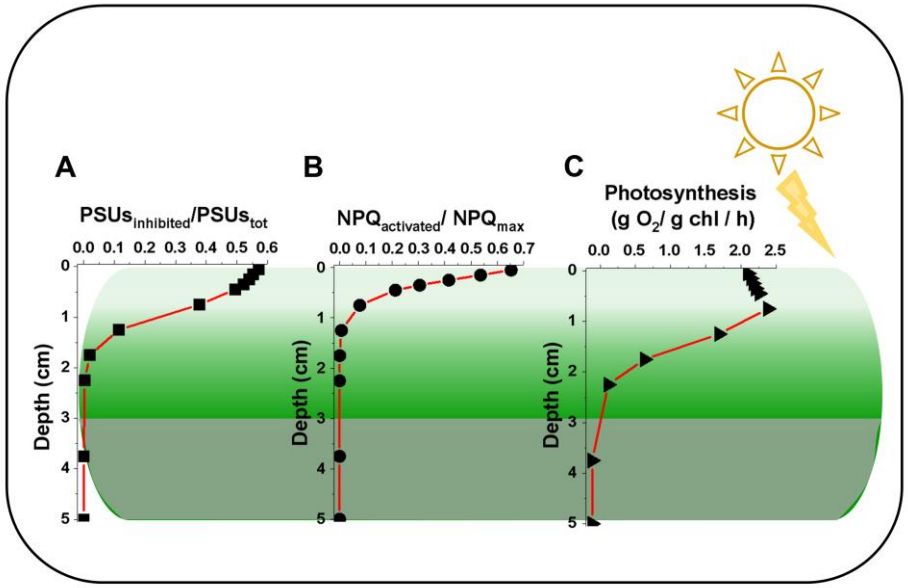


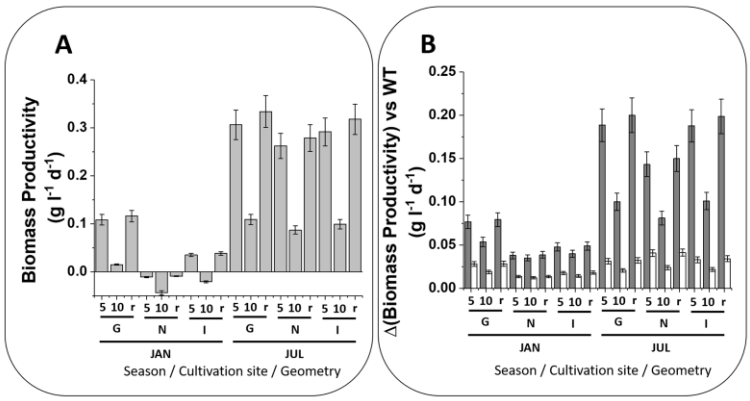
Fig. 3. Quantitative description of photosynthetic activity and induction of photo-regulation in PBR. Prediction of (A) photoinhibition level, expressed as fraction of photo-inhibited PSUs out of the total, (B) NPQ activation, as percentage of its maximal activity, and (C) photosynthetic activity, expressed as oxygen evolution (Perin 2015) for different PBR layers, as function of the PBR depth in centimetres (cm). Predictions come from the model developed in (Perin 2017a) and refer to *Nannochloropsis* cultures with a biomass concentration of 1 g l^{-1} in cylindric lab PBR with 5 cm diameter exposed to 1500 µmol photons m^{-2} s^{-1}. Cultures were kept in semi-continuous mode and thus acclimated to such conditions. Nutrients and CO_2 were provided in excess ensuring that growth was only dependent on PBCE. Photosynthetic activity in different PBR layers was predicted from oxygen evolution as function of incident radiation, later converted as function of the PBR depth according to the light attenuation profile of Fig. 1. The grey area indicates values below the compensation point.



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Fig. 4. Prediction potential of mathematical models. (A) Biomass productivity predictions (g l^{-1} d^{-1}) during a day of cultivation for *Nannochloropsis gaditana* WT strain exposed to irradiances of different seasons/latitudes, assuming a starting biomass concentration of 1.3 g l^{-1}. Error bars represent the estimated uncertainty of different model parameters values. (B) Increase in biomass productivity for two photosynthetic mutants with respect to the parental strain. Mutant #1 (grey) and #2 (white) show 100/5 % NPQ, 75/95 % ASII and 60/85 % Chl content of the parental strain, respectively. Prediction are here represented as a function of season (January (JAN) and July (JUL)), cultivation site (Gibraltar (G), The Netherlands (N), Italy (I)) and PBR geometry (cylindrical with 5 (5), 10 (10) cm diameter or rectangular (r) 4 x 10 cm). For the specific irradiation profile at the different cultivation sites we referred to the following website: http://re.jrc.ec.europa.eu/pvgis/apps4/pvest.php#. Latitude: 36.1 (Gibraltar), 52.4 (The Netherlands), 45.4 (Italy); Longitude: 5.3 (Gibraltar), 4.9 (The Netherlands), 11.9 (Italy).



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